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Histopathological of effect of zinc on liver architecture of mice, *Mus musculus*

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Abstract

The current study was conducted to find out the effect of zinc (Zn) on the liver tissue of adult male white mouse, *Mus musculus* at concentrations (50 and 100 mg/kg/day) and for (30) days. (30) mice were used, divided into three groups of (10) mice, represented by control group, group of (50) mg/kg/day and (100) mg/kg/day. Histopathological changes appeared in the liver of male mice treated with zinc in concentration (50 and 100 mg/kg/day) for 30 days, including deformation and damage to Glisson's capsule, epithelial separation of capsule, in addition to cellular changes represented by hypertrophy of hepatocytes, as seen a swelling necrosis, hypertrophy of nuclei, thickening and degeneration in some hepatocytes, as well as the observed scatter of hepatocytes, and the increasing size and number of Kupffer cells as well as the expansion of sinusoids, as seen hyperplasia of Bile duct cells.

Keywords: Zinc, Liver Histopathology, Mouse.

Introduction

Liver is the main organ which is important in maintaining the internal environment of the body, and suffers from the influence of drugs and others(Kumar *et al*., 2013), there are many chemicals and drugs generally known to be susceptible to hepatotoxicity caused by medical substances, as systems and instructions have been issued by the Food and Drug Administration (FDA) of the risk of liver poisoning caused by medical substances, herbs, weight loss treatments that havbecome very common these days and supplements (Reuben *et al*., 2010). Zinc is an essential ingredient that has an antioxidant and anti-inflammatory effect, affects the molecular functions of many proteins and in the metabolism of cells and signal transmission, also interferes with reproduction and cell differentiation and has profound effects on growth, regeneration and repair of cells, as well as its role in both the acquired immune response Adaptive immunity and innate immunity. Zinc plays a key role in many biochemical and functional processes, and is a key component of more than 300 different enzymes and its catalytic effect is due to its direct participation in substrate conversion and stabilization of its structure, as well as to zinc's extensive roles in immune response at multiple levels including gene expression as well as the differentiation and formation of immune cells(Rink and Haase, 2007).

Materials and Methods

The current study was conducted on 30 white male mice *Mus musculus* obtained from the Center for Drug Control and Research in Baghdad, at the age of 8 weeks. Zinc (Zn) was used in the current study and obtained from America Medic and Science (AMS).This study was conducted on (30) white male mice, where they were divided into three groups, the first group (control) 10 mice were doses with distilled water and for 30 days, the second group (experimental group) 10 mice were taken Zn 50 mg/kg/day concentration for 30 days, group 3 (experimental group) 10 mice taken with Zn concentration 100 mg/kg/day for 30 days. Microscopic slides preparation: The methods used in the preparation of microscopic slides, Liver samples in formalin solution were fixed 10% for 72 hours. Then tissue samples from liver after delivery were prepared for histopathological studies according to paraffin methods(Bancroft and Steven, 1982). Haematoxylin and Eosin Staining was accomplished according to (Humason, 1979). The selected tissue scans were photographed by a MEIJI compound light with a Canon camera, and the tissues were photographed in the Advanced Embryology laboratory at college of Education for Pure Sciences/Ibn Al-Haitham/Baghdad University.

Results and Discussion

Control Group: Results of current study revealed that tissue liver in the control group is surrounded by a Glisson's capsule which formed from connective tissue and represented barriers, divide the liver lobes into lobules (figure 1-A).

Hepatocytes were seen in the visceral tissue of the liver parenchyma with polygonal shape and acidic cytoplasm, and round one or more nuclei, and areas between light liver cells representing vascular channels called sinusoids lined with endothelial cells (figure 1-B). Hepatocytes were circularly organized around the central vein in the middle of the regular lobe, and extended into the lobe in the form of hepatic cords (Figure 1-C). Hepatocytes are organized around the portal area located at the edges of the regular lobe, consisting of the portal vein, which is centerally located, hepatic artery, and bile duct (Figure 1-D).

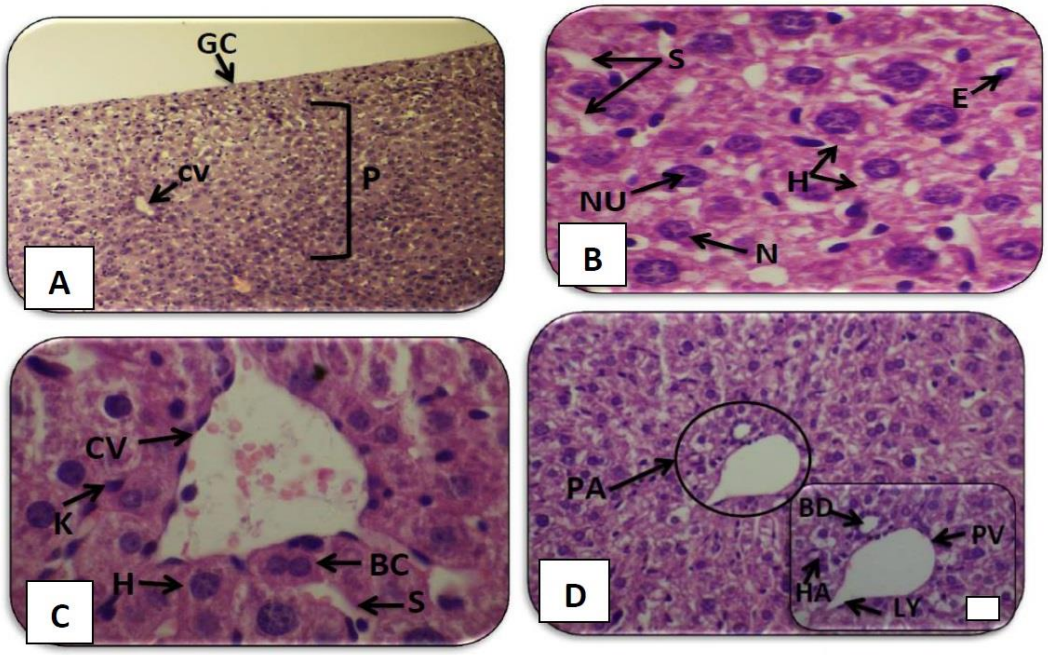

Figure (1): A-Cross section in the liver of male white mouse control group, note: Glisson's capsule (GC), Visceral Tissue of the Liver (P), Central Vein (CV), stained (H & E), 10x. B-note: hepatocytes (H), nuclei (N), nucleolus (NU), sinusoids (S), endothelial cells (E), stained (H &e), 100x. C-note: central vein (CV), hepatocytes (H), Kupffer cells (K), endothelial cells (E), binucleated cells (BC), sinusoids (S), stained (H &E), 100x. D-note: Portal area (PA), portal vein (PV), bile duct (BD), hepatic artery (HA), lymph vessel (LY), stained (H & E), 40x &100x.

The Experimental Group: Liver treated with Zn zinc at a concentration of 50 mg/kg/day for 30 days/dosage), showed damage in Glisson's capsule, representing rupture of the capsule and separation from hepatocytes leading to the appearance of space under the Subcapsular space (Figure 2-A).Cellular changes were also observed represented by the scattering of the radioregulation of hepatocytes architecture (Figure 2-B). Hypertrophy of hepatocytes, and karyomegaly of their nucleus (Figure 2-C). Pyknosis were occurred in some hepatocytes (Figure 2-D), and karyolysis of hepatocytes were also observed (Figure 2-E).

Hydropic degeneration was seen in some hepatocytes, and the increasing number of Kupffer cells (Figure 3-A). as well as the large size of sinusoids found between hepatocytes (Figure 3-B). The phenomenon of hyperplasia was observed in the portal area, and a infiltration was also observed in the portal area (Figure 3-C).

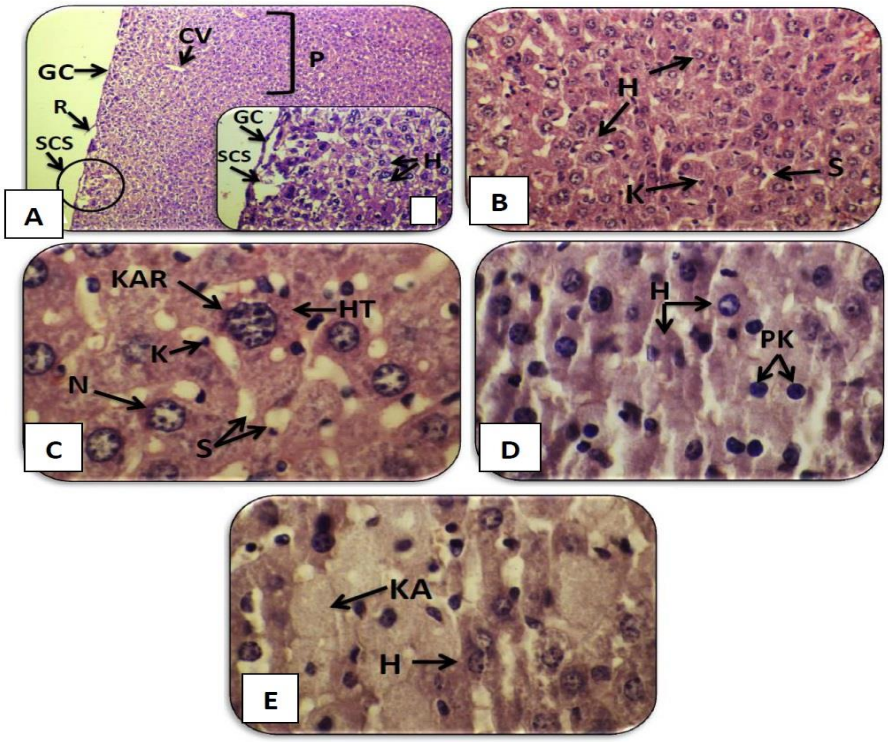

Figure (2): A-Cross section in the white mouse male liver of the group treated with zinc at a concentration of 50 mg/kg/day and for 30 days, note: visceral tissue of the liver (P), Glisson's capsule (GC), rupture of capsule (R), Sub capsular space (SCS), hepatocytes (H), central vein (CV), stained (H & E), 10X & 40X. B-note: scattering hepatocytes (H), kupffer cells (K), sinusoids (S), stained (H&E), 40x. C-note: hyperplasia of hepatocytes (HT), karyomegaly of nucleus (KAR), sinusoids (S), nuclei (N), stained (H&E), 100x. D-note: the occurrence of pyknosis (PK), hepatocytes (H), stained (H & E), 100x. E-note: karyolysis (KA), hepatocytes (H), stained (H&E), 100x.

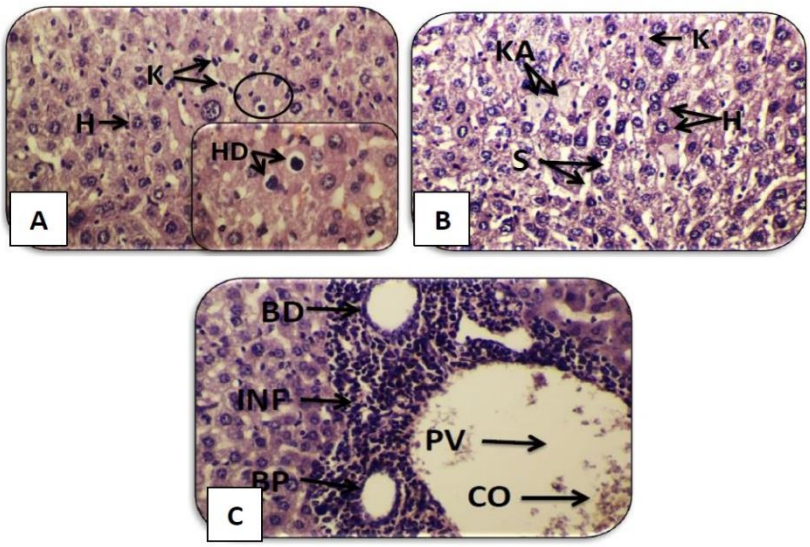

Figure (3): A-note: Hydropic degeneration (HD), hepatocytes (H), kupffer cells (K), stained (H&E), 40x & 100x. B-note: the occurrence of the dilution of sinusoids (S), kupffer cells (K), hepatocytes (H), karyolysis (KA), stained (H & E), 40x. C-note: the proliferation of bile duct cells (BP), bile ducts (BD), infiltration (INF), portal vein (PV), congestion (CO), stained (H & E), 40x.

Results of the group treated with concentration of 100 mg/kg/day of Zinc for 30 days/dose, revealed changes, represented by the Glisson's capsule appearance in comparison with control (Figure 4-A). Cytological changes have also emerged, represented by the scattering of the radioregulation of hepatocytes architecture (Figure 4-B) and hypertrophy is enlarged by some hepatocytes, and Karyomegaly the nuclei of some hepatocytes (Figure 4-C). An increase in pyknosis of hepatocytes nuclei was observed in a darker color and a smaller size (Figure 4-D), and karyolysis nuclei of some hepatocytes (Figure 4-E).

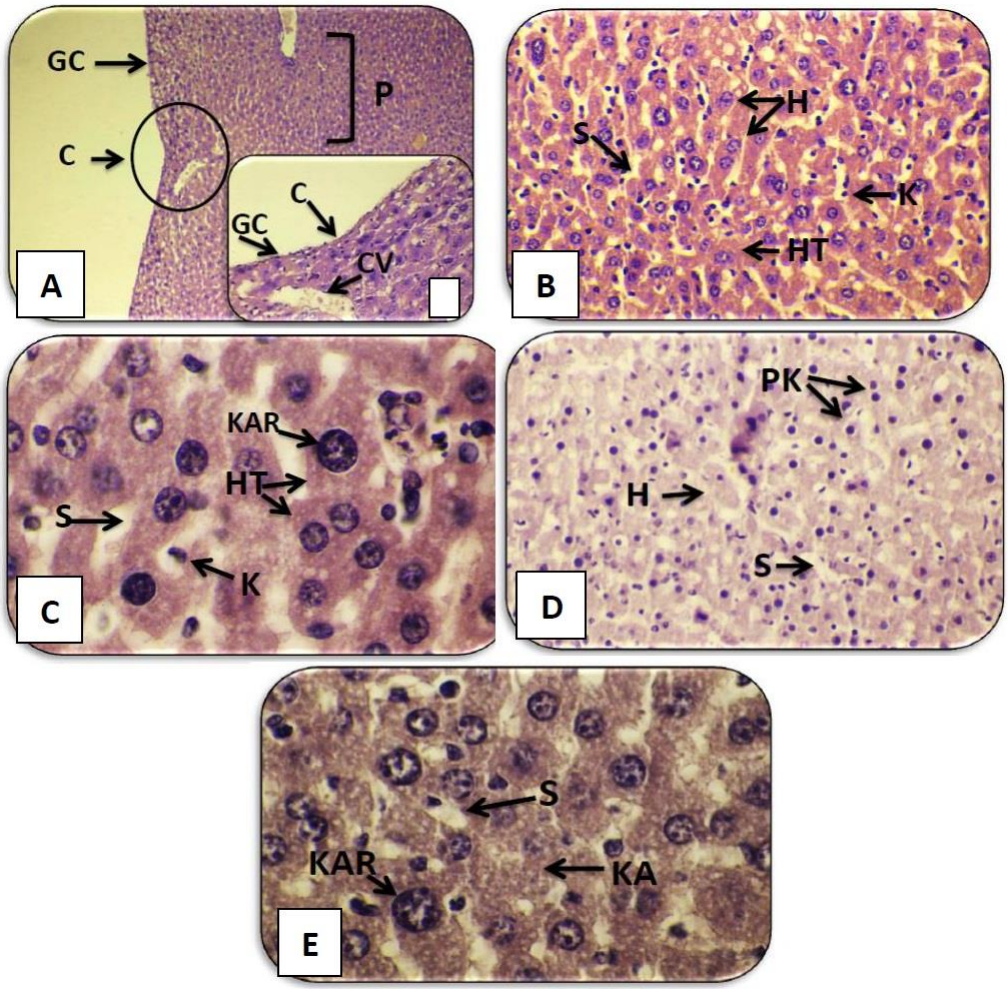

Figure (4): A-Cross section in the liver of the white mouse male of the group treated with zinc at a concentration of 100 mg/kg/day and for 30 days, note: Glisson's capsule (GC) with a incidence of concavity (C) in it, parenchymal tissue of the liver (P), central vein (CV), stained (H & E), 10x & 40X. B-note: scattering hepatocytes (H), kupffer cells (K), sinusoids (S), hypertrophy of hepatocytes (HT), stained (H&E), 40x. C-note: hypertrophy of hepatocytes (HT), Karyomegaly (KAR), kupffer cells (K), sinusoids (S), stained (H & E), 100x. D-note: Pyknosis (PK), hepatocytes (H), sinusoids (S), stained (H &E), 40x. E-note: karyolysis (KA), karyomegaly (KAR), sinusoids (S), stained (H & E), 100x.

Ballooning degeneration also was observed in some hypatocytes (Figure 5-A), and hydropic degeneration was also observed in some hepatocytes (Figure 5-B). The dilation of sinusoids, and the increasing number of Kupffer cells were also observed (Figure 5-C). The phenomenon of hyperprodulation of bile duct proliferation cells found within the portal area was also observed, and a infiltration was observed around the portal vein (Figure 5-D).

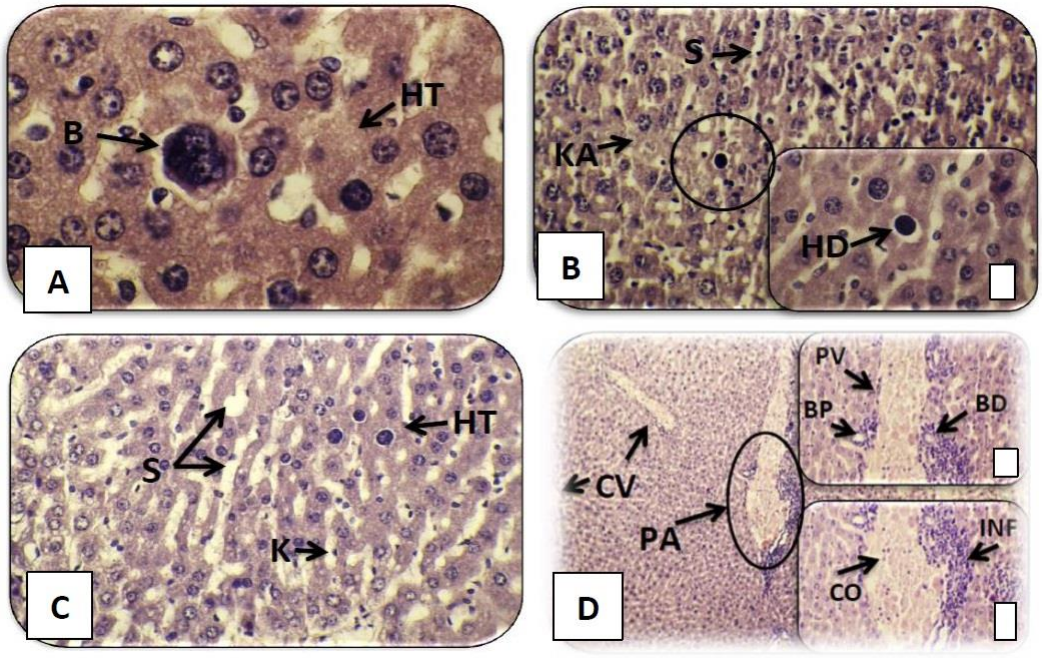

Figure (5): A-Cross section in the liver of the white mouse male of the group treated with zinc at a concentration of 100 mg/kg/day and for 30 days, note: Ballooning degeneration (B), karyomegaly (HT), stained (H&E), 100x. B-note: hydropic degeneration (HD), karyolysis (KA), sinusoids (S), stained (H & E), 40x & 100x. C-observed: the occurrence of the dilution of sinusoids (S), the increasing number of kupffer cells (K), hypertrophy (HT) of hepatocytes, stained (H&E), 40x. D-note: portal area (PA), proliferation of bile duct cells (BP), bile duct (BD), portal vein (PV), congestion (CO), infiltration (INF), central vein (CV), stained (H & E), 10x&40x.

In the current results, the tissue structure of the liver was seen in the control group as it appeared surrounded by a Glisson's capsule of fibrous connective tissue, and hepatocytes were seen in the polyhedral liver parenchyma with acidic cytoplasm, contains one round nucleus and a some of bi-nucleated cells, each has one or more nucleus, and hepatocytes are circularly organized around the central vein located in the center of the hepatic lobules. Hepatocytes are organized around the portal area located at the edges of the hepatic lobe, the portal area consists of the portal vein in the middle of the site, which is the largest of the components of the portal area, an oval hepatic artery liver with thick walls, bile duct with dark cells and circular shape (Baratta, 2009; Mescher, 2018). studied on the cellular organization of the natural mouse liver. The current result also showed different effects on the liver of white male mice treated with zinc in concentration (50 and 100 mg/kg/day) for 30 days of dose when compared to the control group, the following effects showed: Damage of Glisson's capsule was seen in the zinc-treated group with a concentration (50mg/kg/day) when compared to the control group, as space appeared under the sub capsular space, and the shape of the convex natural capsule almost disappeared, with concave areas in the wallet appearing in the 100 mg/kg/day package.This finding is consistent with (Tan et al., 2016; Lee et al., 2008). The cause of damage to the shape of the capsule and the appearance of sub-capsular space may be the negative effect of zinc on this result. Cytological alterations in hepatocytes, represented by hydropic degeneration in hepatocytes, were also observed when treated with concentrated zinc (50 and 100 mg/kg/day) when compared to the control group. This degeneration has led to hypertrophy of hepatocytes, which is consistent with (Damjanov, 2009; Elshama et al, 2013). Ballooning degeneration in hepatocytes has also been observed when treated with zinc at a concentration (100 mg/kg/day), which is a form of apoptosis(Lock et al., 1982) noted through their study on the liver of the rat exposed to hexachlorobutadiene (HCBD) the occurrence of degeneration. (Jaber, 2018) referred to ballooning degeneration in the liver by studying the effect of carbamazepine on the liver of white females.

Changes were also seen on the nuclei of some hepatocytes in the liver of adult white mouse males at concentrations of 50 and 100 mg/kg/day, as they were represented by the karyomegaly that occupied most of the size of the hepatocyte, and this result is consistent with (Jarrar and Taib, 2012). (Mohr et al., 1996; Wang et al , 2017) noted that the karyomegaly of the nuclei of some
cells is due to karyomegaly of hepatocytes caused by enzymatic induction. As seen in this result, pyknosis is a appearance of some hepatocyte's nuclei, as the nuclei appeared in a darker color and smaller compared to the control group, and this result is consistent with (Kumar et al., 2013) indicated that the thickening of the nuclei usually occurs when the cell passes after cellular injury as a result of a toxic substance necrosis.

In addition, the karyolysis nuclei of some hepatocytes was observed in this result, as the cell appeared in a uniform color due to the complete disappearance of the nucleus, and several studies referred to this phenomenon such as (Zamzami and Kroemer, 1999; Pandey et al., 2008; Husain, 2011) . The cause of hepatocytes degeneration and hyperplasia, and the pyknosis and hepatocytes may have affected the normal shape of the liver.

In addition to the damage to hepatocytes that appeared in the current result, zinc concentration (50 and 100 mg/kg/day) affected the sinusoids in terms of the appearance of dilation compared to the control group and this is consistent with (Brancatelli et al., 2018; Marzano et al. , 2015) . The treatment with zinc in concentration (50 and 100 mg/kg/day) in the current result also increased the number of Kupffer cells, in line with what they came up with (Dardouri et al., 2016) during their study on the combined effects of cadmium and mercury on liver and kidney tissues and their functions in mice. (Billiar and Curran, 1990; Fu et al., 2008) noted that increased numbers of kupffer cells. The increase in the number of kupffer cells may be caused by the current result as a result of increased numbers of inflammatory cells.

Also shown in the current result was the bile duct proliferation in the liver of white mouse males, and this is what he found (Slott et al., 1990; Fox et al., 2009). (Maronpot et al., 2010; Al-Bakri et al., 2020; Jaber and Al-Bakri, 2018) noted that the result of the toxicity of foreign substances exposed to the organism increases in the number of bile duct cells. The cause of hyperplasia of bile ducts in this study may be the toxicity of the drug as a foreign substance to the body that leads to a liver reaction leading to hyperplasia.

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