P-ISSN: 2305-6622; E-ISSN: 2306-3599
International Journal of Agriculture and Biosciences
www.ijagbio.com; editor@ijagbio.com
Research Article https://doi.org/10.47278/journal.ijab/2023.064

Possible Effect of Ascorbic Acid against Zinc Oxide Nanoparticles Induced Hepatotoxicity in Swiss Albino Mice

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Article History: 23-382 Received: 01-Jul-2023 Revised: 05-Aug-2023 Accepted: 18-Aug-2023

ABSTRACT

Zinc oxide nanoparticles (ZnO-NPs) has long been used in cosmetics and paints without knowing its possible side effects in human beings. This study was conducted to find whether ZnO-NPs toxicity can be reversed by the administration of ascorbic acid in mouse. Swiss albino mice were divided into 4 groups; control group, ascorbic acid (100mg/kg), ZnO-NPs (30mg/kg) and ascorbic acid + ZnO-NPs for 21 alternate days. After that mice were decapitated and liver was preserved for histopathological analysis. Ascorbic acid groups showed the normal liver structure while ZnO-NPs group exhibited inflammation and necrosis of hepatocytes. The combination of ascorbic acid+ZnO-NPs showed reduced inflammation and necrosis after 28 days of treatment. Ascorbic acid showed hepatoprotective effect against liver fibrosis by increasing the activity of anti-oxidant enzyme and lowering of pro-oxidant effects in mice. This study concluded hepatoprotective effect of ascorbic acid against ZnO-NPs induced liver damage in mice.

Key words: Zinc Oxide Nanoparticles, Ascorbic Acid, Liver, Mouse Model

INTRODUCTION

The utilization of nanoparticles (NPs) has seen a significant rise, yet its potential impact on human remains uncertain due to unavailability of proper research data. NPs have garnered attention owing to their distinctive small size, its ability of non-biodegradability and presence of many reactive surfaces. Their capacity for easy dispersion in the environment without observable consequences is largely attributed to their remarkably small particle size, typically ranging from 1 to 100 nm (Wang et al., 2009). Nanoparticles (NPs) can lead to adverse human health issues, depending upon their origin, level of toxicity, shape and size depending on its chemistry. Metallic NPs cause redox cycling reactions in the body which releases may reactive oxygen and nitrogen species in the human body. Previous data has demonstrated the NPs toxicity, which is closely associated with the type of reactive species produced by them (Chauhan et al., 2022; Zhang et al., 2010). Metallic NPs increased the oxidative stress in the body at the cellular level which in turn increased the oxidation and can be harmful for the human body. Many studies are conducted to find out the toxicity caused by ultrafine NPs in specific relation to oxidative stress (Jiang et al., 2018). The utilization of nanotechnology has increased globally owing to its wide-ranging applications in disease diagnosis, drug delivery technology, paints, food, beauty products and even in electronics (Schilling et al., 2010). The mechanism of toxicity induced by NPs is not fully understood. However, it is considered that NPs chemical and physical properties effect its level of toxicity in human body, either due to the presence of surface groups (carbonyl or thiol group) or reactive sites on the NPs. The reactive sites can either be electron acceptor or electron donor depending on the nature of NPs (Jain et al., 2020).

A lot of NPs are used in the biotechnology and engineering but the Zinc oxide nanoparticles (ZnO-NPs) are used in abundance in many products. This is due to the fact that ZnO-NPs possess the ability to absorb UV radiation without scattering visible light, making them valuable for UV protection. Additionally, they efficiently absorb infrared radiation. ZnO-NPs serve as a versatile microbial agent in the fortification industry, employed as additives and for packaging purposes. In agriculture, they can be utilized as a fungicide, while in drug development, they show promise as anti-cancer agents and in biomedical

Cite This Article as: Anjum R, Hamid M, Khalil R and Ajmal A. 2023. Possible effect of ascorbic acid against zinc oxide nanoparticles induced hepatotoxicity in Swiss albino mice. Int J Agri Biosci, 12(3): 193-198. https://doi.org/10.47278/journal.ijab/2023.064

imaging applications. However, it's important to note that ZnO-NPs can induce adverse effects in mammalian cells. These effects include membrane injuries, inflammation, DNA damage, and apoptosis. Thus, careful consideration is essential while using them in various applications to mitigate potential health risks (Rasmussen et al., 2010). It is believed that ascorbic acid has ability to reduce lipid peroxidation by releasing Vitamin E which is a lipid soluble compound with antioxidant properties (Abdulrazzaq et al., 2019). Being hydrophilic, ascorbic acid serves as an important scavenger of free radicals in the extracellular body fluid. It effectively neutralized radicals and prevent oxidation of membranes from peroxide damage. Ascorbic acid exhibits excellent scavenging abilities against oxygen ions, superoxide, hydroxyl ion, peroxyl radicals and hypochlorous acid. Moreover, ascorbic acid is a potent source of electrons, enabling it to transfer electrons to free radicals, thereby neutralizing their toxic activity. It acts as an important cofactor in numerous biochemical reaction, functioning as either electron donor or acceptor. Researchers have reported that ascorbic acid possesses hepato-protective properties, which are closely associated with its antioxidant capacity. By potentially regenerating other antioxidants like vitamin E, ascorbic acid helps to prevent oxidative damage (Vergara-Jimenez et al., 2017).

The liver is highly susceptible to toxicity due to its significant involvement in the metabolic pathways and detoxification of foreign particles. It acts as clearing medium for the removal of NPs form the body. The liver contains many transforming enzymes that convert these toxic compounds into more water-soluble products, facilitating their elimination. Furthermore, the liver houses antioxidant enzymes that are essential in reducing the formation of reactive species by alleviating oxidative stress within the liver. Zinc is an essential element of cell composition, has been demonstrated to lower the activity of glutathione peroxidase and glutathione peroxidase, which are metabolic enzymes of body (Kumar et al., 2011).

The purpose of this research was to validate hepatoprotective effects of ascorbic acid, which is currently being used without any scientific authentication. In our study, ascorbic acid was used alone in ZnO-NPs-induced hepatic damage in mice. Ascorbic acid was evaluated on the basis of histopathological parameters.

MATERIALS AND METHODS

Pharmaceutical Agents

0.9% NaCl purchased from Ostuka, Pakistan Ltd, F/4.9H.I.T.E, Hub, Balochistan**.** Zinc oxide nanoparticles (18nm) and Ascorbic acid purchased from Sigma Aldrich.

Animals and Diet

The present study was carried out at Physiology and Endocrinology lab, Department of Zoology, Lahore College for Women University (LCWU), Lahore. Thirty-six healthy Swiss albino mice (average 30-45 gm) were purchased from Animal Housing facility of Government College University, Lahore. During the period of habituation, each animal was adapted to normal mice feed. The animals were housed at room temperature under 12-hour light and dark cycle.

Experimental Design

Thirty-six Swiss albino mice were grouped as following;

Group I (n=9): Control group was provided with 0.9% saline daily orally.

Group II (n=9): Ascorbic acid (100mg/kg) was given daily using oral tube for 28 days.

Group III (n=9): Animal were given ZnO-NPs (30mg/kg) orally for 28 days.

Group IV (n=9): Animals were given ascorbic acid (100mg/kg) and after 1-hour ZnO-NPs (30mg/kg) was administered for 28 days.

Sample Collection

After the completion of the experimental study period, the animals were anesthetized using ketamine (100 mg/kg), and all the blood was drained from the mouse's body. An incision was made on the ventral side of the mouse to expose all the organs. The liver was then carefully removed using forceps. Subsequently, the liver was washed in cold phosphate-buffered saline and preserved in paraformaldehyde (10%) for further analysis.

Histopathological Studies

Liver tissues that were previously fixed in paraformaldehyde were utilized for histopathological analysis. The samples were removed from paraformaldehyde and subjected to dehydration using various grades of ethanol, ranging from 30% to absolute, with each grade treated for 1 hour at room temperature. Subsequently, the tissues were impregnated with paraffin by incubating them in paraffin at 100°C for 24 hours. Blocks were then formed using molten paraffin, and these blocks were cut into sections around 4-5 µm thickness using a microtome. The sections were fixed on slides using heat and stained with hematoxylin (H) and eosin (E) crystals. Following staining, the sections were rehydrated with ascending grades of ethanol and then fixed with DPX, a mounting agent. Finally, the prepared slides were observed under a light microscope for further analysis.

RESULTS

Hepatocytes and Sinusoidal Spaces in Control Group

The histology of the animals in the control group throughout the research period did not reveal any evident hepatocyte abnormalities. It demonstrated the clearly defined liver central vein with a consistent layer of hepatocytes and a clearly defined portal vein. A central vein was surrounded by cords of hepatocytes that were covered in epithelial tissue (Figure 1). Hepatocyte cords are separated by sinusoids. Hepatocytes had typical morphology, no vacuoles, and central nuclei. Red blood cell infiltration was not present. The gaps between the hepatocyte sheets were obviously sinusoidal. The hepatic lamina is made up of regular radial hepatic cords that extend from the central vein in that direction.

Ascorbic Acid on Hepatocytes and Sinusoidal Spaces

After providing the ascorbic acid for a continuous 28 days, no histological changes were noticed. The structure is similar to that of the animal in the control group, although there may be some protective effects. There is no

discernible change in size or shape, and the central vein, hepatocytes, and nuclei are all normal (Figure 2).

Toxicity of ZnO-NPs on Hepatocytes and Sinusoidal Spaces

In the mouse liver following ZnO-NPs administration, loss of normal architecture, vacuolization of hepatocytes, and central vein hemorrhage were noted. After receiving therapy with ZnO-NPs, there was deformation of the central vein, some infiltration in the portal vein, uneven hepatocyte sheets, and deformed hepatic lobules (Figure 3). After 28 days of treatment with ZnO-NPs, there were increased infiltrations in central vein, hepatocytes lost their regular shape and sinusoidal spaces increased due to

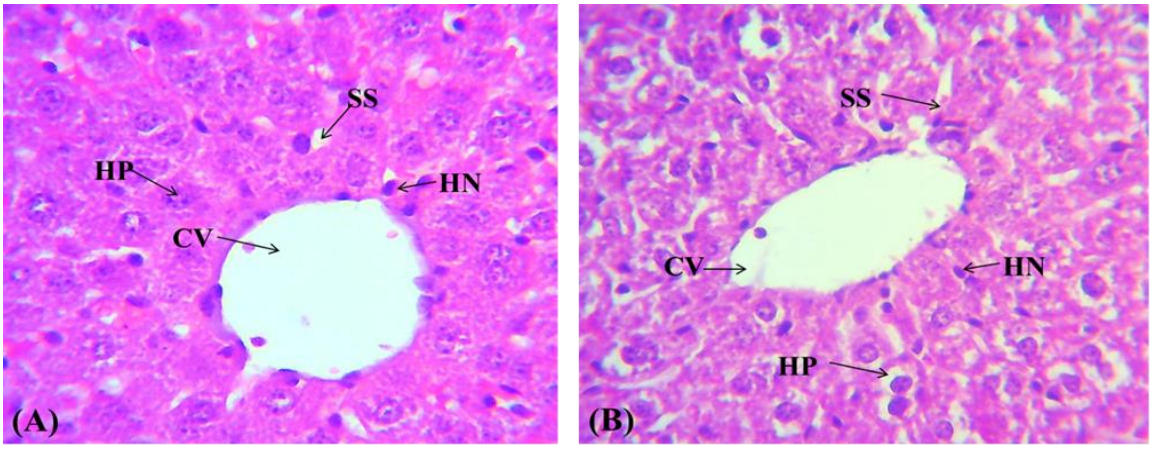

Fig. 1: Cross section of liver of control group of **(A)** 14 days **(B)** 28 days. Central vein (CV), hepatocytes (HP), sinusoidal spaces (SS) and hepatic nuclei (HN). (H & E).

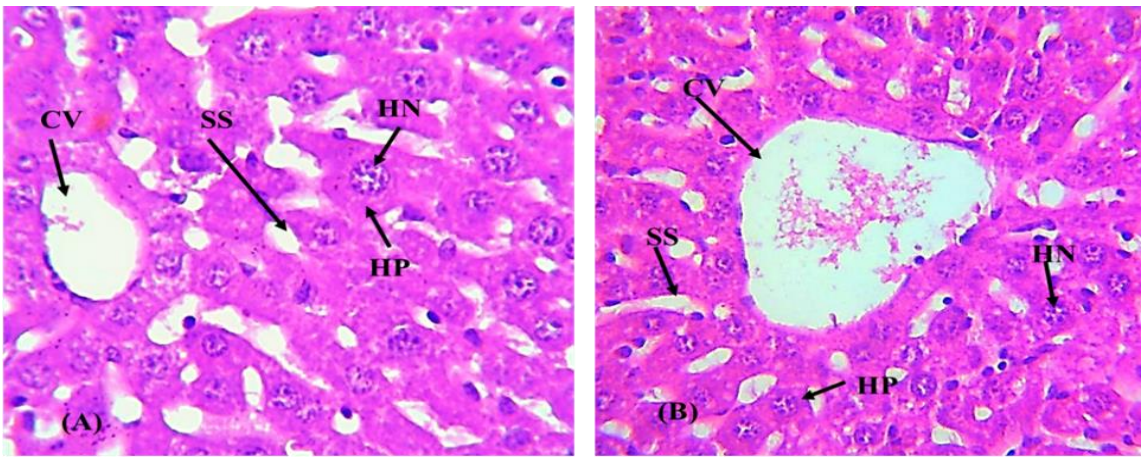

Fig. 2: Cross section of liver of ascorbic acid treated group of **(A)** 14 days **(B)** 28 days. Central vein (CV), hepatocytes (HP), sinusoidal spaces (SS) and hepatic nuclei (HN). (H & E).

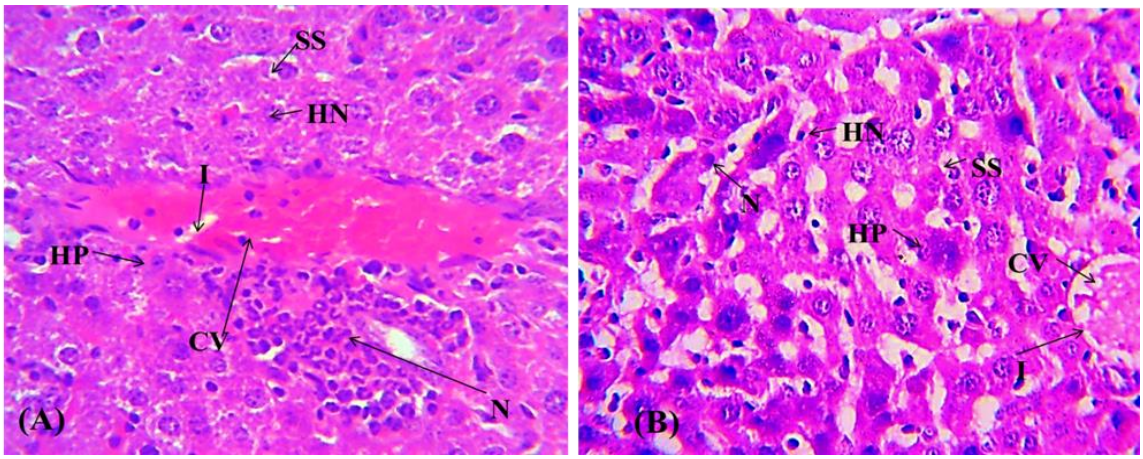

Fig. 3: Cross section of liver of ZnO-NP treated group of **(A)** 14 days **(B)** 28 days. Central vein (CV), hepatocytes (HP), sinusoidal spaces (SS), hepatic nuclei (HN), necrosis (N) and infiltration (I). (H & E).

increased oxidative stress. The vacuolization is typical of lipid accumulation in liver.

Combination of Ascorbic Acid + ZnO-NPs on Hepatocytes and Sinusoidal Spaces

Mice receiving combinations of ascorbic acid and ZnO-NPs first display enlarged hepatocytes, clogged sinusoids, and infiltration in the central vein. Although there were fewer infiltrations, the hepatocytes returned to their normal shape, and there were fewer sinusoidal spaces after 28 days of treatment, which demonstrates the hepatoprotective role of ascorbic acid on the liver of albino mice when the toxicity was caused by ZnO-NPs (Figure 4).

DISCUSSION

In this study, we aimed to investigate the potential protective role of ascorbic acid against liver damage induced by ZnO-NPs. The liver is a vital organ responsible for various metabolic processes and detoxification (Tamargo-Rubio et al., 2023). However, exposure to nanoparticles can lead to liver toxicity and oxidative stress due to the generation of reactive species and cellular inflammation (Flores-López et al., 2019). ZnO-NPs have been shown to induce liver injury in previous studies. Therefore, finding effective strategies to mitigate this toxicity is critical. Ascorbic acid, also known as vitamin C, is a potent antioxidant that scavenges free radicals and protects cells from oxidative damage. It has been extensively studied for its hepatoprotective effects. In our study, we hypothesized that ascorbic acid could attenuate ZnO-NPs-induced liver damage by reducing oxidative stress and inflammation.

To test this hypothesis, we designed an experimental study using a mouse model. Mice were divided into different groups: control, ZnO-NPs only, ascorbic acid only, and ascorbic acid plus ZnO-NPs. The ZnO-NPs group received a single dose of ZnO-NPs, while the ascorbic acid group received a daily dose of ascorbic acid for a specified duration. The ascorbic acid plus ZnO-NPs group received ascorbic acid prior to ZnO-NPs exposure.

The present study shows that, after the administration of ascorbic acid, there was no significant change in the structure of live. By increasing the activity of antioxidant enzymes and reducing prooxidant effects, ascorbic acid had positive effects on liver damage. This is because ascorbic acid interacts with hydroxyl, superoxide, alkoxyl, and peroxyl radicals, scavenging them in the process (Pisoschi et al., 2021). Supplemental ascorbic acid caused the antioxidant defense of the controls to slightly decline. Ascorbic acid's pro-oxidant activity in healthy cells may be the cause of this, as demonstrated by Marik, 2018.

It has been demonstrated that ascorbic acid plays important role in antioxidant property. The present study was indicated that the control group which has given normal diet showed normal structure of liver cells (Anjum et al., 2023). Greń et al., 2012 and Afifi & Embaby, 2016 showed the similar results that ascorbic acid supplementation protects against oxidative damage caused by different pathological conditions through inhibition of ROS production.

Ascorbic acid reduces the changed enzyme level and defends the mouse liver against hepatotoxicity brought on by medications and toxins. Ascorbic acid has a clear hepatoprotective impact on the liver, which greatly reduces enzyme increase in rats by maintaining the structural integrity of the liver (Gumaih et al., 2019). Ascorbic acid had a hepatoprotective effect in the current study for 4 weeks, which demonstrated that it was employed to lessen the liver damage in rats exposed to ZnO-NP toxicity where zinc oxide is a well-known model substance for inducing liver damage.

Oxidative stress increases the activation of stellate cells and collagen production, which is a key factor in the development of liver fibrosis. Hepatotoxins can harm the perivenular region of the hepatic acinus by creating hypoxic conditions. There are less antioxidant factors and antioxidant enzymes in the hepatocytes in the perivenular region. Histological analysis of the liver treated with ZnO-NP revealed hepatocellular alterations as demonstrated by Bashandy et al., 2018 and Ahmed et al., 2022.

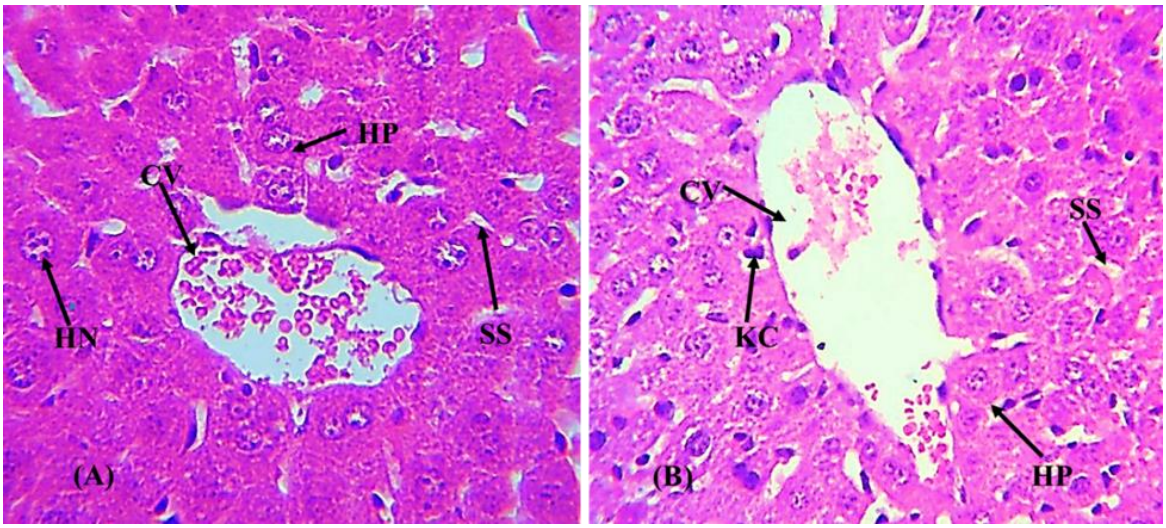

Fig. 4: Cross section of liver of Ascorbic acid+ ZnO-NP treated group of **(A)** 14 days **(B)** 28 days. Central vein (CV), hepatocytes (HP), congested sinusoidal spaces (SS), hepatic nuclei (HN) and infiltration (I). (H & E).

The exact mechanisms underlying the hepatoprotective role of ascorbic acid in the context of ZnO NPs toxicity require further investigation. Nevertheless, several plausible mechanisms can be postulated based on previous research. Ascorbic acid may directly scavenge reactive species and decreased lipid peroxidation, thereby preventing oxidative damage in hepatocytes. Furthermore, ascorbic acid has been reported to enhance endogenous antioxidant defense systems (Farooq et al., 2020), including catalase, superoxide dismutase, and glutathione peroxidase, which could contribute to the observed hepatoprotection in our research.

The treatment of ZnO-NPs and ascorbic acid assisted in reducing the anti-oxidant and anti-fibrotic effects of ascorbic acid as reflected by some blood hemolysis in central vein and moderately organized hepatocytes in cords. In mice, ascorbic acid improves liver fibrosis by increasing anti-oxidant enzyme activity and lowering pro-oxidant effects (Imessaoudene et al., 2022; Sae-Khow et al., 2022).

Overall, our findings suggest that ascorbic acid supplementation exerts a hepatoprotective effect against ZnO-NPs-induced liver damage in the mouse model. Its antioxidant properties may play a crucial role in attenuating oxidative stress, reducing liver enzyme levels, and preserving liver function. These results highlight the potential therapeutic use of ascorbic acid in protecting against nanoparticle-induced liver toxicity. The findings of this study have important implications for the development of potential therapeutic strategies for mitigating the adverse effects of ZnO NPs on liver health. Ascorbic acid, with its well-established safety profile, presents a promising candidate for further investigation and potential clinical applications. Future research should delve deeper into the specific molecular pathways involved in the hepatoprotective effects of ascorbic acid and explore optimal dosing regimens to maximize its efficacy.

Conclusion

The current study showed that ascorbic acid has anti-inflammatory and act as hepatoprotectant and prevent from the toxicity induced by ZnO-NPs. Histological study also showed recovery of liver after the administration of hepatoprotective compounds with induced toxicity of ZnO-NPs proving the protecting and regenerative impact. When the combination of anti-oxidant and zinc oxide nanoparticle were given, recoveries of cells were observed. Ascorbic acid helped to maintain the normal cell structure and its functions. Results demonstrate the positive hepatoprotective effects of ascorbic acid when toxicity was given.

Acknowledgment

The authors thank the Department of Zoology, Government College University Lahore, for providing all facilities for the completion of this study.

Conflict of interest

The authors have declared no conflict of interest.

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