Asian Journal of Basic Science & Research
Volume 7, Issue 3, Pages 207-225, July-September 2025

Protective Effect of Ginger on Liver, Kidney, Heart, and Spleen Toxicity Induced by Zinc Oxide Nanoparticles

Saima Sharif[1*], Arooj Fatima[2], Atiya Iftikhar[3], Eman Fatima[4], Sara Khan[5], Ammara Waris[6], Shagufta Naz[7] & Komal Junaid[8]

[1-7]Department of Zoology, Lahore College for Women University, Lahore, Pakistan. [8]Department of Computer Science, Lahore College for Women University, Lahore, Pakistan. Corresponding Author Email: ssharif1987@gmail.com[*]

DOI: Under Assignment

Article Received: 17 July 2025 *Article Accepted: 23 September 2025* *Article Published: 26 September 2025*

ABSTRACT

Zinc oxide nanoparticles (ZnO NPs) are widely utilized in various industrial and biomedical applications due to their unique physicochemical properties. However, increasing evidence suggests that excessive exposure to ZnO NPs can lead to significant toxicity in vital organs, including the liver, kidneys, spleen, and heart, primarily through oxidative stress, inflammation, and cellular damage. This study investigates the protective potential of ginger (*Zingiber officinale*) against ZnO NP induced toxicity in these organs. Adult rats were divided into control, ZnO NP treated, ginger-treated, and ZnO NP plus ginger-treated groups. Biochemical parameters, histopathological examinations, and oxidative stress markers were analyzed to assess organ function and tissue integrity. Results demonstrated that.

ZnO NP exposure significantly altered liver, kidney, and spleen function markers. Co-administration of ginger ameliorated these effects, restoring biochemical parameters and preserving histological architecture, likely due to its potent antioxidant and anti-inflammatory properties. These findings suggest that ginger may serve as a natural therapeutic agent to mitigate the toxic effects of ZnO NPs on vital organs.

Keywords: Zinc Oxide Nanoparticles; Oxidative Stress; Organ Toxicity; Liver; Kidney; Heart; Spleen; Ginger; Antioxidant; Anti-Inflammatory.

1. Introduction

Nanotechnology, involving the manipulation of materials at the nanoscale, was first conceptualized by Richard Feynman in 1959 [14]. Metallic nanoparticles (MNPs), especially noble metals like gold, silver, and platinum, are being explored for health applications. Their properties depend on synthesis methods, categorized into top-down and bottom-up approaches [35]. Physical methods use thermal or mechanical energy to produce mono-dispersed particles, while chemical methods use reducing agents [20].

Due to their high surface-area-to-volume ratio, nanoparticles offer enhanced mechanical and catalytic features, making them suitable for pharmaceutical use [28]. They are increasingly utilized in drug delivery systems for effective molecular transport [27],[26].

Zinc oxide (ZnO), naturally found as zincite and synthetically produced, is non-toxic and skin- compatible, used in industrial, cosmetic, and pharmaceutical applications [21]. ZnO nanoparticles (ZnO-NPs) exhibit antidiabetic and antibacterial effects by releasing Zn^{2+} ions, generating ROS, and disrupting bacterial cells [22]. They are widely used in sunscreens for UV protection and antimicrobial action [17].

However, ZnO-NPs can become toxic. In vitro and in vivo studies show they produce ROS, leading to oxidative stress, DNA damage, and apoptosis [39],[46]. ZnO-NPs can enter the body through inhalation, ingestion, or injection, causing cell cycle disruptions, including in neurons [19].

In rats, ZnO-NPs mainly affect the liver, causing histological changes like congestion, necrosis, and inflammation [3], linked to oxidative stress [42],[6]. Rat kidneys eliminate metabolic waste and foreign substances [37]. NPs cause kidney damage directly by altering proximal tubular cells or indirectly via liver-generated metabolites [12].

ZnO-NPs induce oxidative stress, damage mitochondria and DNA, suppress antioxidants, and trigger cell death and inflammation. Observed changes include glomerular segmentation, epithelial necrosis, and proximal tubule swelling [48].

The cardiovascular system is also susceptible. ZnO-NPs accumulate in the heart, increasing cardiac injury markers like myoglobin and troponin-T and promoting inflammation [8]. Despite physiological differences, murine hearts share structural features with humans, making them useful models [43]. ZnO-NPs disrupt calcium balance, impair mitochondrial function, and alter heart electrophysiology, potentially causing arrhythmias. They can also cause endothelial dysfunction, vascular leakage, and blood pressure changes.

Toxins also impair immunity. The spleen, the largest peripheral lymphoid organ, is vulnerable to toxins [41]. It consists of red and white pulp, separated by a marginal zone. The white pulp houses T- and B- cell zones; the red pulp filters blood and contains plasma cells [9],[16]. ZnO-NP exposure alters splenic architecture, thickening capsules, reducing white pulp, congesting red pulp, and increasing megakaryocytes [1].

Although ZnO-NP toxicity is well-documented, its specific impact on albino rat livers, kidney, heart and especially spleen remains insufficiently explored. Moreover, although ginger has been suggested to potentially have therapeutic properties against liver, kidney and heart damages, its effectiveness in alleviating zinc oxide nanoparticle-induced hepatotoxicity, nephrotoxicity and immunotoxicity requires further investigation. Thus, this research aims to address these gaps by examining the toxicological effects of zinc oxide nanoparticles on the histology of four different organs of albino rats and evaluating the therapeutic potential of ginger as an intervention.

1.1. Study Objectives

1) To evaluate the effects of ZnO nanoparticles on liver function.
2) To assess kidney function alterations after ZnO NP exposure.
3) To investigate histopathological changes in spleen tissue.
4) To determine dose-dependent effects of ZnO NPs.
5) To compare oxidative stress markers among treated groups.

2. Materials and Methods

This study was conducted at the Animal House of the Zoology Department, Lahore College for Women University (LCWU), Lahore. A total of 108 healthy albino rats (3 weeks old) were obtained from the University of Veterinary and Animal Sciences (UVAS), Lahore. Animals were housed in 20×20 cm^2 wire cages under controlled temperature, natural light–dark cycles, and provided with clean water and a balanced diet. Prior to experimentation, rats were acclimatized for 7 days under standard laboratory conditions.

2.1. Experimental Design and Grouping

The rats were randomly assigned into four treatment groups, each comprising 27 rats (n = 27), with further subdivision into three time-point subgroups (n = 9 each):

- Group I (Control): Received 0.25 mL oral saline daily for three weeks.
- Group II (Ginger-treated): Administered 200 mg/kg ginger powder suspended in 0.25 mL distilled water [10].
- Group III (ZnO NP-treated): Administered 300 mg/kg ZnO nanoparticles orally for three weeks [11].
- Group IV (ZnO + Ginger): Co-treated with 300 mg/kg ZnO nanoparticles and 200 mg/kg ginger powder in 0.25 mL saline.

At the end of each week, three animals per subgroup were sacrificed under chloroform anesthesia for tissue collection.

2.2. Preparation of Ginger Powder

Fresh ginger rhizomes were procured from a local herbal market in Lahore, Pakistan. After washing and peeling, slices were shade-dried for 7–10 days, ground into fine powder using an electric grinder, and stored in airtight containers at 5 °C until use [29].

2.3. Preparation of ZnO Nanoparticles

Zinc oxide nanoparticles in dry powdered form were purchased from a certified scientific supplier. The required doses were freshly suspended in distilled water before each administration to ensure uniform delivery.

2.4. Tissue Sampling and Processing

After dissection, heart, liver, spleen, and kidney tissues were rinsed in 0.85% saline to remove blood and debris. Samples were fixed in a solution of ethanol (60 mL), formaldehyde (30 mL), and glacial acetic acid (10 mL) [44].

Dehydration was carried out in ascending ethanol grades (80%, 90%, 100%), followed by clearing in cedarwood oil. Embedding was done using a toluene–paraplast wax mixture (1:1) at 60 °C. Solidified blocks were trimmed and sectioned using a rotary microtome.

2.5. Sectioning and Staining

Tissue sections of 5 µm thickness were mounted on albumin-coated slides and incubated overnight at 60 °C. Sections were deparaffinized with xylene, rehydrated through descending ethanol grades, and stained with Hematoxylin and Eosin (H&E). After staining, slides were dehydrated, cleared, and mounted with Canada balsam under coverslips.

2.6. Microscopic Evaluation

Slides were examined under a Nikon Ei1-L2 trinocular microscope with an attached digital camera. Histological architecture was evaluated, and photomicrographs were captured at magnifications ranging from 100× to 1000×.

2.7. Body Weight Monitoring

Body weight of each rat was recorded before the start of the experiment and monitored regularly throughout the treatment period. The initial average body weight was 240–250 g (±10 g).

Asian Journal of Basic Science & Research
Volume 7, Issue 3, Pages 207-225, July-September 2025

3. Results

Zinc oxide nanoparticles (ZnO NPs), despite being widely utilized in many industries because of their high reactivity and nanoscale size, are known to pose toxicological concerns to the liver, kidneys, heart, and spleen of albino rats. This study examined ginger's possible protective properties during a three- week period against ZnO NP-induced damage in albino rats. Rats were split into four groups: control, ginger-only, ZnO NPs-only and ZnO NPs + ginger. Histopathological alterations in the liver tissues of each group were monitored once a week. During the research there were no recorded deaths.

3.1. Liver

Rats with normal hepatic histology in this initial week control group showed well-organized hepatic lobules encircling a central vein (Figure 1a). Cellular edema (Figure 1c), Kupffer cell multiplication, lipid peroxidation, mitochondrial damage, fibrosis and portal vein congestion (Figure 1d) were all brought on by ZnO-NP exposure. Histology was better in animals given ginger (Figure 1b). When ZnO- NPs and ginger were administered together the liver suffered less damage with fewer necrotic cells, less inflammation and minor hepatocyte degeneration (Figure 1e).

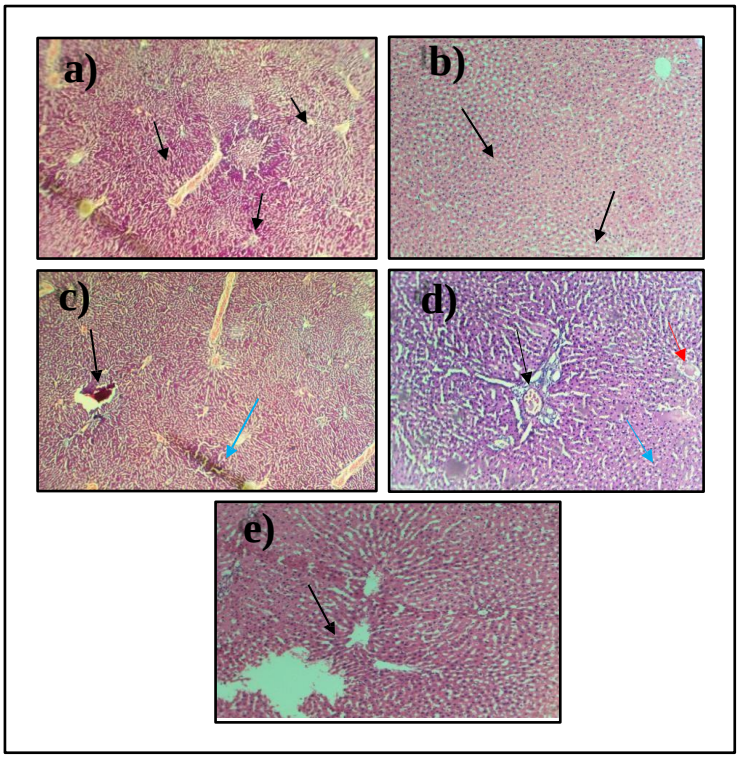

Figure 1. Histopathological examination of liver tissue sections after 1 week (H&E stain): a) section of the control rat liver showing portal tract (black arrow), kupffer cells (red arrow) and central vein (blue arrow) at 100X, b) Section of rat liver treated with ginger indicates normal sinusoidal space (black arrow) and hepatocytes (blue arrow) at 400X, c) Section of liver of Group III showing edema (black arrow) and ballooning degeneration (blue arrow) at 100X, d) Section of liver of Group III showing swelling (red arrow), hyperemia (black arrow) and congestion (blue arrow) of portal vein at 100X, e) Section of rat liver in Group IV indicates the protective effect of ginger against toxicity induced by ZnO-NPs showing lesser hepatocytes degeneration at 100X.

Rats given ZnO-NP showed vacuolization, lipid peroxidation, sinusoidal dilatation and hepatocellular degeneration in the second week (Figure 2a&b). Indicating protection against oxidative damage, ginger co-treatment produced lesser damage including sinusoidal dilatation, decreased inflammation and vacuolization (Figure 2c).

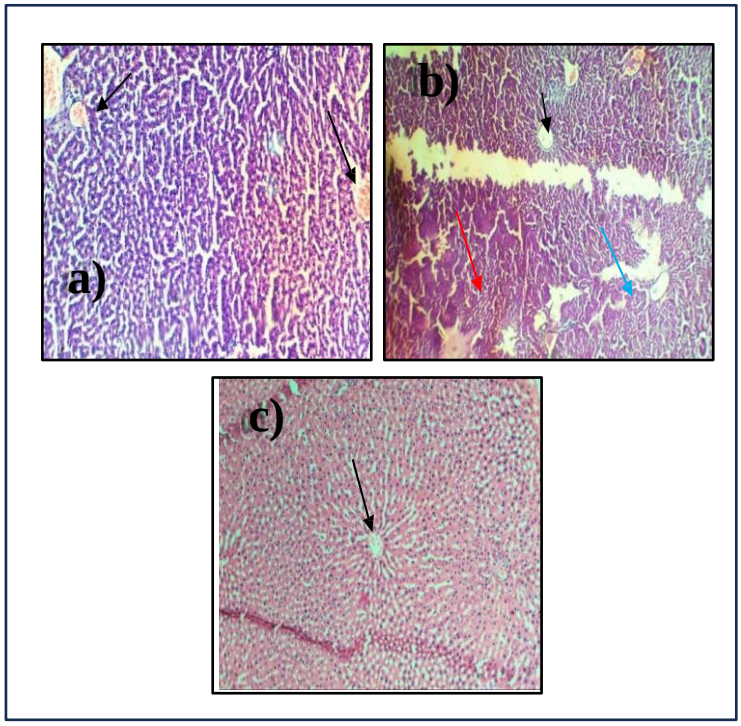

Figure 2. Histopathological examination of liver tissue sections after 2 weeks (H&E stain) : a) Section of liver of Group III showing sinusoidal dilation (black arrow) and vacuolated cells (red arrow) at 400X, b) Group III section of liver showing cellular infiltration (red arrow), inflammation (black arrow) and disruption of hepatic cords (blue arrow) at 100X, c) Section of rat liver in Group IV indicates the protective effect of ginger in hepatocytes, lesser inflammation of hepatocytes at 100X.

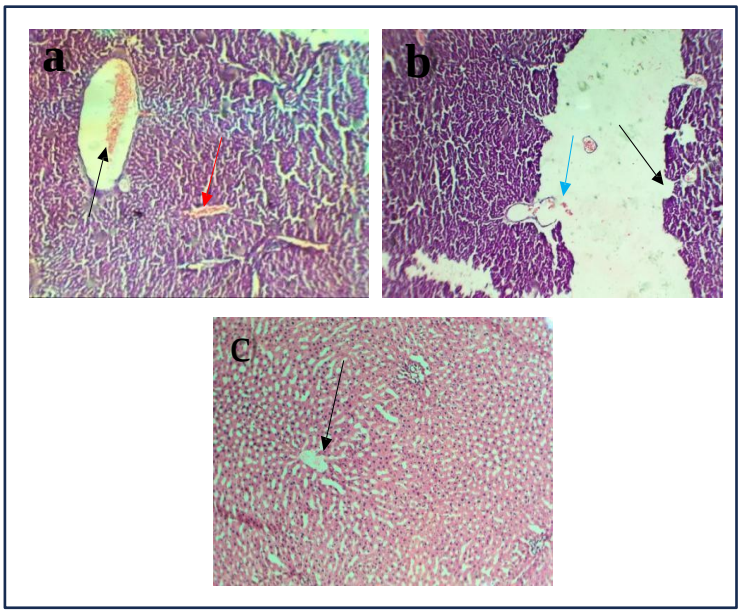

Figure 3. Histological examination of liver tissues after week 3 (H&E stain) : a) Section of liver showing vascular congestion (VC), and intracellular vacuolization (IV) at 400X, b) Section of liver showing apoptosis (A) and

necrosis (N) in Group III at 100X, c) Section of rat liver indicates lesser disruption of hepatocytes (DH) in Group IV at 100X.

Hepatocellular necrosis, inflammation, vacuolization and apoptosis were observed in the third week as a result of prolonged exposure to ZnO-NP (Figure 3a&b). Ginger's beneficial antioxidant activity was demonstrated by the considerable reduction in liver damage, necrosis and inflammation that resulted from co-administration (Figure 3c).

3.2. Kidney

The control group's renal histology was normal after the first week with intact tubules and healthy glomeruli (Figure 4a&b). According to the figure 4c the results of the ginger-only group were comparable suggesting that ginger had no negative consequences. In contrast the ZnO NPs group showed early damage indicators like moderate tubular swelling, epithelial cell vacuolation and inflammatory cell infiltration including macrophages and lymphocytes (Figure 4d). Significantly fewer pathological alterations were seen in the group that received both ZnO NPs and ginger indicating that ginger may help minimize early tissue damage as shown in figure 4e.

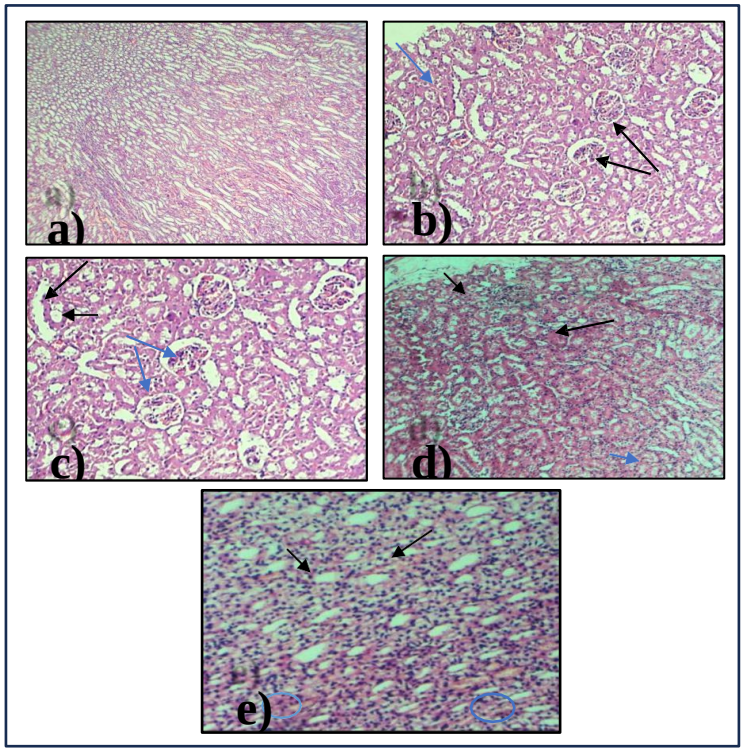

Figure 4. Histopathological examination of kidney tissue sections after 1 week (H&E stain): a) Section of Kidney of Control Group Rat Showing Cells of Renal Cortex (RC) and Renal Medulla (RM) at 100X, b) Kidney Tissues of Control Group Showing Intact Glomerulus Capsule (GC) (black arrows) and Proximal and Convoluted Tubules (PCT) (blue arrow) at 400X, c) Kidney Tissues of Group II Showing Intact Structure of Glomerulus and Proximal Tubules as Shown in Group I at 400X, d) Kidney Tissues of Group III Showing Mild Vacuolization of Tubular Epithelium (blue arrow) and Infiltration of Inflammatory Cells (black arrow) at 100X, e) Kidney Tissues of Group IV Showing Reduction in the Degradation and Vacuolization in the Cytoplasm of Tubular Epithelium (black arrows) and Intact Epithelium of Tubules (blue circles) at 400X.

The ginger group's renal architecture was still normal at the second week. Conditions worsened in the ZnO NPs group resulting in glomerulus damage, dilation and degradation of proximal and convoluted tubules (Figure 5a), endothelial damage in blood vessels, tubule degeneration, neutrophil and eosinophil infiltration (Figure 5b) and eosinophilic debris. With decreased inflammation and improved preservation of renal tissue the ZnO + ginger group saw less severe histological damage and reduced number of inflammatory cells and inflammation due to these C cells and there were also mild to no damage to tubules that can be seen in figure 5c, intact tubules suggests that ginger has a preventive effect against progressive damage there was reduced congestion of blood vessels and more vascular stability and Normal Blood Vessels (Figure 5d).

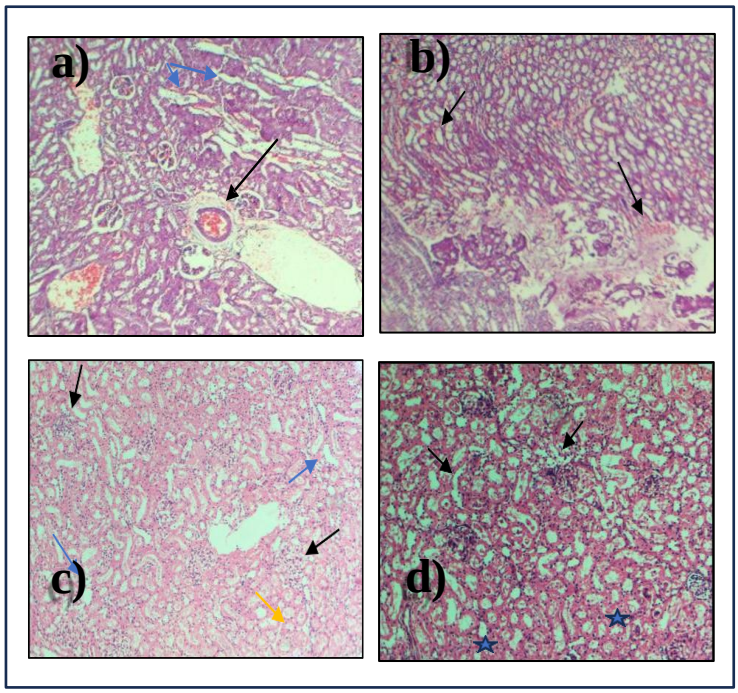

Figure 5. Histopathological examination of kidney tissue sections after 2 weeks (H&E stain) : a) Tissues of Group III Showing Damaged Glomerulus Capillaries (black arrow) due to Capillary Infiltration and Degradation and Dilation of Proximal and Convoluted Tubules (blue arrows) at 400X, b) Tissues of Group III Showing Aggregation of Inflammatory cells, Particularly Mononuclear cells (MC) Around Blood Vessels and Between Renal Tubules (black arrows) at 100X, c) Kidney Tissues of Group IV Showing Reduced Number of Inflammatory Cells and Inflammation Due to These Cells (black arrows) and There is also Mild to no Damage to Tubules that can be seen in this Picture (blue arrows) and Intact Tubules (yellow arrow) at 400X, d) Kidney Tissues of Group IV Showing Reduced Congestion of Blood Vessels and More Vascular Stability (black arrows) and Normal Blood Vessels (blue stars) at 400X.

The kidneys in the ginger treated rats exhibit preserved histo-architecture in the last week. Vascular congestion, glomerular sclerosis (Figure 6a), loss of brush boundaries (Figure 6b), fibrosis and cytoplasmic vacuolation (Figure 6c) were among the severe pathologies seen in the ZnO NPs group. But there was a noticeable histological improvement in the ZnO + ginger group as seen by more intact glomeruli and tubules, less fibrosis and fewer proteinaceous casts (Figure 6d). This demonstrated ginger's restorative and preventive effects in lowering renal damage brought on by ZnO NP.

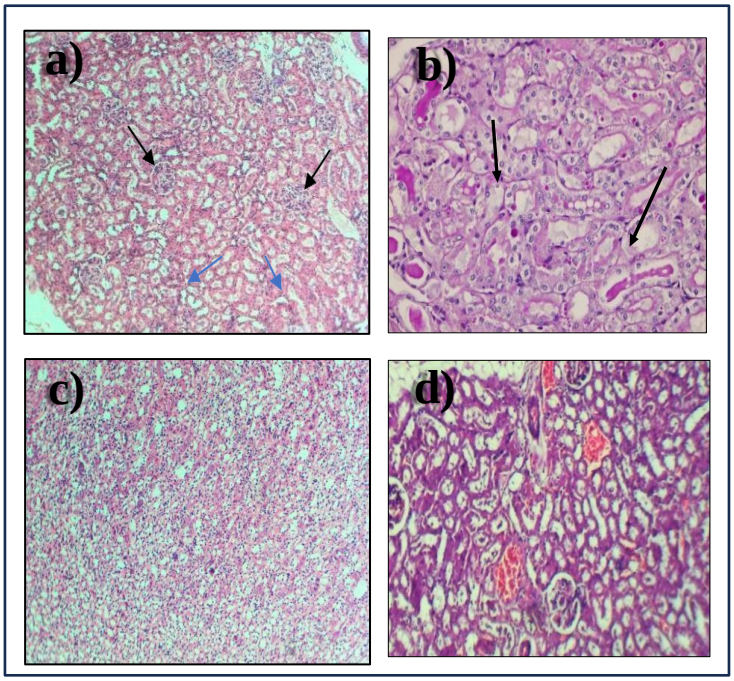

Figure 6. Histological examination of kidney tissues after week 3 (H&E stain): a) group III showing severe glomerulus sclerosis due to extracellular matrix accumulation (black arrows) and severe congestion of blood vessels (blue arrows) at 400X, b) Tissue Section of Kidney Rat Treated with ZnO NPs Showing Loss of PT Brush Border (black arrow) at 400X, c) Group III Showing Prominent Vacuolization of Cytoplasm of Tubules at 100X, d) Kidney Tissues of Group IV Showing Reduced Formation of Proteinaceous Cast Significantly as Compare to Group III at 400X.

3.3. Heart

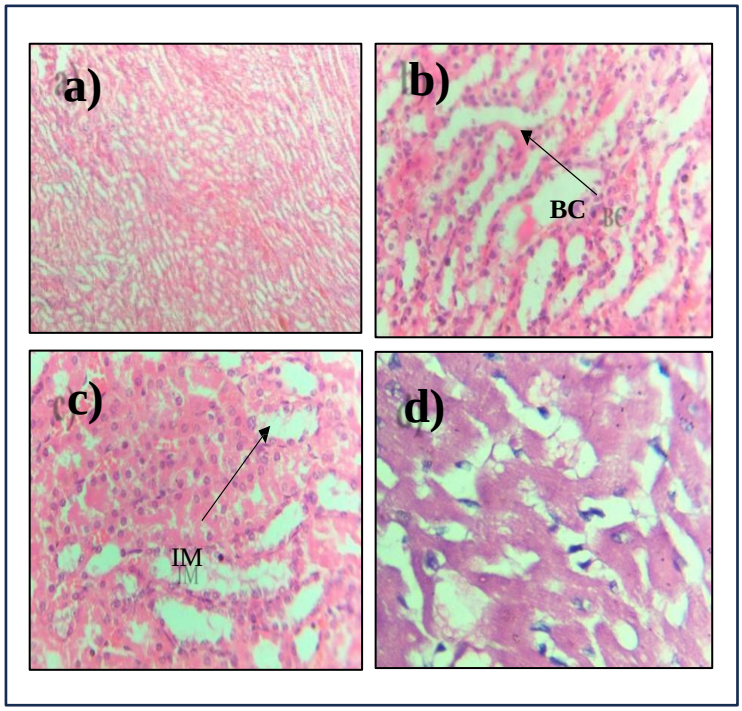

Figure 7. Histopathological examination of heart tissue sections after 1 week (H&E stain): a) Group I at 100X showing no significant histopathological changes, b) Group II at 400X showing branching cells (BC) and nucleus

(N), c) Group III at 400X showing inflammation of mononuclear cells (IM), d) Group IV at 100X showing reduced inflammation.

During the first week, the rats in the control group showed no symptoms of inflammation or stress and had normal cardiac histology (Figure 7a). The antioxidant qualities of ginger are probably the reason the ginger-only group also had healthy cardiac tissue (Figure 7b). On the other hand, fibrosis, myocardial degeneration, and inflammatory cell infiltration were observed in the ZnO-NPs group (Figure 7c). ZnO-NPs and ginger co-treatment resulted in less inflammation and oxidative stress markers, as well as less severe tissue damage, suggesting that ginger has a protective impact (Figure 7d).

During the second week, in the rats given ginger alone, oxidative stress indicators showed a small improvement, although histology remained normal (Figure 8a). Inflammatory indicators and oxidative stress were elevated in ZnO-NPs only rats (Figure 8b). Rats given both ZnO-NPs and ginger, however, showed better heart architecture and far lower levels of stress and inflammation than the ZnO-only group (Figure 8c).

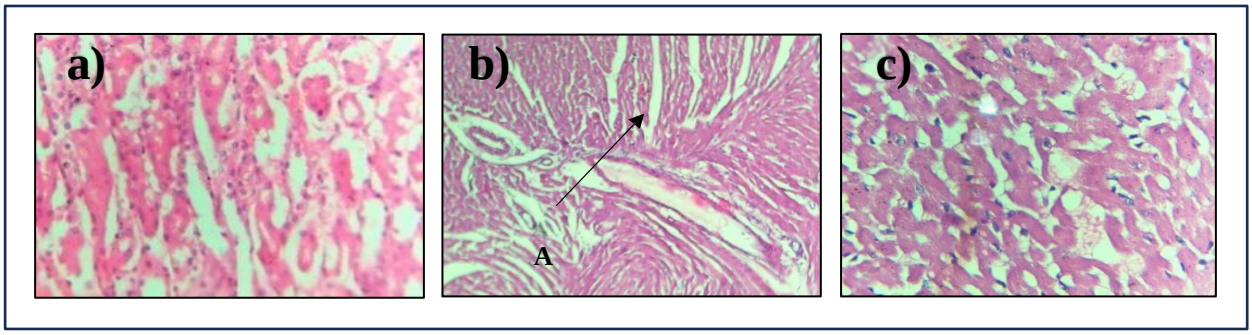

Figure 8. Histopathological examination of heart tissue sections after 2 weeks at 100X magnification (H&E stain): a) Group II showing results similar to the control group, b) Group III treated with zinc oxide nanoparticle showing apoptosis (A) and increased inflammation, c) Group IV showing fewer signs of damage.

Ginger treated rat exhibit preserved histoarchitecture in the third week (Figure 9a). With significant myocardial injury and increased oxidative stress, the ZnO-NPs only group showed even more decline (Figure 9b). Co-treatment animals confirmed the ginger extract's long-lasting protective effect by exhibiting maintained heart architecture and noticeably reduced oxidative damage (Figure 9c).

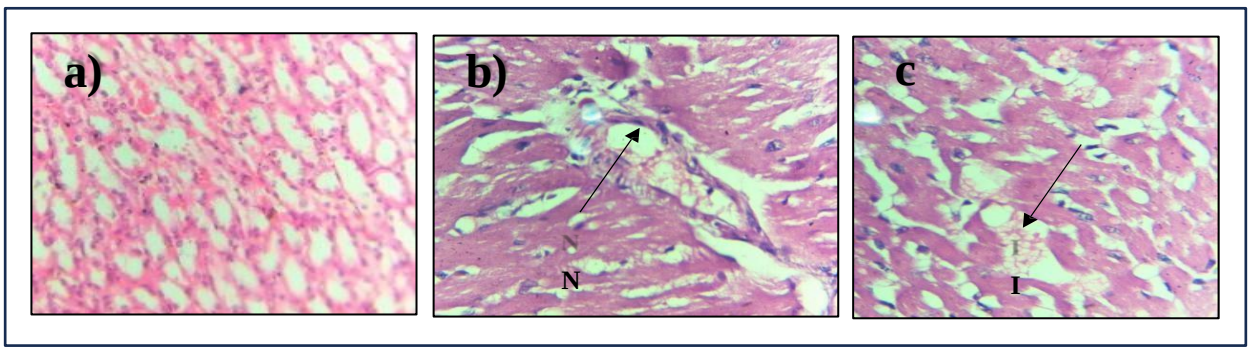

Figure 9. Histopathological examination of heart tissue after 3 weeks (H &E stain): a) Group II at 100X showing normal architecture, b) Group III at 100X showing necrosis (N), c) Group III at 400X revealing mononuclear cell inflammation (IM).

3.4. Spleen

After one week, spleen tissues from the control group (Group I) showed a normal structure with intact red and white pulp (Figure 10a). Ginger-treated rats exhibit preserved histoarchitecture (Figure 10b). In Group III, which received ZnO nanoparticles, moderate degeneration was observed, including capsular fibrosis (Figure 10c), enlarged trabeculae (Figure 10d), and a slight reduction in white pulp lymphocytes, while the red pulp remained mostly unaffected. Group IV, which received both ginger and ZnO-NPs, largely retained overall spleen structure (Figure 10e).

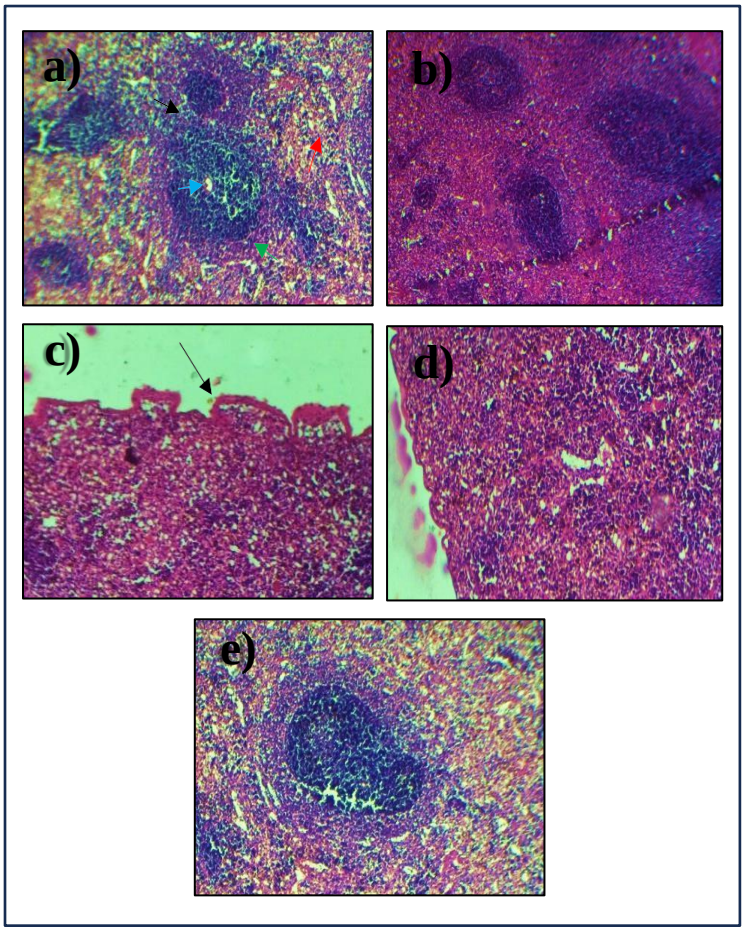

Figure 10. Histopathological examination of spleen tissue sections after 1 week (H&E stain): a) Group I at 400X with normal WP structure: Central arteriole (blue arrow), marginal zone (green arrow), PALS (black arrow) and RP (red arrow), b) Group II at 100X showing no significant damage), c) Group III at 400X with thickened capsule, d) Group III at 400X with thickened trabeculae, e) Group IV at 400X showing white pulp with almost proper structure.

By the second week, ginger treatment alone still showed minimal effects on spleen histology (Figure 11a). However, Group III displayed moderate to severe changes, including red pulp hyperplasia, congestion, inflammatory infiltration, dispersed lymphocyte clusters, increased megakaryocytes (Figure 11b), and distorted white pulp (Figure 11d) with disturbed germinal centers (Figure 11c). Group IV, with ginger and ZnO-NPs showed reduced damage, with less red pulp expansion, milder capsular thickening, and mostly preserved white pulp (Figure 11e).

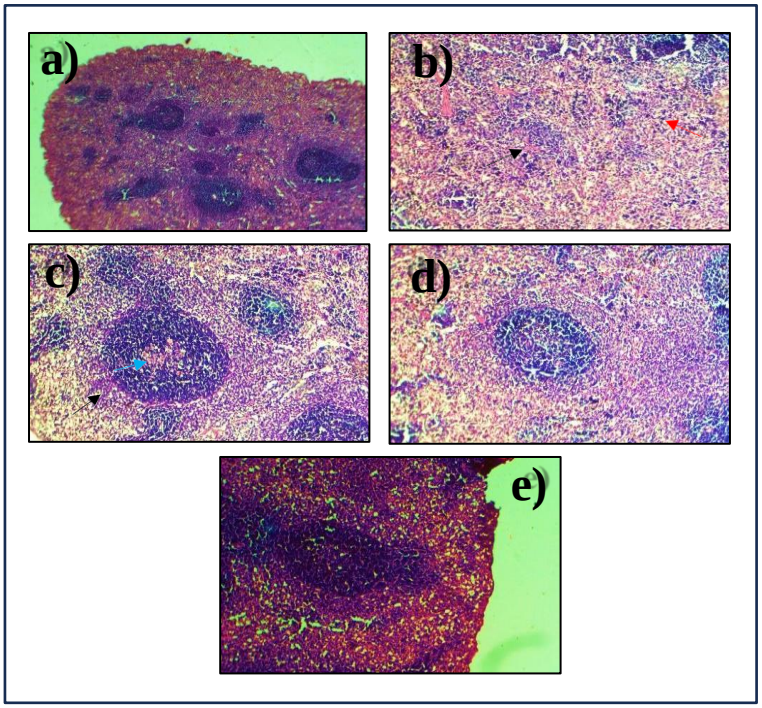

Figure 11. Histopathological examination of spleen tissue sections after 2 weeks (H&E stain): a) Group III at 100X showing capsular fibrosis, destroyed white pulp and widened sinusoids, b) Group III at 400X showing congestion of red pulp with many megakaryocytes (black arrow) and scattered clusters of lymphocytes (red arrow), c) Group III at 400X showing WP interior germinal center containing many macrophages (blue arrow) and depleting lymphocytes (black arrow), d) Group III at 400X showing ill-defined marginal zones and distorted structure of WP, e) Group IV at 400X showing that the capsule thickening and RP congestion is considerably less than Group III, and WP has almost retained its structure.

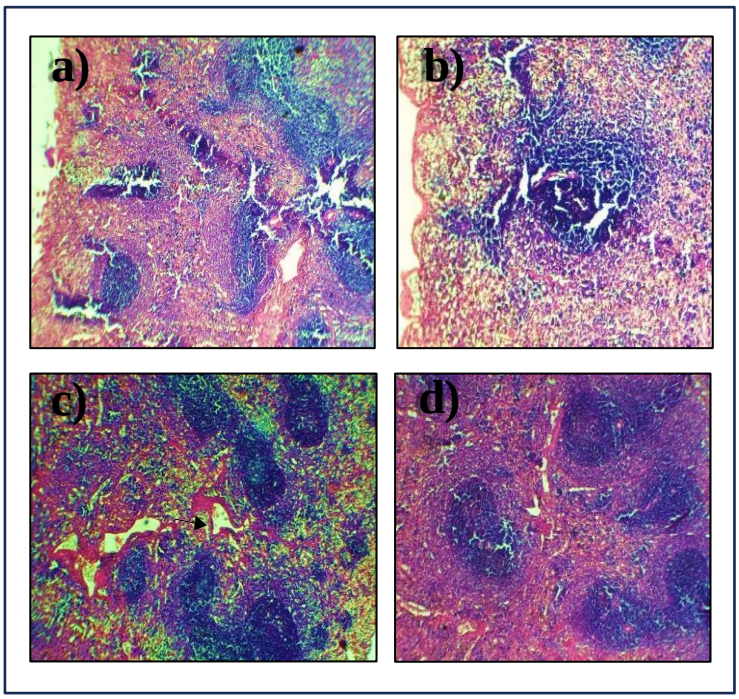

Figure 12. Histopathological examination of spleen tissue after 3 weeks (H &E stain): a) Group III at 100X showing severe damage to architecture of red pulp and white pulp and sinusoids and trabeculae widened, b) Group

III at 400X showing severely distorted structure of white pulp, c) Group III at 100X showing sinusoids and trabeculae have been widened along with degenerated structure of the white pulps, d) Group IV at 100X showing that the ginger has considerably kept the integrity of the spleen structure by combating the ZnO NPs toxicity.

In the third week, ZnO nanoparticles caused significant splenic damage in Group III (Figure 12a), including loss of marginal zones, reduced PALS cellularity, and signs of degeneration and apoptosis. Trabeculae and sinusoids were enlarged (Figure 12c), white pulp appeared scattered (Figure 12b), and lymphocytes and megakaryocytes were dispersed. Conversely, Group IV showed mitigated damage, with more lymphocytes in red pulp, less inflammatory infiltration, and preserved white pulp with lymphoid nodules and loosely arranged lymphocytes (Figure 12d).

4. Discussion

The purpose of this study was to assess the histological effects of zinc oxide nanoparticles (ZnO- NPs) on the liver, kidney, heart, and spleen in albino rats, as well as the protective impact of ginger against ZnO-NP-induced toxicity. Vital organs are at risk from ZnO-NPs, which are widely used in electronics, cosmetics, and medicine. Although ZnO-NPs have antibacterial activity and are used in drug delivery systems, their accumulation in albino rat tissues causes oxidative stress, inflammation, and apoptosis.

Due to their ability to cause oxidative stress, inflammation, and organ toxicity, ZnO-NPs are useful models for studying the protective effects of natural compounds like ginger. Compared to other nanoparticles such as copper oxide, silver nanoparticles (AgNPs), or arsenic oxide, ZnO-NPs are easier to control and monitor. Arsenic, with permanent effects like neurotoxicity and cancer, is extremely toxic and carcinogenic, limiting its relevance in studies on remediative therapies like ginger. Copper oxide nanoparticles (CuO NPs), due to their greater cytotoxicity and high reactivity even at low concentrations—leading to oxidative stress, DNA damage, and cell death—are also less suitable for controlled experiments with protective agents like ginger. ZnO-NPs is more suitable for research on natural product interventions than AgNPs, which are less appropriate due to their high toxicity, environmental concerns, and limited consumer use [34].

For three weeks, the rats in this study were given 300 mg/kg ZnO NPs orally. This resulted in severe histological damage to the liver including hepatocyte degeneration, congestion, vacuolization, inflammatory infiltration and cellular necrosis. According to Hossein [15] and Yahya [45] who reported ROS generation, lipid peroxidation and DNA breakage after ZnO-NPs exposure these results are in line with Salman's [36] observations of oxidative stress-induced inflammation and DNA damage. Yousef [48] confirmed that ZnO-NPs also caused Kupffer cell activation, sinusoidal dilatation and structural disarray in liver tissues. They also found hepatotoxicity and bile duct hyperplasia in rats that received ZnO-NPs treatment. These findings demonstrate the liver's susceptibility to toxicity brought on by ZnO-NPs.

The hepatoprotective, anti-inflammatory, anti-apoptotic, and antioxidant properties of ginger, a medicinal herb, greatly improved liver histology. It has been demonstrated that the bioactive substances in ginger including flavonoids, vitamin C, shogaol, gingerol and paradol improve antioxidant defense systems, lower lipid peroxidation, inhibit inflammatory cytokines and promote liver regeneration [47],[4]. This study's results which are in line with those of Ahmed [5] and Shamsabadi [38] showed that co-administration of ginger and ZnO-NPs

preserved liver architecture and decreased hepatocyte necrosis and inflammatory infiltration. Ginger has been shown to have hepatoprotective potential as evidenced by its protective effects against tissue damage and oxidative stress.

Rats exposed to 300 mg/kg ZnO NPs showed signs of kidney inflammation, glomerular damage, mononuclear cell infiltration and proximal and distal tubule degeneration. These findings are in line with those of Amara [7] who found that rats treated with ZnO or quartz nanoparticles experienced glomerular degeneration and necrosis. Histopathological alterations in the kidneys including the buildup of proteinaceous material and structural abnormalities support the findings of Noori [24] who observed tubular degeneration and eosinophilic infiltration in rats given ZnO NPs. These results are consistent with those of Salman [36] who noted inflammatory infiltration and accumulation of collecting tubules after exposure to ZnO-NPs. It was shown that ZnO-NPs raised ROS levels and caused oxidative stress which resulted in renal shrinkage, necrosis and apoptosis [24].

ZnO-NP-induced kidney damage was significantly prevented by ginger's flavonoids and polyphenols especially gingerol. Rong [32] also observed that the co-administration of ginger and ZnO-NPs protected kidney architecture, decreased glomerular damage and limited glomerulosclerosis symptoms. Ginger's capacity to increase the activity of antioxidant enzymes like catalase and superoxide dismutase helped to reduce inflammation and lipid peroxidation which is consistent with research by Nasri [23] that highlighted the function of shogaol and gingerol in suppressing pro-inflammatory cytokines. Histopathological changes including intact tubular epithelium and decreased inflammation highlight the therapeutic benefits of ginger. Reddy [30] also showed that ginger extract dramatically reduced ZnO-NPs induced kidney damage.

This study examined how ginger extract protected rats' hearts against the toxicity caused by zinc oxide nanoparticles (ZnO NPs). Significant cardiotoxicity was brought on by ZnO NPs, as shown by increased cardiac enzymes, oxidative stress indicators, and histological damage. Through the reduction of oxidative stress, the reduction of enzyme levels (CK, LDH), and the improvement of cardiac tissue structure, ginger extract successfully counteracted these effects.

The main pathway through which ZnO nanoparticles (NPs) exert their toxic effects is oxidative stress, which triggers lipid peroxidation, inflammation, and cellular injury. Bioactive compounds in ginger—such as gingerol, shogaol, and paradol—counteract this by neutralizing reactive oxygen species (ROS), boosting the activity of natural antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT), and maintaining cell membrane stability. Consequently, ginger administration significantly minimizes cardiac damage.

In summary, the study confirms that ZnO NPs cause heart damage mainly through oxidative pathways, while ginger extract provides marked protection due to its strong antioxidant and anti- inflammatory properties. These findings underline ginger's therapeutic potential in preventing nanoparticle-induced cardiac injury.

Rats administered 300 mg/kg ZnO-NPs exhibited capsular fibrosis in the spleen, and prolonged exposure led to red pulp hyperplasia and significant deformation of the white pulp structure. These observations align with Abd El Fadeel [2], who reported that oral ZnO-NP ingestion resulted in spleen tissue alterations such as capsule and trabeculae thickening, white pulp destruction, and red pulp expansion. Similarly, Abbas [1] noted clear

histopathological changes in the spleen, including reduced white pulp compartments and dispersed lymphocyte clusters in the red pulp. They suggested the oxidative/inflammatory pathway as a possible mechanism for ZnO-NPs-induced toxicity.

Ginger and its bioactive compounds help mitigate oxidative damage and inflammation caused by chemical or natural toxins. Safiullah [33] found that ginger increases plasma antioxidant capacity. In the current study, oral administration of ginger alone had no adverse effects on spleen histopathology, and rats treated with ginger displayed relatively normal spleen architecture. Ginger contains antioxidant compounds such as shogaol, paradol, and gingerol, which scavenge free radicals. Catalase, an enzyme present in ginger, plays a key role in neutralizing free radicals and reducing oxidative stress Stoilova [40]. Additionally, Oboh [25] reported that ginger influences various cellular signaling pathways related to inflammation, apoptosis, and oxidative stress responses.

5. Conclusion

In conclusion, this study demonstrates that zinc oxide nanoparticles (ZnO-NPs) induce multi-organ toxicity in the liver, kidneys, heart, and spleen through oxidative stress and inflammation. Prolonged exposure resulted in glomerular and tubular degeneration in kidneys, hepatic necrosis, elevated cardiac markers with myocardial damage, and white pulp depletion with splenic inflammation. These toxic effects were both dose- and duration-dependent. Co-administration of ginger significantly alleviated these alterations by enhancing antioxidant defenses and suppressing inflammatory responses. Overall, ginger demonstrates significant protective effects against ZnO- NP-induced multi-organ toxicity, primarily through antioxidant and anti-inflammatory mechanisms. These results highlight the dual need for regulating ZnO-NP exposure and exploring ginger as a natural protective agent.

6. Future Directions

The following are some future recommendations concerning this study.

1) To evaluate the long-term effects of ZnO-NPs at lower and chronic exposure doses.
2) To investigate gender- and age-related differences in susceptibility to ZnO-NP toxicity.
3) To explore molecular signaling pathways underlying ginger's protective mechanisms.
4) To compare ginger with other natural antioxidants in mitigating nanoparticle-induced damage.
5) To assess the combined impact of ZnO-NPs with other environmental toxicants.
6) To extend findings through pre-clinical and clinical studies for potential therapeutic applications.

Declarations

Source of Funding

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Competing Interests Statement

The authors declare that they have no competing interests related to this work.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

All the authors took part in literature review, analysis, and manuscript writing equally.

Availability of data and materials

Supplementary information is available from the authors upon reasonable request.

Ethical Approval

Based on institutional guidelines.

References

[1] Abass, M.A., Selim, S.A., & Selim, M. (2017). Effect of orally administered zinc oxide nanoparticles on albino rat thymus and spleen. IUBMB Life, 69(8): 528–539. https://doi.org/10.1002/iub.1638.

[2] Abd El Fadeel, S.A.E., & Yassa, H.D. (2022). Effect of zinc oxide nanoparticles on the spleen of adult male albino rat and the possible protective role of selenium: Microanatomical and biochemical study. Egyptian Journal of Medical Research, 3: 87–104. https://doi.org/10.21608/ejmr.2022.253797.

[3] Aboulhoda, B.E., Abdeltawab, D.A., Rashed, L.A., et al. (2020). Hepatotoxic effect of oral zinc oxide nanoparticles and the ameliorating role of selenium in rats: A histological, immunohistochemical and molecular study. Tissue and Cell, 67: 101441. https://doi.org/10.1016/j.tice.2020.101441.

[4] Ahd, K., Dhibi, S., Akermi, S., et al. (2019). Protective effect of ginger (*Zingiber officinale*) against PCB-induced acute hepatotoxicity in male rats. RSC Advances, 9: 29120–29130. https://doi.org/10.1039/c9ra03136g.

[5] Ahmed, M.A. (2013). The protective effect of ginger (*Zingiber officinale*) against adriamycin- induced hepatotoxicity in rats: Histological study. Journal of Life Sciences, 10: 1412–1422.

[6] Almansour, M.I., Alferah, M.A., Shraideh, Z.A., et al. (2017). Zinc oxide nanoparticles hepatotoxicity: Histological and histochemical study. Environmental Toxicology and Pharmacology, 51: 124–130. https://doi.org/10.1016/j.etap.2017.02.015.

[7] Amara, S., Slama, I.B., Mrad, I., et al. (2014). Effects of zinc oxide nanoparticles and/or zinc chloride on biochemical parameters and mineral levels in rat liver and kidney. Human & Experimental Toxicology, 33: 1150–1157. https://doi.org/10.1177/0960327113510327.

[8] Baky, N.A., Faddah, L.M., Al-Rasheed, N.M., et al. (2013). Induction of inflammation, DNA damage and apoptosis in rat heart after oral exposure to zinc oxide nanoparticles and the cardioprotective role of α-lipoic acid and vitamin E. Drug Research, 63: 228–236. https://doi.org/10.1055/s-0033-1334923.

[9] Balogh, P., Horváth, G., & Szakal, A.K. (2004). Immunoarchitecture of distinct reticular fibroblastic domains in the white pulp of mouse spleen. Journal of Histochemistry & Cytochemistry, 52: 1287–1298. https://doi.org/10.1177/002215540405201005.

[10] Bhandari, U., Shamsher, A.A., Pillai, K.K., et al. (2003). Antihepatotoxic activity of ginger ethanol extract in rats. Pharmaceutical Biology, 41: 68–71. https://doi.org/10.1076/phbi.41.1.68.14697.

[11] Chung, H.E., Yu, J., Baek, M., et al. (2013). Toxicokinetics of zinc oxide nanoparticles in rats. Journal of Physics: Conference Series, 429: 012037. https://doi.org/10.1088/1742-6596/429/1/012037.

[12] Fahmy, M.A., Diab, K.A., Abdel-Samie, N.S., et al. (2018). Carbon tetrachloride induced hepato/renal toxicity in experimental mice: Antioxidant potential of Egyptian *Salvia officinalis* L essential oil. Environmental Science and Pollution Research International, 25: 27858–27876. https://doi.org/10.1007/s11356-018-2820-6.

[13] Gantedi, S., & Anreddy, R.N.R. (2017). Toxicological studies of zinc oxide nanomaterials in rats. Toxicological & Environmental Chemistry, 94: 1768–1779.

[14] Haleem, A., Javaid, M., Singh, R.P., Rab, S., & Suman, R. (2023). Applications of nanotechnology in the medical field: A brief review. Global Health Journal, 7: 70–77. https://doi.org/10.1016/j.glohj.2023.02.008.

[15] Hosseini, S.M., Amani, R., Moshrefi, A.H., et al. (2020). Chronic zinc oxide nanoparticles exposure produces hepatic and pancreatic impairment in female rats. Iranian Journal of Toxicology, 14: 145–154. https://doi.org/10.32598/ijt.14.3.626.1.

[16] Ibrahim, R., Salem, M.Y., Helal, O.K., & Abd El-Monem, S.N. (2018). Effect of titanium dioxide nanoparticles on the spleen of adult male albino rats: Histological and immunohistochemical study. Egyptian Journal of Histology, 41: 311–328. https://doi.org/10.21608/ejh.2019.25408.

[17] Islam, F., Shohag, S., Uddin, M.J., et al. (2022). Exploring the journey of zinc oxide nanoparticles (ZnO-NPs) toward biomedical applications. Materials, 15: 2160. https://doi.org/10.3390/ma15062160.

[18] Komorowicz, I., & Hanć, A. (2024). Can arsenic do anything good? Arsenic nanodrugs in the fight against cancer – last decade review. Talanta, 276: 126240. https://doi.org/10.1016/j.talanta.2024.126240.

[19] Król, A., Pomastowski, P., Rafińska, K., et al. (2017). Zinc oxide nanoparticles: Synthesis, antiseptic activity and toxicity mechanism. Advances in Colloid and Interface Science, 249: 37–52. https://doi.org/10.1016/j.cis.2017.07.033.

[20] Kumari, S., Raturi, S., Kulshrestha, S., et al. (2023). A comprehensive review on various techniques used for synthesizing nanoparticles. Journal of Materials Research and Technology, 27: 1739–1763. https://doi.org/10.1016/j.jmrt.2023.09.291.

[21] Mirzaei, H., & Darroudi, M. (2017). Zinc oxide nanoparticles: Biological synthesis and biomedical applications. Ceramics International, 43: 907–914. https://doi.org/10.1016/j.ceramint.2016.10.051.

[22] Mishra, P.K., Mishra, H., Ekielski, A., Talegaonkar, S., & Vaidya, B. (2017). Zinc oxide nanoparticles: A promising nanomaterial for biomedical applications. Drug Discovery Today, 22: 1825–1834. https://doi.org/10.1016/j.drudis.2017.08.006.

[23] Nasri, H., Nematbakhsh, M., Ghobadi, S., et al. (2013). Preventive and curative effects of ginger extract against histopathologic changes of gentamicin-induced tubular toxicity in rats. InternationalJournal of Preventive Medicine, 4: 316–321. https://doi.org/10.4103/ijpvm.ijpvm_316_17.

[24] Noori, A., Karimi, F., Fatahian, S., & Yazdani, F. (2014). Effects of zinc oxide nanoparticles on renal function in mice. International Journal of Biosciences, 5(9): 140–146. https://doi.org/10.12692/ijb/5.9.140-146.

[25] Oboh, G., Akinyemi, A.J., & Ademiluyi, A.O. (2012). Antioxidant and inhibitory effect of red ginger (*Zingiber officinale* var. Rubra) and white ginger (*Zingiber officinale* Roscoe) on Fe^{2+}- induced lipid peroxidation in rat brain in vitro. Experimental and Toxicologic Pathology, 64(1–2): 31–36. https://doi.org/10.1016/j.etp.2010.06.00.

[26] Pandit, C., Roy, A., Ghotekar, S., Khusro, A., Islam, M.N., Emran, T.B., Lam, S.E., Khandaker, M.U., & Bradley, D.A. (2022). Biological agents for synthesis of nanoparticles and their applications. Journal of King Saud University – Science, 34: 101869. https://doi.org/10.1016/j.jksus.2022.101869.

[27] Patra, J.K., Das, G., Fraceto, L.F., Campos, E.V.R., Rodriguez-Torres, M.D.P., Acosta-Torres, L.S., Diaz-Torres, L.A., Grillo, R., Swamy, M.K., Sharma, S., Habtemariam, S., & Shin, H.S. (2018). Nano-based drug delivery systems: Recent developments and future prospects. Journal of Nanobiotechnology, 16(1): 71. https://doi.org/10.1186/s12951-018-0392-8.

[28] Polizu, S., Savadogo, O., Poulin, P., & Yahia, L. (2006). Applications of carbon nanotubes- based biomaterials in biomedical nanotechnology. Journal of Nanoscience and Nanotechnology, 6(7): 1883–1904. https://doi.org/10.1166/jnn.2006.197.

[29] Rahmani, A.H., & Aly, S.M. (2014). Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. International Journal of Physiology, Pathophysiology and Pharmacology, 6: 125.

[30] Reddy, Y.A., Chalamaiah, M., Ramesh, B., Balaji, G., & Indira, P. (2014). Ameliorating activity of ginger (*Zingiber officinale*) extract against lead-induced renal toxicity in male rats. Journal of Food Science and Technology, 51(5): 908–914. https://doi.org/10.1007/s13197-011-0568-9.

[31] Rehman, S.U., Choi, M.S., Choe, K., & Yoo, H.H. (2014/2015). Interactions between herbs and antidiabetics: An overview of the mechanisms, evidence, importance, and management. Archives of Pharmacal Research, 38(7): 1281–1298. https://doi.org/10.1007/s12272-014-0517-z.

[32] Rong, X., Peng, G., Suzuki, T., Yang, Q., Yamahara, J., & Li, Y. (2009). A 35-day gavage safety assessment of ginger in rats. Regulatory Toxicology and Pharmacology, 54(2): 118–123. https://doi.org/10.1016/j.yrtph.2009.03.002.

[33] Safiullah, Chand, N., Khan, R.U., Naz, S., & Khan, M.A. (2019). Effect of ginger (*Zingiber officinale* Roscoe) and organic selenium on growth dynamics, blood malondialdehyde and paraoxonase in broilers exposed to heat stress. Journal of Applied Animal Research, 47(1): 212–216. https://doi.org/10.1080/09712119.2019.1608211.

[34] Sajjad, H., Sajjad, A., Haya, R.T., Sarwar, H., Ali, R., & Baig, M.M.F.A. (2023). Copper oxide nanoparticles: In vitro and in vivo toxicity, mechanisms of action and factors influencing their toxicology. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 271: 109682. https://doi.org/10.1016/j.cbpc.2023.109682.

[35] Sajid, M., & Płotka-Wasylka, J. (2020). Nanoparticles: Synthesis, characteristics, and applications in analytical and other sciences. Microchemical Journal, 154: 104623. https://doi.org/10.1016/j.microc.2020.104623.

[36] Salman, R.A. (2018). Histopathological effect of zinc oxide nanoparticles on kidney and liver tissues in albino male mice. Ibn Al-Haitham Journal for Pure and Applied Science, 31(1): 9–14. https://doi.org/10.30526/31.1.1844.

[37] Seely, J.C., Hard, G.C., & Blankenship, B. (2018). Pathology of the rat: A model for human disease. In Suckow, M.A., Hankenson, F.C., Wilson, R.P., & Foley, P.L. (Eds.), The Laboratory Rat, Pages 125–166, Academic Press. https://doi.org/10.1016/c2017-0-01188-6.

[38] Shamsabadi, S., Nazer, Y., Ghasemi, J., Mahzoon, E., Rahimi, V.B., Ajiboye, B.O., & Askari, V.R. (2023). Promising influences of zingerone against natural and chemical toxins: A comprehensive and mechanistic review. Toxicon, 233: 107247. https://doi.org/10.1016/j.toxicon.2023.107247.

[39] Sruthi, S., Ashtami, J., & Mohanan, P.V. (2018). Biomedical application and hidden toxicity of zinc oxide nanoparticles. Journal of Materials Today Chemistry, 10: 175–186.

[40] Stoilova, I., Krastanov, A., Stoyanova, A., Denev, P., & Gargova, S. (2007). Antioxidant activity of a ginger extract (*Zingiber officinale*). Food Chemistry, 102(3): 764–770. https://doi.org/10.1016/j.foodchem.2006.06.023.

[41] Suttie, A.W. (2006). Histopathology of the spleen. Toxicologic Pathology, 34(5): 466–503. https://doi.org/10.1080/01926230600867750.

[42] Syama, S., Reshma, S.C., Sreekanth, P.J., Varma, H.K., & Mohanan, P.V. (2013). Effect of zinc oxide nanoparticles on cellular oxidative stress and antioxidant defense mechanisms in mouse liver. Toxicological and Environmental Chemistry, 95(3): 495–503. https://doi.org/10.1080/02772248.2013.789606.

[43] Wessels, A., & Sedmera, D. (2003). Developmental anatomy of the heart: A tale of mice and man. PhysiologicalGenomics, 15(3): 165–176. https://doi.org/10.1152/physiolgenomics.00033.2003.

[44] Yadav, R., & Srivastava, D. (2007). Kinetics of the acid-catalyzed cardanol–formaldehyde reactions. Materials Chemistryand Physics, 106(1): 74–81. https://doi.org/10.1016/j.matchemphys.2007.05.020.

[45] Yahya, R.A.M., Azab, A.E., Shkal, K.E.M., & Jbireal, J.M. (2019). Hepatotoxicity induced by copper oxide and zinc oxide nanoparticles and their mixtures in male albino rats. East African Scholars Journal of Medical Sciences, 2(11): 650–660. https://doi.org/10.36349/easms 2019.v02i11.015.

[46] Yang, H., Liu, C., Yang, D., Zhang, H., & Xi, Z. (2009). Comparative study of cytotoxicity, oxidative stress and genotoxicity induced by four typical nanomaterials: The role of particle size, shape and composition. Journal of Applied Toxicology, 29(1): 69–78. https://doi.org/10.1002/jat.1385.

[47] Yassin, N.A., ElRokh, E.M., & El-Shenawy, E., et al. (2010). Study of the hepatoprotective effect of ginger aqueous infusion in rats. Journal of Chemical and Pharmaceutical Research, 2: 476–488.

[48] Yousef, M.I., Mutar, T.F., & Kamel, M.A.E.N. (2019). Hepato-renal toxicity of oral sub-chronic exposure to aluminum oxide and/or zinc oxide nanoparticles in rats. Toxicology Reports, 6: 336–346. https://doi.org/10.1016/j.toxrep.2019.04.003.