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Evaluation of Histological Changes of Liver after Induced Toxity by Zinc Oxide Nanoparticles in Adult Albino Rat

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Annotation: Oreview: Every year, large quantities of zinc oxide nanoparticles (ZnO-NPs) are produced. These particles are utilized extensively in numerous industrial products and are now also being used in medication delivery and bioimaging. Aim: The recent report set out towards explore the impact of ZnO-NPs, or zinc oxide nanoparticles on hepatic tissue in an effort to discuss and comprehend the harmfulness and possible danger of its usage in medicine and diagnosis. Materials and Methods: Seventy-eight healthy male albino excpermenta rats were treated with zinc oxide nanoparticles (ZnO-NPs) and exposed to doses of 50 or 100 µL of ZnO-NPs with particle sizes of 25, 50, and 75 nm over periods of 5 or 10 days. Results: Findings of the recent work demonstrated the potential toxicity of (ZnO-NPs) affecting hepatic tissues. The main characteristics associated with liver cells alterations were chronically dilated central veins, portal and lobular infiltration by chronically inflammatory cells, hydropic degeneration, cloudy expanding, and fatty degradation. Conclusions: In summary, the noticed alterations might indicate that ZnO nanoparticles (NPs) have damaged hepatocytes, making them unable to deal with accumulated wastes as a result of metabolic and structural abnormalities that NPs produce.The changes that occurred were proportional to size, particularly the smallest producing the greatest impacts, and they were

linked to the duration of exposure to ZnO-NPs.

Keywords: Zinc oxide nanoparticles, hepatic tissue, histology, hydropic degeneration, nanotoxicity, rats.

Overview

The scientific field of nanotechnology focuses on creating and developing nanomaterials, that have dimensions smaller under approxmility 100 nm (Bhardwaj e*t al*., 2020). Researchers look at examining nanoparticles because their tiny size, comparatively large surface-to-area proportion, and physical characteristics, such as the fractional volume impact and the atomic size effect (Doolotkeldieva *et al*., 2022). Nanoparticles of zinc oxide (ZnO-NPs) are amongst the greatest extensively produced nanomaterials, ranking third globally, after silicon dioxide in addition to titanium dioxide nanoparticles (Rajput *et al*., 2018). They are utilized because it readily penetrates the tissues of human bodies, in biomedical science, the farming industry, and manufacturing. Moreover, There are uncertainties regarding the potential uses of ZnO-NPs in biological domains, including drug transport, cancer treatment, and biological diagnostics, as well as certain worries regarding their bio- safety (Kalra *et al*., 2022). ZnO-NPs are utilized in the food additive, water, administration of drugs, cosmetic, pharmaceuticals, and food packaging industries due to their antibacterial and antifungal qualities. This ultimately makes it possible to consume ZnO-NPs with ease (Imade *et al*., 2023).

Despite the fact that ZnO-NPs accumulate in the environment, nothing is known about their prolonged effectivness. They employed to alter heart rate, induce inflammation, and metabolize first in hepatic tissues before moving on to other organs such as the kidney, spleen, brain, and lungs (Al-Ali *et al*., 2022). ROS distorted DNA, altered the free radical scavenging mechanism , and harmed proteins, lipids, as well as carbohydrates (Kołodziej *et al*., 2018). Blood indicators decreased or increased due to the formation of the metallic cation (Zn2+) from zinc with free radicals, that altered ionic equilibrium, unbalanced ions, and hindered iron transportation (Khalid *et al*., 2022). Cardiovascular disease, cancer, and aging are all brought on by free radicals (Khalid *et al*., 2022). Free radicals induce aging, cancer as well as atherosclerosis (Phaniendra *et al*, 2015). In order to investigate the potential changes that these particles could cause in the hepatocellular tissue, the current investigation was carried out.

Materials and Methods

Animals

In the present experimental study, seventy eight adult male-albino rats with an average weight of 200-250 ± 50 g and ages were varying between three to four months were used in the study. Experimental animales were acquired from AL-Nahrain University- Biotechnology Research/Center of animal house. The excpermental animals were housed independently in cages in a sideroom with a controlled 12-hour light/dark cycle, maintained at a temperature of 24±2°C and a relative humidity of 55±10%. Previous to the research, they were given a week to acclimatize, throughout which they had constant contact to standard laboratory food and water. Current work performed in accordance with the ethical standards set forth by the animal experimentation of the AL-Nahrain University- Biotechnology Research /Center of animal house respecting international standards and ethics for experimental research on animals (Smith *et al*., 2020). The handling of animals as per the recommendations providing in the Guide for the Care and Use of Laboratory Animals by National Research Council, (2021).

Experimental groups and administration of substances

Experimental rats were divided randomly into seven main groups, control group (6 rats) and experimental groups II , III, IV, V, VI, VII (12 rats each). Each experimental group was divided

into two subgroups each of 6 rats corresponding to 5^{th}, 10^{th} , days as follows:

Group I (the control group): Consists of 6 rats received standard diet only

Group II- subdivided into:

Subgroup IIa: Administered with 50 µl ZnO-NPs with a measurement of 25 nm within a 5-day timeframe (n = 6).

Subgroup IIb: Administered with of 50 µl ZnO-NPs with a measurement of 25 nm within 10 - day timeframe (n = 6).

Group III- subdivided into:

Subgroup IIIa: Administered with of 50 µl ZnO-NPs with a measurement of 50 nm within 5- day timeframe (n = 6).

Subgroup IIIb: Administered with of 50 µl ZnO-NPs with a measurement of 50 nm within 10- days timeframe (n = 6).

Group IV- subdivided into:

Subgroup IVa: Administered with of 50 µl ZnO-NPs with a measurement of 75 nm within 5- day timeframe (n = 6).

Subgroup IVb: Administered with of 50 µl ZnO-NPs with a measurement of 75 nm within 10- day timeframe (n = 6).

Group V- subdivided into:

Subgroup Va: Administered with of 100 µl ZnO-NPs with a measurement of 25 nm within 5- day timeframe (n = 6).

Subgroup Vb: Administered with of 100 µl ZnO-NPs with a measurement of 25 nm within 10 day- timeframe (n = 6).

Group VI- subdivided into:

Subgroup VIa: Administered with of 100 µl ZnO-NPs with a measurement of 50 nm within 5 - day timeframe (n = 6).

Subgroup VIb: Administered with of 100 µl ZnO-NPs with a measurement of 50 nm within10- day timeframe (n = 6.)

Group VII- subdivided into:

Subgroup VIIa: Administered with of 100 µl ZnO-NPs with a measurement of 75 nm within 5- day timeframe (n = 6).

Subgroup VIIb: Administered with of 100 µl ZnO-NPs with a measurement within 75 nm for 10 day timeframe (n = 6).

Dissection of Animals

At the conclusion of the 5 and 10 day experiment, the experimental animals were sacrificed via cervical dislocation in order to prevent chemical damage. The liver quickly removed and fixed immediately for the entire night in 40 g/l formaldehyde polymer in PBS.Then, sequential 5µm hepatic slides were stained with hematoxylin and eosin for histopathological investigation.

Chemicals

Chemical company sigma-Aldrich, Egypt, supplied the nanoparticles of zinc oxide (ZnO-NPs) at a weight percentage of 50% in phosphate buffered saline (PBS). Considering the data that is being provided via manufacturerZnO-NPs nanoparticles are 35 ± 10 nm in size. Deionized water was utilized for the preparation of the biosynthesized the nanocomposite.

ZnO-NPs stock solution formulation

Using an ultrasonic cleaner sonicator (Branson Ultrasonic Corporation, Danbury, Connecticut, USA), The nanoparticles of ZnO-NPS (35 ± 10 nm) were dissolved with 10 mg/mL of distilled water, then sonicated for 20 minutes at room temperature at 230 V. Prior to delivery, the suspension was vortexed at different doses (25, 50, and 75 nm).

Characterization of Nanoparticles of zinc oxide (ZnO)

To investigate the details and morphology of the practicels, ethyl alcohol was used for dissolving the specimens, then the solution that is diluted was deposited onto a copper metal grid. Subsequently, they had been studied by scaning electron microscope (Field Emission Scanning Electron Microspoe Zeiss Sigma 500 VP, Carl Zeiss , Germany) at the College of Science - University of Basra / Scanning Electron Microscope lab (Ben-Slama *et al*., 2015).

Histology changes

Briefly, hepatic tissue was extracted from excpermental ainmales and immediately fixed in 10% formalin. Following fixation, the sampels was administered with standard alcohol and xylene, paraffin-infused besides sliced in sections of 5-8 thickness measured in micrometers. After that, haematoxylin and eosin (H&E) was used to stain the histological sections, and images were captured using a light microscope (Olympus BH 2; Japan) connected to a digital imaging system.4o mini. Sections of stained tissue from each group were examined for signs of histological features, including changes in the structure, portal regions, liver cells, blood vessels, as well as signs of degeneration, necrosis, fat accumulation, and fibrosis in the portal area prescribed by Sakr and Steenkamp . (2021).

Results

General observations

One mortality was reported throughout the experiment from the ZnO-NPs group. Results of all rats of the control group were comparable to each other.

Zinc oxide nanoparticles (ZnO-NPs) characterization

As shown in (Fig 1), scaning electron microscopy (TEM) showed arbitrary distribution of grain size was detected, and they are agglomerative. The ZnO-NPs particles with 25 and 50 nm ZnO-NPs displayed an approximately spherical morphology of ZnO-NPs and their average size 8.62 ± 2.23 nm, 18.16 ± 1.60 nm respectively, whereas particles with 75 nm ZnO-NPs demonstration crystalline, hexagonal structure with an average size 59.88 ± 2.11.

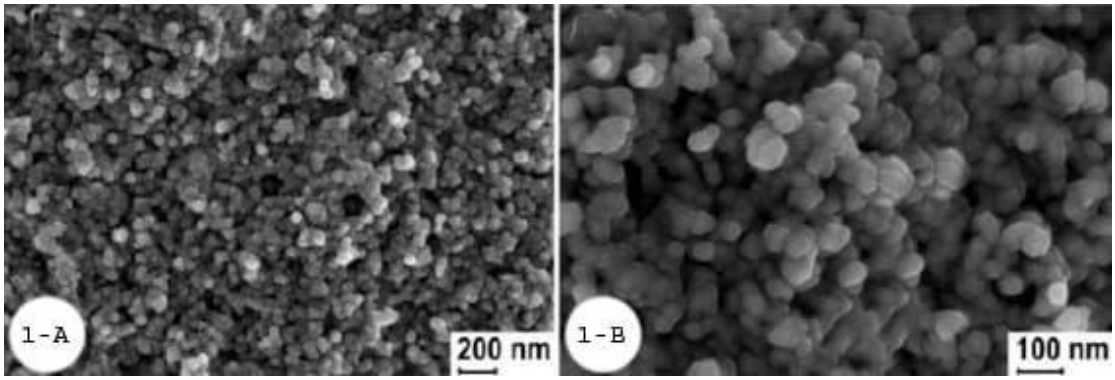


Figure 1 (A, B) : ZnO-NPs photomicrograph under scaning electron microscope (SEM) and particle size distribution of ZnO-NPs.

Microscopic histological examination

None of the experimental groups in this study had any mortality throughout the 5- or 10-day ZnO-NPs administration periods, and neither the appearance nor behavior of the ZnO-NPs -treated rats

differed from that of the control rats. Since these effects, particularly in the liver tissue, have not previously been reported, we would like to clarify the adverse effects caused by intraperitoneal delivery of ZnO-NPs in this work.

Primarily, microscopic assessment of the control rats hepatic sections revealed:normal hepatic arrangements including: normal liver architecture, normal hepatocytes, normal portal triad, and normal central vein (Figure 2). Nevertheless, when compared with the tissue from untreated rats, histological analysis of the hepatic sections from all experimental rats revealed a significant damage in both hepatic tissues (Fig. 2 through Fig. 14; Table 1). Hepatocyte cytoplasm was identified by the presence of darkly pigmented, granular, pink granules and vacuoles,when compared to the control rat, these tissues showed a dilated central vein with vesiculated aberrant and more irregularly shaped nuclei. The major histopathological marker in these tissues decrease in contrast to normal hepatic sections as follows :

The photomicrograph of rats treated with 50 microliters of 25 nanometer nanoparticles over a period of 5 days (Figure 3) exhibited expanded and clogged portal venule with perivenular necrosis infiltration of inflammatory cells, whereas in the liver of rats subjected to with 50 microliter of 25 nanometer particles for 10 days showing sinusoidal dilatation and congestion along with localized hepatocyte depletion with mononuclear inflammatory cell infiltration (Figure 4).

Following a five-day exposure to 50 microliter of 50 nanometer nanoparticles, hepatic tissue of rats exsposed to ZnO-NPs showed a noticeable improvement in hepatic architecture, along with little dilatation and central vein congestion. Additionally, the majority of hepatocytes have acidophilic cytoplasm and seem normal (Figure 5).

On other hand, in a photomicrograph of the hepatic tissues of rats given a dose of 50 microlitier containing 50 nanometer nanoparticles administered over 10 days, the central vascular structure was noticeably dilated and clogged, the blood sinusoids were also dilated and clogged, and the majority of the hepatocytes had hydropic degeneration (Figure 6).

After five days of exposure to 50 microliter of 75 nanometer particles, the hepatic sections displayed extensive lipid accumulation of the hepatic tissues along besides concurrent immune infiltration that infiltrating lobular structures of the liver (Figure 7). In return, photomicrograph of hepatic tissues of rats that receieved a dose of 50 μL of 50 nanometer nanoparticles provided across over 10 days showed different leucocytes, RBC congestion besides fatty deposits (Figure 8).

As displayed in (Figure 9),the results of rats that treated over five consecutive days with 100 microliter of 25 nanometer nanoparticles displayed lobular pro-leukocytes infiltration, congestion, dilatation of central veins besides sinusoids besides activation of kuffer cells, while photomicrograph of hepatic tissues treated rats with 100 microliter of 25 nanometer particles for 10 days revealed the presence of a few binucleated cells, In addition to being bordered by streamlined endothelial cells, along with sinusoidal capillaries that were situated between the cords (Figure 10). Different types of leukocytes were identifiable in the histopathologic images of hepatic tissues that subjected to 100 microliter of 50 nanometer particles for five days (Figure 11). However, binuclear hepatocytes and a noticeable dilation of the clogged portal vein were visible in the photomicrograph of the hepatic tissues of rats that exposed to 100 microliter of 50 nanometer particles over ten consecutive days (Figure 12).

Microscopic investigation of hepatic sections from rats given 100 microliter of 75 nanometer particles over a 5-day period exposed scattered hepatocytes exhibiting cloudy swelling and minor leukocyte infiltration (Figure 13). Conversely, hepatic tissues from rats exposed to the same dosage for 10 days exhibited no discernible pathological modifications. A few hepatocytes were recognized as binucleated or, in rare cases, trinucleated, with no additional anomalies noticed (Figure 14).

The histological changes induced by peritoneal injection of ZnO-NPs were size-dependent; smaller ZnO-NPs induced more efects, and these alterations in tissue were correlated with the

length of exposure to ZnO-NPs.

These histological alterations were observed in figures: 2,3,4,5,6,7,8,9,10,11, 12,13 and 14 as below:

Figure 2: Photomicrograph of hepatic tissues of normal rats (control group) showing: typical appearance of liver histology (H & E; X100).

Figure 3: Photomicrograph of hepatic tissues of treated rats with 50 microliter of 25 nanometer particles for 5 days showing: Engorged portal venule accompanied by perivenular necrosis and infiltration of inflammatory cells (H & E; X200).

Figure 4: Photomicrograph of hepatic tissues of treated rats with 50 microliter of 25 nanometer particles for 10 days showing: Expanded and obstructed vascular channels besides focal hepatocyte depletion with mononuclear cell infiltration (H&E x400).

Figure 5: Photomicrograph of hepatic tissues of treated rats with 50 microliter of 50 nanometer particles for 5 days showing: notable recovery of hepatic architecture with mild dilation and congestion of the central vein; the majority of hepatocytes exhibit a normal appearance with acidophilic cytoplasm and vesicular nuclei. A few cells exhibition hyperchromatic nuclei, and apoptotic nuclei are detected (H & E; X100).

Figure 6: Photomicrograph of hepatic tissues of treated rats with 50 microliter of 50 nanometer particles for 10 days showing: A markedly dilated and congested central vein is observed, along with dilated and congested blood sinusoids and most hepatocytes showing cloudy swelling (H & E; X200).

Figure 7: Photomicrograph of hepatic tissues of treated rats with 50 microliter of 75 nanometer particles for 5 days showing: Localized fatty degeneration of hepatic cells with focal infiltration of inflammatory cells in hepatic lobules; widespread fatty alternations (H & E; X200) .

Figure 8: Photomicrograph of hepatic tissues of treated rats with 50 microliter of 75 nanometer particles for 10 days showing: Neuemerous eucocytes, fat accumulation, red blood cell congestion, and hyperplasia of kupffer cells (H & E; X200) .

Figure 9: Photomicrograph of hepatic tissues of treated rats with 100 microliter of 25 nanometer particles for 5 days showing: congestion, sinusoidal and central vein dilatation, and kuffer cell activation (H&E X200).

Figure 10: Photomicrograph of hepatic tissues of treated rats with 100 microliter of 25 nanometer nanoparticles for 10 consecutive periods showing: Some cells have two nuclei. Flat endothelial cells border the blood sinusoids, which are situated in between the cords. Note that there are some kupffer cells present (H & E; X400) .

Figure 11: Photomicrograph of hepatic tissues of treated rats with 100 microliter of 50 nanometer nanoparticles along 5 consecutive periods showing: It was possible to identify many leukocyte kinds, particularly neutrophils (H & E; X200).

Figure 12: Photomicrograph of hepatic tissues of treated rats with 100 microliter of 50 nanometer particles along 10 days showing: Binuclear hepatocytes; noticeable dilatation of congested portal vein (H & E; X100).

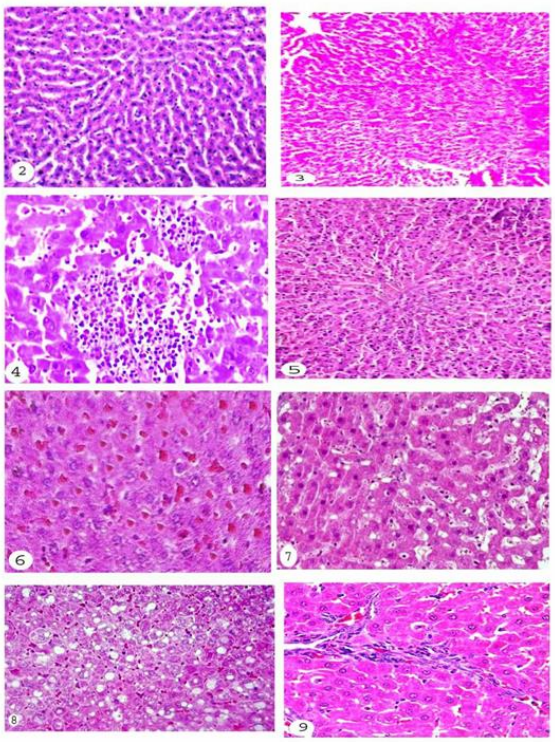
Figure 13: Photomicrograph of hepatic tissues of treated rats with 100 microliter of 75 nanometer nanoparticles along 5 consecutive periods showing: Scattered liver cells cloudy swellings; distributed leukocytes infiltration (H & E; X100).

Figure 14: Photomicrograph of hepatic tissues of treated rats with 100 microliter of 75 nanometer nanoparticles along 10 consecutive days showing. There are no obvious pathologic alterations were identified. Only a few number of hepatocytes showed binucleated or, in rare cases, trinucleation forms, with no further abnormalities detected (H & E; X100).

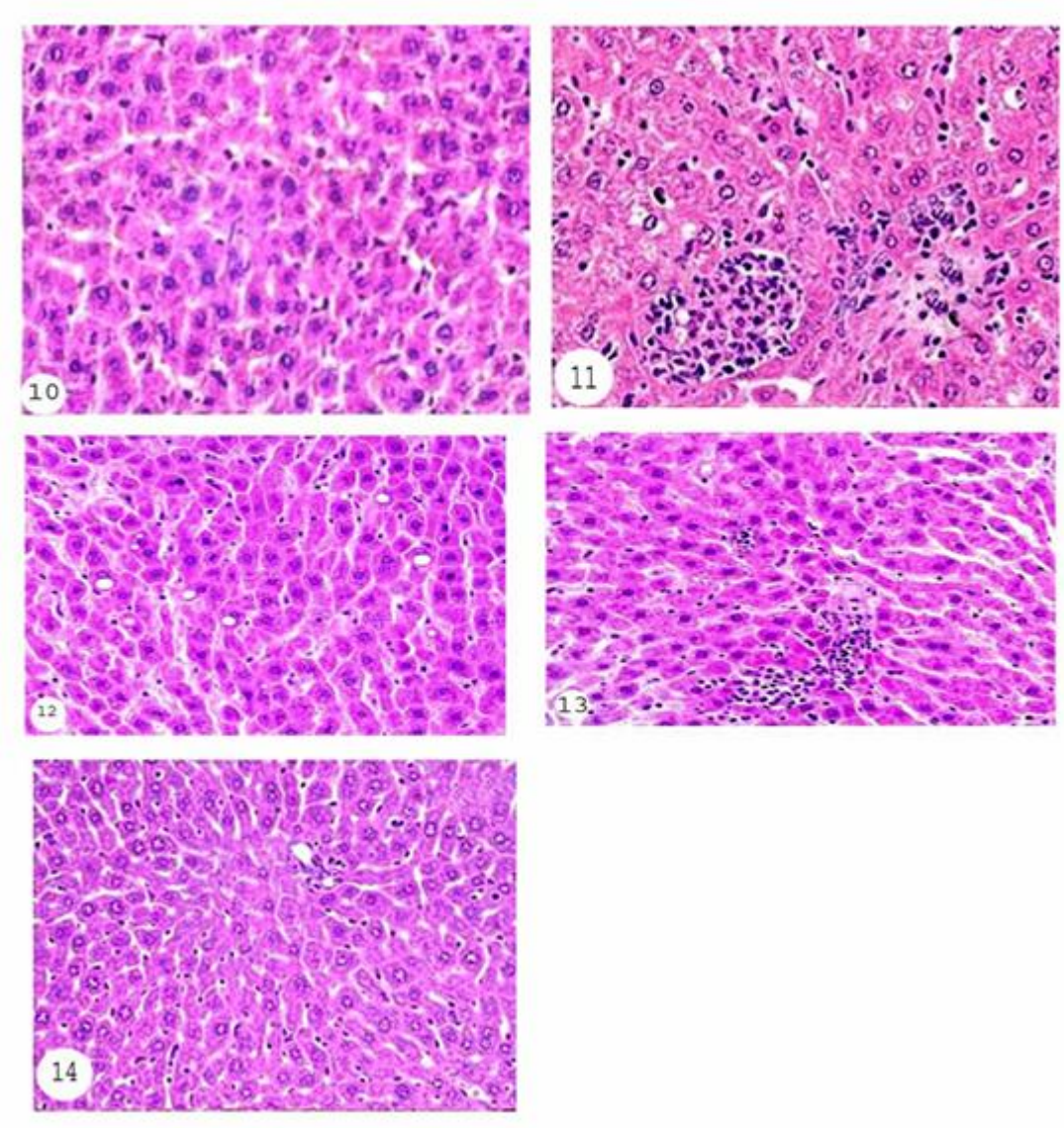
Table 1: Histopathological evaluation of critical occurrences in the hepatic samples of the administered animals

Experimental groups	Group I	Group II		Group III		Group IVa		Group V		Group VI		Group VII	
		IIa	IIb	IIIa	IIIb	IVa	IVb	Va	Vb	VIa VI	VIb	VIIa	VII b
Central vein	(-)	(+)	(++)	(++ +)	(++ +)	(++ ++)	(++)	(++ ++)	(+ +)	(+++)	(++)	(++)	(++ +)
Kupffer cell	(-)	(++)	(++)	(++)	(++ ++)	(++ ++)	(++ ++)	(++ ++)	(+ +)	(++)	(++)	(++)	(++)
Hepatocyte cytoplasm	(-)	(+)	(++)	(++)	(++ ++)	(++ ++)	(++ ++)	(++ ++)	(+ +)	(++)	(++)	(++ ++)	(++)
Hepatocyte nucle	(-)	(+)	(++)	(++ +)	(++ +)	(++ +)	(++ ++)	(++ ++)	(+ +)	(++)	(++)	(++)	(++)
Hemolyzed blood	(-)	++) (	(++ +)	(++)	(++ +)	(++ +)	(++ +)	(++ ++)	(+ +)	(++)	(++)	(++ +)	(++ +)
Narrow sinusoids	(-)	++) (	(++)	(++)	(++ +)	(++ +)	(++ ++)	(++ +)	(+ +)	(++)	(++)	(++)	(++)
Inflammatory cell	(-)	(+)	(++)	(++ +)	(++ ++)	(++ ++)	(++ ++)	(++ ++)	(+ +)	(++)	(++ ++)	(++)	(++)

Histopathological marking symbol: (-) No alteration, (+) slight change, (++) moderate alteration, (+++) moderate to marked alteration, and (++++) marked change.



Figuers (2 until 9): Histological changes induced by peritoneal injection of ZnO-NPs for the different groups at different concentrations throughout specific experimental periods.



Figuers (10 until 14): Histological changes induced by peritoneal injection of ZnO-NPs for the different groups at different concentrations throughout specific experimental periods (continuation of the previous description in figure 2).

Discussion

The TEM consequences obtained from the present study are in agreement with the study carried out by Saeed et al. (2021), who synthesized ZnO-NPs and found that they were crystalline due to peak creation and hexagonal construction. Agglomerated ZnONPs were also found by Imade et al. (2022) as a result of mild forces causing them to associate (Fig 1)

The results of the current study show some hepatic histological changes that are consistent with the findings from earlier research, which found that ZnO-NPs caused inflammation, hepatic necrosis, and deterioration (Kausar *et al.*, 2023). The cloudy swelling may be caused by disturbances of membranes function that result in a significant influx of water and Na+because of ZnO-NPs actions.

The leaking of lysosomal enzymes which results in cytoplasmic damage and macromolecular congestion may accompany cellular swelling. The cause of hydropic degeneration is ion and fluid homeostasis, which increases intracellular water levels (El-Wafaey and Faruk, 2022). According to numerous research, acute and subacute liver damage caused by ZnO-NPs may be shown by Hepatic parenchyma cytoplasmic enlargement with vacuoles enlargematns in the animals subjected to these nanoparticles. Nuclear polymorphism has been linked to hepatic cell dysplasia

and malignancy damage (Al-husseini *et al*., 2020). A defining feature of cellular injury is binucleation, which is a reflection of chromosomal hypergenesis commonly seen in cells that are regenerating. Furthermore, rats given ZnO-NPs showed intermittent infiltration of inflammatory cells in the portal and periportal triad regions.

Lymphocytes and plasma cells made up the bulk of the infiltrate cells. Complying with ten days of therapy, this infiltration became more apparent in animals who received 100 µl as opposed to those that received 50 µl. Inflammatory cells in hepatic tissue may be a sign that ZnO-NPs interfere with the antioxidant defense system by interacting with enzymes along with proteins in the liver interstitial connectivetissue. Reactive oxygen species (ROS) could be produced as a result of this disturbance, potentially causing an inflammatory reaction (Hassan *et al*., 2019).

Sinusoidal Kupffer cells became noticeably more prominent and proliferated after being exposed to ZnO-NPs.In comparison to 50 nm and 75 nm ZnO-NPs, this effect was especially noticeable with 25 nm ZnO-NPs at a dose of 100 µl. Following ten days of theraby, the variations were also more noticeable than in animals that were showing for 5 days. A similar result was reported by Pei et al. (2023)

As a protective detoxification mechanism, the observed Kupffer cell hyperplasia appears to correlate with the severity of hepatic tissue damage caused by ZnO-NPs intoxication, but it also contributes to hepatic oxidative stress. The activation of Kupffer cells suggests that ZnO-NPs improve the phagocytes effectiveness of endothelial cells of sinusoids via rising Kupffer cells count, facilitating the clearance remains accumulating ZnO-NPs through the involvement of lysosomes, which are essential in the intracellular degradation of such nanoparticles into smaller metabolic products (Hassan *et al*., 2021). Exposure to 100 µL of 10 nm ZnO-NPs nanoparticles caused fatty degeneration in the hepatocytes of certain rats. This effect was less evident in rats treated with larger nanoparticle sizes. Besides, the occurrence of liver steatosis was expressively higher among rats Subjected to the effects of the nanoparticles over a 10-day period rather than 5 consecutive days.

As stated by El-Wafaey and Faruk (2022), lipid peroxidation can activate fatty changes in hepatocytes via damaging the rough endoplasmic reticulum, disrupting cytoplasmic lipoprotein organization, and altering normal lipid metabolism. In this study, the abnormal lipid retention observed in hepatocytes subjected to ZnO-NPs might signify nanoparticle-induced hepatic toxicity, presenting as liposis in these cells .

There were sporadic, distinct necrotic patches found in some animal hepatocytes treated with ZnO-NPs nanoparticles. The hepatic tissues of excpermental animals showed this modification when exposed to 25 nm particles, less so when treated with to 50 nm particles, and not at all when treated with 75 nm particles.

Organelles, particularly the mitochondria and the endoplasmic reticulum, may enlarge in conjunction with apoptotic changes. Before nuclear shrinkage and breakdown, these alterations may produce an amorphous eosinophilic cytoplasm, which could be a sign of the liver cell necrosis (Pei *et al*., 2023). Hepatocyte apoptosis seen after prolonged contact with ZnO-NPs nanoparticles might indicate oxidative stress brought on via decreased glutathione in these types of cells.

Conclusions

Histological changes brought on by Subjected to ZnO-NPs, as demonstrated by the current study's findings, may be a sign of damage to hepatocytes due ZnONPs toxic effects, which renders the cells incapable to handle the remains that build as a result of the of ' metabolic along with structural disruptions attributable to these particles. It can be inferred that these changes are influenced by particle size, with smaller ZnO-NPs causing greater damage, particularly with prolonged exposure. The observed cytoplasmic degeneration and nuclear damage in hepatocytes submit that ZnO-NPs could disrupt hepatic proteins and enzymes, impairing the antioxidant defense system. This disruption likely promotes the generation of reactive oxygen species (ROS), which might trigger

oxidative stress, ultimately leading to hepatocyte atrophy and necrosis

To correlate the biomedical usage of ZnO-NPs with the possible risk of their therapeutic and diagnostic use, more ultrastructural and histomorphological studies are required.

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