ZITA MEDICAL MANAGEMENT

www.hsmc.gr/en/pharmakeftiki-journal/

ΦΑΡΜΑΚΕΥΤΙΚΗ, 36, II, 2024 | 89-102 ΕΡΕΥΝΗΤΙΚΗ ΕΡΓΑΣΙΑ

PHARMAKEFTIKI, 36, II, 2024 | 89-102 RESEARCH ARTICLE

# Assessment of zinc acetate overconsumption on hepatic histology and liver enzymes in rats

**Mahmood A. Hussein[1], Maha T. AL-Saffar[2], Karam H. AL-mallah[3]**

*[1]Nineveh Health Directorate, Mosul, Iraq*

*[2]Dental Basic Sciences Department, College of Dentistry, University of Mosul, Iraq*

*[3]Pathology and Poultry Diseases Department, College of Veterinary Medicine, University of Mosul, Iraq*

**KEYWORDS:**
**Zinc, Liver, ALT, AST, Liver pathology, Liver histology.**

**ARTICLE INFO:**
*Received: June 18, 2023*
*Revised: November 21, 2023*
*Accepted: March 11, 2024*
*Available on line: July 19, 2024*

*** CORRESPONDING AUTHOR:**
Mahmood A. Hussein
Nineveh Health Directorate, Mosul, Iraq
Tel.: +9647707490914
Email:
Mahmood.21dep69@student.uomosul.edu.iq

## ABSTRACT

Background: Overconsumption of dietary zinc (Zn) supplements is one of the most common causes of acute Zn poisoning. Zinc is commonly found in commercially available minerals and nutritional supplements. Aims: We sought to investigate the histopathological and biochemical toxic effects of zinc acetate on the liver. Materials and Methods: Twenty-five healthy male Albino rats with an age range of (2-3) months and weights of (250-360 g) were kindly provided from Animal House. The rodents were separated into five equal groups (5 rats each): the control group received normal saline intraperitoneally once every other day for 3 weeks, groups 2,3,4, and 5 received zinc acetate (4,8, 12, and 24mg/kg) respectively, intraperitoneally once every other day for 3 weeks. All the experimental animals of all groups were euthanized on day 22 for biochemical serum assessment and histopathological assessment of the liver. Results: Liver enzymes significantly elevated in a dose-dependent manner particularly in group 5 compared to control and other groups. Histopathological examination of the liver revealed the architectural changes and modulation of liver histology, these changes were dose-dependent and more apparent in group 5 compared to control and other groups. In conclusion: overconsumption of zinc acetate produce significant effects on liver biochemical parameters (Alkaline Phosphatase, Aspartate Aminotransferase, and Alanine Aminotransferase) in all treated groups corresponding with dose and significant effects on liver revealed by histological examination like congestion of portal and central veins, focal lymphocytic infiltration and capsular fibrosis at all treated groups, moreover, the effects were more severe at 4$^{th}$ and 5$^{th}$ groups. Overconsumption of zinc acetate has been found

to have significant effects on liver biochemical parameters, specifically Alkaline Phosphatase, Aspartate Aminotransferase, and Alanine Aminotransferase, in all treated groups. These effects were observed to correspond with the dosage administered. Furthermore, histological examination of the liver revealed notable changes in all treated groups, with more severe effects observed in the 4th and 5th groups. These changes included congestion of the portal and central veins, focal lymphocytic infiltration, and capsular fibrosis. These findings suggest that excessive intake of zinc acetate can lead to liver damage and dysfunction, emphasizing the importance of consuming this compound within safe and recommended limits. Further research is warranted to explore the underlying mechanisms and potential preventive measures to mitigate the adverse effects of zinc acetate overconsumption on liver health.

**Introduction**

Zinc is an essential nutrient since it is required in practically all elements of cellular/biological processes in the human body (e.g., catalytic functions, structural functions, and regulatory functions)[1]. Zinc's structural activities include intracellular component/cell membrane maintenance and stabilization. Zinc's regulatory activities include being a component of DNA/RNA polymerase, many kinases, and ribonuclease[2].

As a mineral, zinc is known as taking some is good while taking more is better and people believes that zinc heals many diseases including, cancer, infection, growth failure, and skin diseases[3]. The excessive consumption of dietary Zn supplements emerges as a prominent culprit behind the dreaded acute Zn poisoning[4]. Those widely available vitamins and nutrition supplements we often rely on, innocently harbouring zinc within their enticing packages. However, a cautionary tale unfolds when we dare to take multiple supplements simultaneously, unaware that we may unwittingly surpass the Recommended Dietary Allowance (RDA) of zinc intake[4].

Recently, zinc has been consumed in high doses to fight diseases via boosting immune response against coronavirus[1,5,6] or as an add-on therapy against chronic diseases, diabetes for example[7,8], irrespective of their possible toxicities.

Due to the abundance of zinc sources in the environment, it's not uncommon to encounter exposure and toxicity issues[7]. These can arise from various factors, such as inhaling zinc particles at work, excessive intake of nutritional supplements, using denture cream, or improperly prepared total parenteral nutrition (TPN). Unfortunately, some of these situations have even resulted in fatalities[9,10]. While zinc is generally considered less hazardous than metals like lead, arsenic, mercury, and cadmium[11], excessive exposure can still lead to both acute and chronic toxicities[12].

However, like many other elements, zinc can have toxic effects when present in excessive amounts. One organ particularly vulnerable to the toxic effects of zinc is the liver[13]. When zinc accumulates in the liver beyond its normal physiological range, it can disrupt liver function and lead to various detrimental effects[14]. One of the primary toxic effects of zinc on the liver is hepatotoxicity, which refers to the damage or injury inflicted upon liver cells. High levels of zinc can induce oxidative stress, resulting in the production of reactive oxygen species (ROS) that

can damage liver cells and impair their normal functioning[15,16]. Additionally, excessive zinc accumulation can interfere with the metabolism of other essential metals such as copper and iron, leading to imbalances and further liver damage. Zinc toxicity can also disrupt the synthesis and secretion of bile, an essential fluid involved in the digestion and absorption of fats[17]. This disruption can cause cholestasis, a condition characterized by the accumulation of bile in the liver, leading to inflammation and liver dysfunction. Furthermore, prolonged exposure to high levels of zinc can trigger fibrosis, the excessive buildup of scar tissue in the liver, which can ultimately progress to cirrhosis, a severe and irreversible condition. In summary, while zinc is vital for numerous physiological processes, its excessive accumulation can have toxic effects on the liver, including hepatotoxicity, oxidative stress, disruption of metal metabolism, bile synthesis impairment, and fibrosis[15,16].

Surprisingly, there is a lack of comprehensive studies on the toxicity of zinc, and only limited information exists regarding its systemic effects[19]. Therefore, the purpose of this study is to delve into the hepatotoxic effects of zinc acetate at different doses in rats, shedding light on this relatively unexplored aspect.

**Materials and Methods**

Design of experiment: Twenty-five healthy male Albino rats with an average age of (2-3) months and a weight of (250-360 g) were obtained from the Animal House at the University of Mosul's College of Veterinary Medicine. All experimental methods were carried out in compliance with the standards for the care and use of laboratory animals established by the University of Mosul, College of Dentistry[20,21]. At room temperature (22°C±2°C) and humidity (55%±5%), homogenized wood shavings were used as bedding. In a typical light environment (12-hour light/12-hour dark cycle), food and drink were provided ad libitum. The Research Ethics Committee of the College of Dentistry/University of Mosul (UoM.Dent/ A.88/ 22) approved this study.

Study groups: The rats were divided into five equal groups of five rats each: control group received normal saline intraperitoneally once every other day for 3 weeks, groups 2,3,4, and 5 received zinc acetate (4,8, 12, and 24mg/kg) respectively, intraperitoneally once every other day for 3 weeks.

All the experimental animals of all groups were euthanized by cervical dislocation on day 22 for biochemical and histopathological assessment of the liver.

Chemicals: Zinc acetate $(CH_2.COO)_2Zn._2H2O$ crystalline powder (hydrate, 98% pure, solid) was prepared by BDH laboratory reagents, England and was obtained from the chemical laboratory of the Department of Dental and Basic Science, College of Dentistry, University of Mosul.

Dosage preparation and Administration: Zinc acetate was dissolved in distilled water and injected intraperitoneally into the rats at different concentrations through a syringe (1 ml). The volume of injection depends on the weight of the animals and the dose of drugs.

Biochemical assays: Fresh blood was obtained from each rat at the time of euthanization for the examination of biochemical parameters. The serum was then separated by centrifuge (800 electric, China) for About 15 minutes at a pace of 3000 cycles per minute (cpm). The separated 1.5 ml of serum was then transferred to Eppendorf tubes and frozen at (-20°C) to the time of analysis. by using a spectrophotometer (Agilent Technologies, USA). The enzyme activities in the serum were measured using the commercial kits (supplied from Biolabo, France) for aspartate aminotransferase (AST, LP80605), alanine aminotransferase (ALT, K4507), and alkaline phosphatase (ALP, 92314).

Liver tissue histological preparations involve the careful and systematic process of preparing liver tissue samples for microscopic examination. The process begins with the collection of liver tissue samples, which can be obtained through biopsy or autopsy. Once collected, the tissue is fixed using formalin, which helps preserve the cellular structure and prevent decay. After fixation, the tissue is dehydrated using a series of alcohol solutions, gradually replacing water with alcohol. This step is crucial as

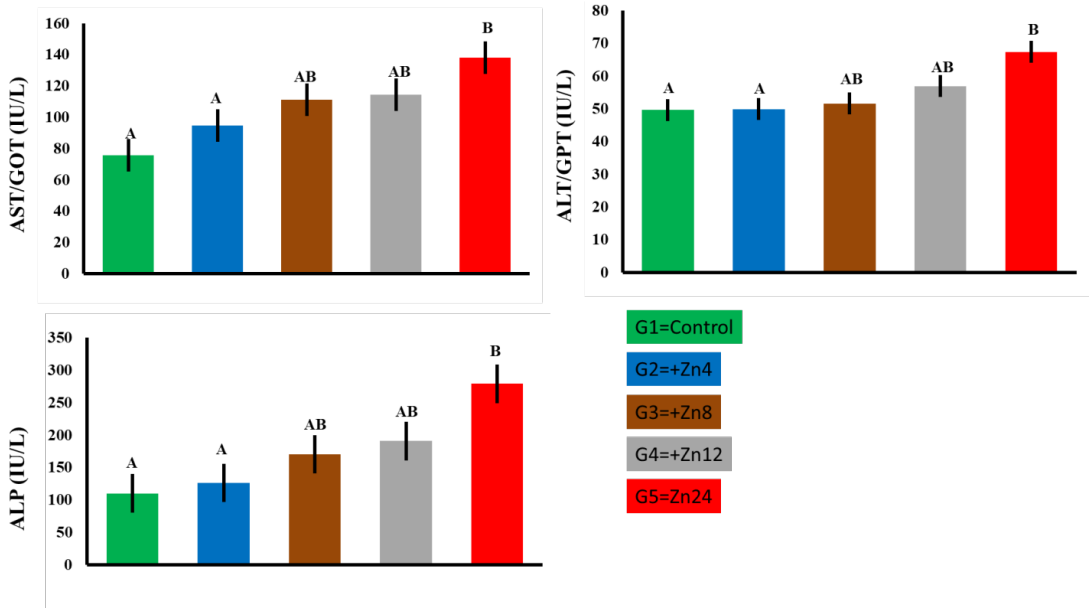


***Figure 1.*** *Liver function test of the rat exposed to different doses of zinc. Data expressed as mean±SD. Different letters between groups indicated significant differences (p<0.05) with an unpaired t-test. AST/GOT=aspartate aminotransferase, ALT/GPT=alanine transaminase, ALP= alkaline phosphatase. G1= received vehicle with no Zn, G2= received IP Zn 4mg every other day, G3= received IP Zn 8mg every other day, G4= received IP Zn 12mg every other day, G5= received IP Zn 24mg every other day. The study duration 3 weeks.*

it prepares the tissue for embedding in a solid medium. Once dehydrated, the tissue is infiltrated with a clearing agent, such as xylene, which removes the alcohol and renders the tissue transparent. The next step involves embedding the tissue in paraffin wax, which provides support and facilitates thin sectioning. The tissue is placed in a mold, surrounded by molten wax, and allowed to solidify. Once solidified, the tissue block is trimmed and sectioned into thin slices using a microtome. These thin sections, also known as histological slides, are mounted onto glass slides and subjected to a series of staining techniques. Staining is essential for enhancing the contrast and visibility of different cellular components within the tissue. Hematoxylin and eosin staining (H&E) is one of the most commonly used staining methods, where hematoxylin stains the cell nuclei blue and eosin stains the cytoplasm pink. After staining, the slides are coverslipped using a mounting medium to protect the tissue and preserve the staining. Finally, the prepared slides are examined under a microscope[22-24].

Statistical analysis: Statistical analysis was performed using SPSS software tool for Windows (version 22, USA). The one-way analysis of variance (ANOVA) and DUNCAN tests were used to compare the results. The level of significance was set at ($p<0.05$).

## Results

Biochemical parameters: Liver function test results demonstrated a substantial rise in aspartate aminotransferase concentration in group 5 when compared to the other groups, as well as a significant difference in aspartate aminotransferase concentration between groups at P-value 0.05. There was a significant variance in alanine aminotransferase concentration in group 5 in relation with other groups. There was a noticeable increase in alanine aminotransferase concentration between groups at P-value ≤ 0.01. There was a substantial increase. in Alkaline Phosphatase in group 5 in relation to groups

**Table 1.** Hepatic lesion parameters between the different groups of the experiment.

| Group | Hepatic lesion score | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Vacuolar degeneration | Coagulative necrosis | Hepatic cell apoptosis | Kuepfer cell proliferation | Congestion of portal vein | Congestion of central vein | Focal lymphocytic infiltration | Capsular fibrosis |
| G1 Control | 0.26±0.11 A | 0.00±0.00 B | 0.13±0.09 C | 0.40±0.13 A | 0.73±0.18 C | 0.53±0.13 A | 0.20±0.10 B | 0.20±0.14 C |
| G2 +Zn4 | 0.93±0.18 B | 0.26±0.45 B | 0.26±0.45 C | 1.20±0.22 B | 1.26±0.24 C | 0.93±0.22 AB | 0.60±0.16 BC | 0.86±0.27 CD |
| G3 +Zn8 | 1.73±0.15 C | 0.26±0.45 B | 0.80±0.22 CD | 1.46±0.23 BC | 1.33±0.27 C | 1.46±0.21 BC | 0.93±0.26 C | 0.86±0.30 CD |
| G4 +Zn12 | 1.93±0.18 CD | 1.06±0.79 C | 1.13±0.32 D | 1.86±0.16 CD | 2.13±0.21 D | 1.73±0.20 C | 1.66±0.30 D | 1.06±0.28 D |
| G5 +Zn24 | 2.4±0.19 D | 1.66±1.11 D | 1.26±0.34 D | 2.1±0.25 D | 2.53±0.19 D | 2.60±0.16 D | 1.73±0.31 D | 1.20±0.29 D |

Data expressed as mean±SE
The different letter means statistically significant ($p<0.05$) difference between values.

(Control and 2) at P-value ≤ 0.01 (Figure 1).

Histopathological findings: The examination under a light microscope revealed, normal hepatic cells, normal hepatic cords organization, normal hepatic circulatory system including normal portal blood vessels, normal central veins and sinusoids. Liver sections in the second group received 4 mg /kg zinc acetate and showed mild pathological changes including mild vacuolar degeneration, focal lymphocytic inflammatory infiltrations at periportal areas and acute cell swelling under the hepatic capsule. In the 3rd group treated with 8 mg/kg zinc acetate, the congestion of central veins, sinuses and portal vessels with periportal oedema, hepatic cords disorganization, acute swelling of hepatocytes, periportal inflammatory cells infiltrations and foci of coagulative necrosis were noticed under the hepatic capsule.

More severe changes were observed in the 4th group, which received 12 mg/kg of zinc acetate, those included moderate to severe vacuolar degeneration, stages of apoptosis were noticed at the hepatic cells pericentral and periportal inflammatory cells infiltrations and thickening of the hepatic capsule. Liver sections from the 5th group rats, who received 24 mg/kg of zinc acetate showed diffused vacuolar degeneration, subacute venous congestion, Kuepfer cells proliferation, multifocal coagulative necrosis of hepatocytes, multifocal inflammatory cells infiltrations and thickening of capsule (Figures 2-10, Table 1).

## Discussion

Among the most sensitive indicators for liver damage and toxicity, we find ALT and AST. These remarkable enzymes, originally confined within the cytoplasm, are set free into the bloodstream when a cellular injury occurs. Notably, their levels surge during necrosis, degeneration, hepatitis, and inflammatory conditions[19]. The study's results unveiled a significant uptick in the levels of ALT, AST, and ALP enzymes across all groups (2, 3, 4, and 5) when compared to the control group.

Jung et al. (2010) demonstrated an a considerable elevation in the activity of ALT and AST in the blood of mice treated with ZnO nanoparticles compared to the control group, which was consistent with the present study[25].

Ben-Slama et al. (2015) demonstrated that subacute oral administration of a moderate dose of ZnO nanoparticles (10 mg/kg) increased plasma AST and ALT enzymes and explained that increased amounts

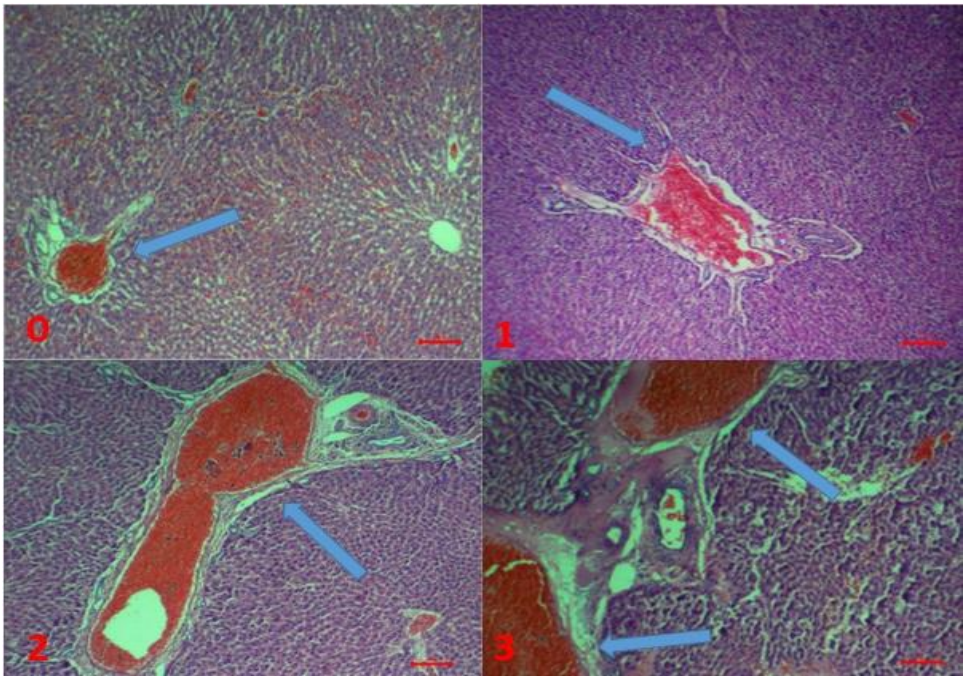


***Figure 2.*** *Central vein congestion in rats' liver: 0=Normal view, 1=Mild congestion, 2=moderate congestion, 3=Sever congestion. Staining H&E, Magnification 100 X . Calibration bar 100 µm.*

of transaminases, which are primarily found in the cytosol of liver cells, is a sign of impairment that leads to liver dysfunction in treated rats[26]. Wang et al. (2006) demonstrated that taking 5g zinc/kg body weight as micro or nano ZnO particles orally for 14 days significantly raised serum GOT and GPT activity. Higher amounts of enzymes were frequently seen in the animals, indicating that micro and nano zinc caused liver injury[27]. Sharma et al. (2012) discovered that oral exposure to 300 mg/kg nanoZnO particles increased serum levels of ALT and ALP as well as pathological abnormalities in the liver. The authors argued that the liver damage was caused by nanoparticle buildup, which caused oxidative stress, DNA damage, and apoptosis[28]. In addition to the release of AST and ALT into the serum from the cell cytoplasm as a result of cellular necrosis, elevated AST and ALT activities have been linked to high liver microsomal membrane fluidity, free radical production, and liver tissue modification[29].

Elevated ALP activity can stem from various factors, including biliary injury or a blockage in the biliary tree[29]. These conditions disrupt the blood flow to the liver, leading to increased ALP levels [30]. Interestingly, Surekha et al. (2012) discovered something unexpected in their research. They observed a non-significant change in the levels of ALT and AST enzymes, which contradicted their initial expectations. One possible explanation for this discrepancy could be attributed to the different methods used to administer ZnO-nanoparticles (ZnO-NPs)[31]. Ding et al. (1998) showed that high dietary zinc caused liver damage in mice and inhibited the activity of GOT in mouse liver homogenate[32] which contradicts the current study.

These biochemical findings were supported by histopathological examination, which revealed the presence of pathological changes manifested by vacuolar degeneration, coagulative necrosis, hepatic cell apoptosis, Kuepfer cell proliferation, congestion of portal and central veins, focal lymphocytic infiltration, and capsular fibrosis in all treated groups, but

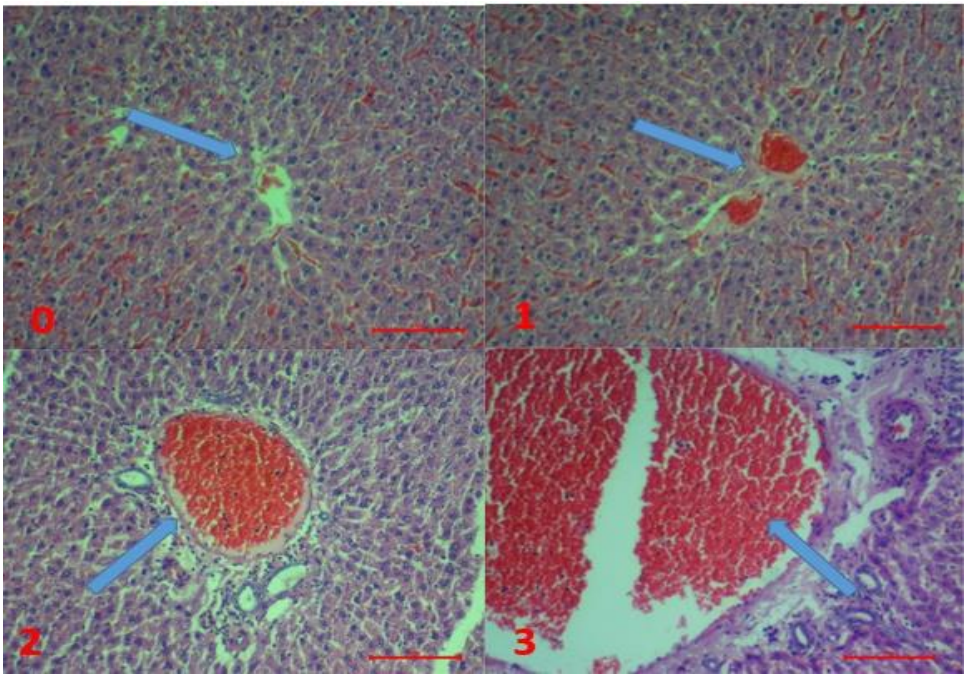


***Figure 3.*** *Portal vein congestion and periportal oedema in rats liver: 0=Normal view, 1=Mild congestion, 2=moderate congestion, 3=Sever congestion. Staining H&E, Magnification 40 X . Calibration bar 100 µm.*

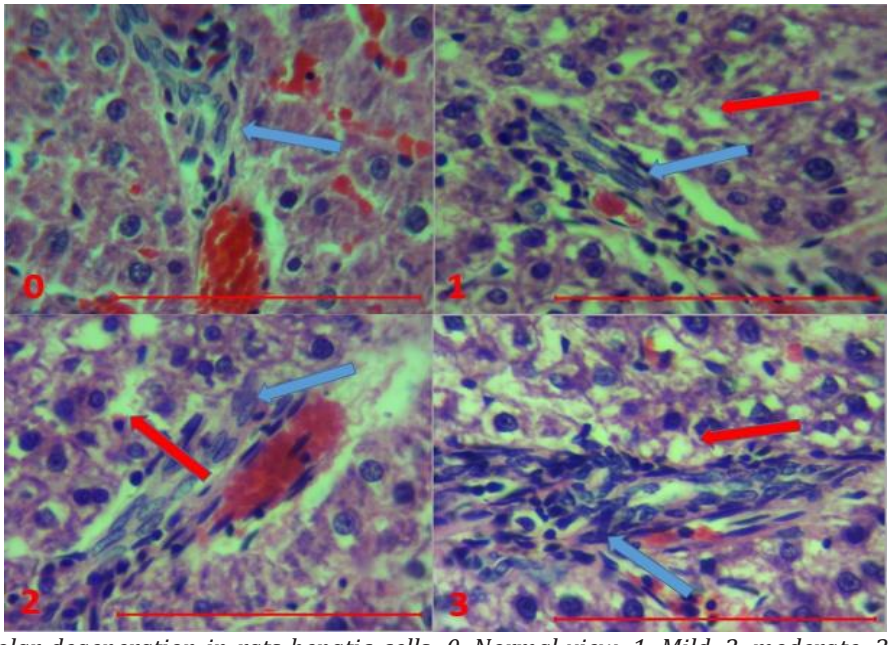


***Figure 4.*** *Vacuolar degeneration in rats hepatic cells: 0=Normal view, 1=Mild, 2=moderate, 3=Sever vacuolar degeneration.Staining H&E, Magnification 100 X . Calibration bar 100 µm.*

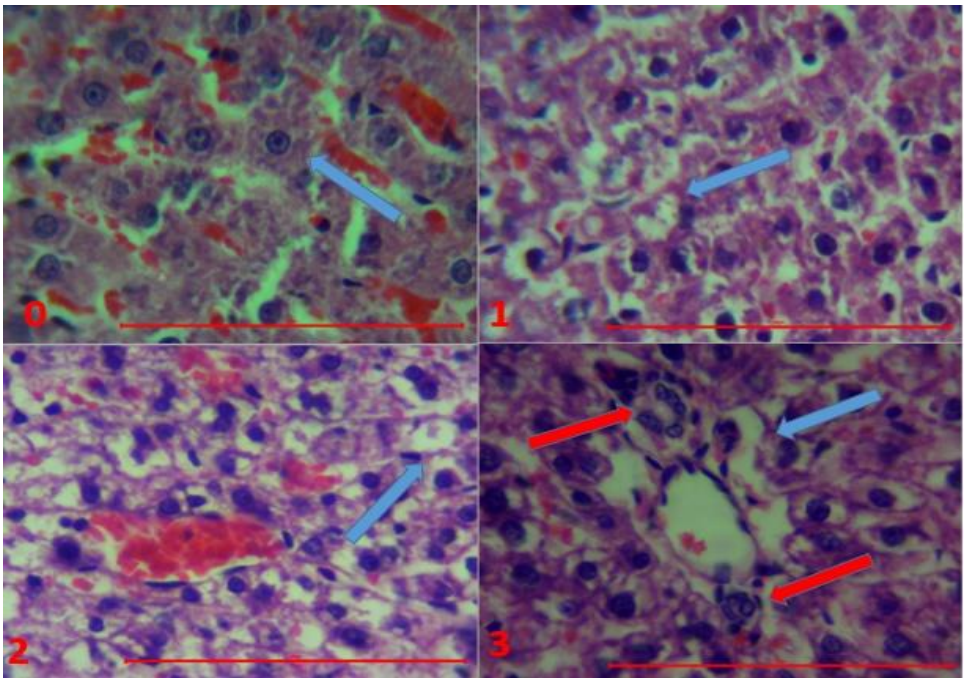


***Figure 5.*** *Vacuolar degeneration in rats hepatic cells: 0=Normal view, 1=Mild, 2=moderate, 3=Sever vacuolar degeneration (Blue arrows) and Giant cell deposition (Red arrows).Staining H&E, Magnification 400 X . Calibration bar 100 µm.*

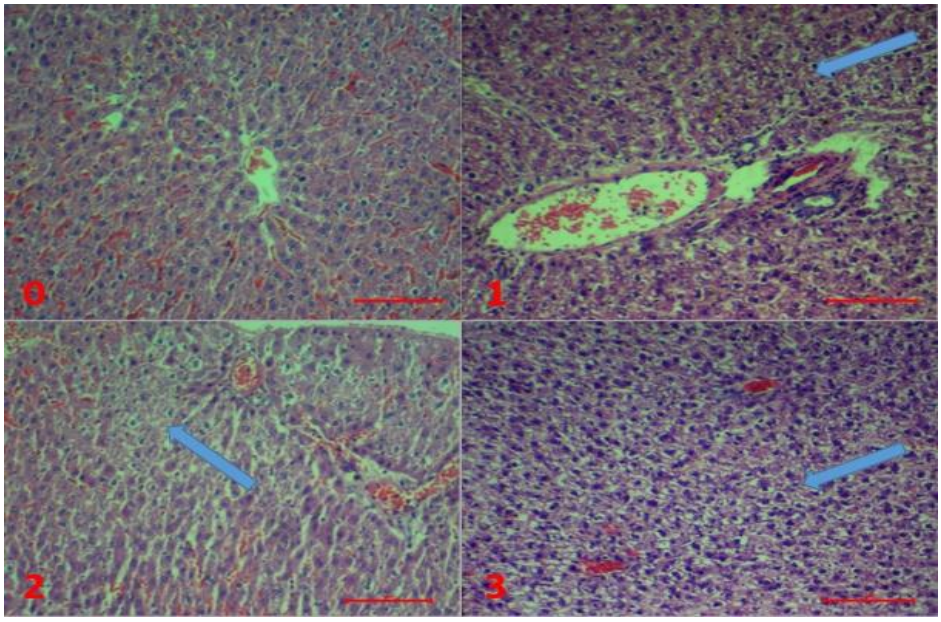


***Figure 6.*** *The proliferation of Kuepfer cells at rats' hepatic sinusoids: 0=Normal view, 1=Mild, 2=moderate, 3=Sever Kuepfer cells proliferation (Blue arrows) and stages of vacuolar degeneration (Red arrows).Staining H&E, Magnification 400 X . Calibration bar 100 µm.*

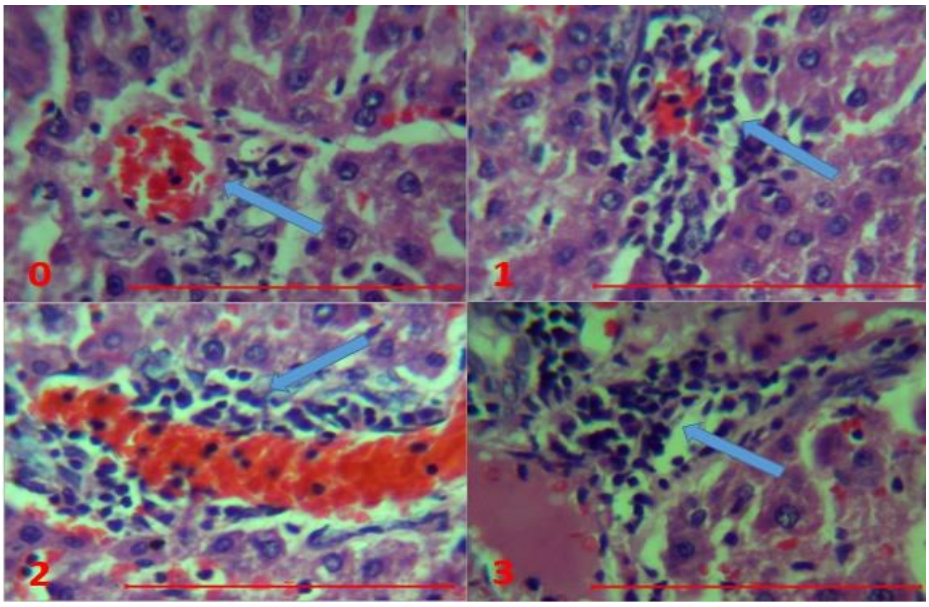


***Figure 7.*** *Focal Lymphocytic inflammatory infiltrations in rats' liver sections: 0=Normal view, 1=Mild, 2=moderate, 3=Sever lymphocytic infiltration (Blue arrows).Staining H&E, Magnification 400 X . Calibration bar 100 μm.*

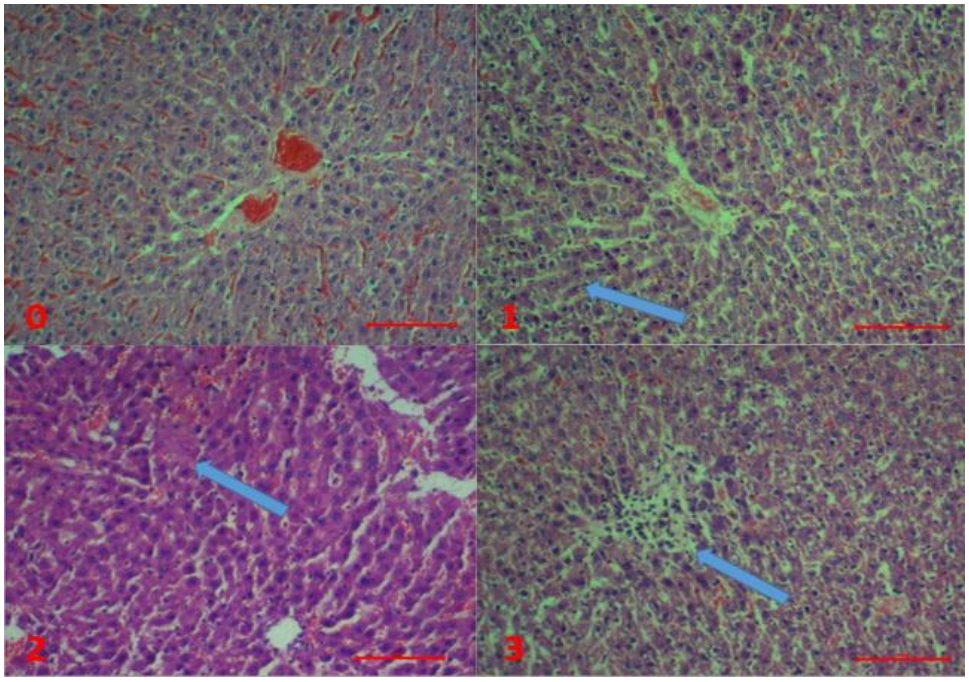


***Figure 8.*** *Focal Lymphocytic inflammatory infiltrations in rats' liver sections: 0=Normal view, 1=Mild, 2=moderate, 3=Severe lymphocytic infiltration (Blue arrows). Staining H&E, Magnification 100 X . Calibration bar 100 μm.*

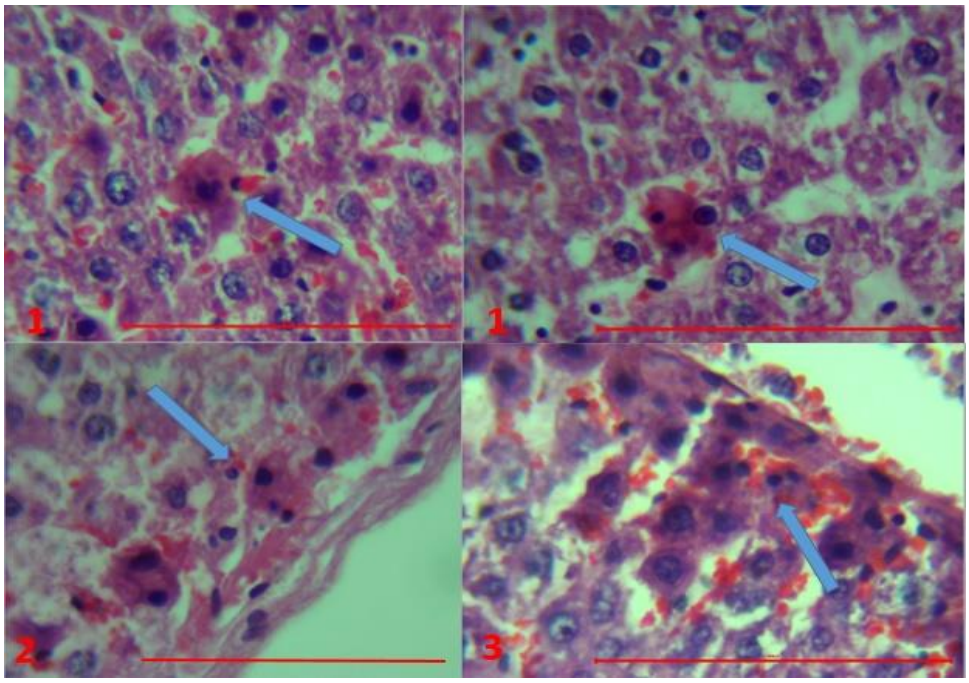


***Figure 9.*** *Apoptotic figures in rats liver sections: 1=Mild, 2=moderate, 3=Massive apoptotic figures (Blue arrows).Staining H&E, Magnification 400 X . Calibration bar 100 μm.*

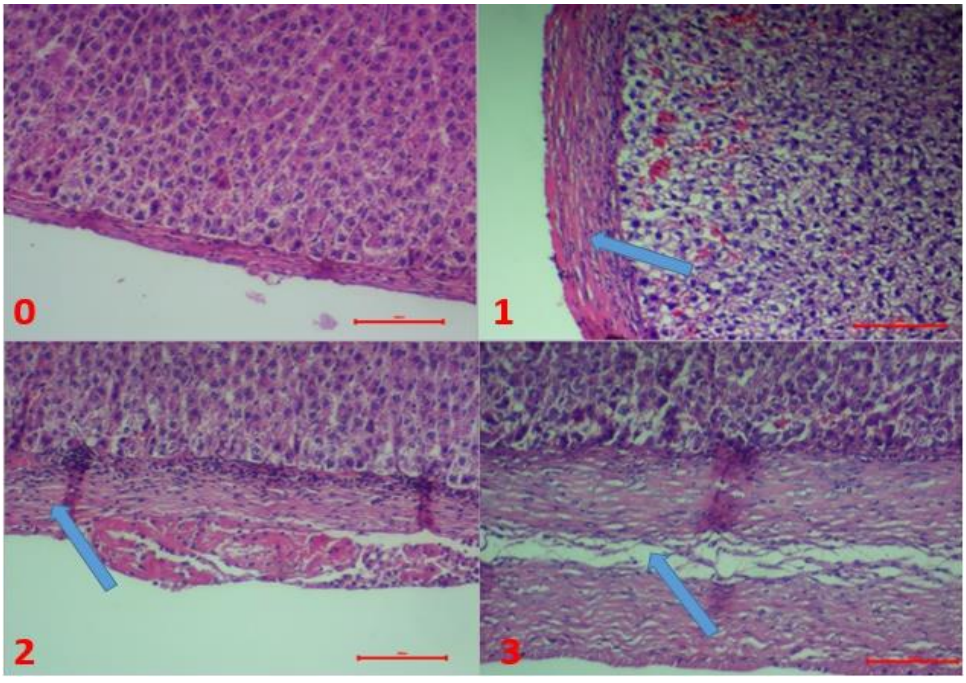


***Figure 10.*** *Hepatic capsular thickening of rats liver sections: 0=Normal view, 1=Mild, 2=moderate, 3=Sever capsular thickening and fibrosis (Blue arrows). Staining H&E, Magnification 100 X . Calibration bar 100 μm.*

more severe in the fourth and fifth groups. These findings correspond with those of Karnakar et al. (2014) and Mansouri et al. (2015) on rats and mice. They observed swelling and vacuolization of hepatocytes, hepatic parenchyma degradation of hepatocytes, and hepatic cell necrosis following ZnO NP administration, as well as severe sinusoidal congestion, hemorrhage, and inflammatory cell infiltration[33,34].

Sharma et al. (2012) found that subacute oral exposure to ZnO nanoparticles (300 mg/kg) for 14 days caused hepatic necrosis[28]. They also found that ZnONPs cause ROS production, oxidative DNA damage, and cell death via apoptosis in human liver cells. According to Abdel-Warith et al. (2011), histological changes in specimens exposed for one and four weeks to 2,4 and 6 mg/L, indicating a toxic response, including cellular swelling and congestion of blood vessels, implying that exposure of fish to different zinc concentrations causes significant stress and causes severe changes in their histology[35]. Cellular swelling happens either directly as a result of the denaturation of volume-regulating ATPases or indirectly as a result of disturbance of the cellular energy transfer pathways required for ionic regulation[35]. Wang et al. (2006) demonstrated that after 2 weeks of treatment, the livers of mice in both the micro-Zn and nano-Zn groups displayed clinical histological alterations such as oedema, hydropic degeneration, and minor necrosis of hepatocytes surrounding the central vein[27].

The observed elevation in the levels of AST and ALT blood enzymes in treated groups, as well as histological alterations in treated group liver sections, show the detrimental and toxic effects of zinc acetate supplied at various dosages and consistent with Moatamed et al. (2019), who showed that zinc build-up in liver tissue following a subacute i.p injection of ZnONPs every other day for 10 days triggered ROS generation and produced oxidative stress in hepatocytes[36].

In a study conducted by Al-Hamdani in 2013, it was revealed that the liver can suffer from extensive damage due to a surge in cellular oxidative stress. This phenomenon wreaks havoc on vital liver functions, including the disruption of superoxide dismutase and glutathione peroxidase activity, as well as a notable increase in lipid peroxidation. Al-Hamdani's research points to these factors playing a pivotal role in the development of pathological liver conditions[37].

In a study conducted by Almansour et al. (2017), male albino rats were subjected to an intriguing experiment involving the administration of ZnO NPs at a daily dose of 2 mg/kg for 21 days. The results of this experiment revealed a myriad of degenerative alterations in these rats. One of the most intriguing findings of this study was the occurrence of hepatic cell death and vacuolar degeneration in the rats. The researchers delved deeper into the mechanism behind these alterations and discovered the biochemical destruction of antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase. This destruction caused a state of oxidative stress-induced cell death, shedding light on the intricate relationship between ZnO NPs and cellular health. Furthermore, the study shed light on the explanation behind vacuolation in hepatocytes. The researchers proposed that disruptions in the membrane function of these cells resulted in an enormous influx of water and $Na^+$. This phenomenon is directly attributed to the effects of ZnO NPs, aligning perfectly with the present study and further solidifying the importance of these findings.[38]

The latest investigation has made an intriguing discovery, revealing a notable surge in collagen deposition among the groups treated with zinc. This finding aligns with the research conducted by Meyer et al. (2011), who also observed significant hepatic fibrosis when using ZnO NPs. The development of this fibrosis can be attributed to the oxidative stress induced by ZnO NPs. Remarkably, reactive oxygen species (ROS) have been implicated in the progression of hepatic fibrosis as they stimulate the proliferation of fibroblasts and the production of collagen[39]. Furthermore, our investigation unveiled the presence of inflammatory cellular infiltrations within the hepatic tissues of the groups treated with zinc. This finding is consistent with the studies conducted by Landsiedel et al. (2010) and John et al. (2010), both of which demonstrated that ZnO NPs have the poten-

tial to enhance neutrophil counts. The infiltration of inflammatory cells by ZnO NPs suggests a dynamic interaction between these particles and the interstitial hepatic tissues, resulting in a diverse range of immunological responses[40,41]. However, the toxicity of zinc could vary between the studied groups or no toxicities could be noticed when applied to human, these variations in the outcome could be potentially linked to the localized cell milieu or released trophic factors by cells in response to localized environments[42,43].

Despite of these aforementioned studies depicting zinc toxic impact on liver; however, zinc play a great role biochemical and physiological performances of human body. Research has demonstrated that low dose zinc supplementation can help reduce liver inflammation by modulating the expression of pro-inflammatory cytokines and inhibiting the activation of inflammatory pathways. Moreover, zinc has been found to enhance liver regeneration and repair. It stimulates the proliferation of liver cells, known as hepatocytes, and promotes their differentiation into functional liver tissue[44,45].

## Conclusion

In conclusion, the subacute toxic effects of zinc acetate have been found to have a significant impact on liver biochemical parameters, specifically Alkaline Phosphatase, Aspartate Aminotransferase, and Alanine Aminotransferase, across all treated groups. The severity of these effects corresponded with the administered dose, indicating a dose-dependent relationship. Furthermore, histological examination of the liver tissue revealed several notable findings. These included congestion of both the portal and central veins, focal lymphocytic infiltration, and capsular fibrosis. These histological changes were observed in all treated groups, suggesting a consistent impact on liver structure and function. Notably, the severity of these histological alterations was more pronounced in the 4th and 5th groups, indicating a potential cumulative effect of zinc acetate toxicity over time. Taken together, these findings highlight the significant and detrimental effects of subacute exposure to zinc acetate on liver health, both biochemically and histologically. □

**Conflict of interests:** None.

**Acknowledgement:** The authors are grateful to the College of Dentistry/University of Mosul for their provided facilities to accomplish this study.

**Funding:** Self-funded

**References**

1. Althanoon Z.A., Merkhan M.M. Effects of zinc supplementation on metabolic status in patients with metabolic syndrome. *Acta Pol. Pharm.* 78(4), 2021.
2. Ivanova I.D., Pal A., Simonelli I., Atanasova B., Ventriglia M., Rongioletti M., Squitti R. Evaluation of zinc, copper, and Cu: Zn ratio in serum, and their implications in the course of COVID-19. *J. Trace Elem. Med. Biol.* 71, 126944, 2022.
3. Aoki C., Imai K., Owaki T., Kobayashi-Nakano T., Ushida T., Iitani Y., Kotani T. The possible effects of zinc supplementation on postpartum depression and anemia. *Medicina,* 58(6), 731, 2022.
4. Richards C.D., Burke R. Local and systemic effects of targeted zinc redistribution in Drosophila neuronal and gastrointestinal tissues. *Biometals,* 28, 967-974, 2015.
5. Merkhan M.M., Abdulrazzaq G.M., Al-Taii H.A. Coronavirus (COVID-19): preventive measures and potential interventions. *Eur. J. Mol. Clin. Med.* 7(10), 1388-99, 2021.
6. Darweesh O., Abdulrazzaq G.M., Al-Zidan R.N., Bebane P., Merkhan M., Aldabbagh R., AlOmari N. Evaluation of the pharmacologic treatment of COVID-19 pandemic in Iraq. *Curr. Pharmacol. Rep.* 7, 171-178, 2021.
7. Younis H.Y., Imad A. Effect of zinc as an add on to metformin therapy on serum lipid profile and uric acid in type 2 diabetes mellitus patients. *Curr. Top. Pharmacol.* 25, 51-59, 2021.
8. Younis H.Y., Thanoon I.A., Fadhil N.N., Merkhan M.M. Effect of zinc as an add-on to metformin therapy on glycemic control, serum insulin, and c-peptide levels and insulin resistance in type 2 diabetes mellitus patient. *Res. J. Pharm. Technol.* 15(3), 1184-1188, 2022.
9. Grissinger M. A fatal zinc overdose in a neonate: confusion of micrograms with milligrams. *Pharm. Therap.*36(7), 393-409, 2011.
10. Hantson P., Lula F., Mahieu P., Lievens M. Extremely high plasma zinc following zinc chloride ingestion. Journal of Toxicology: *Clin. Toxicol.* 36(4), 375-377, 1998.
11. Claverie C., Corbella R., Martin D., Diaz C. Protective effects of zinc on cadmium toxicity in rodents. *Biol. Trace Elem. Res.* 75, 1-9, 2000.
12. Barceloux DG. Zinc. Journal of Toxicology: *Clin. Toxicol.* 37(2), 279-292, 1999
13. Himoto T., Masaki T. Associations between zinc deficiency and metabolic abnormalities in patients with chronic liver disease. Nutrients, *10*(1), 88, 2018.
14. Ito T., Ishigami M., Ishizu Y., Kuzuya T., Honda T., Ishikawa T., Fujishiro M. Correlation of serum zinc levels with pathological and laboratory findings in patients with nonalcoholic fatty liver disease. *Eur. J. Gastroenterol. Hepatol. 32*(6), 748-753, 2020.
15. McCord M.C., Aizenman E. The role of intracellular zinc release in aging, oxidative stress, and Alzheimer's disease. *Front. Aging Neurosci. 6*, 77, 2014.
16. Jain R., Srivastava S., Solomon S., Shrivastava A.K., Chandra A. Impact of excess zinc on growth parameters, cell division, nutrient accumulation, photosynthetic pigments and oxidative stress of sugarcane (Saccharum spp.). *Acta Physiologiae Plantarum*, *32*, 979-986, 2010.
17. Mohammad M.K., Zhou Z., Cave M., Barve A., McClain, C.J. Zinc and liver disease. *Nutr. Clin. Pract. 27*(1), 8-20, 2012.
18. Cassel G.H. Zinc: a review of current trends in therapy and our knowledge of its toxicity. *Del. Med. J.* 50(6), 323-328,1978.
19. Stockham S.L., Scott M.A. (2013) Fundamentals of veterinary clinical pathology. John Wiley & Sons, 2013.
20. Al-Shakarchi W., Saber Y., Merkhan M., Mustafa Y. Acute toxicity of coumacines: An in vivo study. *Georgian Med. News* (338), 126-131, 2023.
21. Al-Shakarchi W., Saber Y., Merkhan M.M., Mustafa Y.F. Sub Chronic Toxicity Study of Coumacines. *Pharmacogn. J. 15*(1), 160-164, 2023.
22. Mahmood M.D., Younes M.A., Saarti M. Pathophysiological Electrolyte Changes Connoted via Antagonism of Serotonin Receptor in Experimental Animals. *Pharmacogn. J. 14*(5), 2022.
23. Abdullah S.I., Al-Bayti A.A., Salih M.J., Merkhan, M.M. Histological and Biochemical Changes Associated with Blocking of Serotonin Receptor. *Trop.*

*J. Nat. Prod. Res.6*(8), 1189-1192, 2022.
24. Abdulqader S.W., Faisal I.M., Saeed M.G., Merkhan M.M. Fluvoxamine suppressed oxidative stress associated with tissue erosion. *Res. J. Pharm. Technol.15*(2), 819-824, 2022.
25. Jung W.C., Kim S., Lee H.J. Acute Toxicity of Nano-Scale Zinc Oxide Powder in ICR Mice. *J. Biomed. Res.* 11(4), 219-224, 2010.
26. Ben-Slama I., Mrad I., Rihane N., Mir L.E., Sakly M., Amara S. Sub-acute oral toxicity of zinc oxide nanoparticles in male rats. *J. Nanomed. Nanotechnol.* 6(3), 284, 2015.
27. Wang B., Feng W.Y., Wang T.C., Jia G., Wang M., Shi J.W., Chai Z.F. Acute toxicity of nano-and micro-scale zinc powder in healthy adult mice. *Toxicol. Lett.* 161(2), 115-123, 2006.
28. Sharma V., Singh P., Pandey A.K., Dhawan A. Induction of oxidative stress, DNA damage and apoptosis in mouse liver after sub-acute oral exposure to zinc oxide nanoparticles. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* 745(1-2), 84-91, 2012.
29. Ibrahim N.M., Eweis E.A., El-Beltagi H.S., Abdel-Mobdy Y.E. Effect of lead acetate toxicity on experimental male albino rat. *Asian Pac. J. Trop. Biomed.* 2(1), 41-46, 2012.
30. Xie F., Xie L. Serum zinc level is associated with liver dysfunction caused by white smoke inhalation. *Gastroenterol. Rep.* 6(4), 304-307, (2018).
31. Surekha P., Kishore A.S., Srinivas A., Selvam G., Goparaju A., Reddy P.N., Murthy P. B. Repeated dose dermal toxicity study of nano zinc oxide with Sprague-Dawley rats. *Cutan. Ocul. Toxicol.* 31(1), 26-32, 2012.
32. Ding H., Peng R., Chen J. Effects of high dietary zinc on liver function, hepatic drug metabolism enzymes and membrane fluidity in mice. *J. Hyg.* 27(3), 180-182, 1998.
33. Karnakar R.Y., Saritha C. H., Sridhar Y., Shankaraiah P. Naringenin prevents the zinc oxide nanoparticles induced toxicity in Swiss Albino mice. *J. Pharmacol. Clin. Toxicol,* 2, 1021, 2014.
34. Mansouri E., Khorsandi L., Orazizadeh M., Jozi Z. Dose-dependent hepatotoxicity effects of zinc oxide nanoparticles. *Nanomed. J.* 2(4), 273–282, 2015.
35. Abdel-Warith A.A., Younis E.M., Al-Asgah N.A., Wahbi O.M. Effect of zinc toxicity on liver histology of Nile tilapia, Oreochromis niloticus. *Sci. Res. Essays.* 6(17), 3760-3769, 2011.
36. Moatamed E.R., Hussein A.A., El-Desoky M.M., Khayat Z.E. Comparative study of zinc oxide nanoparticles and its bulk form on liver function of Wistar rat. *Toxicol. Ind. Health.* 35(10), 627-637, 2019.
37. AL-Taee S.K.I., AL-Hamdani AHA. Pathological study of lethal concentration of N-ZnO in common carp Cyprinus carpio. *Bas. J. Vet. Res.* 12(1), 200-207, 2013.
38. Almansour M.I., Alferah M.A., Shraideh Z.A., Jarrar B.M. Zinc oxide nanoparticles hepatotoxicity: histological and histochemical study. *Environ. Toxicol. Pharmacol.* 51, 124-130, 2017.
39. Meyer K., Rajanahalli P., Ahamed M., Rowe J.J., Hong Y. ZnO nanoparticles induce apoptosis in human dermal fibroblasts via p53 and p38 pathways. *In Vitro Toxicol.* 25(8), 1721-1726, 2011.
40. Landsiedel R., Ma-Hock L., Van Ravenzwaay B., Schulz M., Wiench K., Champ S., Oesch F. Gene toxicity studies on titanium dioxide and zinc oxide nanomaterials used for UV-protection in cosmetic formulations. *Nanotox.* 4(4), 364-381, 2010.
41. John E., Laskow T.C., Buchser W.J., Pitt B.R., Basse P.H., Butterfield L.H., Lotze, M.T. Zinc in innate and adaptive tumor immunity. *J. Transl. Med.* 8(1), 1-16, 2010.
42. Merkhan M.M., Shephard M.T., Forsyth N.R. Physoxia alters human mesenchymal stem cell secretome. *J. Tissue Eng.* 12, 20417314211056132, 2021.
43. Shephard M.T., Merkhan M.M., Forsyth, N.R. Human Mesenchymal Stem Cell Secretome Driven T Cell Immunomodulation Is IL-10 Dependent. *Int. J. Mol. Sci.* 23(21), 13596, 2022.
44. Mousa, N., Abdel-Razik A., Zaher A., Hamed M., Shiha G., Effat N., Eldars W. The role of antioxidants and zinc in minimal hepatic encephalopathy: a randomized trial. *Therap. Adv. Gastroenterol,* 9(5), 684-691, 2016.
45. Perera M., El Khoury J., Chinni V., Bolton D., Qu L., Johnson P., Ischia J. Randomized controlled trial for high-dose intravenous zinc as adjunctive therapy in SARS-CoV-2 (COVID-19) positive critically ill patients: trial protocol. *BMJ open,* 10(12), e040580., 2020.