Original Article

The Potential Hepatoprotective role of Resveratrol in ameliorating the Zinc Oxide Nanoparticles Induced Hepatic Tissue Injury in Rats: A Histological and Immunochemical Study

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ABSTRACT

Introduction: Zinc oxide nanoparticles are widely used in industries and medications. This elevated the chances of exposure which may have harmful effects on different body organs as the liver. So it was of a great value to search for natural protective element against this effect.

Aim of the Work: Determine the effect of ZnO NPs on the structure and function of the liver of albino rats and to evaluate the possible protective role of Resveratrol.

Material and Methods: 40 adult male albino rats, were divided into four groups 10 rats each; Group I (Control group), Group II (ZnO NPs group), Group III (Resveratrol group) and Group IV (ZnO NPs + Resveratrol group) with administration of drugs by oral gavage for four weeks.

At the end of the experiment, blood samples were collected for measuring liver enzymes. Livers were harvested and processed for light microscopic examination.

Results: Liver sections from (Group II) showed disorganized hepatic architecture, hepatocytes with darkly stained nuclei, and deep acidophilic cytoplasm were observed. Central veins and sinusoids were congested. The portal tracts were expanded with congested portal vein and proliferation of the bile ducts. An increase in the collagen deposition around the portal tracts and depletion of glycogen content within the cytoplasm. Hepatocyte apoptosis was evident.

Sections of (Group IV) showed the restoration of normal hepatic lobules where most hepatocytes appeared normal, while few appeared with pyknotic nuclei. Most of the sinusoids and portal tracts returned to their normal sizes. Sections showed minimal collagen deposition. Also Resveratrol restored the glycogen content depleted in (Group II). Resveratrol reduced the necrosis of hepatocytes.

Deterioration of liver enzymes was observed in (Group II), with marked improvement in (Group IV).

Conclusion: Resveratrol can protect against the degenerative effect of ZnO NPs on the histological structure and function of the liver.

Received: 04 September 2023, **Accepted:** 29 November 2023

Key Words: Alanine; hepatocytes; Hepatoprotective; resveratrol; ZO nanoparticles.

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ISSN: 1110-0559, Vol. 48, No. 1

INTRODUCTION

The application of nanoparticles in various fields has expanded recently in the last years which caused an increase in the research in that field as despite their beneficial properties, the risks posed by nanoparticles are still poorly understood and researchers are looking at how they might harm living things[1].

Nanoparticles are extremely small particles, measuring one to 100 nm[2]. They can be directly ingested through water, foods, or drugs that are taken orally[3]. After its absorption by the gastrointestinal tract, nanoparticles are distributed in the spleen, kidney, and liver[4].

After iron, zinc oxide nanoparticles (ZnO NPs) are the most widely utilized metal oxide because they are affordable, secure, and simple to make[5]. ZnO NPs are prepared in the form of white powder[6]. It is widely used for industrial purposes and daily supplies, such as paint, coatings, cosmetics, pharmaceuticals, and electronics. The expanded usage of ZnO NPs increases the possibility of their accumulation in the environment, leading to hazardous effects on different organisms[7].

Even though the body needs the zinc ion, too much of it can cause proteins and lipids to be destroyed by oxidation due to the production of reactive oxygen species (ROS)[8].

Based on previous studies, a high dose of ZnO NPs ranging (1–5 g/kg body weight) can trigger serious oxidative stress[9,10]. The primary organ that toxic substances such as ZnO NPs target is the liver. The substance will accumulate in the liver, and cause damage to its tissue[11].

A non-flavonoid phenol, resveratrol (3, 5, 4-trihydroxystilbene) is a substance that occurs naturally[12]. Resveratrol is detected in hundreds of safe to eat plants

DOI: 10.21608/ejh.2023.231716.1941

as grapes, berries, and peanuts. Plants produce resveratrol as a defense mechanism against any fungal infection, microbial injury or environmental stress. By minimizing liver fibrosis, it can be beneficial for both the protection and treatment of liver illnesses[13].

Several studies demonstrate that a variety of beneficial pharmaceutical uses for resveratrol exists as acting as an antioxidant, anti-inflammatory, antidiabetic, antitumor, hepatoprotective, and cardioprotective substance[12,13].

Exposure to ZnO NPs comes with an elevated risk and their possible harmful effects on the liver are augmented as well, thus the need for possible hepatoprotective products such as Resveratrol becomes mandatory.

MATERIAL AND METHODS

Chemicals

1. Zinc oxide nanoparticles (ZnO NPs) had been bought from Nanotech, a Company developing photo-electronics communication center using nanotechnology. 6th of October city, Al-Giza, Egypt. The particles appeared to be white powder in sphere-like form and the size on average was 30 ±5 nm. The drug was dissolved in a freshly sterile saline solution before use[14].
2. Resveratrol was provided in the form of capsules. The drug was purchased online from UAE by Biovea. The content of the capsule was emptied and dissolved in sterilized physiological saline solution before use[15].

Study design

Forty adult male albino rats, 3-6 months old, with weight ranging from 150-200 grams, were brought from the animal house at the Faculty of Medicine, Ain Shams University, Research Institute (MASRI). They were preserved in accordance with the rules of the Committee of Animal Research Ethics (CARE). Two rats were housed in each cage (stainless steel made) where the rodents were maintained. Before any interference, they were given a week to adapt to experimental circumstances. They were introduced to a 12-hour light/dark cycle and given access to a regular feed (rat chew) and free water, as well as good ventilation and a suitable environment.

There were four groups of rats as follows:

Group I: (control group): included ten male albino rats, which had free access to water and food only.

Group II: (ZnO NPs group): ten rats were in the group, and they were treated with zinc oxide nanoparticles (50 mg /kg body weight/day) for four weeks via oral gavage[14].

Group III: (Resveratrol group): included ten rats that received Resveratrol (20 mg/kg body weight/day) for four weeks via oral gavage[16].

Group IV: (ZnO NPs + Resveratrol group): included ten rats that were co-treated with ZnO NPs and Resveratrol in the same doses and the same route as in groups II and III for four weeks, respectively.

Biochemical analysis

Using the retro orbital way, (2 ml) samples of blood were taken from all animals via the ophthalmic venous plexus. In order to perform the liver function tests (AST, ALT, and ALP), the clear serum had to be separated from the blood samples by centrifugation at 5000 rpm for ten minutes[3].

Collection of samples

Animals were sacrificed at the conclusion of the experiment by being given a lethal dose of anesthetic and their liver tissues were carefully dissected and obtained. The specimens were processed for the preparation of paraffin blocks.

Tissue processing for light microscopy

Liver tissue samples were processed and set in 10% neutral formol saline over 48 hours. After that, the tissues were dehydrated and integrated within paraffin blocks. Five µm-thick serial sections were sliced. Hematoxylin and eosin (H&E), Masson's trichrome, Periodic acid-Schiff (PAS), and immunohistochemical detection of active caspase-3 using particular antibodies were all used to stain the sections[17]. Then a light microscope was used to examine the sections.

Morphometric study (Image analysis)

Light microscope measured parameters were carried out using an image analyzer at the Faculty of Medicine, Ain Shams University, Histology and Cell Biology department applying the Leica Q win program that has been loaded on a Dell PC (Texas, USA), which was linked to a microscope (Leica microsystems, Heerburg, Switzerland).

The following morphometric values were assessed per cut section of the liver tissue using an image analyzer: at a magnification of x400. Analysis was done on the area percentage of collagen fiber deposition, the area percentage of cells expressing caspase-3 in immunohistologically stained sections, and the optical density of PAS stain in the liver sections.

Statistical Analysis

To determine the differences between the research groups, statistical analysis was conducted using the SPSS program (Statistical Package for Social Studies-version 13.0). The means of the various groups were compared using one-way analysis of variance (ANOVA). The Bonferroni Post Hoc test was employed to determine whether there was a difference between each pair of groups. Probability (*P-value*) was used to determine the relevance of the data. $P > 0.05$ was regarded as insignificant. Furthermore, $P \leq 0.05$ was expected to be significant and $P \leq 0.001$ was supposed to be highly significant[18]. MS Excel 2013 was used to create tables and histograms that reflected the data.

RESULTS

Histological Results

Group I (Control group): Examination of sections of the adult male albino rats' livers from group I stained using hematoxylin and eosin demonstrated the typical hepatic lobules. The lobule was made up of hepatocytes arranged in plates, smoothly anastomosing with one another and organized in a radial pattern surrounding a central vein (Figure 1). The hepatocytes had a polygonal form, with basophilic spherical nuclei and acidophilic cytoplasm. There were also binucleated hepatocytes found. Liver sinusoids filled the gaps between the hepatic plates and contained phagocytic Kupffer cells (Figure 1).

The liver was covered by a thin connective tissue capsule, according to Masson's trichrome stain. Around the main vein, between the hepatocytes, a very small amount of collagen fibers showed up (Figure 2). The cytoplasm of the parenchymal hepatocytes showed a significant positive PAS response in liver sections stained with Periodic acid Schiff (Figure 3).

Inspection of Caspase-3 immunohistochemical reaction showed an apparent negative reaction in most hepatocytes (Figure 4).

Group II (ZnO NPs group): The liver obtained from the ZnO NPs group's paraffin sections underwent study and revealed that the hepatic architecture was disorganized. The central vein was dilated, deformed and somewhat congested, with a separate area of endothelial lining (Figure 5). In addition, the blood sinusoids appeared irregular and congested (Figure 6). The hepatocytes were markedly affected. The majority of the nuclei appeared atrophic and heavily stained with deep acidophilic cytoplasm. There were apoptotic shrunken hepatocytes with dark stained nuclei and were surrounded by clear halos. While other cells revealed finely granular cytoplasm with cytoplasmic vacuolation (Figures 5,6). Nevertheless, apparently intact vesicular nuclei within hepatocytes were seen.

Expanded portal tracts were often observed at the margins of most hepatic lobules. They revealed enlarged and congested branches of the portal venule, proliferation of bile ducts. Blood sinusoids appeared enlarged and congested and mononuclear cellular invasion were also noticed (Figure 7).

Examination of sections stained by Masson's trichrome showed an apparent obvious increased collagen fibers in the vicinity of portal tracts (periportal fibrosis) and in between the hepatic cords (Figure 8).

Periodic acid-Schiff stained liver sections revealed diffuse hepatic glycogen depletion with a marked decrease in PAS positive glycogen granules inside hepatocytes. Most hepatocytes were seen with negative PAS reactions (Figure 9).

Immunohistochemical examination showed that most of the hepatocytes had a strong positive reaction to caspase-3 immuno-stain (Figure 10).

Group III (Resveratrol group): Studying the liver sections stained by H&E from the Resveratrol group showed the similar histological structure as in the control group (Figure 11).

Masson's trichrome stained sections demonstrated an apparent considerable amount of collagen fibers between the hepatocytes and around the central vein. (Figure 12).

Most hepatocytes had a significant PAS positive response in their cytoplasm, as seen in periodic acid-Schiff stained sections as a result of the increase in the glycogen content in all zones of hepatic lobule (Figure 13).

Most hepatocytes revealed in their immunohistochemical examination a negative reaction in their cytoplasm to caspase- 3 immunostain (Figure 14).

Group IV (ZnO NPs+Resveratrol group): Inspection of sections stained by H&E obtained from the ZnO NPs + Resveratrol group showed that the liver restored its normal appearance. The classic hepatic lobules could be seen containing central veins in the center and peripheral portal tracts. Most hepatocytes were observed healthy with polygonal form, acidophilic cytoplasm and centralized prominent round nuclei (Figure 15). While few hepatocytes were either having dark stained pyknotic nuclei or hydropic degeneration of the cytoplasm. Binucleated hepatocytes were also observed (Figure 16). Blood sinusoids backed to their normal size but some sinusoids appeared congested and dilated also portal tracts returned to the normal size (Figure 16).

Sections stained by Masson's trichrome indicated few collagen fibers between the hepatic cords and around the normal-sized portal tract (Figure 17).

Most hepatocytes in sections stained by Periodic acid-Schiff had a robust positive response in their cytoplasm. Weak PAS reactions on a few hepatocytes were seen (Figure 18).

Immunohistochemical examination showed an apparent negative reaction to caspase- 3 immunostain in the cytoplasm of most hepatocytes (Figure 19).

Morphometric Results

Assessment of the mean area percentage of interstitial collagen fibers deposition in sections stained by Masson's trichrome demonstrated that the percentage in ZnO NPs group increased in an extremely significant way contrasted to the control group, while the group taking Resveratrol revealed a non - significant difference compared to the control group, however, ZnO NPs + Resveratrol group indicated a significant increase when compared to the control group. But showed a highly significant decrease compared with the ZnO NPs group. (Histogram 1, Table I).

Assessment of the mean area percentages of caspase 3 immunostain expression revealed a highly significant increase in the ZnO NPs group and ZnO NPs + Resveratrol group, in comparison to the control group. Nevertheless,

the ZnO NPs + Resveratrol group indicated a significant decrease in contrast to the ZnO NPs group.

Moreover, the Resveratrol group revealed an extremely significant decrease in comparison to the control group. (Histogram 2, Table I).

Assessment of the optic density of PAS stains showed that there was a remarkably significant decrease in the ZnO NPs group, compared with the control group.

However, the Resveratrol group and ZnO NPs +Resveratrol group revealed a highly statistically significant increase in contrast to the control group. The group receiving ZnO NPs + Resveratrol also showed a highly significant increase when compared to the ZnO NPs group. (Histogram 3, Table I).

Liver function tests

Estimation of liver enzyme levels within the four groups demonstrated a remarkably significant increased ALT, AST and ALP levels in the ZnO NPs group and ZnO NPs + Resveratrol group when compared to the control group. The ZnO NPs + Resveratrol group, on the other hand, exhibited a hugely significant decline in contrast to the ZnO NPs group alone. While the Resveratrol group revealed an insignificant variation in the levels of AST and ALT in relation to the control group but showed an extremely significant decrease in ALP level in relation to the control group. (Histogram 4).

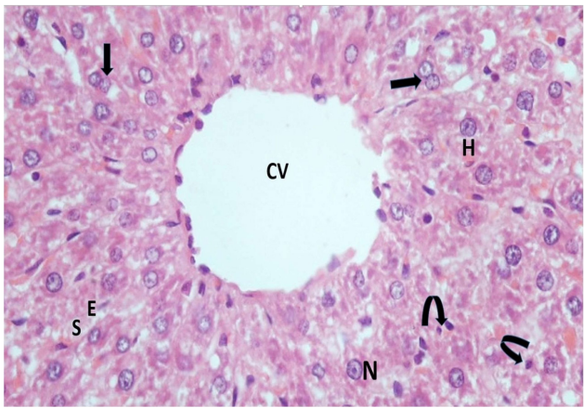


Fig. 1: A photomicrograph of a section of a rat's liver from group I (the control group) showing anastomosing cords of hepatocytes (H) radiating from the central vein (CV). Hepatocytes appear polygonal in shape with acidophilic cytoplasm and central rounded vesicular nuclei (N). Binucleated hepatocytes are seen (arrow). Hepatocytes are separated by irregular slits like blood sinusoids (S) lined with endothelial cells (E). Notice the Kupffer cells (curved arrow) with dense nuclei. H&E X400

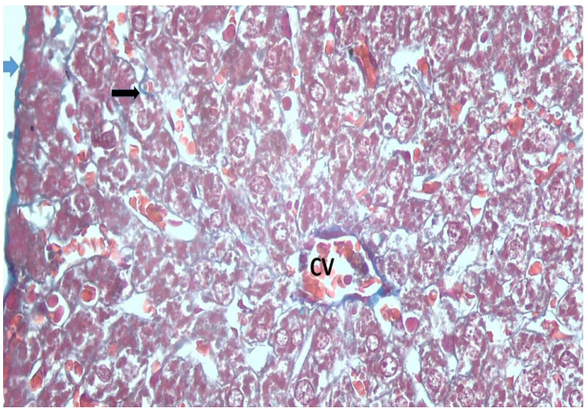


Fig. 2: A photomicrograph of a section of a rat's liver from group I showing the central vein (CV) surrounded by few collagen fibers. Minimal collagen fibers are also seen in between the hepatic cords (black arrow). Notice the thin connective tissue capsule (blue arrow). Masson's trichrome X400

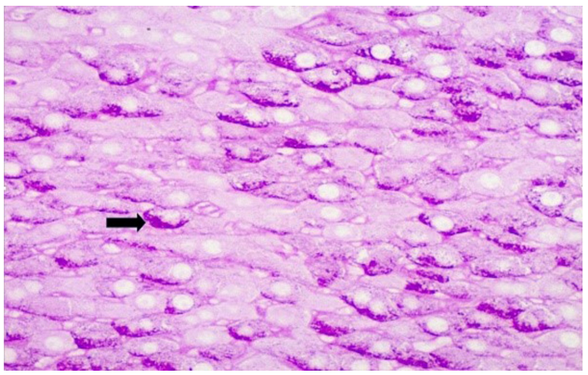


Fig. 3: A photomicrograph of a section of a rat's liver from group I showing a strong positive PAS reaction within the cytoplasm of hepatocytes (arrow). PAS X400

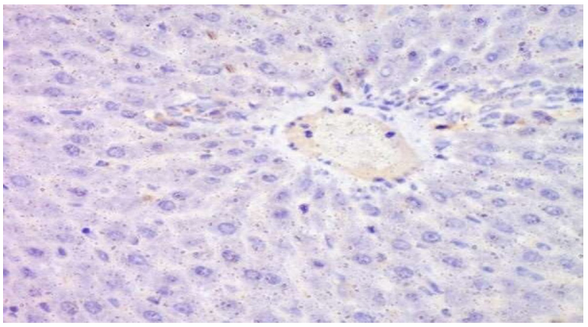


Fig. 4: a photomicrograph of a section of a rat`s liver from group I showing an apparently negative caspase-3 reaction in the cytoplasm of hepatocytes. Caspase-3 immunohistochemical reaction X400

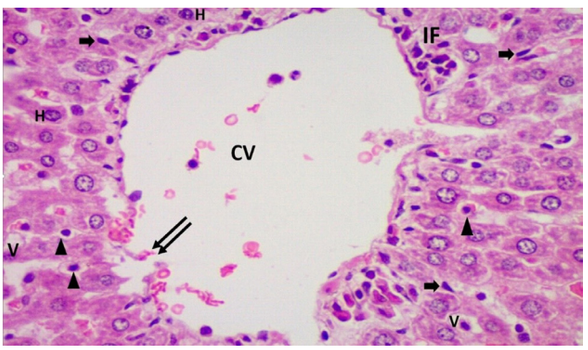


Fig. 5: A photomicrograph of a section of a rat's liver from group II (the ZnONPs-treated group) showing a distorted, dilated central vein (CV), with an area of detached endothelial lining (double arrow). A mononuclear cellular infiltration (IF) is seen around the central vein. Some hepatocytes with darkly stained nuclei (H) and vacuolated cytoplasm (V) are detected while other apoptotic shrunken hepatocytes (arrow head) are seen as well. Notice an apparent increased number of Kupffer cells (arrow) H&E X400.

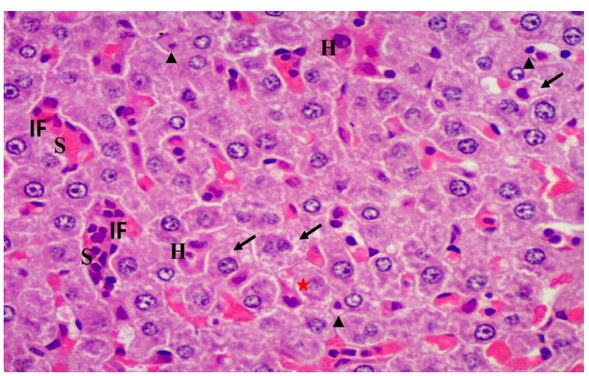


Fig. 6: A photomicrograph of a section of a rat's liver from group II showing necrotic hepatocytes with darkly stained nuclei and deeply stained acidophilic cytoplasm (H). Other cells revealed finely granular cytoplasm with cytoplasmic vacuolation (arrow) and hydropic degeneration (star). There are also some apoptotic cells with clear halos around them (arrow head). Congested blood sinusoids (S) with mononuclear cellular infiltration (IF) are also detected. H&E X400

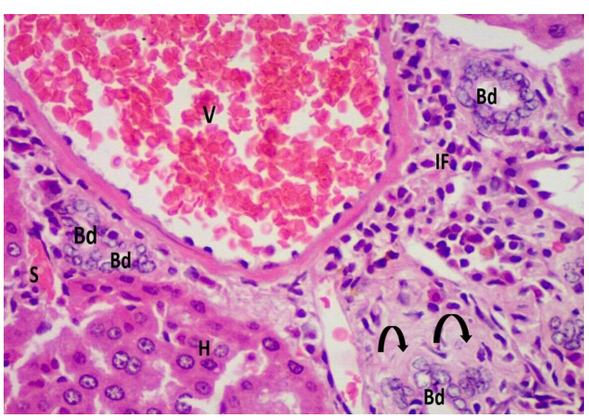


Fig. 7: A photomicrograph of a section of a rat's liver from group II showing the dilated congested portal venule (V), proliferation of the bile ductules (Bd), and mononuclear cellular infiltration (IF). An apparent increase in the amount of collagen fibers (Curved arrow) in the portal tract, congested blood sinusoids (S) and hepatocytes with darkly stained nuclei (H) could be detected. H&E X400

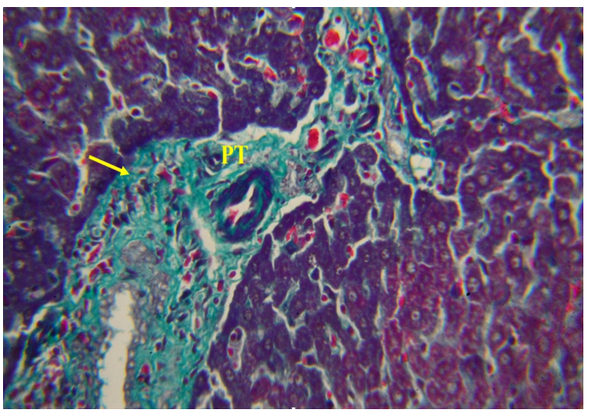


Fig. 8: A Photomicrograph of a section of rat's liver from group II showing marked deposition of collagen fibers (periportal fibrosis) (arrow) in the expanded portal tract (PT). Masson's trichrome X400

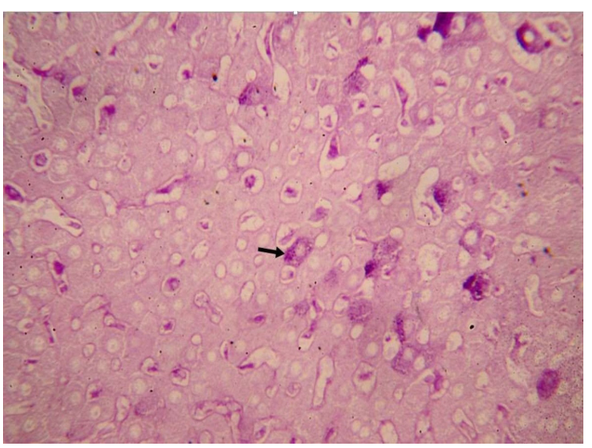


Fig. 9: A photomicrograph of a section of a rat's liver from group II showing that most of the hepatocytes have a negative PAS reaction. Some hepatocytes are still showing mild PAS positive reactions (arrow). PAS X400

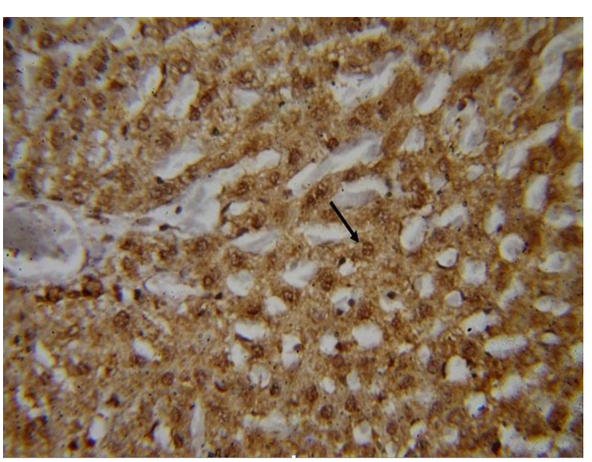


Fig. 10: A photomicrograph of a section of a rat's liver from group II showing a strong positive caspase-3 reaction in the cytoplasm of most hepatocytes (arrow). Caspase-3 immunohistochemical reaction X400

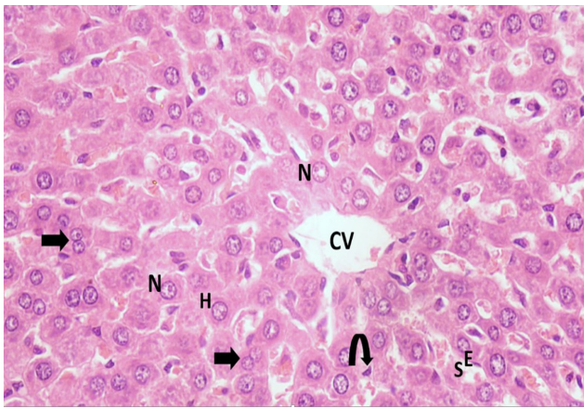


Fig. 11: A photomicrograph of a section of a rat's liver from group III (the Resveratrol group) showing anastomosing cords of hepatocytes (H) radiating from the central vein (CV). Hepatocytes are seen polygonal in shape with acidophilic cytoplasm and central rounded vesicular nuclei (N). Binucleated hepatocytes are seen (arrow). Hepatocytes are separated by irregular slits like blood sinusoids (S) lined with endothelial cells (E). The Kupffer cells (curved arrow) are also noticed. H&E X400

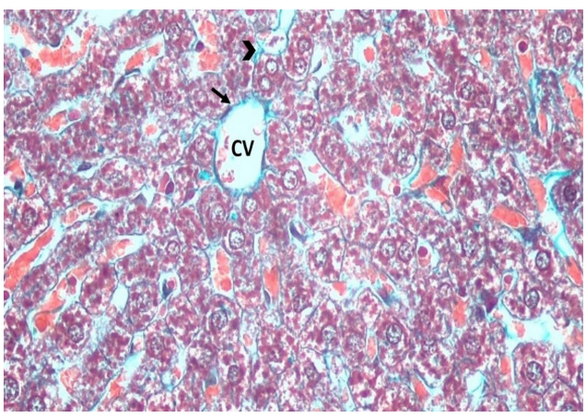


Fig. 12: A photomicrograph of a section of a rat's liver from group III showing the central vein (CV) surrounded by a moderate amount of collagen fibers (arrow). Few collagen fibers are seen in between the hepatic cords (arrow head) . Masson's trichrome X400.

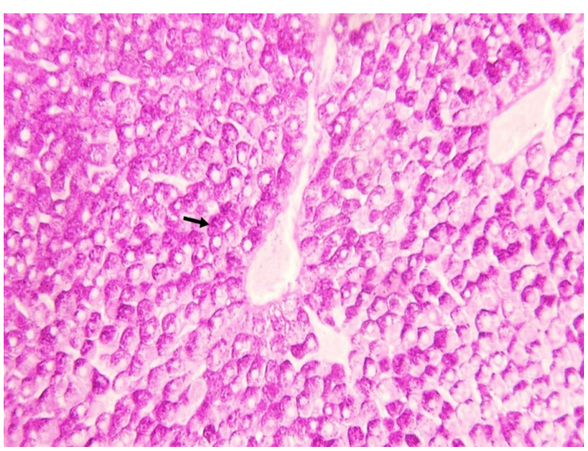


Fig. 13: A photomicrograph of a section of a rat's liver from group III showing strong positive PAS reaction in cytoplasm of hepatocytes (arrow). PAS X400

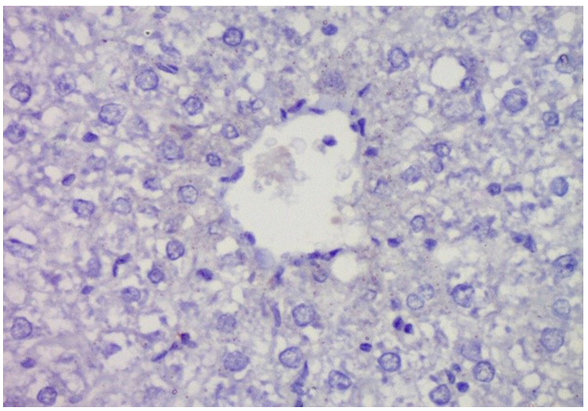


Fig. 14: A photomicrograph of a section of a rat's liver from group III showing a negative caspase-3 reaction in the cytoplasm of hepatocytes. Caspase-3 immunohistochemical reaction X400

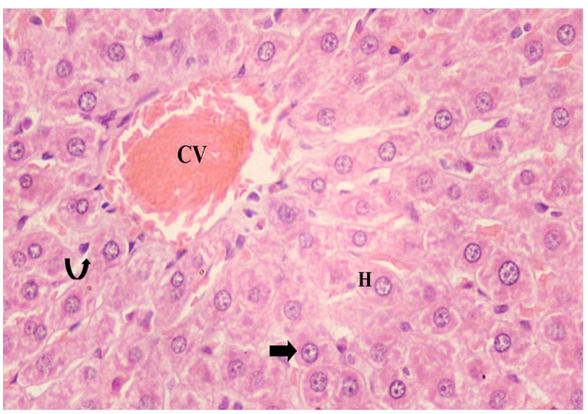


Fig. 15: A photomicrograph of a liver section from group IV (the ZnO NPs +Resveratrol group) showing anastomosing cords of healthy hepatocytes (H) radiating from a dilated, congested central vein (CV). The hepatocytes are seen polygonal in shape, with acidophilic cytoplasm, and central rounded vesicular nuclei (arrow). Notice Kupffer cells with dark nuclei (curved arrow). H&E X400

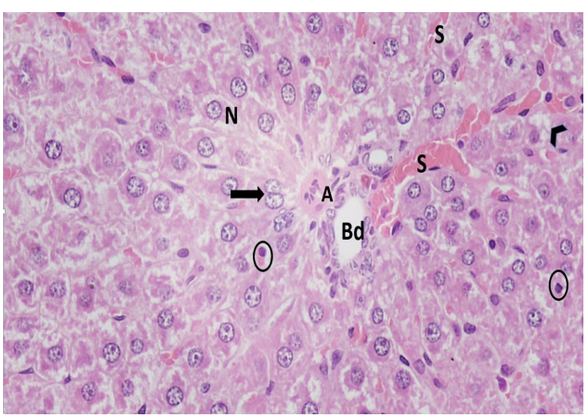


Fig. 16: A photomicrograph of a liver section from group IV showing the components of the portal tract; an arteriole (A), and bile ductule (Bd). Most of the hepatocytes are healthy with vesicular nuclei (N). Some hepatocytes have pyknotic nuclei (circle) or vacuolated cytoplasm (arrow head). Binucleated hepatocytes could be seen (arrow). Notice the congested sinusoids (S). H&E X400

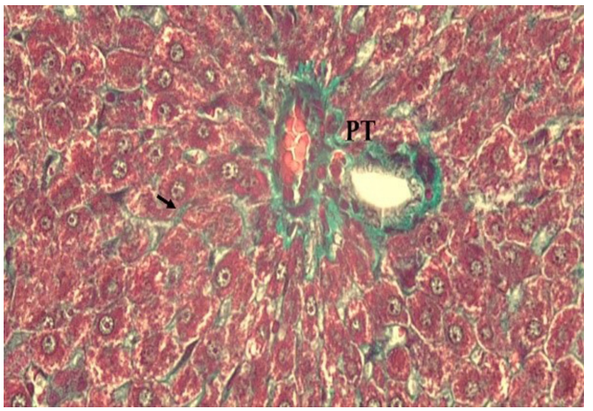


Fig. 17: A photomicrograph of a section of rat's liver from group IV showing the portal tract (PT) surrounded by a few collagen fibers. Few collagen fibers are also seen in between the hepatic cords (arrow). Masson's trichrome X400

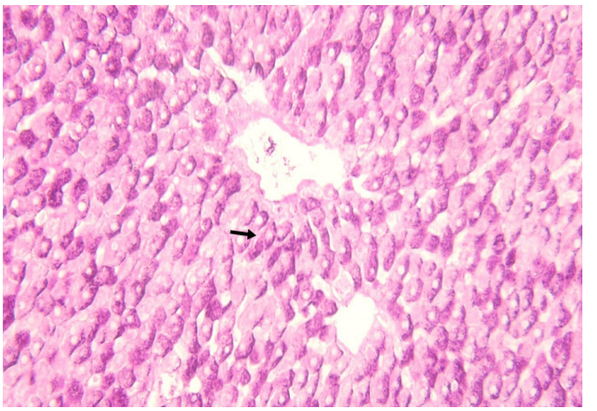


Fig. 18: A photomicrograph of a section of rat's liver from group IV showing strong positive PAS reactions in the cytoplasm of hepatocytes (arrow). PAS X400

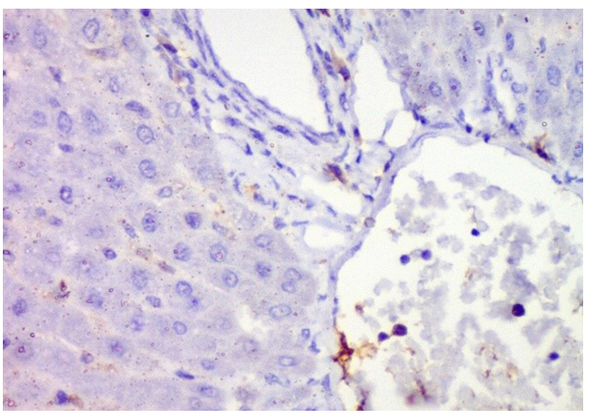
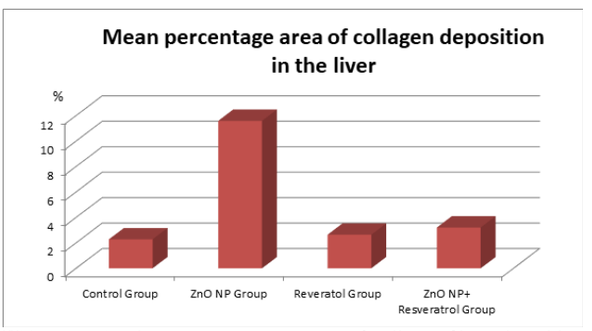


Fig. 19: A photomicrograph of a section of a rat's liver from group IV showing apparently negative caspase-3 reactions in the cytoplasm of hepatocytes. Caspase-3 immunohistochemical reaction X400

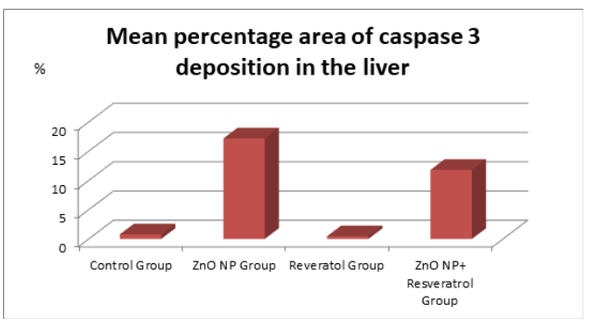
Table I: Histomorphometric parameters in the four study groups

Histological Parameters	Control group	ZnO NPs group	Resveratrol group	ZnO NPs +Resveratrol group
Area percentage of collagen deposition	2.278± 0.61987	11.587±3.08026**	2.639± 0.423489	3.208± 0.7615452*
area percentages of caspase -3 expression	0.775± 0.10814085	17.175±4.88534**	0.451± 0.103435b	11.805± 3.265442**
Optic density percentage of PAS stain	80.929±1.49466793	59.99 ±0.536324943b	85.473±1.770668737**	84.142±1.263072093**

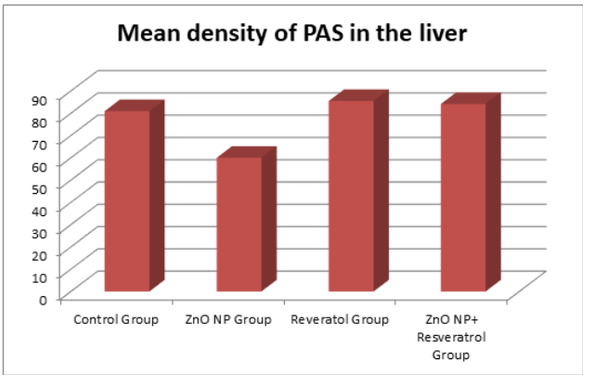
* denote a significant increase $P \leq 0.05$.
**denote a highly significant increase $P \leq 0.001$.
a denote a significant decrease ≤ 0.05.
b denote a highly significant decrease $P \leq 0.001$.



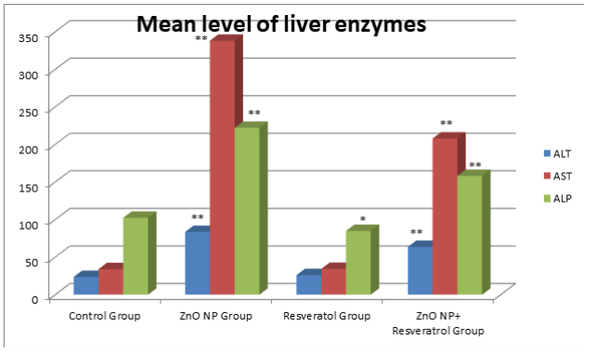
Histogram 1: The mean area percentage of collagen fibers deposition across the four research groups.



Histogram 2: The area percentage of caspase -3 expression across the four research groups.



Histogram 3: The mean optic density of PAS stain across the four research groups.



Histogram 4: comparison of Means of ALT, AST and ALP in the four groups of the study

* denote a highly significant decrease $P \leq 0.001$

**denote a highly significant increase $P \leq 0.001$.

DISCUSSION

Over the past few decades, the numerous applications of nanoparticles led to a rapid increase in active research in this area[1].

Regarding our study, the findings showed considerable alterations in the histological structure of rats' livers treated with ZnO NPs. The central veins appeared dilated and congested with areas of detached endothelial lining. Blood sinusoids showed dilatation and congestion. Similarly, Alferah,[19] and Moatamed *et al*.,[20] found the blood sinusoids dilated and congested moderatley among the hepatic cords in groups given the ZnO NPs at dosages of 50 and 100 mg/kg BW. Puche *et al*.,[21] attributed sinusoidal dilatation to the activation of perisinusoidal cells which had contractile properties.

Moreover, mononuclear cellular infiltration was seen scattered around central veins and in portal tracts with an apparent raised number of Kupffer cells, these findings were in accordance with the studies of Ibrahim *et al*.,[22] and Aboulhoda *et al*.,[23], They described how the ZnO NPs' contact with the hepatic tissues promoted inflammatory cell infiltration, which might trigger different immunological reactions in the liver.

Additionally, the hepatocytes of rats in the ZnO NPs group showed variable degrees of cellular damage. Some hepatocytes showed signs of hydropic degeneration; others hepatocytes had small apoptotic nuclei surrounded by clear halos. Some hepatocytes appeared necrotic. These findings were similar to those of the researches carried out by Mansouri *et al*.,[24] and Reddy *et al*.,[25] who observed foamy degeneration and necrosis of hepatocytes of rats exposed to ZnO NPs.

Aboulhoda *et al*.,[23] assumed that the hepatic cellular injury by ZnO NPs could happen due to disruptions of hepatocyte cell membrane function resulting in an enormous influx of water and sodium. In addition to release of lysosomal hydrolytic enzymes that causes the cytoplasmic degeneration or it is justified by the toxicity of zinc nanoparticles to different cell organelles, including the nucleus, mitochondria and endoplasmic reticulum altering their activity.

Moreover, Abbasalipourkabir *et al*.,[26] proposed that the accumulation of ZnO NPs in the tissue of the liver and stimulation of intracellular ROS generation, leads to diminished mitochondrial membrane potential in addition to raised apoptotic protein.

In the present study, expanded portal tracts were frequently seen at the periphery of most hepatic lobules of ZnO NPs treated group. An infiltration of mononuclear cellular cells, marked increase in the collagen fibers deposition, proliferation of the bile ductules, and congested portal venules were observed. These findings were in parallel with those of Alferah,[19].

Morphometrically, there was a significant increase of the mean percents of the area of collagen fibers deposition in the ZnO NPs group in comparison with the control group. Agreeing results were postulated by Hegazy *et al*.,[3] and Aboulhoda *et al*.,[23], they revealed that ROS, generated within the hepatocytes by exposure to ZnO NPs, could activate collagen production in hepatic stellate cells in a paracrine manner. Therefore, induction of hepatic fibrosis developed.

In the ZnO NPs group there was diffuse hepatic glycogen depletion and most of the hepatocytes revealed a negative PAS reaction. Only a few hepatocytes showed a mild PAS reaction. This was confirmed by the morphometric results, which showed a highly significant drop in the optic density of PAS stain in the group receiving ZnO NPs in relation to the control group. Such findings were in agreement with the studies of Almansour *et al*.,[27] and Alferah,[19], who observed that hepatocytes of rats administered ZnO NPs exhibited glycogen depletion in addition to negative PAS reaction.

Hepatocytes with a strong positive Caspase-3 cytoplasmic response appeared to be more prevalent, according to immunohistochemical examination. The morphometric results supported the histological observations, which showed a remarkable significant

increase in the area percentages of caspase-3 expression in the ZnO NPs group compared to the control group. Hegazy *et al*.,[3] also observed the same statistical results. Sizova *et al*.,[28] and Aboulhoda *et al*.,[23] also noted a significant elevation in caspase-3 expression in contrast to the control group, especially with the high doses of ZnO NPs, which was a sign of cellular stress, DNA destruction and cell cycle arrest.

Resveratrol group showed no histopathological alteration of hepatic tissue as in the control group. Such finding was conforming to Abdu and Al-Bogami,[16] & Al-Baqami and Hamza,[29], who stated that groups given Resveratrol appeared normal physiologically, that is an indicator of the safety of Resveratrol.

Histomorphometric analysis results also showed that Resveratrol treated group caused a non-significant elevation in the mean percents of the area of collagen fibers deposition in contrast to control group. Resveratrol caused an increase in the glycogen content of hepatocytes. This was proved statistically by the extremely significant increase in the optic density of PAS stain in Resveratrol group versus the control group. Similarly, Abd-Elhafiz and Issa,[15] reported the same results.

By morphometric analysis, the Resveratrol group also revealed a greatly significant decrease in the mean area percentages of caspase-3 expression in relation to the control group, which was matched with the hepatoprotective effect of Resveratrol.

Our study results showed the protective role of Resveratrol against hepatotoxicity caused by ZnO NPs, which was evidenced by the restoration of normal classic hepatic lobules. With center rounded vesicular nuclei and acidophilic cytoplasm, the majority of hepatocytes showed signs of health. While few hepatocytes appeared degenerated. Most of the blood sinusoids and portal tracts returned to their normal sizes and showed no proliferation of the bile duct in contrast to ZnO NPs treated group alone, with a marked decrease in inflammatory cell infiltration. These findings were conforming to HAMADI *et al*.,[30], who reported that liver sections prepared from a combination group (Streptozotocin + Resveratrol) restored their structure to an almost normal picture.

Similarly, Al-Baqami and Hamza,[29] also found that with the co-treatment with the Cadmium and Resveratrol together, the hepatocytes seemed normal with the onset of modest hypertrophy and the emergence of binucleated hepatocytes.

A marked decrease in collagen fibers deposition, especially around the portal tract was detected. This is matched with histomorphometric analysis results which indicated a highly significant decrease in the area percentage of collagen deposition, in the group given a combination of ZnO NPs and Resveratrol compared to ZnO NPs group alone. This was explained by Abdu and Al-Bogami,[30] who stated that Resveratrol significantly reduced hydroxyproline level which is known as an amino acid primarily found in collagen and is believed to be a marker for fibrosis. They also reported that Resveratrol possesses a strong potential to block the collagen deposition induced by dimethylnitrosamine.

Additionally, Abd-Elhafiz and Issa,[15] stated Resveratrol administration caused a significant drop in the percents of the area of Masson's trichrome stain in the wall of the blood vessels and the portal tract versus the cisplatin group.

Another interesting finding in our study was detected, where resveratrol restored the glycogen content of hepatocytes that was depleted in the ZnO NPs group. This was marked by a strong positive reaction of the hepatic cells in the PAS stained sections with a few hepatocytes showing weak PAS positive reactions in the group treated by ZnO NPs and Resveratrol together. The histomorphometric study also indicated that the Resveratrol and ZnO NPs group had a highly significant raise in the optic density of PAS reactions in the hepatic cells in contrast to the ZnO NPs treated group alone. Izzo *et al*.,[31] illustrated that Resveratrol can elevate the glycogen synthase, lower the glycogen phosphorylase which will eventually cause an increase in the glycogen content in the liver. This was confirmed by Abd-Elhafiz and Issa,[15], who also found that the Resveratrol led to a significant increase in the percents of the area of PAS reaction in the hepatic cells in relation to the cisplatin group.

Resveratrol significantly reduced the degeneration and necrosis of hepatocytes. This was evident in our study histologically by an apparent negative Caspase-3 reaction in the cytoplasm of the majority of hepatocytes in ZnO NPs and Resveratrol treated group and morphometrically by a statistically significant drop in the mean area percentage of caspase -3 expression compared to ZnO NPs treated group. This was matched with Abd-Elhafiz and Issa,[15], who reported that the Resveratrol administration led to a significant decline in the number of caspase-3 immunopositively cells when compared to the cisplatin group.

In the present work, assessment of the liver function demonstrated a marked deterioration in the ZnO NPs group versus the protected group by Resveratrol and the control group as well.

Liver function was evaluated by measuring the levels of hepatic enzymes ALT, AST, and ALP. Aboulhoda *et al*.,[23] stated that biomarkers with the highest sensitivity are ALT and AST, that can assess the severity of liver injury and toxicity.

In the present study, statistical analysis of the level of ALT, AST, and ALP demonstrated an extremely significant increase in ZnO NPs treated group in contrast with the control group that matched with the histological damage detected. El Shemy *et al*.,[32], Ansar *et al*.,[4], Aboulhoda *et al*.,[23] Ibrahim *et al*.,[22] and Ramadhan and Ghareeb,[33] all confirmed these results and found that ZnO NPs

resulted in a significant elevation in the serum levels of ALT, AST, and ALP.

Statistical analysis of the level of ALT, AST, and ALP in the protected group (ZnO NPs +Resveratrol), indicated a remarkably significant elevation versus the control group. Furthermore, it revealed a highly significant drop compared to ZnO NPs treated group alone. Al Humayed,[34] also found that resveratrol and quercetin improved blood levels of liver damage enzymes, with a substantial decrease in ALT and AST levels as compared to the group receiving acetaminophen treatment. They also increased tissue levels of antioxidants.

Resveratrol group alone showed an insignificant difference when compared to the control group with the statistical analysis of AST and ALT levels, while the statistical analysis of ALP level showed an extremely significant drop in relation to the control group. This confirmed the anti-inflammatory and hepatoprotective roles of Resveratrol. Al-Baqami and Hamza,[29] also stated that AST, ALT, and ALP levels did not change substantially under treatment with Resveratrol compared to the control group, but they did decrease considerably in the Cadmium + Resveratrol group compared to the Cadmium group alone.

CONCLUSION

The present results revealed that the ZnO NPs exposure causes histological and functional changes in the liver, which prompts several queries on the possible harm to human health from using ZnO NPs in diverse applications. In the present study, we provided evidence that Resveratrol can reduce ZnO NPs induced hepatotoxicity.

RECOMMENDATION

The intake of Resveratrol as a prophylactic natural agent against liver injury, especially in the areas of endemic liver diseases.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التأثير الوقائي الكبدي المحتمل للريسفيراترول على جزيئات أكسيد الزنك النانوية المسببة لإصابة الأنسجة الكبدية في الجرذان

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مقدمه: تستخدم جزيئات أكسيد الزنك النانوية على نطاق واسع في الصناعات والأدوية. مما يزيد من فرص التعرض لها والتي قد يكون لها آثار ضارة على أعضاء الجسم المختلفة مثل الكبد. لذلك كان من المفيد جدًا البحث عن عنصر وقائي طبيعي ضد هذا التأثير.

الهدف من البحث: تحديد تأثير جزيئلت أكسيد الزنك النانوية على بنية ووظيفة كبد الجرذان البيضاء وتقييم الدور الوقائي المحتمل للريسفيراترول.

مواد وطرق البحث: تم تقسيم ٤٠ فأرًا أبيضًا ذكرًا بالغًا إلى أربع مجموعات، كل منها ١٠ فئران؛ المجموعة الأولى (مجموعة التحكم)، المجموعة الثانية (جزيئات الزنك النانوية) المجموعة الثالثة (مجموعة الريسفيراترول) والمجموعة الرابعة (جزيئات الزنك النانوية مع الريسفيراترول) يتم إعطاء الأدوية عن طريق الفم لمدة أربعة أسابيع. وفي نهاية التجربة تم جمع عينات الدم لقياس إنزيمات الكبد. تم أستخراج الكبد ومعالجته للفحص المجهري.

النتائج : أظهرت مقاطع الكبد من (المجموعة الثانية) بنية كبدية غير منظمة، ولوحظت خلايا كبدية ذات نوى داكنة اللون، وسيتوبلازم عميق مائل للحموضة. كانت الأوردة المركزية والجيوب الكبدية مزدحمة. تم توسيع المسالك البابية مع الوريد البابي المزدحم وانتشار القنوات الصفراوية. زيادة في ترسب الكولاجين حول المسالك البابية واستنزاف محتوى الجليكوجين داخل السيتوبلازم. وكان موت الخلايا المبرمج في خلايا الكبد واضحا.

أظهرت أقسام (المجموعة الرابعة) استعادة الفصيصات الكبدية الطبيعية حيث ظهرت معظم الخلايا الكبدية بشكل طبيعي، في حين ظهر عدد قليل منها مع نوى تخللية. عادت معظم الجيوب الكبدية والمسالك البوابية إلى حجمها الطبيعي. أظهرت الأقسام الحد الأدنى من ترسب الكولاجين. كما قام ريسفيراترول باستعادة محتوى الجلايكوجين المنضب في (المجموعة الثانية). ريسفيراترول يقلل من نخر خلايا الكبد.

ولوحظ تدهور إنزيمات الكبد في (المجموعة الثانية)، مع تحسن ملحوظ في (المجموعة الرابعة).

خلاصة البحث: يمكن للريسفيراترول أن يحمي من التأثير التنكسي لأكسيد الزنك على التركيب النسيجي و وظيفة الكبد.