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ORIGINAL ARTICLE

Ameliorative effects of Copper(II) albumin complex against zinc oxide nanoparticles induced oxidative DNA damage in Sprague Dawley rats

Aya M. Abdelnaem[1] · Hala Fathy[1] · Doha Yahia[2] · Marwa F. Ali[3] · Ahmed Y. Nassar[4] · Doaa Almaz[1]

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Abstract

Objective The present study was carried out to investigate the possible protective role of copper (II) albumin against zinc oxide nanoparticles (ZnONPs) provoked DNA damage and hepatotoxicity in rats.

Methods Forty adult male Sprague Dawley rats were randomly divided into five groups; Group I: the control group maintained on a regular diet, Group II: received 1 ml/day of milk as the solvent of Cu (II) albumin complex, Group III: received Cu (II) albumin at 0.03 µg/gm, Group IV: exposed to ZnONPs (400 mg/kg/day), and Group V: exposed to 400 mg/kg/day ZnONPs plus Cu (II) albumin. All treatments were administered for 30 days. At the end of the experiment, animals were euthanized for collection of blood and liver samples. DNA damage in blood and liver was evaluated by using comet assay, while hepatotoxicity was evaluated from histopathological changes of hepatic tissue and liver enzymes (ALT and AST). The oxidative status parameters including nitric oxide (NO), glutathione peroxidase, malondialdehyde (MDA), and total antioxidant capacity (TAC) were also measured.

Results The results showed that ZnONPs induced oxidative stress through a significant increase in MDA and NO activities, a significant decrease in TAC, and slight decrease in glutathione peroxidase. Significant DNA damage, a significant increase in AST, and a slight increase in ALT were accompanied by histological changes in the liver ZnONPs exposed group. Concurrent Cu (II) albumin supplement to ZnONPs-treated rats in Group IV reversed most of the histopathological changes and DNA damage, significantly lowered ALT and AST levels as well as MDA and NO, and elevated the TAC and GPx.

Conclusion Based on these results, it can be concluded that Cu (II) albumin effectively protects against ZnONPs-induced hepatic dysfunction and DNA damage in rats.

Keywords ZnONPs · Copper(II) albumin · DNA damage · Oxidative stress

Introduction

Nanoparticles (NPs) have gained a lot of interest in pharmaceutical and biotechnological applications due to their unique physicochemical properties [40, 78].

Zinc oxide nanoparticles (ZnONPs) protect against UV rays so they are commonly found in sunscreens and moisturizers. Furthermore, they are used as food additives and packaging due to their antibacterial properties [41]. Because of their extensive use, ZnONPs can be introduced to the body through different routes i.e., ingestion, inhalation, and parenteral injection with little evidence of transdermal penetration but in case of injured skin as sunburnt skin, they can cross to a minimum extent [47]. The lung, liver, kidneys, and heart are the main organs affected by ZnONPs, however, regardless of the route of exposure, the liver is the primary target of NPs as rich in macrophages where phagocytosis of NPs

✉ Doha Yahia
dohayahia@yahoo.com

[1] Department of Forensic Medicine and Toxicology, Faculty of Medicine, Assiut University, Assiut 71516, Egypt

[2] Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Assiut University, Assiut 71516, Egypt

[3] Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Assiut University, Assiut 71516, Egypt

[4] Department of Biochemistry, Faculty of Medicine, Assiut University, Assiut 71516, Egypt

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results in trapping the particle materials within the organ resulting in excess reactive oxygen species (ROS) production caused by abnormal mitochondrial respiration [27, 75].

Mitochondria appear to be the primary target of ZnONPs, which can disrupt protein balance, causing membrane damage or decreasing functionality by modifying the electron transport chain or activating the (NADPH) oxidase enzymes within the organelle [25].

ROS can bind covalently to DNA molecules by reacting with the nucleophilic centers of cells, causing single-strand breaks, double-strand breaks, and the production of DNA adducts, resulting in DNA damage [38].

ZnONPs, besides ROS production, are hepatotoxic via inhibiting the activity and expression of hepatic cytochrome P450 (CYP450) enzymes, which results in liver histopathological abnormalities. Furthermore, zinc accumulates in the liver and inhibits the clearance of other medicines and substances, resulting in their accumulation and toxicity [68, 72]. There is no widely accredited treatment but some agents showed efficacy in protection against ZnONPs toxicity as Silymarin [24], Naringenin [20], and Withania somnifera [44]. According to our knowledge, it is the first study to investigate the potential role of copper complexes against the toxic effects of ZnONPs which we proposed to be beneficial.

Copper displays considerable biochemical action either as an essential trace metal (bounded to ceruloplasmin, albumin, and other proteins) or as a component of various synthesized compounds which are administered exogenously (bounded to ligands of various types forming complexes) [34], [53].

Copper is important for the integrity of collagen and elastin as required for resistance to nonspecific proteinases that cause elastolysis and collagenolysis and enhancing lysyl oxidase function which is responsible for the establishment of lysine-derived cross-links in connective tissue [59].

Copper complexes are used as antimicrobials, antivirals, anti-inflammatory, enzyme-inhibitors, or chemical nucleases [34]. Copper complexes have an anti-cancer effect by inducing apoptosis and cleavage of DNA in cancer cells [57].

This study aims to evaluate the toxic effects of ZnONPs on DNA, oxidative status, and the liver and to assess the ameliorative role of Cu (II) albumin on ZnONPs-induced toxicity.

Materials and methods

Chemicals

Copper (II) albumin complex was obtained from Prof. Ahmed Yassin Nassar—who was awarded a patent for developing such a complex, according to the patent cooperation treaty (PCT) in the International Bureau of World Intellectual Property (WIPO), Geneva, Switzerland / World Organization (WO) 2008/028497.

Zinc oxide nanoparticles (ZnONPs) were prepared at the Faculty of Science, Assiut University as follows:

Zinc Chloride ($ZnCl_2$) (Alpha Chemika, India) was purchased as a white odorless fine powder with particle size < 100 nm and molecular weight: 81.39 g/mol, ZnONPs were synthesized by hydrothermal method using $ZnCl_2$ and NaOH precursors [30].

Animals and treatment

Forty adult male rats weighing between 180 and 220 g were obtained from the Animal House, Faculty of Medicine, Assiut University, and received humane care in compliance with the animal guidelines and ethical regulations of Assiut University. The study was conducted between January 2021 and August 2021 at the Faculty of Medicine, Assiut University, Egypt. All rats were left to acclimatize 14 days before the experiment in plastic cages free from any source of chemical contamination under controlled conditions and maintained at a natural light-dark cycle. The rats had free access to tap water and a balanced diet before and during the experiment.

Experimental design

Rats were randomly divided into five groups as follows:

Group I (control) (n=5): each rat received a regular diet and tap water for 30 days.

Group II (n=5): animals received 1 ml/day of milk (solvent of Cu (II) albumin complex) orally by gavage, in addition to regular diet and tap water for 30 days.

Group III (copper (II) albumin complex): (n=10) rats received copper (II) albumin complex dissolved in milk (1 ml/day) with dosage adjusted to contain 0.03 µg/g body weight gavaged once daily for 30 days.

Group VI (ZnONPs): (n=10) rats received (400 mg/kg/day) of Zinc oxide nanoparticles dissolved in 1 ml distilled water orally by gavage for 30 days. This dose represents 1/15 of the oral LD50 of ZnONPs according to [77].

Group V (ZnONPs + Copper (II) albumin complex): (n = 10) rats received copper(II) albumin complex dissolved in milk (1 ml/day) with dosage adjusted to contain 0.03 µg/g body weight 1 h later, rats received ZnONPs (400 mg/kg/day) dissolved in 1 ml distilled water both gavaged once daily for 30 days. All animals were observed after administration for any morbidity or mortality.

Necropsy and sampling

At the end of the experiment, the body weight for each rat was recorded then animals were euthanized under diethyl

ether anesthesia. Blood samples were collected from the descending aorta. Whole blood samples (1 ml) were collected in Ethylene Diamine Tetra Acetic acid (EDTA) tubes [74] and used immediately for the comet assay which was done at the Forensic Laboratory of Forensic Medicine and Toxicology Department at the Faculty of Veterinary medicine. Blood samples (3 ml) were collected in sterile plain tubes, and centrifuged at 3000 rpm for 15 min to separate the serum that was maintained at −20 °C till biochemical analysis [74].

Liver samples were collected, weighted, and inspected for gross abnormalities then recorded for calculation of the absolute and relative liver weight for each rat in all groups. Relative liver weight (%) was calculated by the equation (absolute liver weight/total body weight × 100).

Liver samples then were divided into two portions. One was used immediately for comet assay; the other part was preserved in 10% buffered formalin for histopathological examination.

DNA damage detection by using comet assay (single-cell gel electrophoresis)

DNA damage in blood and liver was determined using comet assay according to [63] as follows:

Sample homogenization

Liver specimens (0.5 g) were separately minced, suspended in a homogenizing buffer (0.075 M NaCl, 0.024 M Na2EDTA, pH 7.5), and then gently homogenized using bench-top homogenizer (PRO Scientific, USA) at 800 rpm. The homogenate was then centrifuged for 10 min at 0 °C at 1500 rpm to obtain the nuclei. The precipitate was resuspended in a chilled homogenizing buffer and then left to settle for 1–2 min. Whole blood samples (20 µl) were mixed with 2% low melting agarose and then spread on the frosted slides.

Preparation of frosted slides

Frosted slides were layered twice with 100 µL of 1% normal agarose. Seventy-five microliter of nuclear suspension (supernatant) was mixed with 75 µL of 2% low melting agarose (LMA) and the mix was layered on the slide. Finally, on the surface, 100 µL of normal agarose was quickly layered and covered with another slide, and left to gel.

Lysing and electrophoresis

The slides were immersed in a chilled lysis solution (2.5 M NaCl, 100 mM Na4EDTA,10 mM Tris, 0.1% sodium lauryl sulfate (SDS), 10% dimethyl sulfoxide, and Triton X-100) and held for 60 min in the dark at 4 °C. The slides were put on a horizontal gel electrophoresis platform (Cleaver Scientific Ltd., UK) in chilled electrophoresis solution (300 mM NaOH and 1 mM Na2EDTA, pH 13) for 10 min. Electrophoresis was performed in the dark for 15 min at 25 V and approximately 300 mA, then the slides were rinsed with 400 mM Tris buffer to neutralize the excess alkali at pH 7.5 for 7 min. The neutralized slides were allowed to dry at room temperature.

Examination of the slides

Slides were stained with 50 µL (20 µg/mL) ethidium bromide (Wako Pure Chemical Industries, Ltd., Japan), then examined by a fluorescence microscope (Olympus BX-43, Japan) equipped with a green filter, the nuclei on the slides were viewed at a 200-fold magnification. At least 150 nuclei per sample were analyzed by the Comet Assay Software Project (CASP).

Serum biochemical parameters

Oxidative stress parameters

Determination of serum glutathione peroxidase (GPx) activity The serum levels of GPx were estimated using a commercially available kit (catalog number: E-BC-K096-S.), according to the manufacturer's instructions (Elabscience Biotechnology Co., Ltd., Houston, TX, USA) by spectrophotometer (Colorimetric method) by the method of [56].

Assay of malondialdehyde (MDA) MDA was measured by spectrophotometric method; using a kit applied by Biodiagnostic, Egypt: catalog number: MD 25 29; according to the method described by [39].

Assay of total antioxidant capacity (TAC) Total antioxidant capacity (TAC) was measured by spectrophotometric method; using a kit supplied by Biodiagnostic, Egypt; catalog number TA 25 13 according to the method described by [43].

Assay of Nitric oxide Nitric oxide (NO) was measured by spectrophotometric method; using a kit supplied by Bio Diagnostics, Egypt; catalog number: NO 25 33; according to the method described by [52].

Liver function tests

Aspartate aminotransferase (AST) AST was measured by colorimetric method using kit supplied by Spectrum Diagnostics, Egypt; catalog number: 260 001 according to the method described by [23].

Assay of Alanine aminotransferase (ALT) ALT was measured by colorimetric method using kit supplied by Spectrum Diagnostics, Egypt; catalog number: 264001 according to the method described by [23].

Histopathology

After fixation, all tissue samples were routinely processed for conventional histopathological examination according to [70] as follows:

Tissue samples were washed in tap water and then kept in 70% ethyl alcohol overnight. Dehydration of the specimens was done by immersion in ascending grades of ethyl alcohol (70%, 80%, 90%, and 100%) for half an hour each. Tissue samples were cleared with xylene and embedded in paraffin wax and then blocked by fresh molten paraffin. 5-µm thick sections were cut and stained with hematoxylin and eosin stain for histopathological examination by light microscopy. (CX31; Olympus, Tokyo Japan) and photographed using a digital camera (Topview, LCMos10000KPA, China).

In addition, all the microscopic lesions of the liver for each group were presented in tables to demonstrate the type of lesion and severity according to [26] as demonstrated in (Table 1)

Statistical analysis

Statistical analyses were conducted using SPSS software package version 26.0. Data were analyzed by using one-way analysis of variance (ANOVA) followed by *post hoc* lowest significant difference (LSD) multiple range test for comparison between control and exposed groups. All data were expressed as mean ± SE for all experimental and control animals. $P < 0.05$ was considered significant compared to control.

Table 1 Scoring of inflammation and destruction in liver:

Pathological lesions	Score
No lesions	0
Hyperemia	1
Cloudy swelling of hepatocytes	1
Vacuolar degeneration	2
Mononuclear cell infiltration	2
Necrosis in hepatocytes (1–3 hepatocytes)	2
Necrosis in hepatocytes (> 3 hepatocytes)	3
Hemorrhage	3

Results

There were no apparent clinical signs observed throughout the experiment in all groups. There were also no gross abnormalities within the liver samples of all groups.

Liver weight

There were no significant changes in the absolute and relative weight of rats' livers in all experimental groups (Table 2).

DNA damage parameters (Comet assay)

The results showed that there were nonsignificant differences regarding mean values of all DNA damage parameters among control group and group II detected either in blood or liver or between the control group and copper (II) albumin exposed group either in blood or liver by ANOVA test followed by post hoc (LSD), so we used the control group as a standard reference in comparison with other treated groups. DNA damage in each group is expressed in Fig. 1 while different degrees of DNA damage found in the ZnONPs-exposed group are expressed in Figs. 2 and 3.

DNA damage parameters in blood

The results showed a significant ($P<0.001$) increase in the mean values of both DNA damage parameters (tail DNA% and tail length (µm) in the blood of the ZnONPs-exposed group when compared with the control group. Treatment of the exposed animals with copper (II) albumin (ZnONPs + copper (II) albumin) significantly ($P<0.001$) reduced the

Table 2 Absolute and relative weight of liver in all experimental groups

Groups	Control $N=5$	Group (II) $N=5$	Copper (II) albumin $N=10$	ZnONPs $N=10$	ZnONPs + copper (II) albumin $N=10$
absolute weight (g)	5.20 ± 0.55^{a}	4.80 ± 0.13^{a}	4.46 ± 0.24^{a}	4.30 ± 0.31^{a}	6.34 ± 0.49^{a}
Relative weight (%)	3.09 ± 0.31^{a}	2.86 ± 0.05^{a}	2.44 ± 0.40^{a}	2.47 ± 0.12^{a}	3.03 ± 0.16^{a}

Data are expressed as mean ± SE. *N* number of rats

[a] Non significant changes among groups

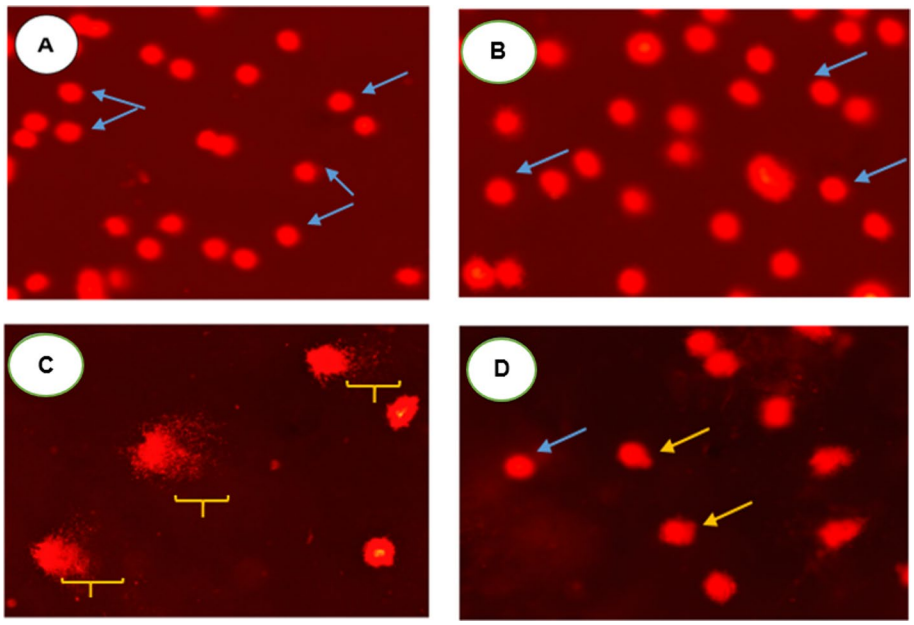

Fig. 1 Comet assay in control and exposed groups. **A** control group showing intact circular nuclei (blue arrows), **B** copper(II) albumin group showing intact circular nuclei (blue arrows), **C** ZnONPs-exposed group showing different degrees of DNA damage (yellow braces), **D** ZnONPs + copper(II) albumin group showing intact circular nuclei (blue arrows), as well as short tail comets (yellow arrows)

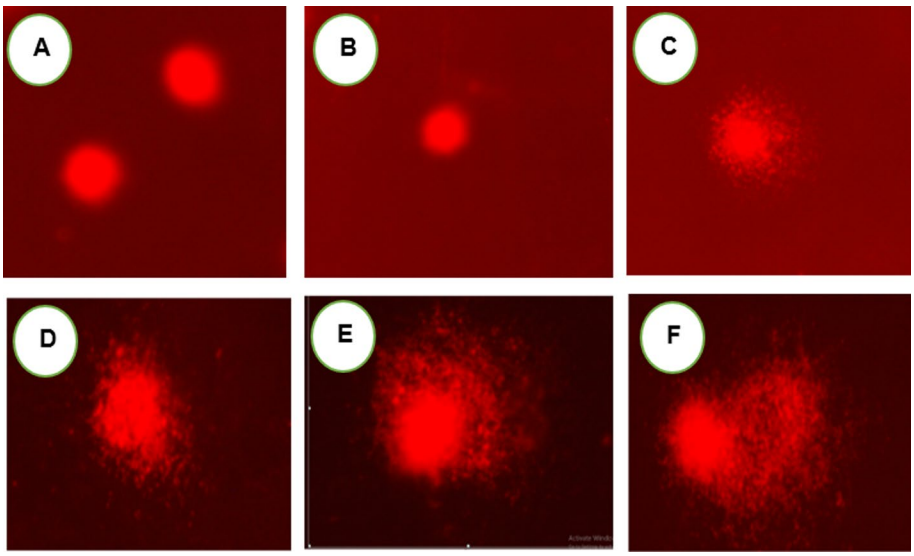

Fig. 2 Different degrees of DNA damage from zero to 4 in blood and liver. **A** and **B** showing intact circular nuclei (0 degree), **C**: mild DNA damage (degree 1), **D**: moderate DNA damage (degree 2). **E** and **F** severe DNA damage degree 3 and 4)

DNA damage parameters in blood when compared with the ZnONPs-exposed group. ZnONPs+ copper (II) albumin group has no significant changes when compared with control indicating that DNA damage has improved to be near normal status (Table 3).

Hepatic DNA damage parameters

The results showed a significant ($P<0.001$) increase in the mean values of both DNA damage parameters (tail DNA%, tail length (μm)) in the liver of the ZnONPs-exposed group when compared with the control group. Treatment of the exposed animals with copper (II) albumin (ZnONPs + copper (II) albumin) significantly ($P<0.001$) reduced the DNA damage parameters in the liver when compared with the ZnONPs-exposed group (Table 4).

Serum biochemical parameters

Oxidative stress parameters The results showed nonsignificant ($P>0.05$) differences regarding mean values of all

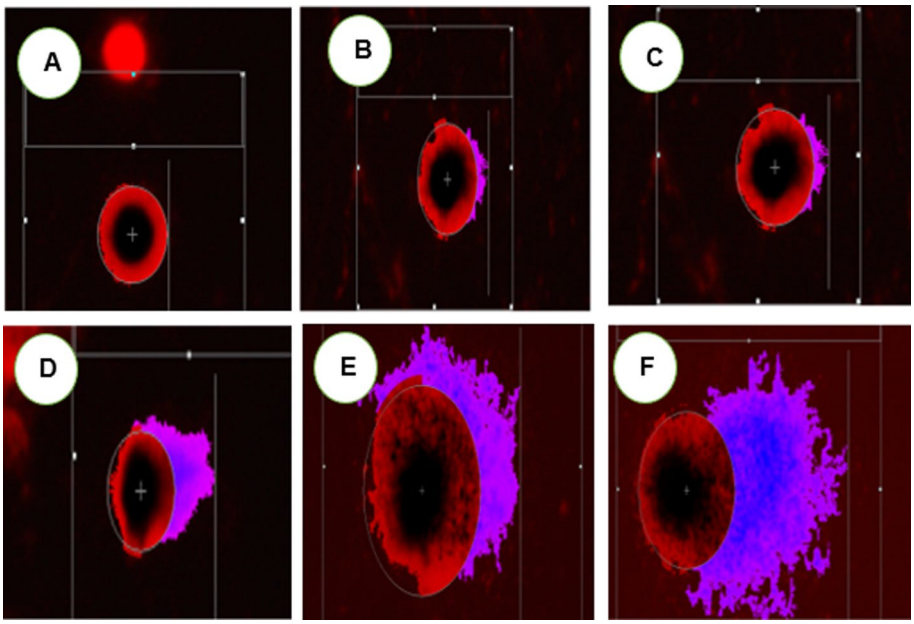

Fig. 3 Different degrees of DNA damage by using Comet Assay Software Project (CASP) software. The blue area represents the comet tail, while the dark red circle represents the comet head boundaries. **A** and **B** no DNA migration in undamaged cell. **C** Mild increase in DNA migration to the tail. **D** Moderate increase in DNA migration. **E** and **F** Severe DNA damage with increased DNA migration and tail length

Table 3 DNA damage parameters (tail DNA%, tail length (μm)) in blood of control group, copper (II) albumin exposed group, ZnO nanoparticles exposed group and ZnO nanoparticles + copper (II) albumin exposed group

DNA damage parameters	Control $N=5$	Copper (II) albumin $N=10$	ZnONPs $N=10$	ZnONPs + copper (II) albumin $N=10$
Tail DNA%	0.79 ± 0.06^{a}	0.80 ± 0.07^{a}	2.57 ± 0.17^{b}	1.17 ± 0.07^{a}
Tail Length	3.74 ± 0.49^{a}	4.20 ± 0.53^{a}	10.03 ± 0.94^{b}	4.81 ± 0.61^{c}

Data are expressed as mean ± SE. N = number of rats

In each row, matching of the letters between 2 groups indicates that there is no significant change between them ($P > 0.05$), while different letters (a, b, c) indicate significant changes among groups

Table 4 Hepatic DNA damage parameters (tail DNA %, tail length (μm)) in control, copper (II) albumin exposed group, ZnO nanoparticles exposed group and ZnO nanoparticles + copper (II) albumin exposed group

DNA damage parameters	Control $N=5$	Copper (II) albumin $N=10$	ZnONPs $N=10$	ZnONPs + copper (II) albumin $N=10$
Tail DNA%	0.65 ± 0.14^{a}	0.59 ± 0.13^{a}	2.70 ± 0.34^{b}	1.57 ± 0.18^{c}
Tail Length	3.48 ± 1.0^{a}	3.96 ± 0.91^{a}	13.08 ± 2.85^{b}	6.33 ± 1.30^{c}

Data are expressed as mean ± SE. N = number of rats

In each row, matching of the letters between 2 groups indicates that there is no significant change between them ($P > 0.05$), while different letters (a, b, c) indicate significant changes among groups

parameters of oxidative stress among control group and group II by ANOVA test followed by post hoc (LSD), so we used control group as a standard reference in comparison with other treated groups.

Glutathione peroxidase (GPx) (U/mL) The results showed a nonsignificant decrease ($P>0.05$) in the mean value of (Gpx) in the ZnONPs-exposed group when compared with the control group but there was a significant increase ($P<0.001$) in the mean value of (Gpx) in ZnONPs+ copper (II) albumin group when compared with ZnONPs-exposed group.

Table 5 Oxidative stress parameters (glutathione peroxidase (GPx), nitric oxide (NO), malondialdehyde (MDA) and total antioxidant capacity (TAC)) in serum of control group, copper (II) albumin exposed group, ZnO nanoparticles exposed group and ZnO nanoparticles + copper (II) albumin exposed group

	Control $N=5$	Copper (II) albumin $N=10$	ZnONPs $N=10$	ZnONPs + copper (II) albumin $N=10$
Gpx	97.40 ± 4.30^{a}	150.20 ± 11.05^{b}	63.16 ± 4.35^{a}	157.66 ± 22.21^{b}
N.O	27.46 ± 1.32^{a}	25.96 ± 0.76^{a}	33.40 ± 0.45^{b}	24.16 ± 1.56^{a}
MDA	9.06 ± 0.27^{a}	8.83 ± 0.18^{a}	11.23 ± 0.13^{b}	9.90 ± 0.05^{c}
TAC	1.43 ± 0.08^{a}	1.46 ± 0.06^{a}	0.28 ± 0.04^{b}	0.60 ± 0.05^{c}

Data are expressed as mean ± SE. N = number of rats

In each row, matching of the letters between 2 groups indicates that there is no significant change between them ($P > 0.05$), while different letters (a, b, c) indicate significant changes among groups

Nitric oxide (NO) (µmol/ml) The results showed a significant increase (P<0.05) in the mean value of (NO) in the ZnONPs-exposed group when compared with the control group while there was a significant decrease ($P<0.001$) in the mean value of (NO) in ZnONPs+ copper (II) albumin group when compared with ZnONPs-exposed group. ZnONPs+ copper (II) albumin group has no significant changes when compared with control group indicating that NO has turned to near normal levels (Table 5).

Malondialdehyde (MDA) (nmol/ml) The results showed a significant increase ($P<0.001$) in the mean value of (MDA) in the ZnONPs-exposed group when compared with the control group while there was a significant decrease ($P<0.05$) in the mean value of (MDA) in ZnONPs+ copper (II) albumin group when compared with ZnONPs-exposed group (Table 5).

Total antioxidant capacity (TAC) (µMol/L) The results showed a significant decrease ($P<0.001$) in the mean value of TAC in the ZnONPs-exposed group when compared with the control group while there was a significant increase ($P<0.05$) in the mean value of TAC in ZnONPs+ copper (II) albumin group when compared with ZnONPs-exposed group (Table 5).

Liver function tests

The results showed nonsignificant ($P>0.05$) differences regarding mean values of ALT and AST between control group (I) and group (II) were detected by ANOVA test followed by post hoc (LSD) or between group I and copper (II) albumin exposed group, so we used the control group (I) as a reference in comparison with other treated groups.

AST (U/L)

The results showed significant increase (P<0.05) in the mean value of AST of ZnONPs-exposed group (III) when compared with group I while there was significant decrease (P<0.05) in the mean value of AST in ZnONPs+ copper (II) albumin group when compared with ZnONPs-exposed group (Table 6).

ALT (U/L)

The results showed nonsignificant increase in the mean values of ALT in ZnONPs-exposed group when compared with group I and a nonsignificant decrease ($P<0.05$) in the mean value of ALT in ZnONPs+ copper (II) albumin group when compared with ZnONPs-exposed group (Table 6).

Table 6 ALT and AST levels in serum of control group, copper (II) albumin exposed group, ZnO nanoparticles exposed group and ZnO nanoparticles + copper (II) albumin exposed group

	Control $N=5$	Copper (II) albumin $N=10$	ZnONPs $N=10$	ZnONPs + Copper (II) albumin $N=10$
ALT	16.33 ± 0.88^{a}	16.66 ± 1.76^{a}	17.66 ± 1.76^{a}	16.66 ± 0.66^{a}
AST	20.66 ± 2.18^{a}	25.00 ± 5.29^{a}	35.66 ± 3.17^{b}	21.00 ± 1.15^{a}

Data are expressed as mean ± SE. N = number of rats

In each row, matching of the letters between 2 groups indicates that there is no significant change between them ($P > 0.05$), while different (a, b, c) letters indicate significant changes among groups

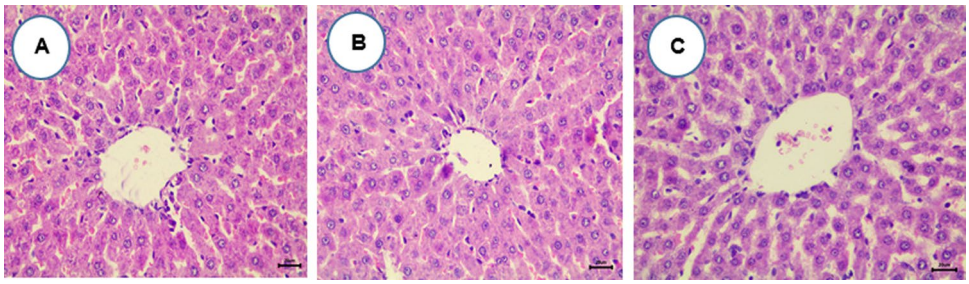

Fig. 4 Histopathological liver sections of control groups and copper (II) albumin complex group, **A** Liver of control group showing normal hepatic architecture, hepatic cords radiated around the central vein H&E. **B** Liver of group (II) showing normal hepatic architecture H&E. **C** Liver of copper (II) albumin complex group showing normal hepatic architecture H&E Bar = 20

Histopathological findings

There were no gross pathological abnormalities among different groups detected by inspection.

Group I: control The liver of the control group showed normal hepatic architecture on microscopic examination of H&E stained tissue sections from the livers of both control group and group II. Hepatic cords are radiated from the central vein, separated by blood sinusoids (Fig. 4A, B). Hepatocytes are polyhedral in shape with acidophilic cytoplasm and vesicular nuclei.

Group III: copper (II) albumin complex-treated group Microscopic examination of H&E stained tissue sections from the liver post administration of copper (II) albumin complex only revealed normal hepatic architecture (Fig. 4C).

Group IV (Zinc oxide nanoparticles (ZnONPs) Histopathological examination of the liver of rats exposed to Zinc oxide nanoparticles revealed some changes throughout the hepatic lobules of all examined rats. These changes were expressed by the focal proliferation of Kupffer cells between hepatic cords (Fig. 5A). The inflammatory reaction was clear as stated by perivascular infiltration of mononuclear inflammatory cells was extensive in some cases (Fig. 5B), while was minimal in other cases. Periportal infiltration of inflammatory cells was also observed (Fig. 5C, D).

The angiopathic changes were very obvious in this group, such as the formation of mixed thrombus consisting of WBCs, RBCs, platelets, and fibrin (Fig. 5F) associated with perivascular infiltration of inflammatory cells (Fig. 5E, F).

Congestion of central veins and some vessels of the portal area were seen as characteristic vascular changes in the liver accompanied by vacuolar degeneration of hepatic tissue affecting a considerable number of hepatocytes (Fig. 6A, B). Interstitial hemorrhage between hepatic cords was also observed (Fig. 6C). In other serial examined sections, the dissociation of hepatic cords around the damaged central vein which suffered from sloughed lining layer of endothelial cells was very evident (Fig. 6D).

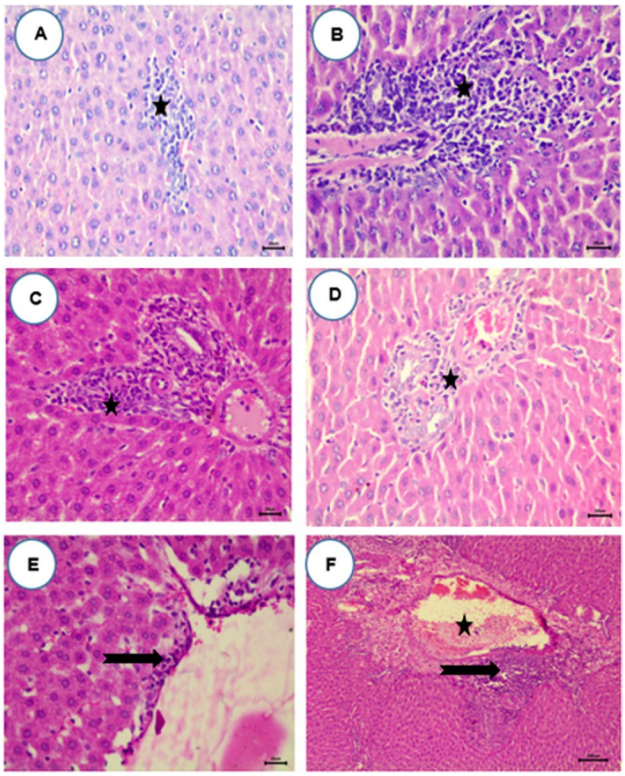

Fig. 5 Histopathological Liver sections of zinc oxide nanoparticles exposed group showing, **A** focal proliferation of Kupffer cells between hepatic cords (star). **B** perivascular infiltration with mononuclear inflammatory cells (star). **C** and **D** portal areas infiltrated with mononuclear inflammatory cells (star). **E**: perivascular lymphocytic infiltration (notched arrow) H&E, bar = 20. **F** mixed thrombosis (star), perivascular lymphocytic infiltration (notched arrow). H&E, bar = 100

Group V (ZnONPs+copper (II) albumin complex) Microscopic examination of H&E stained liver tissue sections

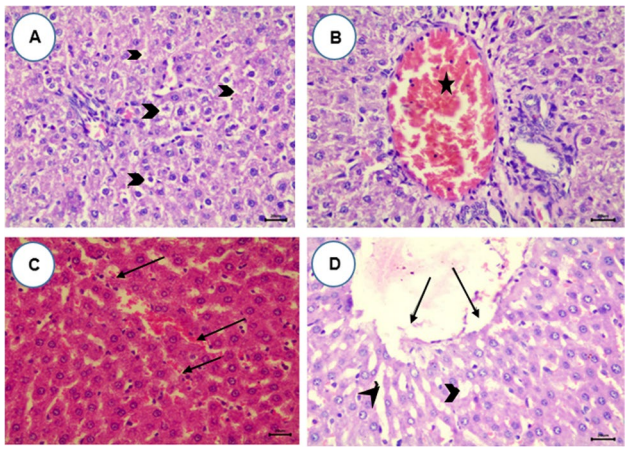

Fig. 6 Histopathological Liver sections of zinc oxide nanoparticles exposed group showing, **A** vacuolar degeneration of hepatic tissue (arrow heads). **B** congestion of blood vessels (star), **C** hemorrhage between hepatic cords (arrows). **D** dissociation of hepatic cords (arrow heads) and sloughing of endothelial cells of central vein (arrow). H&E, bar = 20

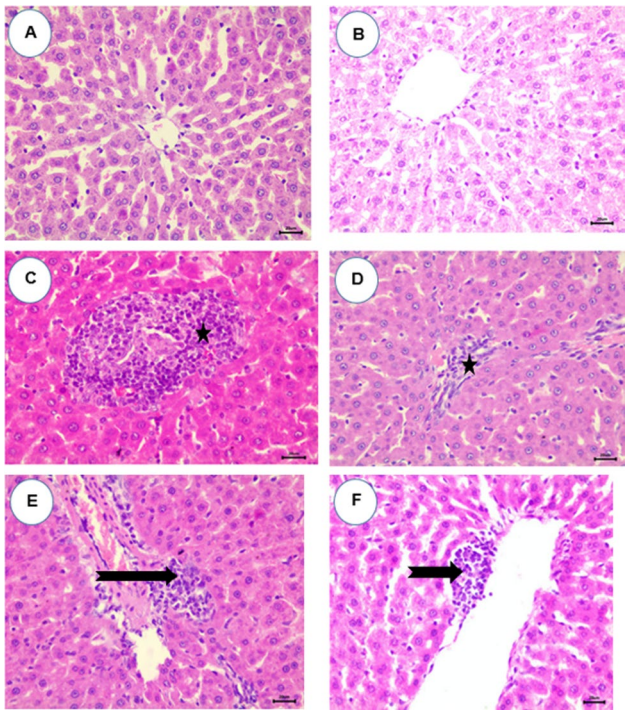

Fig. 7 Histopathological Liver sections of zinc oxide nanoparticles intoxicated groups treated with copper (II) albumin showing **A** normal hepatic architecture. **B** granular degeneration of hepatic cords. **C** portal areas infiltrated with mononuclear inflammatory cells (star). **D** Focal proliferation of Kupffer cells between hepatic cords (star). **E** and **F** perivascular lymphocytic infiltration (notched arrow). H&E, bar = 20

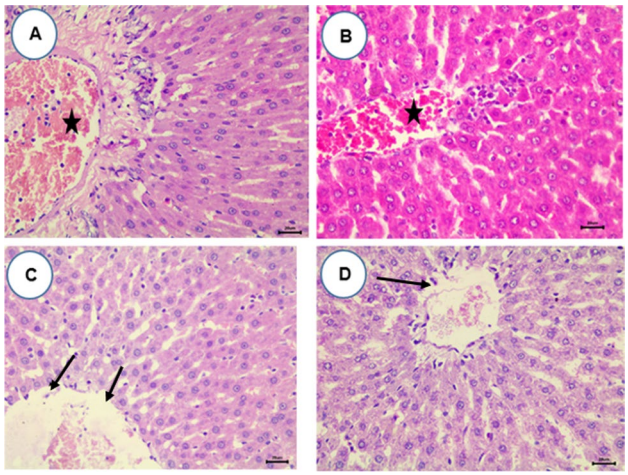

Fig. 8 Histopathological Liver sections of zinc oxide nanoparticles intoxicated groups treated with copper (II) albumin showing **A** congestion of blood vessel (star). **B** thrombus formation (star). **C** and **D** sloughing of endothelial cells of central vein (arrow). H&E, bar = 20

from the ZnONPs + copper (II) albumin group revealed fewer microscopic changes as follows:

Most of the hepatic cords are normal in their architecture (Fig. 7A). There were mild alterations such as focal areas of granular degeneration in some hepatic cords (Fig. 7B). Portal area infiltration with monocytes (Fig. 7C). Focal proliferation of Kupffer cells between hepatic cords (Fig. 7D). Perivascular lymphocytic infiltration was also observed in a low number of cases (Fig. 7E, F).

Angiopathic changes were minimal. These changes were manifested by slight congestion of some blood vessels (Fig. 8A). Thrombus formation was also detected in some blood vessels (Fig. 8B). These diagnostic lesions were associated with the sloughing of endothelial cells of the central vein (Fig. 8C, D).

Histopathological scoring of hepatic lesions in different groups:

There was a significant increase ($P<0.001$) in hepatic lesions in the ZnONPs group when compared with the control group However, the hepatic lesions were significantly decreased ($P<0.001$) in the ZnONPs + copper (II) albumin group when compared with ZnONPs group. In comparison between the control group, group (II), and copper (II) albumin groups there were no significant changes ($P>0.05$) in hepatic lesions. The histopathological scoring is presented in (Table 7).

Table 7 Statistical comparison of histopathological score of hepatic lesions observed by light microscope in all experimental groups

Groups	Control	Group (II)	Copper (II) albumin	ZnONPs	ZnONPs + copper (II) albumin
Hepatic lesions	0.6 ± 0.4^{a}	0.37 ± 1.09^{a}	0.90 ± 0.23^{a}	9.1 ± 0.31^{b}	5.0 ± 0.25^{c}

Data are expressed as mean ± SE

In each row, matching of the letters between 2 groups indicates that there is no significant change between them ($P > 0.05$), while different (a, b, c) letters indicate significant changes among groups

Discussion

ZnONPs are frequently utilized in paints, coatings, and semiconductors, as well as cosmetics including sunscreens, foot care, ointments, and a variety of other topical applications [14].

The present study used the comet assay (single-cell gel electrophoresis) for the detection of the possible DNA damage in the blood and liver of rats quantified by comet tail length and the percentage of DNA in the tail. Tail length is the distance of DNA migration from the body of the nuclear core [15, 73]. The results of the present study showed that exposure of rats to ZnONPs increased both DNA damage parameters in blood and liver. This damage has a direct relation with oxidative stress parameters as MDA and NO increased while GPx and TAC decreased and this result may confirm the rule of oxidative stress in induction of DNA damage.

These results are similar to several studies that also confirmed the DNA damage on different levels and proved its relation to oxidative stress as the oxidative stress-mediated mechanisms are thought to be responsible for ZnONPs' genotoxicity [6, 65].

Sharma et al. [65] reported a significant increase in the DNA damage parameters in rats livers exposed to 300 mg/kg ZnONPs for 14 days. Furthermore, ZnONPs generate an oxidative environment that depletes antioxidant cell repair mechanisms.

The present results are in harmony with most of the previous reports that have revealed the potential DNA damage of ZnONPs; however, a number of studies reported negative results in which ZnONPs demonstrated no or minor DNA damage [4, 13]. This contrast can be explained by the differences in species as these studies were applied to Drosophila melanogaster. Also, Kwon et al. [45] study had shown that ZnONPs did not induce DNA damage in rats when the administered doses were (500, 1000, and 2000 mg/kg) three times by gavage at 0, 24, and 45 h. The short period of exposure could be the reason for the contrasting results.

It should be noted that several previous studies have demonstrated the positive effect of Cu (II) albumin complex on oxidative stress induced by various toxicants, however, and to the extent of our knowledge, there are no publications available evaluating the effect of such complex following the exposure to ZnONPs particularly or its effect on DNA damage resulting from any toxicant.

The present study found that the use of copper (II) albumin complex along with ZnONPs (group IV) significantly reduced the DNA damage parameters.

This result may be attributed to the role of copper as it is a suitable cofactor for a wide range of enzymes and compounds because it participates in redox and catalytic chemistry [34].

In the current study oxidative stress triggered by ZnONPs was indicated by the significant rise of both MDA and NO levels along with decreased levels of both GPx and TAC in the serum of ZnONPs-exposed rats.

Similar findings have been described in which ZnONP cytotoxicity was induced by oxidative stress, resulting in antioxidant depletion as evidenced by high amounts of lipid peroxidation end products and reduced antioxidant status in the liver [83], brain [6], testis [33], and lungs [32] of rats exposed to ZnONPs.

The study of Syama et al. [71] revealed that on treatment of bone marrow mesenchymal stem cells isolated from mice with ZnONPs at a dosage of 70 mg/ml, ZnONPs exhibited cytotoxicity and significantly high ROS induction.

Also, Mansouri et al. [48] reported that the Injection of ZnONPs resulted in a significant rise in oxidative stress in the liver tissue, owing to an increase in lipid peroxidation (MDA) and a significant decrease in superoxide dismutase and glutathione peroxidase enzyme activities.

Previous studies have found that ZnONPs-enhanced oxidation is linked to a reduced antioxidant state and suppressed antioxidant enzyme activity in the rats' livers [5, 31, 48, 83]. According to [49], the formation of ROS by ZnONPs has been related to their nanoscale and semiconductor properties.

In the present study, rats received copper (II) albumin complex along with ZnONPs, both MDA and NO were significantly decreased, while both TAC and Gpx serum levels were increased. These findings can be attributed to the antioxidant activity of the copper (II) albumin complex. The reducibility of Cu as a micronutrient and its bioactive peptide complex form, which plays an important role in endogenous antioxidant defense mechanisms, could also explain the elevation in TAC with copper (II) albumin complex treatment [9].

These findings are in accordance with the study of Mohamed et al. [51] that reported a significant decrease in MDA and an increase in TAC in rats exposed to 10% total body surface area full-thickness thermal burn and treated with copper (II) albumin complex to evaluate its efficacy on thermal wound healing.

Moreover, Abo-Hiemad et al. [2] reported improvement in the levels of GSH, MDA, and NO which were adversely affected in the aflatoxicated rat group.

Also, the levels of antioxidant enzymes (GP_x, GR, GST, SOD, and ceruloplasmin) in Bromobenzene-intoxicated rats were reversed to normal levels, and also NO and lipid peroxidation levels were significantly decreased after copper (II) albumin complex treatment [1].

These findings also agree with other studies using similar copper complexes as Shatat et al. [66] who reported a decrease in NO and lipid peroxides levels in hepatic tissue and an increase in antioxidant cellular chemicals such as GSH and total thiols when the copper complex was used to assess its protective role against aflatoxicosis.

Copper complexes can enhance the activity of the superoxide dismutase enzyme (SOD), reducing oxidative stress [11, 16, 17, 37, 79]. Cu complexes can be regarded as scavengers of oxygen-free radicals because of their SOD-mimetic action, or by promoting SOD synthesis because Cu complexes are good suppliers for SOD biosynthesis. Cu–zinc and manganese are essential components for the two major SOD enzymes' accelerated biosynthesis [42] which explains the positive effects on oxidative stress status of the complex on ZnONPs + copper (II) albumin group.

Clinically, an increase in serum hepatic enzymes (ALT and AST) suggests serious hepatic dysfunction or injury [67, 82]. AST is primarily a mitochondrial enzyme that is utilized particularly with other enzymes to diagnose and confirm the course of hepatotoxicity [76]. ALT, on the other hand, is found largely in the cytoplasm of hepatocytes so its elevation is an early biomarker of cellular necrosis and hepatotoxicity [60].

In the current study the results showed that there was a significant increase in liver transaminases in the group treated with ZnONPs when compared to the control group. Similar results were reported when rats were given ZnONPs at a dose of (10 mg/kg/day) for 28 days orally [80].

Ben-Slama et al. [8] reported that after 5 days of subacute oral administration of ZnONPs (10 mg/kg) to rats, an increase in AST and ALT enzymes was observed.

Similarly, significant increase in hepatic transaminases was found in mice given ZnONPs (300 mg/kg) for 14 days [65], rats given 100 mg/kg ZnONPs orally on a daily basis for 75 days [83], rats receiving 100 mg/kg ZnONPs intraperitoneal every other day for 10 days [50] and rats given 100, 200, 300 mg/kg ZnONPs orally on a daily basis for 14 days [3].

In chicken given a nutritional supplement of ZnONPs, ALT, and AST levels were unlikely lowered [22]. The diversity in animal species could explain the conflicting results. On the other hand, other study indicated no statistically significant changes in the ALT and AST enzyme levels upon exposure of rats to ZnONPs [69]. This disagreement could be explained by the difference in ZnONPs administration routes, as the authors reported their findings using ZnONPs administered dermally.

In the present study Treatment of rats by copper (II) albumin complex along with ZnONPs, showed a prominent decrease in liver transaminases. Similarly, copper (II) albumin complex had been reported to restore the elevated levels of both ALT and AST and the depressed levels of total proteins and albumin to near normal levels reversing the hepatotoxic effect of Bromobenzene [1] and aflatoxin [2].

These results are in accordance with Salama et al. [62] that reported significant decrease in liver transaminases and alkaline phosphatase when copper complex was applied on a nonalcoholic fatty liver rat model and when it was used to evaluate its protective effects against aflatoxicosis [66].

Similar findings, treatment with copper nicotinate complex resulted in an improvement, indicating that the copper complex has an antioxidant and hepatocellular protective action [19]. Moreover, the alterations in transaminases were a positive indication for the copper complex's preventive impact against hepatic tissue collapse caused by ZnONPs.

The hepatoprotective effect of Cu complexes maybe attributed to the anti-inflammatory, anticancerous, antifibrotic, and antioxidative mechanisms of action [57, 59, 12].

The current study investigated the effect of ZnONPs on rat liver as it is a target organ where the majority of nanoparticles tend to accumulate [35].

In the current study, the architecture of the livers of rats exposed to ZnONPs was altered, with angiopathic changes such as dilated congested veins and sinusoidal congestion, thrombosis, and extravasation of blood. Sinusoidal dilatation in hepatic lesions was attributed to hepatocytic atrophy and necrosis or as a result of sinusoidal endothelial injury [55].

The observed vascular changes were accompanied by dispersed inflammatory cellular infiltration in the lobular and portal triads as the proliferation of Kupffer cells and lymphocytic infiltration. This is in line with the findings of Landsiedel et al. [46], who found that ZnONPs can also raise neutrophil counts. The infiltration of inflammatory cells caused by ZnONPs could indicate that these particles interact with interstitial hepatic tissues, resulting in a variety of immunological responses [36]. Kupffer cell hyperplasia following ZnONPs exposure was also observed in [3, 28] that could be a defensive mechanism against hepatic oxidative stress caused by ZnONPs [54, 61].

ZnONPs caused an inflammatory profile in the hearts and lungs of rats [58] in pancreas, liver, and stomach [21] and pathological changes in mice liver as hepatocytic necrosis, kidney as segmentation of glomeruli, epithelial hydropic degeneration, tubular epithelial cells necrosis, and swelling of the proximal tubular epithelium [10].

In this study, hepatocytic vacuolization, hydropic degeneration, and focal regions of hepatic necrosis were also observed. The hydropic hepatocyte degeneration and vacuolization observed suggests that ZnONPs may alter fluid homeostasis, increasing intracellular water permeability and a huge influx of water and Na [5, 64]. Furthermore, hepatocyte degeneration may follow lysosomal hydrolytic enzyme leakage that leads to enhanced cytoplasmic degeneration [5].

The development of cell lysis and cell debris may be the basis of necrotic foci, which could trigger phagocytic infiltration [18]. The development of ROS by NPs is reported to induce cell death and hepatocyte injury through necrosis [81]. Moreover, the necrotic effects of ZnONPs have been linked to hepatocyte vacuolization [29].

Similar results were reported when rats were given ZnONPs at a dose of (10 mg/kg/day) for 28 days orally [80],100, 200, 300 mg/kg body weight for 14 days orally [3], 300 mg/kg body weight for 14 days [48] 1 g/kg body weight for 5 consecutive days [29], and 100 mg/kg body weight every second day for 10 days [50].

In this study rats that received copper (II) albumin complex along with ZnONPs showed a significant decrease in the hepatic lesions when compared with the ZnONPs group. Similarly, livers from Cu (II) albumin complex-treated groups maintained their normal structure of the central vein and portal area with only very small foci of lymphoid cells, and the hepatocyte necrosis was markedly regressed in contrast to the histopathological pattern in the livers of aflatoxicated rats [2].

These results were also confirmed by the improvement to a nearly normal state exerted by Cu (II) albumin complex on hepatitis indicated as (leukocytic infiltration and blood vessels congestion), fatty degeneration, fatty change (steatosis), hepatic necrosis, and nuclear changes in Bromobenzene exposed rats' liver [1].

The observed hepatoprotective effect of Cu (II) albumin complex may be attributed to the anti-inflammatory, antioxidant / radical scavenging activities exerted by the complex [1, 66], [2]. Also, Cu complexes have SOD-mimetic activity, enhance the function of lysyl oxidase and improve Cu–Zn-SOD synthesis which are all necessary for the repair of connective tissue elements.

Some authors thought that the cytoprotective actions of copper complexes are due to their capacity to limit the release of nitric oxide through NOS down-regulation [7].

Conclusion

Sub-chronic exposure of rats to ZnONPs-induced DNA damage, oxidative stress and hepatotoxicity. Cu (II) albumin complex has an ameliorative role in preventing DNA damage, and maintaining the normal structure of the hepatic tissues owing to alleviating the oxidative stress induced by ZnONPs.

Author contributions AMA was involved in preparation of the protocol, performed the experiment, collection of samples, statistical analysis, and writing the article. HF contributed to preparation of the protocol, writing, and revision of the manuscript. DY was involved in comet assay analysis, writing and revision of the manuscript, data analysis, and interpretation of results. MFA contributed to sample collection and preparation for histological examination, photographing, and interpretation of results. AYN was involved in preparation of Copper (II) Albumin Complex. DA contributed to data analysis, preparation, and revision of the manuscript.

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Data availability Data are available if requested by the journal.

Declarations

Conflict of interest Aya M. Abdelnaem, Hala Fathy, Doha Yahia, Marwa F. Ali, Ahmed Y. Nassar, Doaa Almaz declare that they have no conflict of interest.

Consent to participate All authors participated in the preparation of the current study.

Consent to publish All authors agree to publish this article.

Ethical approval The study protocol was approved by the ethical committee of the Faculty of Medicine, Assiut University, Egypt (No. 17101068,4-2020).

References

1. Abdel-Magiud DS, Nassar AY, Shatat AR, Mohamed AS (2019) Radical scavenging activities of albumin-copper complex against bromobenzene induced hepatotoxicity. J Clin Toxicol 9(1):1–10
2. Abo-Hiemad HM, Nassar AY, Shatat AR, Mohamed MA, Soliman M, Abdelrady YA, Sayed AM (2022) Protective effect of copper II-albumin complex against aflatoxin B1- induced hepatocellular toxicity: the impact of Nrf2, PPAR-γ, and NF-kB in these protective effects. J Food Biochem 46(11):e14160. https://doi.org/10.1111/jfbc.14160
3. Aboulhoda BE, Abdeltawab DA, Rashed LA, Abd Alla MF, Yassa HD (2020) Hepatotoxic effect of oral zinc oxide nanoparticles and the ameliorating role of selenium in rats: a histological, immunohistochemical and molecular study. Tissue Cell 67:101441
4. Alaraby M, Annangi B, Hernández A, Creus A, Marcos R (2015) A comprehensive study of the harmful effects of ZnO nanoparticles using Drosophila melanogaster as an in vivo model. J Hazard Mater 296:166–174

5. Almansour MI, Alferah MA, Shraideh ZA, Jarrar BM (2017) Zinc oxide nanoparticles hepatotoxicity: histological and histochemical study. Environ Toxicol Pharmacol 51:124–130
6. Attia H, Nounou H, Shalaby M (2018) Zinc oxide nanoparticles induced oxidative DNA damage, inflammation and apoptosis in rat's brain after oral exposure. Toxins *6*(2), Article 2
7. Baquial JGL, Sorenson JRJ (1995) Down-regulation of NADPH-diaphorase (nitric oxide synthase) may account for the pharmacological activities of Cu(II)2(3,5-diisopropylsalicylate)4. J Inorg Biochem 60:133–148
8. Ben-Slama I, Mrad I, Rihane N, Mir LE, Sakly M et al (2015) Sub-acute oral toxicity of zinc oxide nanoparticles in male rats. J Nanomed Nanotechnol 6:284
9. Berger MM (2006) Antioxidant micronutrients in major trauma and burns: evidence and practice. Nutr Clin Pract 21(5):438–449
10. Bogiswariy S, Jegathambigai R, Marimuthu K (2008) Effect of acute exposure of cadmium chloride in the morphology of the liver and kidney of mice. In: Proceedings of the international conference on environmental research and technology (ICERT), pp 28–30
11. Buchtík R, Trávníček Z, Vančo J (2012) In vitro cytotoxicity, DNA cleavage, and SOD-mimic activity of copper(II) mixed-ligand quinolinonato complexes. J Inorg Biochem 116:163–171
12. Bukhari B, Memon S, Tahir MM, Bhanger MI (2009) Synthesis, characterization and antioxidant activity copper–quercetin complex. Spectrochim Acta Part A Mol Biomol Spectrosc 71(5):1901–1906
13. Carmona ER, Inostroza-Blancheteau C, Rubio L, Marcos R (2016) Genotoxic and oxidative stress potential of nanosized and bulk zinc oxide particles in Drosophila melanogaster. Toxicol Ind Health 32(12):1987–2001
14. Chupani L, Niksirat H, Lünsmann V, Haange S-B, von Bergen M, Jehmlich N, Zuskova E (2018) Insight into the modulation of the intestinal proteome of juvenile common carp (*Cyprinus carpio* L.) after dietary exposure to ZnO nanoparticles. Sci Total Environ 613–614:62–71
15. Collins AR, Dobson VL, Dušinská M, Kennedy G, Štětina R (1997) The comet assay: what can it really tell us? Mutat Res Fundam Mol Mech Mutagen 375(2):183–193
16. Dumitru I, Ene CD, Ofiteru AM, Paraschivescu C, Madalan AM, Baciu I, Farcasanu IC (2012) Identification of [CuCl(acac)(tmed)], a copper(II) complex with mixed ligands, as a modulator of Cu, Zn superoxide dismutase (Sod1p) activity in yeast. J Biol Inorg Chem 17(6):961–974
17. Duncan C, White AR (2012) Copper complexes as therapeutic agents. Metallomics 4(2):127–138. https://doi.org/10.1039/c2mt00174h
18. Edinger AL, Thompson CB (2004) Death by design: apoptosis, necrosis, and autophagy. Curr Opin Cell Biol 16(6):663–669
19. El-Saadani MAM (2004) A combination therapy with copper nicotinate complex reduces the adverse effects of 5-fluorouracil on patients with hepatocellular carcinoma. J Exp Therapeut Oncol 4:19–24
20. El-wafaey DI, Nafea OE, Faruk EM (2022) Naringenin alleviates hepatic injury in zinc oxide nanoparticles exposed rats: impact on oxido-inflammatory stress and apoptotic cell death. Toxicol Mech Methods 32(1):58–66
21. Esmaeillou M, Moharamnejad M, Hsankhani R, Tehrani AA, Maadi H (2013) Toxicity of ZnO nanoparticles in healthy adult mice. Environ Toxicol Pharmacol 35(1):67–71
22. Fathi M, Haydari M, Tanha T (2016) Effects of zinc oxide nanoparticles on antioxidant status, serum enzymes activities, biochemical parameters and performance in broiler chickens. J Livestock Sci Technol 4(2):7–13
23. Friedman RB, Young DS (1989) Effects of disease on clinicallaboratory tests. 4th ed. AACC Press, Washington, D.C.
24. Ghareeb OA (2021) Toxicopathological effects of zinc oxide nanoparticles on the liver function and preventive role of silymarin in vivo. Indian J Forensic Med Toxicol 15(2):3212–3217
25. Giordo R, Nasrallah GK, Al-Jamal O, Paliogiannis P, Pintus G (2020) Resveratrol inhibits oxidative stress and prevents mitochondrial damage induced by zinc oxide nanoparticles in zebrafish (*Danio rerio*). Int J Mol Sci 21(11), Article 11
26. Gökakın AK, Atabey M, Deveci K, Sancakdar E, Tuzcu M, Duger C, Topcu O (2014) The effects of sildenafil in liver and kidney injury in a rat model of severe scald burn: a biochemical and histopathological study. Ulus Travma Acil Cerrahi Derg 20(5):319–327
27. Guo Z, Luo Y, Zhang P, Chetwynd AJ, Qunhui Xie H, AbdolahpurMonikh F, Tao W, Xie C, Liu Y, Xu L, Zhang Z, Valsami-Jones E, Lynch I, Zhao B (2020) Deciphering the particle specific effects on metabolism in rat liver and plasma from ZnO nanoparticles versus ionic Zn exposure. Environ Int 136:105437
28. Hassan ME, Hassan RR, Diab KA, El-Nekeety AA, Hassan NS, Abdel-Wahhab MA (2021) Nanoencapsulation of thyme essential oil: a new avenue to enhance its protective role against oxidative stress and cytotoxicity of zinc oxide nanoparticles in rats. Environ Sci Pollut Res 28(37):52046–52063
29. Hegazy AA, Ahmed MM, Shehata M (2018) Changes in rats' liver structure induced by zinc oxide nanoparticles and the possible protective role of vitamin E. Int J Hum Anat 1(3):1–16
30. Hjiri M, Aida MS, Neri G (2019) NO_2 selective sensor based on αFe_2O_3 nanoparticles synthesized via hydrothermal technique. Sensors 19(1), Article 1
31. Hosseini SM, Amani R, Moshrefi AH, Razavimehr SV, Aghajanikhah MH, Sokouti Z (2020) Chronic zinc oxide nanoparticles exposure produces hepatic and pancreatic impairment in female rats. Iranian J Toxicol 14(3):145–154
32. Huang C-C, Aronstam RS, Chen D-R, Huang Y-W (2010) Oxidative stress, calcium homeostasis, and altered gene expression in human lung epithelial cells exposed to ZnO nanoparticles. Toxicol In Vitro 24(1):45–55. https://doi.org/10.1016/j.tiv.2009.09.007
33. Hussein MMA, Ali HA, Saadeldin IM, Ahmed MM (2016) Querectin alleviates zinc oxide nanoreprotoxicity in male albino rats. J Biochem Mol Toxicol 30(10):489–496
34. Iakovidis I, Delimaris I, Piperakis SM (2011) Copper and its complexes in medicine: a biochemical approach. Mol Biol Int 2011:1–13
35. Jeon Y-M, Kim W-J, Lee M-Y (2013) Studies on liver damage induced by nanosized-titanium dioxide in mouse. J Environ Biol 34(2):283–287
36. Johar D, Roth JC, Bay GH, Walker JN, Kroczak TJ, Los M (2004) Inflammatory response, reactive oxygen species, programmed (necrotic-like and apoptotic) cell death and cancer. Rocz Akad Med Bialymst 1995(49):31–39
37. Joseph J, Nagashri K, Rani GAB (2013) Synthesis, characterization and antimicrobial activities of copper complexes derived from 4aminoantipyrine derivatives. J Saudi Chem Soc 17(3):285–294
38. Kang DH (2002) Oxidative stress, DNA damage, and breast cancer. AACN Adv Crit Care 13(4):540–549
39. Kei S (1978) Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 90(1):37–43
40. Khan I, Saeed K, Khan I (2019) Nanoparticles: properties, applications and toxicities. Arab J Chem 12(7):908–931
41. Kim I, Viswanathan K, Kasi G, Thanakkasaranee S, Sadeghi K, Seo J (2020) ZnO nanostructures in active antibacterial food packaging: preparation methods, antimicrobial mechanisms, safety issues, future prospects, and challenges. Food Rev Int 0(0):1–29

42. Klotz L-O, Kröncke K-D, Buchczyk DP, Sies H (2003) Role of copper, zinc, selenium and tellurium in the cellular defense against oxidative and nitrosative stress. J Nutr 133(5):1448S-1451S
43. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V (2001) Method for the measurement of antioxidant activity in human fluids. J Clin Pathol 54(5):356–361
44. Kumar J, Mitra MD, Hussain A, Kaul G (2019) Exploration of immunomodulatory and protective effect of *Withania somnifera* on trace metal oxide (zinc oxide nanoparticles) induced toxicity in Balb/c mice. Mol Biol Rep 46(2):2447–2459
45. Kwon JY, Lee SY, Koedrith P, Lee JY, Kim K-M, Oh J-M, Yang SI, Kim M-K, Lee JK, Jeong J, Maeng EH, Lee BJ, Seo YR (2014) Lack of genotoxic potential of ZnO nanoparticles in in vitro and in vivo tests. Mutat Res Genet Toxicol Environ Mutagen 761:1–9
46. Landsiedel R, Ma-Hock L, Van Ravenzwaay B, Schulz M, Wiench K, Champ S, Schulte S, Wohlleben W, Oesch F (2010) Gene toxicity studies on titanium dioxide and zinc oxide nanomaterials used for UV-protection in cosmetic formulations. Nanotoxicology 4(4):364–381
47. Liao C, Jin Y, Li Y, Tjong SC (2020) Interactions of zinc oxide nanostructures with mammalian cells: cytotoxicity and photocatalytic toxicity. Int J Mol Sci 21(17), Article 17
48. Mansouri E, Khorsandi L, Orazizadeh M, Jozi Z (2015) Dose-dependent hepatotoxicity effects of zinc oxide nanoparticles. Nanomed J 2(4):273–282
49. Mir AH, Qamar A, Qadir I, Naqvi AH, Begum R (2020) Accumulation and trafficking of zinc oxide nanoparticles in an invertebrate model, *Bombyx mori*, with insights on their effects on immunocompetent cells. Sci Rep 10(1):1–14
50. Moatamed ER, Hussein AA, El-desoky MM, Khayat ZE (2019) Comparative study of zinc oxide nanoparticles and its bulk form on liver function of Wistar rat. Toxicol Ind Health 35(10):627–637
51. Mohamed AO, Nassar AY, Mandour MAM, Saad Eldien HM, Mohammed GME (2012) Effects of Copper (II) albumin complex on thermal skin burn. Bull Egypt Soc Physiol Sci 32(2):12
52. Montgomery HACDJ, Dymock JF (1961) Determination of nitrite in water. Analyst 86(102):414
53. Mukherjee S, Sarathi Mukherjee P (2013) Cu II-azide polynuclear complexes of Cu_4 building clusters with Schiff-base co-ligands: synthesis, structures, magnetic behavior and DFT studies. Dalton Trans 42(11):4019–4030
54. Neyrinck A (2004) Modulation of Kupffer cell activity: physiopathological consequences on hepatic metabolism. Bull Mem Acad R Med Belg 159(5–6):358–366
55. Oligny LL, Lough J (1992) Hepatic sinusoidal ectasia. Hum Pathol 23(8):953–956
56. Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 70(1):158–169
57. Palanimuthu D, Shinde SV, Somasundaram K, Samuelson AG (2013) In vitro and in vivo anticancer activity of copper bis(thiosemicarbazone) complexes. J Med Chem 56(3):722–734
58. Pasupuleti S, Alapati S, Ganapathy S, Anumolu G, Pully NR, Prakhya BM (2012) Toxicity of zinc oxide nanoparticles through oral route. Toxicol Ind Health 28(8):675–686
59. Philips N, Hwang H, Chauhan S, Leonardi D, Gonzalez S (2010) Stimulation of cell proliferation and expression of matrixmetalloproteinase-1 and interluekin-8 genes in dermal fibroblasts by copper. Connect Tissue Res 51(3):224–229
60. Ramaiah SK (2007) A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. Food Chem Toxicol 45:1551–1557
61. Sadauskas E, Wallin H, Stoltenberg M, Vogel U, Doering P, Larsen A, Danscher G (2007) Kupffer cells are central in the removal of nanoparticles from the organism. Part Fibre Toxicol 4(1):10
62. Salama RHM, Nassar AYA, Nafady AAM, Mohamed HHT (2007) A novel therapeutic drug (copper nicotinic acid complex) for non-alcoholic fatty liver. Liver Int 27:454–464
63. Sasaki YF, Izumiyama F, Nishidate E, Matsusaka N, Tsuda S (1997) Detection of rodent liver carcinogen genotoxicity by the alkaline single-cell gel electrophoresis (Comet) assay in multiple mouse organs (liver, lung, spleen, kidney, and bone marrow)1This paper is dedicated to the memory of the late Prof. Kiyosi Tutikawa, National Institute of Genetics, Mishima, Japan.1. Mutat Res Genet Toxicol Environ Mutagen 391(3):201–214
64. Schrand AM, Rahman MF, Hussain SM, Schlager JJ, Smith DA, Syed AF (2010) Metal-based nanoparticles and their toxicity assessment. WIREs Nanomed Nanobiotechnol 2(5):544–568
65. Sharma V, Singh P, Pandey AK, Dhawan A (2012) Induction of oxidative stress, DNA damage and apoptosis in mouse liver after subacute oral exposure to zinc oxide nanoparticles. Mut Res Genet Toxicol Environ Mutagen 745(1):84–91
66. Shatat AR, Eldien HMS, Nassar MY, Mohamed AO, Hussein AHM, El-Adasy A-BA, Khames AA, Nassar AY (2013) Protective effects of copper (I)-nicotinate complex against aflatoxicosis. Open Toxicol J 6(1):1–10
67. Singh SP, Lars B, Luca V, Jian C, Falck JR, Attallah K et al (2016) Downregulation of PGC-1α prevents the beneficial effect of EET-heme oxygenase-1 on mitochondrial integrity and associated metabolic function in obese mice. J Nutr Metab 2016:1–15. https://doi.org/10.1155/2016/9039754
68. Spaggiari D, Geiser L, Daali Y, Rudaz S (2014) A cocktail approach for assessing the in vitro activity of human cytochrome P450s: an overview of current methodologies. J Pharm Biomed Anal 101:221–237
69. Surekha P, Kishore AS, Srinivas A, Selvam G, Goparaju A, Reddy PN, Murthy PB (2012) Repeated dose dermal toxicity study of nano zinc oxide with Sprague-Dawley rats. Cutan Ocul Toxicol 31(1):26–32
70. Suvarna KS, Layton C, Bancroft JD (2018) Bancroft's theory and practice of histological techniques E-Book. 7th ed. Elsevier Health Sciences, p 75–100
71. Syama S, Sreekanth PJ, Varma HK, Mohanan PV (2014) Zinc oxide nanoparticles induced oxidative stress in mouse bone marrow mesenchymal stem cells. Toxicol Mech Methods 24(9):644–653
72. Tang H-Q, Xu M, Rong Q, Jin R-W, Liu Q-J, Li Y-L (2016) The effect of ZnO nanoparticles on liver function in rats. Int J Nanomed 11:4275–4285
73. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Sasaki YF (2000) Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 35(3):206–221
74. Tuck MK, Chan DW, Chia D, Godwin AK, Grizzle WE, Krueger KE, Brenner DE (2009) Standard operating procedures for serum and plasma collection: early detection research network consensus statement standard operating procedure integration working group. J Proteome Res 8(1):113–117
75. Valentini X, Rugira P, Frau A, Tagliatti V, Conotte R, Laurent S et al (2019) Hepatic and renal toxicity induced by TiO_2 nanoparticles in rats: a morphological and metabonomic study. J Toxicol 2019:5767012. https://doi.org/10.1155/2019/5767012
76. Vozarova B, Stefan N, Lindsay RS, Saremi A, Pratley RE, Bogardus C, Tataranni PA (2002) High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. Diabetes 51(6):1889–1895
77. Wang B, Feng W, Wang M et al (2008) Acute toxicological impact of nano- and submicro-scaled zinc oxide powder on healthy adult mice. J Nanopart Res 10:263–276. https://doi.org/10.1007/s11051-007-9245-3

78. Wang L, Xu H, Qiu Y, Liu X, Huang W, Yan N, Qu Z (2020) Utilization of Ag nanoparticles anchored in covalent organic frameworks for mercury removal from acidic waste water. J Hazard Mater 389:121824
79. Weder JE, Dillon CT, Hambley TW, Kennedy BJ, Lay PA, Biffin JR, Regtop HL, Davies NM (2002) Copper complexes of non-steroidal anti-inflammatory drugs: an opportunity yet to be realized. Coord Chem Rev 232(1):95–126
80. Yahya RAM, Azab AE, Shkal KEM, Jm J (2019) Hepatotoxicity induced by copper oxide and zinc oxide nanoparticles and their mixtures in male albino rats. East Afr Sch J Med Sci 2:11
81. Yao Y, Zang Y, Qu J, Tang M, Zhang T (2019) The toxicity of metallic nanoparticles on liver: the subcellular damages, mechanisms, and outcomes. Int J Nanomed 14:8787–8804
82. Yakubu OE, Olawale O, Arowora KA et al (2017) Biochemical changes in haematological and liver function parameters in intoxicated male albino rats treated with hymenocardia acida leaves ethanolic extract. Insights Biomed 2(2):10
83. Yousef MI, Mutar TF, Kamel MAE-N (2019) Hepato-renal toxicity of oral sub-chronic exposure to aluminum oxide and/or zinc oxide nanoparticles in rats. Toxicol Rep 6:336–346