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Protective effects of Vitamin E against Zinc Oxide nanoparticle--induced Oxidative stress, genotoxicity, and histotoxicity of hepatic and testicular tissues in male albino rats.

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Abstract

Nano zinc oxide has the potential to be harmful, so it is important to assess its effects on human health and the biological system. In the current study, vitamin E (100 mg/kg) was used to explore its antioxidant role in mitigating the potential toxicity of ZnO NPs (50 and 200 mg/kg) in male albino rats' tissues. ZnO NPs were synthesized and characterized by transmission electron microscope (TEM). Sixty adult male albino rats weighing 180-200 g were arbitrarily divided into six groups: G1: control group, G2: vita E (100 mg/kgb.w) group, G3: Zn NPs (50 mg/kg b.w) group, G4: Zn NPs (200mg/kg b.w), G5: vita E + Zn NPs(50 mg/kg b.w) group, and G6:Vita E + Zn NPs (200mg/kg b.w) group. Giving both Vita and ZnO NPs daily by oral gavage for 4 weeks. Detailed analysis of the liver and testicular tissue of rats treated with 50 and 200 mg/kg ZnO NPs revealed many adverse effects of nanoscale particles on tissue structure, accompanied by focal necrosis, inflammatory cellular infiltration in liver tissues, and distorted seminiferous tubules with disorganized germ cells in testicular tissues, increased lipid peroxidation, DNA damage, and reduced levels of antioxidant enzymes. Due to their tiny size, which allows them to penetrate physiological barriers, ZnO NPs can enter, translocate within, and damage living organisms. Nevertheless, co-administration of ZnO NPs with Vita E significantly ($p<0.05$) reversed the biochemical alterations associated with ZnO NPs administration and led to improvement of the histopathological picture of hepatic and testicular tissues. Findings related to Vita E may either inhibit the activity of (ROS) molecules and prevent their binding to the DNA structure and /or scavenging peroxyl lipid radicals, inducing DNA-damaging products. So, the present results indicated that Vita E effectively attenuates the adverse effects of ZnO NPs and could mitigate or prevent their toxicity.

Keywords: Zinc nanoparticles - Vitamin E - comet assay -stress markers -optical microscopy

1. Introduction

ZnO NPs are nanomaterials demanded in biomedicine, agriculture, and industry due to their easy invasion of human body tissues [1]. In addition, there are question marks about the potential applications of ZnO NPs in biomedical fields, such as molecular diagnostics, drug delivery systems, and cancer treatment, and some questions about the biosafety of ZnO NPs [2].

It was found that ZnO NPs boost the generation of reactive oxygen species (ROS) that disrupts the antioxidant system and intracellular metabolic activities. The size and surface area of nanoparticles enhance their capability to produce ROS [3]. Reactive oxygen species (ROS) cause damage to cell

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membranes and macromolecules through lipid peroxidation, oxidative stress, DNA fragmentation, and protein oxidation [4].

ZnO NPs have harmful effects on human health, particularly in the reproductive system. Understanding the impacts of this nanometal on the male reproductive organs will help realize and solve the problem of male infertility. Little studies exist on the changes that ZnO NPs can induce in the rat testicular tissues [5; 6]. According to the research, the liver is the primary organ where ZnO NPs accumulate. This resulted in alterations in the expression activity of hepatic enzymes and histological changes in rats' liver tissues [4]. Rats were chosen as the in vivo experimental animals due to their resemblance to human biochemical, metabolic, and physiological processes [7]. Low dosages of ZnO NPs (5 mg/kg b.wt and 20 mg/kg b.wt) benefit male rats' reproduction and improve their antioxidative and growth performance [8, 9]. Several studies, however, found that ZnO NPs caused testicular toxicity in doses ranging from 50 - 350 mg/kg b.wt at rodents testes [10; 11].

Low doses of ZnO NPs have a noticeable hepatoprotective effect on liver tissues by promoting antioxidant activities that detoxify free radicals [12]. The oral administration of high dosages of ZnO NPs (1–5 g/kg b.wt) causes harmful effects and damages the liver tissues [13].

Nanoparticle toxicity could be estimated by biochemical changes that are considered determinant factors. Huang et al. (2001) and Sharma et al. (2009) [14,15] reported that the DNA-damaging ability of ZnO NPs was associated with ROS generation, which triggers genotoxicity in cells exposed to ZnO NPs. Vitamin E is an antioxidant that can neutralize free radicals, which can initiate or propagate the oxidation of the lipid chain either directly or indirectly. It can also moderate gene expression and signal transduction through its antioxidant and non-antioxidant properties [16].

Nowadays, the harmful effects of Zn NPs in human tissues and cells require more research and investigation. The preceding reports on its toxicological impacts prompted us to investigate Zn NP toxicity and the enhanced liver and testes enzymes and oxidative stress index in the male albino rats as an animal model.

This study evaluates the toxicological impact of ZnO NP administration on experimental male albino rats on various biochemicals, stress markers, genotoxicity, and histopathological parameters in the liver and testes cells. Besides, we evaluated Vit. E's potent antioxidant effect enhances these toxic effects.

2. Materials and methods

2.1. Ethics statement

All experiments adhered to the standards set by the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) and were approved by the Institutional Animal Care and Use Committee (IACUC) at Alexandria University, Egypt (Approval No.: AU 04221220302). The study also followed the ARRIVE guidelines and complied with the National Research Council's guide for the care and use of laboratory animals.

2.2. Characterization of the Zinc nanoparticles

Ultra-thin sections (0.06:0.07 µm) were coated on 200 mesh naked copper grids for transmission electron microscopy analysis. The grids were stained with uranyl acetate for 30 min & lead citrate for 20–30 min [17]. Electron micrographs and scoping of the grid were captured at various magnifications. Using a Jeol 100 CX TEM (JEM1400 plus; JEOL Ltd., Akishima, Tokyo, Japan) available at the E.M. Unit - Faculty of Science, Alexandria University, Egypt.

2.3 Chemicals

ZnO NPs were purchased from Inframat Advanced Materials (Product No 30N-080, Manchester, United States), nanoparticles of 30 nm size with > 99.7 purity. Vitamin E was obtained from PHARCO Pharmaceuticals (Alexandria, EGYPT). Sodium dodecyl sulfate (SDS), 2-Thiobarbituric acid (TBA),

GSH, glutathione reductase, NADPH, hydrogen peroxide, agarose, and ethidium bromide were provided by Sigma Aldrich, USA. All other required chemicals were of high purity grade.

2.3.1 ZnO NP doses

Abbasalipourkabi et al. (2015) [5] showed that treating rats with ZnO NPs at 50 mg/kgt b.wt for 10 days had no toxic effects on the liver. Preceding studies stated that ZnO NPs at doses of 200 to 600 mg/kg triggered substantial harms on liver enzymes, hematological factors, oxidative stress, and histological parameters of the liver [18; 19]. We used the doses 50 (ZnO NP_{50}) and 200 mg(ZnO NP_{200})/kg b.wt of ZnO NPs to treat male albino rats orally for four weeks

2.3.2. Vit. E dose

The oral doses of Vit. E (100 mg/kg b.w) was used, according to [20].

2.4. Animals and Experimental Design

Healthy adult male albino rats (n=60), weighing between 180 and 200 grams, were used for this study. The rats were sourced from the Animal House at the Medical Research Institute in Alexandria, Egypt. They were housed in plastic cages and kept under standard temperature conditions (23±1°C) and humidity levels (55–60%), following a 12-hour light/dark cycle. All animals were provided with a standard rodent diet and water. The experimental design of this study adhered to animal care guidelines, ensuring that the rats did not experience suffering at any point during the experimentation, in accordance with the Medical Ethical Committee of the Faculty of Science at Alexandria University, Egypt.

The rats were grouped arbitrarily into six groups (**Gs**) (10 rats each) as follows: **G1**: Control rats (received no treatment); **G 2**: Animals were given Vit. E (100 mg/kg b.w)); **G3**: Rats were subjected to treatment with ZnO NP_{50} (50 mg/kg b.wt, suspended in dH_2O); **G4**: Animals were treated orally with ZnO NP_{200} (200 mg/kg b.wt, suspended in dH_2O); **G 5**: Rats were co-administrated with ZnO NP_{50} (50 mg/kg b.wt, suspended in dH_2O) and Vit. E (100 mg/kg b.w) and **G6**: Rats were treated with ZnO NP_{200} (200 mg/kg b.wt, suspended in dH_2O) and Vit. E (100 mg/kg b.wt). For four weeks, all groups received daily oral treatments.

2.5. Blood and tissue sampling

After treatment, the animals were starved overnight, and the blood samples were collected from the retro-orbital venous plexus using diethyl ether as an anesthetic. In a cooling centrifuge, all blood samples were centrifuged at 3000 rpm/15 min to separate serum for metallothionine, LH, total testosterone measurements, free testosterone, and liver function parameters (ALT, AST, ALP, ACP, LDH, T. bilirubin, D. bilirubin, and total protein). Additionally, a small piece of the animal's testes and liver was fixed in formalin for histological examination. Other specimens from the liver and testes were isolated, washed in cold saline, and kept in the freezer at -70 °C until used to determine the MDA, CAT, and GPx activities and for measuring the concentration of zinc.

2.5.1. Preparation of hepatic and testicular tissue homogenates

Homogenate preparations of liver and testicular tissue were conducted to measure glutathione peroxidase (GPx), catalase (CAT), and malondialdehyde (MDA). A tissue homogenate of approximately 10% (W/V) was created in a 50 mM potassium phosphate buffer (pH 7.4), 150 mM KCl, and 200 mM EDTA using a tissue homogenizer, followed by centrifugation at 15,000 rpm for 20 minutes at 4 °C. The supernatant was utilized for subsequent biochemical analyses.

2.6. Biochemical measurements in the liver

2.6.1. Assessment of serum liver enzyme activities

The serum enzymes, Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were assayed spectrophotometrically, using methods based on the laboratory procedures of Biosystems kits, Spain. Acid phosphatase (ACP), alkaline phosphatase (ALP), and lactate dehydrogenase

(LDH) were determined spectrophotometrically using SPINREACT Laboratories kits, Spain.

2.6.2. Protein content

Total protein was measured in the liver homogenates by the method of [21]. Total bilirubin, direct bilirubin, and total protein in the rat's sera were measured spectrophotometrically using Spectrum kits, Germany.

2.7. Sex hormones evaluation

Luteinizing hormone (LH), total and free testosterone were measured in rats' sera by the ELISA using the commercial kits (Catalog No. LS-F20636, LSBio, ab108666, Abcam, and Catalog No. CSB-E05097r CUSABIO, respectively).

2.8. Measurement of Metallothionine (MT) Levels

Metallothionine was analyzed using a commercial ELISA kit (Catalog No. LS-F27514, LSBio).

2.9. Oxidative stress biomarkers

2.9.1. Assessment of Lipid peroxidation in tissues: liver and testis

Lipid peroxidation in liver and testes tissues was estimated calorimetrically by measuring Malondialdehyde (MDA) (as nmol/mg protein) by the thiobarbituric acid assay [22].

2.9.2. Antioxidants assay in liver and testes homogenates

Hepatic and testicular catalase and glutathione peroxidase activities were measured (as U/mg protein) using the spectrophotometer following the methods of [23] and [24], respectively.

2.10. Zinc content assessment

A suitable quantity of the hepatic and testicular tissues (1 gm) was digested in nitric acid. 5 ml of concentrated nitric acid was added to each sample, heated at 90 °C until white fumes were well observed. Then, solutions were cooled, and 25 ml dist. Water was added, followed by filtration of the solution. The Zn contents were measured by a spectrophotometer (model AA-6650 – Shimadzu AA).

2.11. Sperm count and sperm motility

Semen content from the cauda epididymis was collected to estimate sperm count and motility using optical microscopy, following the method described in [25]. Sperm counts were obtained with a hemocytometer after dissecting the caudal epididymis and squeezing its contents into warm, sterile distilled water. The sperm suspension from the testes was diluted in 10% buffered formalin (1:20) before being examined under a light microscope at ×100 magnification, resulting in a measurement of million/ml of suspension. Sperm motility was assessed by counting motile spermatozoa per unit area under the microscope at ×100 magnification, and the percentage of motile sperm was recorded.

2.12. Comet assay

Comet analysis was conducted to evaluate the in vivo genotoxic potential of ZnO nanoparticles in the livers and testes of rats. The tissues were minced into small pieces using small dissecting scissors and allowed to sit for 5 minutes. Cells were centrifuged to obtain a pellet. After mixing the cells with low-melting-point agarose, the mixture was spread over a frosted slide that had been pre-coated with normal-melting agarose. The loaded samples were placed horizontally on the slides for 30 minutes in a dark environment. Following this, low-melting-point agarose was pipetted over the slides, which included the samples. The slides were then hardened at 4 °C for half an hour before being placed in lysis buffer for one hour. Afterward, the slides were immersed in a fresh alkaline unwinding buffer at 20 °C for one hour. The slides were then electrophoresed at 0.8 V/cm and 300 mAmps for 30 minutes to assess DNA damage. Image analysis was performed using a Leitz Orthoplan epifluorescence microscope. A computer-based image analysis system (Comet Assay IV software, Perspective Instruments) was used in conjunction with the microscope. Approximately 100 randomly selected cells per slide were scored for comet formation. DNA damage was assessed based

on tail length, percentage of tail DNA, and tail moment.

2.13. Histopathological examination

According to Drury and Wallington (1980) [27], the liver and testes were kept in 10 % formalin and fixed in paraffin. Thin sections (5 µm) of the different Gs were prepared using a microtome and mounted on slides following overnight drying at 37 °C. After de-waxing in xylene, the tissue sections were hydrated in a graded solution of alcohol and stained with hematoxylin and eosin to evaluate histological changes in the tissues.

2.14. Statistical analysis

SPSS (version 21) software was used to analyze the data of biochemical parameters, oxidative stress biomarkers, sex hormones, DNA damage, and sperm count and sperm motility assays. Differences between the Gs were evaluated by analysis of variance (ANOVA) followed by the Tukey test. All significant results were reported based on the probability of $P < 0.05$.

3. Results

3.1. The characterization of ZnO NPs

The characterization of ZnO NPs was illustrated to analyze the physical characteristics. In electron micrographs, ZnO NPs appeared as single spherical or aggregated particles ranging from18.35 to 25.23 nm (Figure 1).

3.2. Liver Functions (**Biochemical Parameters and protein levels**

No mortality was recorded in the rats treated with Vit. E or ZnO NPs.

Table 1 showed that administering Vit. E to ZnO NP-intoxicated rats with either low or high doses, 50 and 200 mg/kg b.wt, significantly reduced serum liver enzyme levels compared with intoxicated rats. Liver function protein levels (T. bilirubin and D. bilirubin) were relatively similar in rats treated with ZnO NPs plus Vit. E than in a control group. Moreover, total protein levels were reduced considerably (P<0.05) in groups of ZnO NPs compared with control rats.

3.3. Testis Functions (Fertility hormones and sperm characterizations)

Hormone levels (LH, total testosterone, and free testosterone) in ZnO NPs administered rats were decreased dramatically with significant differences (P<0.01) at the high dose (200 mg/kg) of the nanoparticles in comparison to the control rats (Table 2). This was accompanied by a significant improvement in the Vit. E + ZnO NPs co-administered groups. Moreover, sperm count and sperm motility in male rats exposed to ZnO NPs were reduced considerably (P<0.01) compared with control rats. Conversely, LH, total testosterone, free testosterone, sperm count, and motility in male rats in G5 and G6 significantly increased compared to those in **G4** exposed to 200 mg/kg ZnO NPs.

3.4. Lipid peroxidation and Oxidative stress markers in liver and testis tissues

The protective effect of Vit. E on the activity level of MDA, CAT, and GPx in liver and testes tissues in rats intoxicated with low or high doses is shown in Table 3.

As a result of lipid peroxidation, the activity level of MDA was significantly increased in **G4** compared to other groups, although it decreased significantly in **G6**. Furthermore, CAT and GPx activity levels in rats exposed to ZnO NPs increased dose-dependently. The lowest activity levels were observed at the high dosages of the nanoparticles. Treatment of rats with low or high doses of ZnO NPs along with Vit. E markedly decreased the activity of MDA. It increased the activities of CAT and GPx more than those in rats treated with ZnO NPs.

3.5. Zinc contents and metallothionein Levels in liver and testes

The influence of Vit. E on zinc (Zn) and metallothionein **(**MT) concentrations in liver and testes tissues of male rats exposed to ZnO NPs is illustrated in Table 4. Male rats exposed to ZnO NPs showed considerable elevation in Zn and MT concentrations in a dose-dependent way, where their levels were elevated, especially at 200 mg/kg of ZnO

NPs. Administration of rats with ZnO NPs plus Vit. E significantly (P<0.05) reduced the Zn and MT contents when Vit. E was given to rats exposed to the low ZnO NPs only. Additionally, the concentration of Zn and MT was decreased slightly in Gs (5 and 6) compared to rats exposed only to ZnO NPs in G3 and G4.

3.6. Cytotoxicity assays (Comet assay)

Figures 2 and 3 demonstrate the prevention impact of Vit. E against ZnO NPs triggered DNA damage in the liver and testes tissues of male rats. The incidence of tail DNA%, tail length, and olive tail moment in testes and liver tissues of male rats treated with Vit. E was relatively close to that in control rats. Nevertheless, the incidence of tail length, tail DNA%, and olive tail moment in liver and testes tissues of male rats exposed to 50 and 200 mg/kg b.wt. of ZnO NPs was increased significantly (P<0.05, P<0.01, respectively) compared with control rats. In contrast, the treatment of ZnO NPs-exposed rats with Vit. E decreased considerably (P<0.05) the incidence of tail length, tail DNA%, and olive tail moment in liver and testes tissues compared to rats exposed to ZnO NPs only.

3.7. Histopathological examination

Findings of the biochemical markers were confirmed by histopathological observations in the liver tissues of the experimental rats stained with HE. This examination showed that male albino rats receiving 50 mg/kg b.wt of ZnO NPs showed many hepatocytes with karyolysis.

Figure 4 shows the histopathological lesions in the liver tissues of male rats induced by ZnO NPs and the potential protective role of Vit. E. Under the light microscope, liver sections of the control rats displayed the normal histological morphology of the hepatic lobule (Fig. 4 A). Hepatocytes were also observed spreading from the centrilobular venule into the hepatic lobule's periphery. They were densely packed, pink in color, and had spherical violet nuclei with conspicuous nucleoli. The Vit. E treatment showed almost the same histological structure (Fig.4 B). Observing liver sections from rats exposed to a low ZnO NPs dose (Fig. 4 C&D) showed dilated and congested central vein, microvesicular and macrovesicular steatosis. Liver sections obtained from rats treated with a high ZnO NPs dose (Fig.4 E&F) showed disturbed hepatic lobular architecture with cytoplasmic vacuolization of the hepatocytes, focal necrosis, inflammatory cellular infiltration, and dilated congested central vein. However, liver tissues from rats exposed to a low dose of ZnO NPs plus Vit. E (Fig. 4 G) showed no apparent histopathological abnormalities compared to rats exposed to a low dose of ZnO NPs only. Moreover, rats exposed to a high dose of ZnO NPs plus Vit. E (Fig. 4 H) showed a reduction in the histological changes with few inflammatory cellular infiltrations compared with rats exposed to a high dose of ZnO NPs only.

The histopathological lesions in testes tissues of male rats induced by ZnO NPs and the potential suppression effect of Vit. E are illustrated in Figure 5. Examining testes sections of the control group (Fig. 5 A) presented normal histology, where most of the seminiferous tubules contained normal spermatozoa and spermatogenic cell layers. The seminiferous tubule structures and spermatogenic cell series in rats treated with Vit. E (Fig. 5 B) showed a similar structure to the control groups. While the testes of rats exposed to a low dose of ZnO NPs (Fig.5 C) showed degenerated spermatogenic cells, and vacuoles replaced some cells. Rats exposed to a high dose of ZnO NPs (Fig. 5 D&E) exhibited obvious damage, such as distorted seminiferous tubules with disorganized germ cells with deeply stained pyknotic nuclei, sloughing, many Sertoli cells, and interstitial vacuolation. Analyzed sections from rats subjected to a low dose of ZnO NPs plus Vit. E presented an improvement in the histopathological picture (Fig. 5 F), revealing apparently normal seminiferous tubules. Testis of rats treated with a high dose of ZnO NPs plus Vit. E (Fig. 5 G) showed mild degenerated spermatogenic cells and aggregation of multinucleated giant cells.

Table 1: Effect of Vit. E treatments on some biochemical parameters and protein levels in liver and testes of ZnO NP-intoxicated male albino rats

Parameters	Treatment groups					
	Control	Vit. E_{100}	ZnO NPs_{50}	ZnO NP_{200}	ZnO NP_{50}+ Vit. E_{100}	ZnO NP_{200}+ Vit. E_{100}
ALT (IU/L)	37.8 ± 4.3^{e}	40.8 ± 4.1^{de}	101.4 ± 10.3^{b}	143.7 ± 8.2^{a}	51.8 ± 5.4^{d}	67.7 ± 4.6^{c}
AST (IU/L)	61.6 ± 3.8^{e}	69 ± 5.9^{de}	148.0 ± 8.1^{b}	194.9 ± 6.5^{a}	78.9 ± 5.9^{d}	103.2 ± 9.1^{c}
ALP (IU/L)	137.8 ± 7.1^{e}	153.7 ± 10.2^{d}	268.4 ± 5.3^{b}	401.1 ± 7.5^{a}	155.0 ± 8.7^{d}	225.0 ± 6.9^{c}
ACP (IU/L)	6.3 ± 1.1^{c}	5.9 ± 1.1^{c}	9.9 ± 1.1^{b}	19.0 ± 1.6^{a}	7.5 ± 0.9^{bc}	11.4 ± 1.7^{b}
LDH (IU/L)	108.0 ± 6.7^{e}	105.0 ± 9.4^{e}	235.2 ± 16.6^{b}	288.2 ± 15.5^{a}	130.4 ± 5.2^{d}	175.0 ± 7.5^{c}
T. Proteins (gm/dl)	8.3 ± 0.3^{a}	7.9 ± 0.8^{a}	6.8 ± 0.3^{ab}	6.0 ± 0.4^{b}	7.4 ± 0.6^{a}	6.8 ± 0.2^{ab}
T. Bilirubin (mg/dl)	0.6 ± 0.01^{c}	0.7 ± 0.1^{bc}	1.0 ± 0.1^{b}	2.2 ± 0.2^{a}	0.7 ± 0.1^{bc}	0.8 ± 0.1^{bc}
D. Bilirubin (mg/dl)	0.3 ± 0.1^{b}	0.4 ± 0.1^{b}	0.4 ± 0.1^{b}	0.6 ± 0.1^{a}	0.4 ± 0.1^{b}	0.5 ± 0.1^{ab}

G 1, control group; **G** 2, Vit. E_{100}; **G 3**, ZnO-NP 50 mg; **G 4**, ZnO NP_{200} mg; **G 5**, ZnO NP_{50}+ Vita E_{100} and **G 6**, ZnO NP_{200} + Vita E_{100}. All values are expressed as means ± SEM. ^{a,b,c,d} Mean values within treatments with unlike superscript letters were significantly different ($P< 0.05$).

Table 2. Effect of Vit. E treatments on fertility hormones, sperm count, and motility in ZnO NP-intoxicated male albino rats.

Parameter	Treatment groups					
	Control	Vit. E_{100}	ZnO NP_{50}	ZnO NP_{200}	ZnO NP_{50}+ Vit. E_{100}	ZnO NP_{200}+ Vit. E_{100}
LH (mIU/ml)	7.5 ± 0.04^{a}	7.9 ± 0.07^{a}	6.7 ± 0.12^{b}	5.2 ± 0.06^{c}	7.1 ± 0.063^{a}	6.5 ± 0.11^{b}
Total testosterone (ng/ml)	8.8 ± 0.04^{a}	8.9 ± 0.03^{a}	7.2 ± 0.03^{b}	6.2 ± 0.05^{c}	7.8 ± 0.152^{ab}	7.3 ± 0.03^{b}
Free testosterone (pg/ml)	0.88 ± 0.03^{a}	0.94 ± 0.03^{a}	0.62 ± 0.02^{b}	0.53 ± 0.04^{c}	0.71 ± 0.027^{b}	0.64 ± 0.03^{b}
Sperm count (million/ml)	109.9 ± 4.07^{a}	109.8 ± 3.23^{a}	78.4 ± 4.60^{c}	49.8 ± 5.27^{d}	87.1 ± 5.2^{b}	71.1 ± 5.51^{c}
Sperm motility %	86.1 ± 4.01^{a}	86.4 ± 4.12^{a}	64.8 ± 3.39^{c}	53.4 ± 3.13^{d}	74.7 ± 4.2^{b}	62.0 ± 2.58^{c}

G 1, Control group; **G** 2, Vit. E_{100}; **G 3**, ZnO-NP_{50} ; **G 4**, ZnO NP_{200} ; **G 5**, ZnO NP_{50}+ Vit E_{100} and **G 6** ZnO NP_{200} + Vit E_{100}. All values are expressed as means ± SEM. ^{a,b,c,d} Mean values within treatments with unlike superscript letters were significantly different ($P< 0.05$).

Table 3: Effect of Vit. E treatments on oxidative stress markers and enzymes in the liver and testes in ZnO NP-intoxicated male albino rats.

Parameter	Treatment groups					
	Control	Vit. E_{100}	ZnO NP_{50}	ZnO NP_{200}	ZnONP_{50}+ Vit. E_{100}	ZnO NP_{200}+ Vit. E_{100}
Liver MDA (nmol/mg protein)	4.5 ± 0.51^{c}	4.7 ± 0.10^{c}	8.2 ± 0.27^{b}	11.0 ± 0.24^{a}	7.4 ± 0.37^{b}	8.9 ± 0.37^{ab}
Testes MDA (nmol/mg protein)	1.24 ± 0.01^{d}	1.26 ± 0.30^{d}	4.9 ± 0.20^{b}	6.1 ± 0.47^{a}	2.38 ± 0.17^{c}	3.7 ± 0.33^{bc}
Liver CAT (U/mg protein)	159.4 ± 3.11^{a}	166.6 ± 10.74^{a}	$129.6\pm 6.48b^{c}$	81.2 ± 3.49^{d}	141.2 ± 3.36^{b}	117.2 ± 6.23^{c}
Testes CAT (U/mg protein)	128.2 ± 4.25^{a}	137.7 ± 6.42^{a}	107.3 ± 4.23^{b}	85.9 ± 3.28^{c}	113.04 ± 5.46^{b}	106.8 ± 7.32^{b}
Liver GPx (U/mg protein)	170.9 ± 5.17^{a}	188.4 ± 5.81^{a}	122.1 ± 5.97^{c}	92.5 ± 7.41^{d}	139.4 ± 7.25^{b}	117.9 ± 7.94^{c}
Testes GPx (U/mg protein)	124.0 ± 7.18^{a}	128.8 ± 7.24^{a}	87.8 ± 8.04^{cd}	74.2 ± 5.96^{d}	107.1 ± 5.57^{b}	90.8 ± 6.75^{c}

G 1, Control group; **G** 2, Vit. E_{100}; **G 3**, ZnO-NP_{50}; **G 4**, ZnO NP_{200}; **G 5**, ZnO NP_{50}+ Vit. E_{100} and **G 6**, ZnO NP_{200} + Vit. E_{100}. All values are expressed as means ± SEM. ^{a,b,c,d} Mean values within treatments with unlike superscript letters were significantly different ($P< 0.05$).

Table 4: Effect of Vit. E treatments on zinc contents and metallothionein levels in liver and testes in ZnO NP-intoxicated male albino rats.

Parameter	Treatment					
	Control	Vit. E_{100}	ZnO NP_{50}	ZnO NP_{200}	ZnO NP_{50}+ Vit. E_{100}	ZnO NP_{200}+ Vit. E_{100}
Liver zinc content (µgm/gm)	12.4 ± 1.88^{d}	12.9 ± 2.17^{d}	26.1 ± 1.27^{b}	32.3 ± 1.21^{a}	20.7 ± 1.31^{c}	24.9 ± 0.95^{bc}
Testes zinc content (µgm/gm)	8.4 ± 0.9^{c}	7.07 ± 1.21^{c}	13.8 ± 0.85^{b}	31.5 ± 1.0^{a}	11.9 ± 0.78^{b}	27.1 ± 0.548^{a}
Liver Metallothionein (ng/ml)	6.6 ± 0.41^{c}	7.9 ± 0.58^{c}	11.4 ± 0.62^{b}	16.4 ± 0.82^{a}	9.7 ± 0.53^{bc}	12.4 ± 0.63^{b}
Testes Metallothionein (ng/ml)	6.1 ± 0.35^{c}	7.2 ± 0.47^{c}	10.2 ± 0.55^{b}	14.8 ± 0.78^{a}	8.5 ± 0.39^{bc}	11.7 ± 0.49^{ab}

G 1, control group; **G** 2, Vit. E_{100}; **G 3**, ZnO-NP_{50}; **G 4**, ZnO NP_{200}; **G 5**, ZnO NP_{50}+ Vit. E_{100} and **G 6**, ZnO NP_{200} + Vita E_{100}. All values are expressed as means ± SEM. ^{a,b,c,d} Mean values within treatments with unlike superscript letters were significantly different ($P< 0.05$).

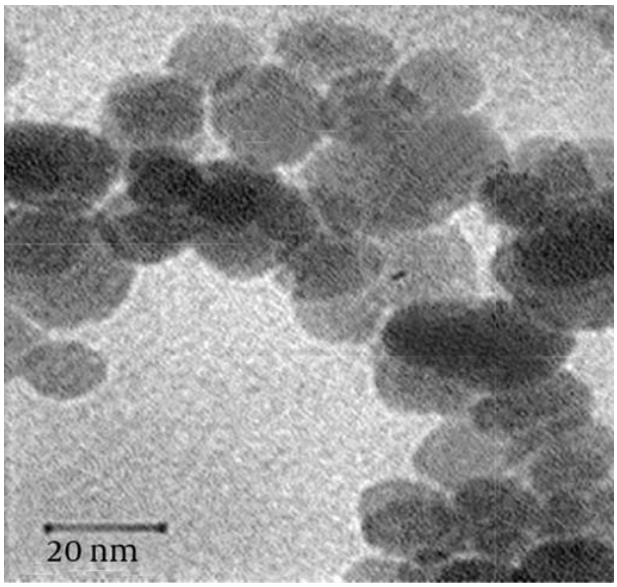

Fig 1. TEM image of ZnO nanoparticles

A

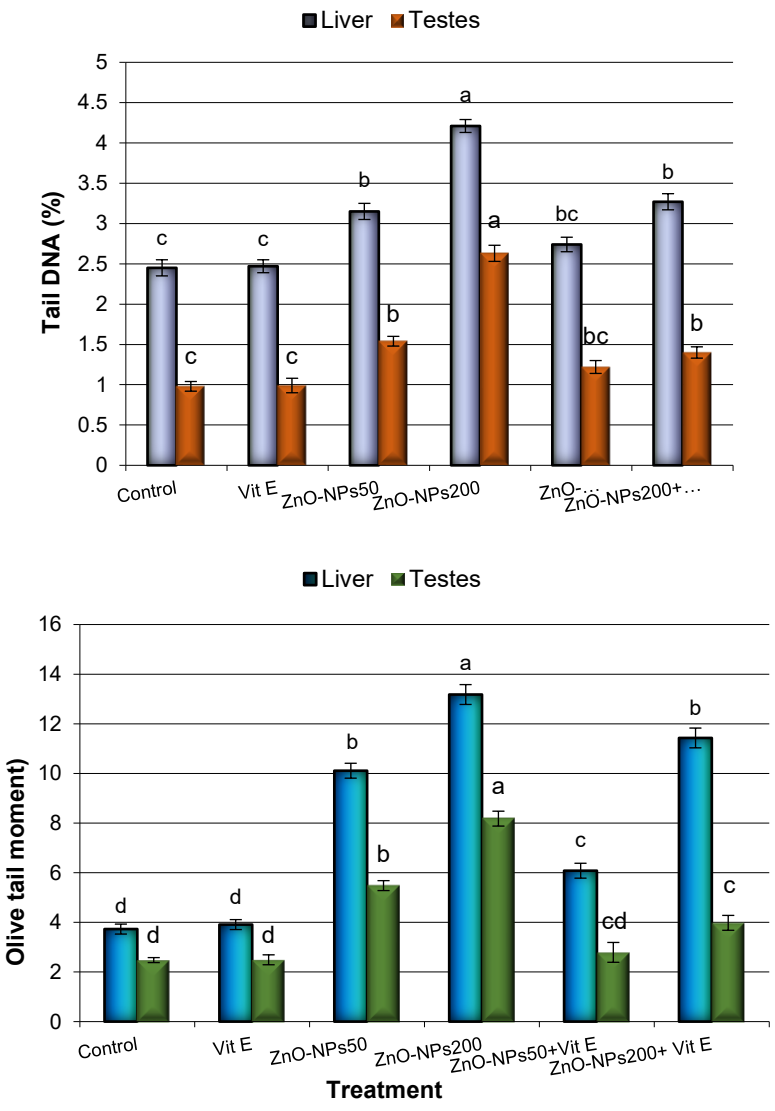

Fig. 2. Effect of Vit. E on DNA levels of liver and testes tissues in ZnO NP-intoxicated male albino rats. A: tail length, B: tail DNA% and C: olive tail moment. Data are presented as mean ± SEM. [a,b,c,d] Mean values within treatments with unlike superscript letters were significantly different ($P< 0.05$).

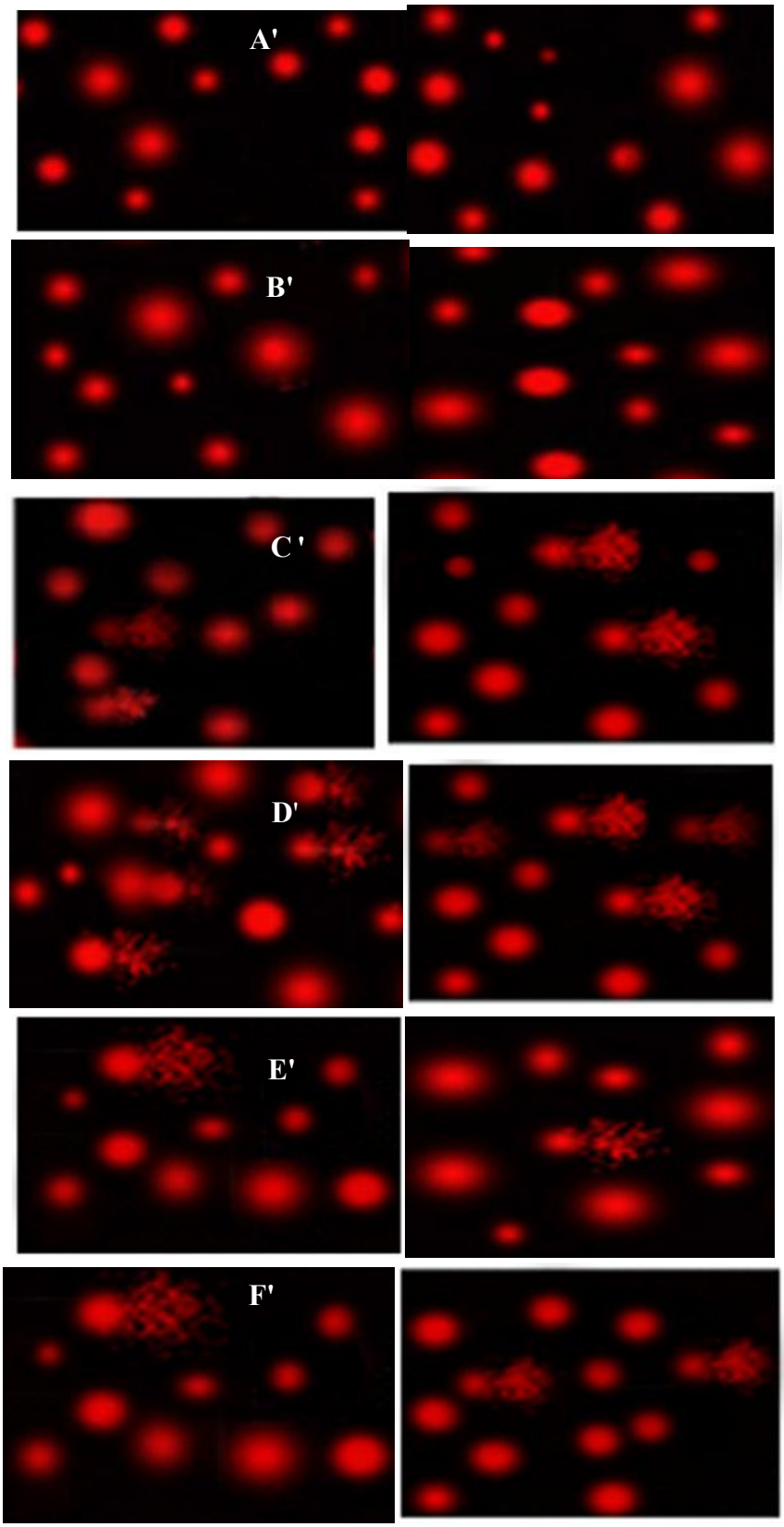

Fig. 3. Effect of Vit. E against ZnO NP-induced DNA damage. A and A': liver and testis tissues of the control group, showed intact DNA. B and B': liver and testis tissues of the Vit E group, respectively, showed intactDNA. C and C': liver and testis tissues of the ZnO-NP_{50} group, respectively, showed damaged DNA. D and D': liver and testis tissues of the ZnO-NP_{200} group, respectively, showed a high incidence of damaged DNA. E and E': liver and testis tissues of the ZnO-NP_{50}+Vit E group, respectively, showed less damaged DNA compared to those treated with NPs only at low doses. F and F': liver and testis tissues of the ZnO-NP_{200}+ Vit E group showed less damaged DNA than those treated with NPs only at high doses.

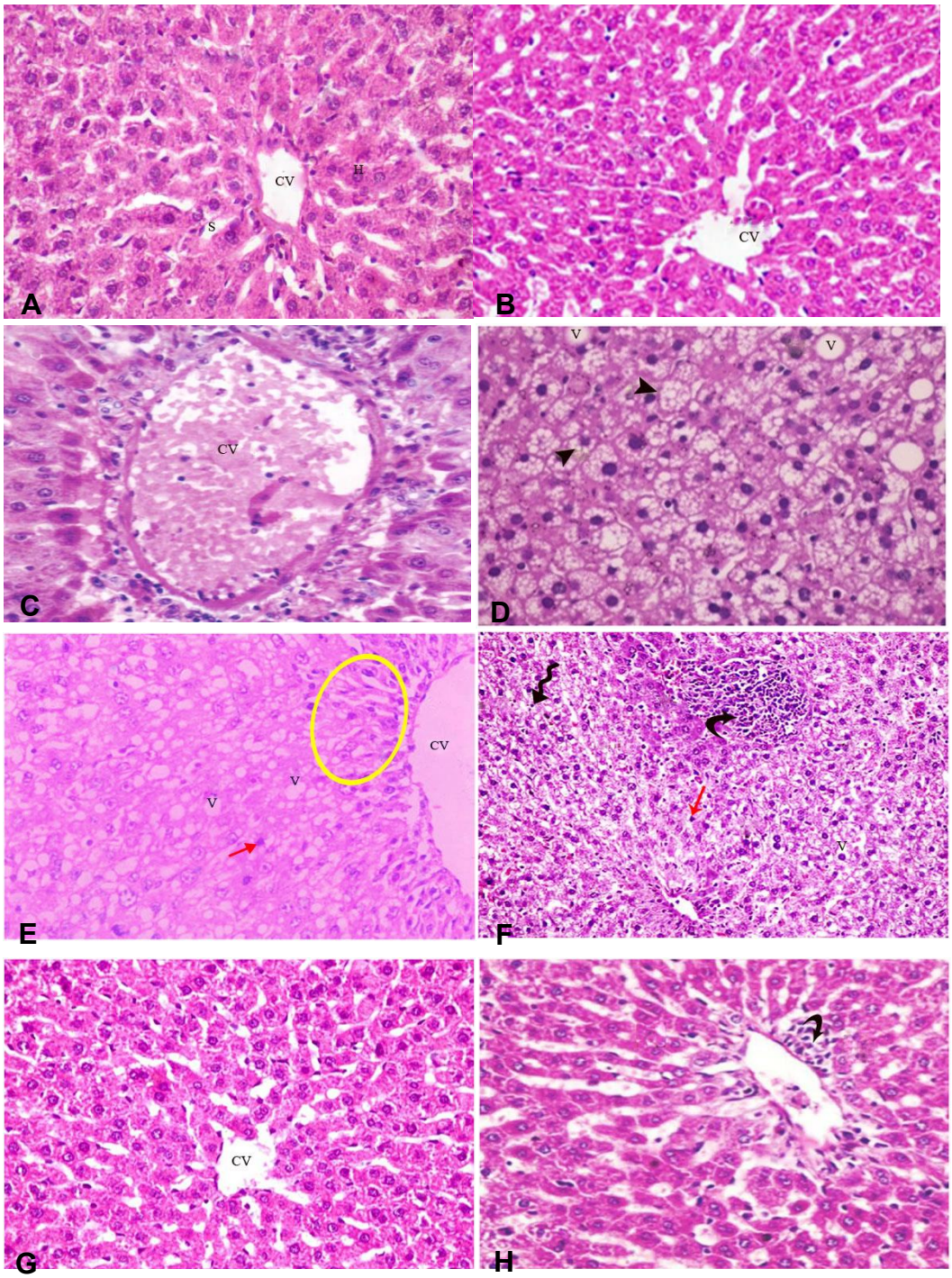

Fig. 4. Photomicrograph with H&E stain from different experimental groups showing transverse sections of liver tissues of male rats treated with ZnO NPs or Vit. E. A: Control rat liver section showing the normal histological structure of the liver: hepatocytes (h), Central Vein (CV) and sinusoids (s) (H&E x400). B: Vit. E treated rat liver section showing the normal histopathological appearance of liver tissue structure (H&E x400). C&D: ZnO NP_{50} treated rat liver section showing congested and dilated central vein, microvesicular (head arrow) and macrovesicular steatosis (V) (H&E x400). E&F: ZnO NP_{200} treated rat liver section showing dilated central vein, disruption of the normal architecture of hepatocytes with areas of necrosis and infiltration of inflammatory cells (yellow circle), macrovesicular steatosis (V), ballooning of hepatocytes, nuclei of most hepatocytes are small pyknotic (red arrows), mononuclear cell infiltration (curved arrow) and numerous Kupffer cells (waved arrow) (H&E x200). G: ZnO NP_{50}+Vit. E treated rat liver section showed near-normal liver tissue structure (H&E x400). H: ZnO NP_{200}+Vit. E treated rat liver section showed improvement in hepatic tissue with mild infiltration of inflammatory cells (H&E x400).

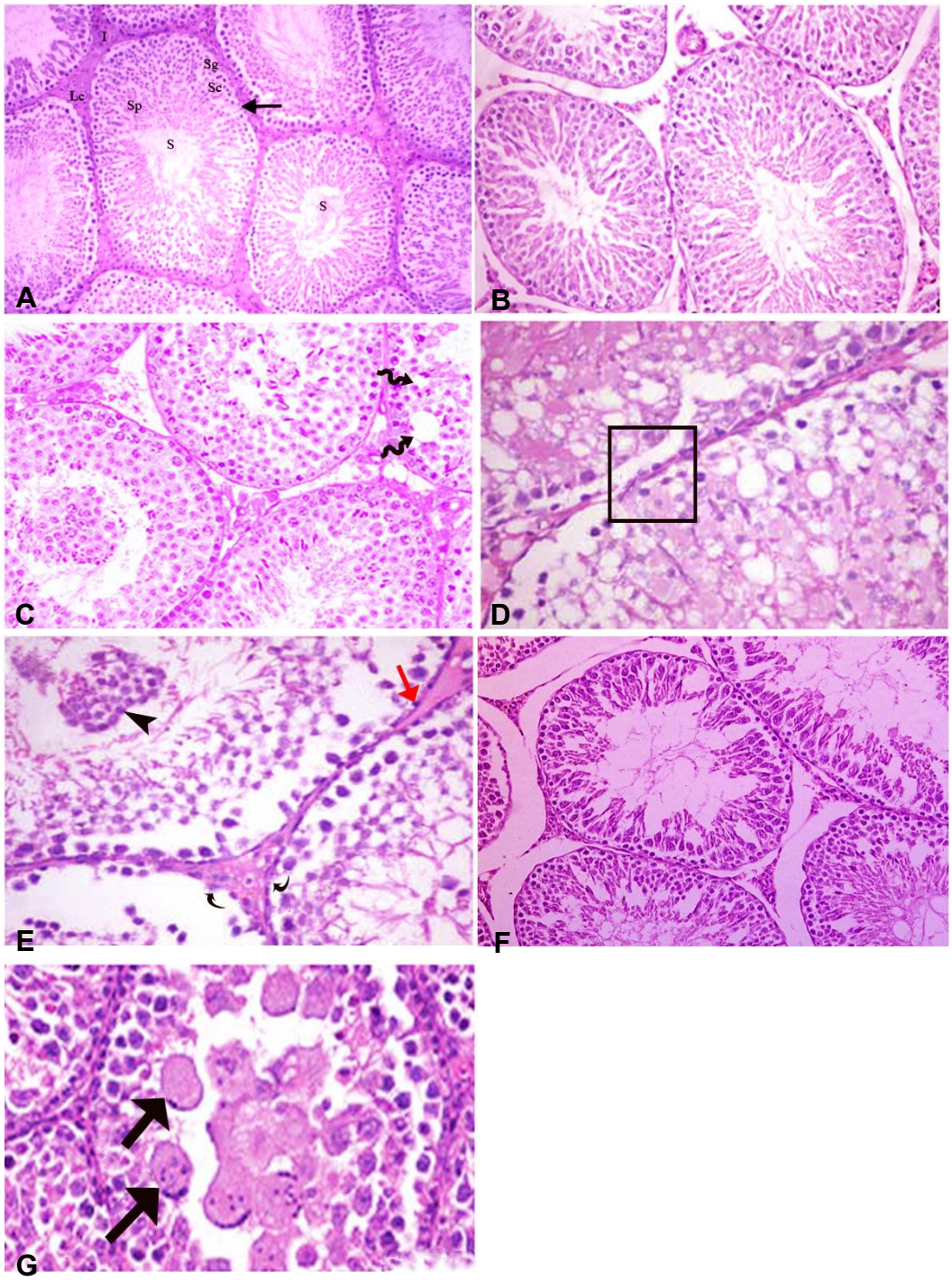

Fig. 5. Photomicrograph with H&E stain from different experimental groups showing transverse sections of testes tissues of male rats treated with ZnO NPs and or Vit. E. A: Control rat testes section showing normal seminiferous tubule (S) containing different types of germ cells; spermatogonia (Sg) lying on the basement membrane (arrow), spermatids spermatozoa (Sp) with normal interstitial tissue (I) in between, and somatic Sertoli cells (Sc). The interstitial tissues between seminiferous tubules contain interstitial cells, Leydig cells (Lc) (H&E x200). B: Vit. E treated rat testes section showing testicular histoarchitecture (H&E x200). C: ZnO NP50-treated rat testes section showing sloughing of spermatocytes, spermatids, and tubular vacuolation (zigzag arrow). D&E: ZnO NP200-treated rat testes section showing distortion of the seminiferous tubules with a marked disorganized germinal epithelium and interstitial vacuolation (D); spermatogenic cells with dark pyknotic nuclei (red arrow), sloughed spermatogenic cells (arrowhead) into the lumen of the seminiferous tubules, and many Sertoli cells (curved arrow) (E) (H&E x400). F: ZnO NP_{50}+Vit. E treated rat testes section showed nearly normal testicular histoarchitecture (H&E x400). G: ZnO NP_{200}+Vit. E treated rat testes section showing mild degeneration of spermatogenic cells with formation of spermatid giant cells in the seminiferous tubules (arrows) (H&E x400).
Dilated central vein, disruption of the normal architecture of hepatocytes with areas of necrosis and infiltration of inflammatory cells (yellow circle), macrovesicular steatosis (V), ballooning of hepatocytes, nuclei of most hepatocytes are small pyknotic (red arrows), mononuclear cell infiltration (curved arrow), and numerous Kupffer cells (waved arrow) (H&E x200). G: ZnO NPs_{50}+Vit. E treated rat liver section showed near-normal liver tissue structure (H&E x400). H: ZnO NP_{200}+Vit. E treated rat liver section showed improvement in hepatic tissue with mild infiltration of inflammatory cells (H&E x400).

4. Discussion

Metal oxide nanoparticles (MONPs) play a role in many areas of life. Once the animal is exposed to NPs, they can move to various organs and tissues due to their extremely small diameter, affecting several biochemical parameters [28]. ZnO NPs are an easily accumulated nanomaterial; this accumulation varies according to the tissue type. It was found that the toxicity of this nanometal increased as its size decreased and its concentration increased [29].

The wide range of toxic nanoparticles depends not only on the size and dose but also on the administration route and timing of nanoparticle exposure [30]. It is well known that ZnO NPs are used in food packaging, consumer products, as coatings, and dermatological uses. There is always a risk of ingestion when they are used [31]. Hence, oral doses of ZnO NP administration are recommended for the studied rats.

Lynch et al. (2005) and Adeyemi and Adewumi (2014) [32] and [33] reported that the reliable 'markers' enzymes of liver damage (ALT, AST, ALP, ACP, and LDH) rise in serum levels during hepatitis, degeneration, necrosis, and inflammatory conditions. Pasupuleti et al. (2012) and Mansouri et al. (2015) [34] and [35] recorded a significant rise in the levels of ALT and AST in ZnO NP-treated groups (50 and 300 mg/kg). Moreover, Kavaz et al. (2021) [36] remarked a considerable alteration in serum level markers (ALT, ALP, and AST) of the liver in rats. In their study, rats were exposed to 40 to 80 mg/kg of ZnO NP concentrations for 4 weeks.

In the present study, ALT, AST, ALP, ACP, and LDH enzyme activities were elevated significantly in male albino rats treated with 200 mg/kg for 4 weeks, much more than those treated with 50 mg/kg of ZnO NPs. Several studies showed that the activities of these enzymes in treated liver tissues with ZnO NPs were significantly increased [37]. The current study detected decreased enzyme activity levelsin rats treated with ZnO NPs plus Vit. E doses. In support of these results, Hegazy et al. (2018) [38] reported that the changes in AST and ALT levels were reduced in ZnO NPs plus Vit. E group compared to the ZnO NP groups. Welson et al. (2021) [39] noticed that Vit. E lowered serum liver enzymes side by side to decrease liver tissue oxidative stress. This may be because Vit. E can normalize concentrations of these enzymes [39].

A significant decrease in blood total proteins and an increase in total and direct bilirubin levels in groups treated with ZnO NPs compared to the normal control group are additional indicators of liver tissue injury. (Table 1). On the other hand, Kavaz et al. (2021) [36] reported that male rats treated with nano zinc (80 mg/kg for 28 consecutive days oral administration) showed an increase in total protein and bilirubin levels in blood samples compared with control rats. A recent study revealed that ZnO NPs induced elevated T. protein and bilirubin levels, which could be attributed to increasing red blood cell hemolysis beyond the hepatic function capacity [36]. In the current study, the relatively normal values detected were in groups exposed to ZnO NPs plus Vit. E. It is confirmed that Vit. E is regarded hepatoprotective agent against ZnO NP toxicity [39]. Vit. E isimportant in improving immune and metabolic disorders associated with liver damage.

ZnO NPs cause toxic and destructive disruptions in human health, particularly in the reproductive system, and remain a concern. Some reports have demonstrated the reproductive toxicity of ZnO NP [10; 40].

Lanzafame et al. (2009) [41] declared the role of oxidative stress in male infertility. This is because free radicals and ROS can damage sperm functions. After ZnO NP treatment, this stress was recorded in different studied animals [34]. ZnO NPs are considered a testicular toxicant at dosages ranging from 50 to 350 mg kg in rodents [10; 11].

The current study showed that male albino rats exposed to both doses, 50 mg and 200 mg ZnO NPs, reduced the levels of luteinizing hormone (LH), free and total testosterone in rats compared with the control group. Zhang et al. (2006) [43] mentioned

that metal NP, including ZnO NPs, can traverse the blood-testis barrier (BTB).

Exposure to NP may cause a systemic inflammatory response in the host, damaging Leydig cells and lowering testosterone blood levels, weakening the BTB's integrity [44]. It is critical to understand that any change in BTB could pose a significant risk to spermatogenesis [45]. A marked decrease was recorded in testosterone levels in ZnO NP-treated groups, which correlates with the reduced proliferation and activity of Leydig cells. The same results were established by the studies of 20, [46,47]. The results obtained indicate that sperm count and sperm motility in male rats treated with ZnO NPs, particularly at the high dose of 200 mg/kg, were significantly reduced ($P<0.01$) compared to control rats. ZnO NPs lead to a decrease in the number, motility, and quality of sperm cells, while also improving morphological abnormalities such as double heads, double tails, and amorphous heads. Consequently, ZnO NPs may cross the blood-testis barrier, resulting in testicular toxicity and affecting reproductive hormones and sperm quality. The current study demonstrated that male rats exposed to ZnO NPs in conjunction with Vitamin E (100 mg/kg) for four weeks showed increased levels of LH, total testosterone, free testosterone, sperm count, and sperm motility compared to those exposed to ZnO NPs alone. Aydilek et al. (2004) noted that Vitamin E is essential for animal reproduction. Previous studies by Karanth et al. (2003) and Li et al. (2016) reported that Vitamin E enhances reproductive hormone production, including LH, and offers protective effects against testicular injury in mice. Additionally, Yin et al. (2012) found that mice treated with Vitamin E at a dose of 100 mg/kg significantly increased the secretion levels of LH and testosterone, as well as improved sperm quality [49-52].

Oxidative stress reveals the imbalance between reactive oxygen species (ROS) generation and the biological response of an animal that detoxifies mechanisms. The presence of zinc nanoparticles (Zn-NPs) led to a significant increase in various antioxidant enzymes and gene expression levels [53].

The effect of ZnO NPs on the enzyme activity levels was evaluated in this study, and proved that the activity levels of MDA were increased. Still, the activity levels of CAT and GPx were decreased in the liver and testis (Table 3). The outcomes agree with 65, who documented that ZnO NP affected the CAT and GPx levels that were drastically reduced as lipid peroxidation was considerably increased, as shown by the MDA level in tests tissues.

In line with the present study, Abbasi et al. (2018) [54] reported that doses of more than 100 mg/kg ZnO NPs reduced the GPX and CAT activities and increased MDA levels. Syama et al. (2014) [55] suggested that the release of Zn++ ions and rising ROS generation might cause oxidative stress in liver tissues.

In the current study, GPx and CAT activities increased significantly in Vit. E-treated groups 5 and 6. This vitamin's protective effect is due to its essential role in peroxyl radical scavenging, terminating lipid peroxidation, and enhancing the glutathione-antioxidant system [56].

Metallothionein (MT) serves as a zinc pool, and its deficiency causes a noticeable upsurge in zinc accretion in the different tissues [57]. Male rats exposed to ZnO NPs showed a considerable elevation in Zn and MT contents in the liver and testicular tissues. Wang et al. (2017) [58] reported that ZnO NPs significantly enhanced the MT levels in mice tissues by promoting their gene expression. Swain et al. (2019) [59] mentioned that the expression of MT was proportional to the intake of dietary zinc, which serves a sequestration function to decrease metal toxicity. Increased MT expression in the liver compared to the testis tissues might be attributed to the better bioavailability of zinc from nano zinc particle sources and related to different physiological activities needed of the tissues of the studied animals, which greatly contributes to Zn homeostasis.

ROS can damage genetic materials in cells exposed to different metals. The reaction of ROS with DNA molecules has caused significant damage that can affect purine, pyrimidine and DNA backbones [60]. DNA damage is useful for evaluating the impact of metal exposure in the environmental matrix and in model research [61; 62]. By quantifying breakage in the DNA chain, the comet assay offers a sensitive, rapid, and multipurpose technique to evaluate the damage produced by various metals. It is an essential example of cellular genotoxicity. Several studies have assessed the potential genotoxic impacts of ZnO NPs in several tissues using the comet assay [63]. Current data proved that the ZnO NPs-triggered oxidative stress in the liver and testes coincided with severe DNA damage assessed by the comet assay. Compared with the control group, a remarkable improvement was recorded in the tail length (TL) and DNA % in the tail of livers and testis of rats intoxicated with the high dose of ZnO NPs. This damage was more pronounced in rat livers ingesting a high dose of the NPs than in testis tissues (Figs 2 and 3). According to Singh et al. (1988) [26], two signals indicate the amount of DNA breakage: the length and intensity of the comet tail. A remarkable increase in DNA% and tail length, as in our study, has been recorded by [64] in the livers of rats intoxicated with ZnO NPs (600 mg/kg/day) for 5 consecutive days.

In hepatocytes and testes tissues, ZnO NPs caused oxidative damage of DNA and ROS-triggered mitochondrial-mediated apoptosis, according to several studies [65]. In the same way, liver injury was observed after oral exposure to ZnO NPs (300 mg kg−1) for 14 successive days [66]. Nanomaterials can induce intracellular oxidative stress by disturbing the equilibrium between oxidant and antioxidant activities [67]. The rats supplemented with ZnO NPs plus Vit. E declined DNA damage in the liver and testis compared to those exposed to ZnO NPs only. The same results were reported by [68] and [69].

Badgujar et al. (2017) [70] declared that Vit. E supplemented in rats protects DNA from the attack of free radicals that cause damage to DNA molecules and acts by one of the two mechanisms as follows: (a) it inactivates ROS molecules and prevents their binding with DNA structure, or (b) scavenges peroxyl lipid radicals, which breaks the lipid peroxidation chain reaction that results in DNA-damaged products. Nassar et al. (2017) [71] suggested that the protective role of Vit. E against ZnO NPs induced toxicity in rats could be attributed to its capability to suppress inflammatory mediators' expression. So, the anti-inflammatory action of Vit. E might be associated with the trapping of ROS and mitigation of oxidative damage, as well as a decrease in the expression levels of IL-6, TNF-α, and CRP that are induced by ZnO NPs [72]. Consistent with these conclusions, co-administration of the 100 mg/kg Vit. E to ZnO NPs intoxicated doses efficiently protected livers and testes tissues from DNA injury, indicated by a reduction in DNA % and tail length compared to intoxicated rats.

The histopathological inspection of liver and testicular cells substantiated the stress markers and genotoxicity results. The liver tissues showed disturbed hepatic lobular architecture with cytoplasmic vacuolization of the hepatocytes, focal necrosis, inflammatory cellular infiltration, and dilated, congested central vein. In line with these, Johar et al. (2004) [73] suggested that ZnO NP possibly exert an oxidative stress mechanism on rat tissues, which may induce an inflammatory response. Among others, Schrand et al. (2010) and Babele (2019) [74] and [75] found that ZnO NPs increased reactive oxygen species (ROS) within mammalian cells, leading to necrosis.

Studies have shown that necrosis is stimulated by toxicants that hurt the cell organelles (endoplasmic reticulum, mitochondria, and cell nuclei), upsetting their activities [76]. It also leads to glutathione depletion, decreases in catalase and superoxide dismutase activity [77].

Regarding the testis, tissue lesions in groups exposed to ZnO NPs showed distorted seminiferous tubules with disorganized germ cells with pyknotic nuclei, sloughing, and many Sertoli cells and interstitial vacuolation. These findings agree with those obtained by [46] and [40], who described rats exposed to ZnO NPs had severe changes in seminiferous tubules, sloughing, and reduction in the germinal epithelium and pyknosis of spermatogenic cells. Talebi et al. [10] found that rats given 300 mg/kg ZnO NP experienced sloughing or even atrophy of the epithelium of the seminiferous tubules after 35 days of treatment. Johnson (2014) [78] suggested that the sloughing of the germinal epithelium that partially or even connects the entire tubule might be related to the toxic effect on the Sertoli cell cytoskeleton. Moreover, disturbance in physical interactions between Sertoli and other germ cells leads to sloughing and detachment of the germ cells [79]. Halawa (2010) [80] added that nanoparticles can damage and even disrupt the underlying membranes, germ cells, and Sertoli cells, leading to further destruction of the damaged cell. These observations align with the earlier reports of [81] and [82].

Figure 8 showed a moderate histopathological change in the liver and testis tissues of male albino rats exposed to ZnO NPs and Vit. E compared to control animals. A potent anti-apoptotic outcome of the co-administration of Vit. E with ZnO NPs to rats may lead to down-regulating the increase in liver caspase 3 activity (03). Al-Rasheed et al. (2012) [64] concluded that by inhibiting lipid peroxidation, Vit. E maintains cell membranes' integrity, function, and flexibility. Also, this vitamin plays an important role in reducing inflammation. Also, Abdallah et al. (2018) [83] remarked that supplementation of Vit. E during ZnO NP exposure, light has protective effects against tissue dysfunction. Pearce et al. (2019) and Lazzarino et al. (2019) [84] and [85] reported that vitamin E may play a role in enhancing the quality of semen and, as a result, improving fertility. This could happen by improving sperm quality and minimizing DNA disintegration and apoptosis.

Treatment of ZnO NPs intoxicated male albino rats with Vit. E, pointedly boosted most of the diverged biochemical, genotoxicity, and histopathological alterations and effectively prevented the destructive effect caused by ZnO NPs intoxication, which was established microscopically in the histological structures of liver and testes cells.

5. Conclusions

In conclusion, the present study concluded that oral zinc oxide nanoparticles (50 and 200mg/kg) generate excessive oxidative stress-triggered alterations in biomarker enzymes, destructive genotoxicity, and cytotoxicity in the liver and testicular cells. Supplementation of Vit. E during exposure to zinc oxide nanoparticles may offer protection against their damaging effects. Hence, Vit. E might be applied with other treatments to enhance human health, such as exposure to different ZnO NPs.

Authorship contribution statement

Nessrin Kheirallah: conceived and designed the experiments, conceptualization, supervision, Validation, formal analysis, writing - original draft, reviewing, and editing the draft.

Hussein K. Hussein: supervision, revising the draft.

Hamasa Adam Ali: performed the experiments, Formal analysis, and analyzed the data. Data curation, Investigation

Amel El-Gendy: conceived and designed the experiments, conceptualization, supervision, writing - original draft, reviewing, and editing the draft.

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Data availability statement: Derived data supporting the results of this study are available on request.

Conflict of interest: no conflict of interest

Ethical clearance: The authors declare that they have followed all guidelines and regulations required in the present study.

Animal ethics approval: AU 04221220302

References

1- Djebbi, E., Bonnet, D., Pringault, O., Tlili, K., and Yahia, M.N.D., 2021. "Effects of nickel oxide nanoparticles on survival, reproduction, and oxidative stress biomarkers in the marine calanoid copepod *Centropages ponticus* under short-term exposure". *Environmental science and pollution research international* 28(17): 21978-21990.

2- Islam, F. Shohag, S. Uddin, M.J. Islam, M.R. Nafady, M.H. Akter, A. Mitra, S. Roy, A. Emran, T.B., and Cavalu, S. 2022. "Exploring the Journey of Zinc Oxide Nanoparticles (ZnO-NPs) toward Biomedical Applications". *Materials (Basel)* 15(6): 2160.

3- Møller, P., Jacobsen, N.R., Folkmann, J.K., Danielsen, P.H., and Mikkelsen, L., 2010. "Role of oxidative damage in toxicity of particulates". *Free radical research* 44(1): 1-46

4- Moatamed, E.R., Hussein, A.A., El-Desoky, M.M., and El Khayat, Z., 2019. "Comparative study of zinc oxide nanoparticles and its bulk form on liver function of Wistar rat". *Toxicology and Industrial Health* 35(10): 627-637.

5- Abbasalipourkabir, R., Moradi, H., Zarei, S., Asadi, S., Salehzadeh, A., Ghafourikhosroshahi, A., Mortazavi, M., Ziamajidi, N., et al., 2015. "Toxicity of zinc oxide nanoparticles on adult male Wistar rats". *Food and Chemical Toxicology* 84: 154-60.

6- Radhi, M.J., Adnan, G., and Al-bairuty, G. 2019. "Effect of Zinc oxide nanoparticles (ZnO-NPs) on weights of some reproductive organs and sperm abnormalities in the tail of epididymis of albino mice". *Journal of Pharmaceutical Sciences and Research* 11: 243-246.

7- Argmann, C.A., Chambon, P., and Auwerx, J. 2005. "Mouse phenogenomics: the fast track to systems metabolism". *Cell Metabolism* 2: 349-360.

8- Zhao, C.Y. Tan, X.S. and Xiao, X.Y., 2014. "Effects of dietary zinc oxide nanoparticles on growth performance and antioxidative status in broilers". *Biological Trace Element Research* 160(3): 361-367.

9- Morsi, M.H., 2015. "Physiological effects of Nano-zinc on Reproductive performance and Immunity of Male Rat". Thesis (Ph.D.), Faculty of Veterinary Medicine, Alexandria University, Egypt, 2015.

10- Talebi, A.R., Khorsandi, L., Moridian, M., 2013. "The effect of zinc oxide NPs on mouse spermatogenesis". *Journal of Assisted Reproduction and Genetics* 30: 1203-1209.

11- Moridian, M., Khorsandi L., and Talebi, A.R. 2015. "Morphometric and stereological assessment of the effects of zinc oxide NPs on the mouse testicular tissue". *Bratislava Medical Journal* 116(5): 321-325.

12- Atef, H.A., Mansour, M.K., and Ibrahim, E.M., 2016. "Efficacy of Zinc Oxide Nanoparticles and Curcumin in Amelioration the Toxic Effects in Aflatoxicated Rabbits". *International Journal of Current Microbiology and Applied Sciences* 5(12): 795-818.

13- Wang, B., Feng, W., Wang, M., Wang, T., Gu, Y., Zhu, M., et al., 2008. "Acute toxicological impact of nano-and submicro-scaled zinc oxide powder on healthy adult mice". *Journal of Nanoparticles Research* 10: 263-276.

14- Huang, T.H., Lee, H., and Kao C.T., 2001. "Evaluation of the genotoxicity of zinc oxide eugenol-based, calcium hydroxide-based, and epoxy resin-based root canal sealers by comet assay". *Journal of Endodontics* 27: 744-748.

15- Sharma, V., Shukla, R.K., Saxena, N., Parmar, D., Das, M., and Dhawan, A., 2009. "DNA damaging potential of zinc oxide nanoparticles in human epidermal cells". *Toxicology Letters*185(3): 211-8.

16- Ford, T.C., Downey, L.A., Simpson, T., McPhee, G., Oliver, C., Stough, C., et al., 2018. "The Effect of a High-Dose Vitamin B

Multivitamin Supplement on the Relationship between Brain Metabolism and Blood Biomarkers of Oxidative Stress: A Randomized Control Trial" *Nutrients* 10(12):1860.

17- Reynolds, E.S., 1963. "The use of lead citrate at high pH as an electron-opaque stain in 539 electron microscopy". *Journal of Cell Biology* 17: 208-212.

18- Hong, J.S. Park, M.K., and Kim, M.S., 2014. "Prenatal development toxicity study of zinc oxide nanoparticles in rats". *International Journal of Nanomedicine* 9: 159-171.

19- Rajput, V.D., Minkina, T.M., and Behal, A., 2018. "Effects of zinc-oxide nanoparticles on soil, plants, animals and soil organisms: a review". *Environmental Nanotechnology, Monitoring and Management* 9: 76-84.

20- Shati, A.A., 2014. "Ameliorative effect of vitamin E on potassium dichromate-induced hepatotoxicity in rats". *Journal of King Saud University-Science* 26 (3): 181-189.

21- Lowry, O.H. Rosebrough, N.J. Farr, A.L. and Randall, R.J., 1951. "Protein measurement with Folin phenol reagent". *Journal of Biological Chemistry* 193: 265-75.

22- Ohkawa, H., Ohishi, N., and Yagi, K., 1979. "Assay for lipid peroxidase in animal tissue by thiobarbituric acid reaction". *Analytical Biochemistry* 95: 351-358.

23- Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105: 121-6.

24- Flohe, L., Gunzler, W.A., 1984. "Assay of glutathione peroxidase methods". *Enzymology* 105, 114-21.

25- Makler, A., 1980. "The improved ten-micrometer chamber for rapid sperm count and motility evaluation". *Fertility and Sterility* 33: 337-8.

26- Singh, N.P. McCoy, M.T. Tice, R.R. and Schneider, E.L., 1988. "A simple technique for quantitation of low levels of DNA damage in individual cells". *Experimental Cell Research* 175: 184-191.

27- Drury, R.A. and Wallington, E.A. 1980. "Preparation and fixation of tissues". Carleton's histological technique. 41-54.

28- Yameny, A. A Comprehensive Review on Nanoparticles: Definition, Preparation, Characterization, Types, and Medical Applications. *Journal of Medical and Life Science*, 2024; 6(4): 663-672. doi: 10.21608/jmals.2024.419629

29- Chen, A., Feng, X., Sun, T., Zhang, Y., An, S., Shao, L., et al., 2016. "Evaluation of the effect of time on the distribution of zinc oxide NPs in tissues of rats and mice: A systematic review". *IET Nanobiotechnology* 10: 97-106.

30- Lee, I.C., Ko, J.W., Park, S.H., Lim, J.O., Shin, I.S., Moon, C., Kim, S.H., Heo, J.D., Kim, J.C., et al., 2016. "Comparative toxicity and biodistribution of copper nanoparticles and cupric ions in rats". *International Journal of Nanomedicine* 11: 2883-900.

31- Choi, J., Kim, H., Kim, P., Jo, E., Kim, H. M., Lee, M.Y., Jin, S.M., and Park, K., 2015. "Toxicity of zinc oxide nanoparticles in rats treated by two different routes: single intravenous injection and single oral administration". *Journal of Toxicology and Environmental Health* part A. 78(4): 226-43.

32- - Lynch, E.D., Gu, R., Pierce, C., and Kil, J., 2005. "Combined oral delivery of ebselen and allopurinol reduces multiple cisplatin toxicities in rat breast and ovarian cancer models while enhancing anti-tumor activity". *Anticancer Drugs* 16(5): 569-79.

33- Adeyemi, O. and Adewumi, I., 2014. "Biochemical evaluation of silver NPs in wistar rats". *International Scholarly Research Notices* 2014: 1-8.

34- Pasupuleti, S., Alapati, S., Ganapathy, S., Anumolu, G., Pully, N.R., Prakhya B.M., et al.,

2012. "Toxicity of zinc oxide NPs through oral route". *Toxicology and Industrial Health* 28(8): 675-86.

35- Mansouri, E. Khorsandi, L. Orazizadeh, M. and Jozi, Z., 2015. "Dose-dependent hepatotoxicity effects of zinc oxide nanoparticles". *Journal of Nanobiotechnology* 2 (4): 273-282.

36- Kavaz, D. Abubakar, A.L. Rizaner, N. and Umar, H., 2021. "Biosynthesized ZnO Nanoparticles Using *Albizia lebbeck* Extract Induced Biochemical and Morphological Alterations in Wistar Rats". *Molecules* 26(13): 3864.

37- Shakeel, M., Jabeen, F., Qureshi, N.A., et al., 2016. "Toxic Effects of Titanium Dioxide Nanoparticles and Titanium Dioxide Bulk Salt in the Liver and Blood of Male Sprague-Dawley Rats Assessed by Different Assays". *Biological Trace Element Research* 173: 405-426.

38- Hegazy, A.A., Ahmed, M.M., Shehata, A.M., and Abdelfattah, M.M., 2018. "Changes in Rats' Liver Structure Induced by Zinc Oxide NPs and the Possible Protective Role of Vit. E". *International Journal of Human Anatomy* 1(3): 1-16.

39- Welson, N.N., Rofaeil, R.R., Ahmed, S.M., Gaber, S.S., Batiha, G.E., Shahataa, M.G., et al., 2021. "Vitamin E protects against gabapentin-induced chronic hepatic and renal damage associated with the inhibition of apoptosis and tissue injury in rats". *Life Science* 267: 118940.

40- Ahmed, A.F. Ibrahim, I.A. Mokhtar, H.L. and Morsy, M., 2019. "Effect of Zinc Oxide Nanoparticles on the Structure of Testis of Adult Albino Rats and the Possible Protective Role of Naringenin". *Medical Journal of Cairo University* 87: 3469-83.

41- Lanzafame, F.M., La Vignera, S., Vicari, E., and Calogero, A.E., 2009. "Oxidative stress and medical antioxidant treatment in male infertility". *Reproductive Biomedicine* 19(5): 638-659.

42- Lazzarino, G., Listorti, I., Bilotta, G., Capozzolo, T., Amorini, A., Longo, S., Bilotta, P., et al., 106-2019. "Water-and fat-soluble antioxidants in human seminal plasma and serum of fertile males". *Antioxidants* 8(4): 96.

43- Zhang, Y. Ling, F. and Zhu, Q., 2006. "New development of study on the toxicity of nano materials". *Nanoscience* 11: 137-141.

44- Lan, Z., and Yang. W.X., 2012. "Nanoparticles and spermatogenesis: How do nanoparticles affect spermatogenesis and penetrate the blood-testis barrier". *Nanomedicine* 7: 579-596.

45- Leclerc, L., Klein, J.P., Forest, V., Boudard, D., Martini, M., Pourchez, J., Blanchin, M.G., Cottier, M., et al., 2015. "Testicular biodistribution of silica-gold NPs after intramuscular injection in mice". *Biomedical Microdevices* 17(4): 66.

46- Mozaffari, Z., Parivar, K., Roodbari, N.H., and Irani, S., 2015. "Histopathological Evaluation of the Toxic Effects of Zinc Oxide (ZnO) Nanoparticles on Testicular Tissue of NMRI Adult Mice". *Advanced Studies in Biology* 7(6): 275-291.

47- Hussein, M.A., Ali, H.A., Saadeldin, I.M., and Ahmed, M.M., 2016. "Querectin Alleviates Zinc Oxide Nanoreprotoxicity in Male Albino Rats". *Journal of Biochemical and Molecular Toxicology* 30: 489-496.

48- Ahmed, D. H., El-Beih, N. M., El-Hussieny, E. A. & El-Sayed, W. M. 2024 Zinc Oxide Nanoparticles Induced Testicular Toxicity Through Inflammation and Reducing Testosterone and Cell Viability in Adult Male Rats. *Biological Trace Element Research. /doi.org/10.1007/s12011-024-04330-1*

49- Aydilek, N., Aksakal, M., and Karakilçik, A.Z., 2004. "Effects of testosterone and Vit. E on the antioxidant system in rabbit testis". *Andrologia* 36(5): 277-81.

50- Karanth, S., Yu, W.H., and Mastronardi, C.A., 2003. "Vitamin E stimulates luteinizing hormone-releasing hormone and ascorbic acid

release from medial basal hypothalami of adult male rats". *Experimental Biology and Medicine (Maywood)* 228(7): 779-785.

51- Li, D.J., Xu, Z.S., and Zhang, Z.H., 2006. "Antagonistic effects of vitamin E on the testicular injury by cyclophosphamide in mice". *Zhonghua Nan Ke Xue* 12(4): 318-322.

52- Yin, H.P., Xu, J.P., Zhou, X.Q., and Wang, Y., 2012. "Effects of vitamin E on reproductive hormones and testis structure in chronic dioxin-treated mice". *Toxicology and Industrial Health* 28(2): 152-61.

Saddick, S., Afifi Osama, M.A., and Zinada, A., 2017. "Effect of Zinc Nanoparticles on Oxidative Stress-Related Genes and Antioxidant Enzymes Activity in the Brain of *Oreochromis Niloticus* and *Tilapia Zillii*". *Saudi Journal of Biological Sciences* 24 (7):1672-1678.

53- Abbasi, O.E. Mirzaei, F., and Mirzaei, A., 2018. "Effects of ZnO nanoparticles on intestinal function and structure in normal/high fat diet-fed rats and Caco-2 cells". *Nanomedicine* (Lond). 13(21): 279-816.

54- Syama, S., Sreekanth, P.J., Varma, H.K., and Mohanan, P.V., 2014. "Zinc oxide nanoparticles induced oxidative stress in mouse bone marrow mesenchymal stem cells". *Toxicology Mechanisms and Methods* 24(9): 644-653.

55- Santos, S.A., Silva, E.T., Caris, A.V., Lira, F.S., Tufik, S., Dos Santos, R.V., et al., 2016. "Vita E supplementation inhibits muscle damage and inflammation after moderate exercise in hypoxia, Journal of Human Nutrition and Dietetics". *The Official J British Dietetic Association* 29: 516-522.

56- Davis, S.R., McMahon, R.J., and Cousins, R.J., 1998. "Metallothionein knockout and transgenic mice exhibit altered intestinal processing of zinc with uniform zinc-dependent zinc transporter-1 expression". *Journal of Nutrition* 128: 825-831.

57- Wang, C., Cheng, K., Zhou, L., He, J., Zheng, X., Zhang, L., Zhong, X., Wang, T., et al., 2017. "Evaluation of Long-Term Toxicity of Oral Zinc Oxide Nanoparticles and Zinc Sulfate in Mice. Biol". *Trace Elements Research* 178(2): 276-282.

58- Swain, P.S., Rao, S.B.N., and Rajendran, D., 2019. "Effect of Supplementation of Nano Zinc Oxide on Nutrient Retention, Organ and Serum Minerals Profile, and Hepatic Metallothionein Gene Expression in Wister Albino Rats". *Biological Trace Element Research* 190: 76-86.

59- Martinez, G.R., Loureiro, A.P., Marques, S.A., Miyamoto, S., Yamaguchi, L.F., Onuk, J., Almeida, E.A., Garcia, C.C., Barbosa, L.F., Medeiros, M.H., DI Mascio, P., et al., 2003. "Oxidative and alkylating damage in DNA". Mutation Research 544:115-127.

60- Demir, F.T., and Yavuz, M., 2020. "Heavy metal accumulation and genotoxic effects in levant vole (*Microtus guentheri*) collected from contaminated areas due to mining activities". *Environmental Pollution* 256: 113378.

61- Jiang, X., Chang, Y., Zhang, T., Qiao, Y., Klobucar, G., and Li, M., 2020. "Toxicological effects of polystyrene microplastics on earthworm (*Eisenia fetida*)". *Environmental Pollution* 259:113896.

62- Nagar, V., Singh, T., Tiwari, Y., Aseri, V., Pandit, P.P., Chopade, R. L., Pandey, K., Lodha, P., and Awasthi, G. 2022. ZnO Nanoparticles: Exposure, toxicity mechanism, and assessment. Materialstoday: Proceedings 69(1): 56-63.

63- Al Rasheed, N., Abdel Baky, N.A., Al Rasheed, N., Shebly, W., Ahmed A. M., and Faddah, L. M., 2012. "Effect of vitamin E and α-lipoic acid on nano zinc oxide induced renal cytotoxicity in Rats". *African Journal of Pharmacy and Pharmacology* 6(29): 2211-2223.

64- Deepa, S., Murugananthkumar, R., Gupta, Y.R., Gowda, K.S.M., and Senthilkumaran, B., 2019. "Effects of zinc oxide nanoparticles and zinc sulfate on the testis of

common carp, *Cyprinus carpio*". *Nanotoxicology* 13(2): 240-257.

65- Esmaeillou, M., Moharamnejad, M., Hsankhani, R., Tehrani, A.A., and Maadi, H., 2013. Toxicity of ZnO nanoparticles in healthy adult mice. Environ. Toxicol. Pharmacol. 35, 67-71

66- Shi, Y., Pilozzi, A.R., and Huang, X., 2020. "Exposure of CuO Nanoparticles Contributes to Cellular Apoptosis, Redox Stress, and Alzheimer's Abeta Amyloidosis". *International Journal of Environmental Research and Public Health* 17(3):1005.

67- Al-Rasheed, N.M., Al-Rasheed, N.M., Abdel Baky, N-.A., Faddah, L.M., Fatani, A.J., Hasan, I.H., Mohamad R.A., et al., 2014. "Prophylactic role of alpha-lipoic acid and vitamin E against zinc oxide nanoparticles induced metabolic and immune disorders in rat's liver". European Review for Medical and Pharmacological Sciences 18(12): 1813-28.

68- Niedernhofer, L.J., Daniels, J.S., Rouzer, C.A., Greene, R.E., and Marnett, L.J., 2003. "Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells". *Journal of Biological Chemistry* 278: 31426-31433.

69- Badgujar , P.C., Selkar , N.A., Chandratre, G.A., Pawar, N.N., Dighe, V.D., Bhagat, S.T., Telang, A.G., Vanage , G.R., et.al., 2017. "Fipronil-induced genotoxicity and DNA damage in vivo: Protective effect of vitamin E". *Human and Experimental Toxicology* 36(5): 508-519.

70- Nassar, S.A., Ghonemy, O.I., Awwad, M.H., Mahmoud, M.S.M., and Alsagati, Y.M.B., 2017. "Cyto and Genotoxic Effects of Zinc Oxide Nanoparticles on Testicular Tissue of Albino Rat and the Protective Role of Vitamin E". *Transylvanian Review* 25(22): 5809-5819.

71- Faddah, L.M., Baky, N.A., Al-Rasheed, N.M., Fatani, A.J., Atteya, M., et al., 2012. "Role of quercetin and arginine in ameliorating nano zinc oxide-induced nephrotoxicity in rats". *BMC Complementary and Alternative Medicine* 12 (1): 1062.

72- Johar, D., Roth, J.C., Bay, G.H., Walker, J.N., Kroczak, T.J., Los, M., et al., 2004. "Inflammatory response, reactive oxygen species, programmed (necrotic-like and apoptotic) cell death and cancer". *Roczniki Akademii Medycznej w Bialymstoku* 49: 31-39.

73- Schrand, A.M., Rahman, M.F., Hussain, S.M., Schlager, J.J., Smith, D.A., Syed, A. F., et al., 2010. "Metal-based nanoparticles and their toxicity assessment". *Wiley interdisciplinary reviews. Nanomedicine and nanobiotechnology* 2(5): 544-68.

74- Babele, P.K., 2019. "Zinc oxide nanoparticles impose metabolic toxicity by de-regulating proteome and metabolome in Saccharomyces cerevisiae". *Toxicology* Reports 6: 64-73.

75- Sharma, V., Singh, P., Pandey, A.K., and Dhawan, A., 2012. "Induction of oxidative stress, DNA damage and apoptosis in mouse liver after sub-acute oral exposure to zinc oxide NPs". Mutation Research - Genetic Toxicology and Environmental Mutagenesis 745: 84-91.

76- Shukla, R.K., Sharma, V., Pandey, A.K., Singh, S., Sultana, S., and Dhawan, A., 2011. "ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells". *Toxicology in Vitro* 25(1): 231-41.

77- Johnson, K. J., 2014. "Testicular histopathology associated with disruption of the Sertoli cell cytoskeleton". *Spermatogenesis* 4 (2014): 979106

78- Erkanlı Şentürk, G., Ersoy Canillioğlu, Y., Umay, C., Demiralp-Eksioglu, E., and Ercan, F., 2012. "Distribution of Zonula Occludens-1 and Occludin and alterations of testicular morphology after in utero radiation and postnatal hyperthermia in rats. *International Journal of Experimental Pathology*" 93 (6): 438-449.

79- Halawa, A.M., 2010. "Effect of sildenafil administration on ischemia/reperfusion of the testis in adult Albino rat light and electron microscopic study". *Egyptian Journal of Histology* 33: 380-395.

80- Almansour, M.I., Alferah, M.A., Shraideh, Z.A., and Jarrar, B.M., 2017. "Zinc oxide nanoparticles hepatotoxicity: histological and histochemical study". *Environmental Toxicology and Pharmacology*. 51: 124-130.

81- Pinho, A.R. Rebelo, S. and de Lourdes Pereira, M. 2020. "The Impact of Zinc Oxide Nanoparticles on Male (In) Fertility". *Materials* 13" 849.

82- Abdallah, E., Abdelwahab, M.M., and Omran, B. H., 2018. "A study of Subchronic Genotoxic effects of Zinc Oxide Nanoparticles and protective Role of Vitanin E on the Stomach and Pancreas in Adult Albino Rats". *Egyptian Journal of Forensic Sciences and Applied Toxicology* 18 (3): 2018.

83- Pearce, K.L., Hill, A., and Tremellen, K.P., 2019. "Obesity related metabolic endotoxemia is associated with oxidative stress and impaired sperm DNA integrity". *Basic and Clinical Andrology* 29(1): 6.

84- Lazzarino, G., Listorti, I., Bilotta, G., Capozzolo, T., Amorini, A., Longo, S., Bilotta, P. 2019. "Water-and fat-soluble antioxidants in human seminal plasma and serum of fertile males". Antioxidants 8(4): 96.