Silymarin Ameliorates Hepatotoxic Effect of Cisplatin: A Structural and Ultrastructural study of Adult Albino Rats

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Abstract: Objectives: To determine structural and ultrastructural changes in adult rat liver induced by cisplatin (Cis) and to evaluate the impact of drug withdrawal and the effect of silymarin on cisplatin heptotoxicity. **Material & Methods:** Forty male albino rats were divided into 4 equal groups: Control group kept on normal diet without medications, Positive control (Cis-1) group received cisplatin every other day for 10 injections, Withdrawal (Cis-2) group received cisplatin every other day for 10 injections followed by drug withdrawal for 4 weeks from the last injection and Study (Cis-3) group received cisplatin every other day for 10 injections plus a daily oral dose of silymarin. Cisplatin was injected intraperitoneally in a dose of 1mg/kg but silymarin was given orally in a dose of 16 mg/kg dissolved in 2 ml distilled water and given orally by gastric tube. Both control and study groups were sacrificed, liver specimen were obtained and prepared for light (L/M) and electron (E/M) microscopic examination. **Results:** L/M examination of Cis-1 specimens showed loss of normal hepatic architecture with diffuse cytoplasmic vacuolations of most hepatocytes, some hepatic nuclei are vacuolated and eccentric with areas of focal necrosis of hepatocytes, focal hemorrhage and small areas of degeneration among hepatocyte. Connective tissue is increased around the dilated central vein (CV) and portal tract (PT). PAS staining showed vacuolated hepatocytes with absence of glycogen granules. Cis-2 specimens showed restored hepatic cords with congested CV, connective tissue is increased around CV and extended to nearby blood sinusoids. Most hepatocytes have good positive PAS for glycogen granules. In Cis-3 specimens, few hepatocytes were vacuolated, PT appeared normal and hepatocyte cytoplasm showed good positive PAS reaction for glycogen granules. E/M examination of Cis-1 specimens showed marked intranuclear and intracytoplasmic vacuolations, decreased number of organelles and the nuclei of some hepatocytes are shrinked with irregular outlines. In Cis-2, hepatocytes' architecture was normal with rounded nuclei and cytoplasm contains numerous intact mitochondria. Most hepatocytes contain few vacuoles; however some hepatocytes show many vacuoles. Cis-3 group showed normal hepatocytes with rounded nucleus and nucleolus, cytoplasm contains numerous organelles and numerous intact mitochondria. **Conclusion:** Oral administration of silymarin ameliorated the deleterious hepatic structural and ultrastructural toxic effects of cisplatin. Cytotoxic drug withdrawal allowed partial restoration of hepatic architecture so silymarin administration was recommended till complete hepatic convalescence to augment the effect of drug withdrawal.

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Keywords: Cisplatin, Silymarin, Hepatotoxicity, Drug withdrawal

1. Introduction

The liver is covered with Glisson's capsule that branches and extends throughout the substance of the liver as septae with sheets of connective tissue that divides the parenchyma of the liver into lobules. Hepatic lobule consists of stacks of anastomosing plates of hepatocytes (one cell thick) separated by the anastomosing system of sinusoids that perfuse the cells with the mixed portal and arterial blood. At the center of the lobule is a relatively large venule, the terminal hepatic venule (central vein), into which the sinusoids drain. Portal triad occupies a potential space at each of the six corners of the lobule and a portal venule, a branch of the hepatic artery, and bile ductile. Hepatocytes are arranged in cords, plates or columns radiating from the central vein to the periphery and are always perpendicular to the central vein **(Michael, 2006, Ovalle & Nahirney, 2008)**.

The nuclei of the hepatocyte are large and spherical and its cytoplasm is eosinophilic, mainly because of the large number of mitochondria and some smooth (sER) and rough endoplasmic reticulum (rER). Other organelles recognized in the cytoplasm include Golgi apparatus, lyosomes, peroxisomes and multivesicular bodies. Hepatocyte cytoplasm also contains glycogen and fat droplets as cell inclusions. Von-Kupffer cells are large branched phagocytic cells lining the liver blood sinusoids. They are rich in lysosomes and play a role in destruction of senile red blood cells and formation of bile pigments. Space of Disse is the space that separates the hepatocytes from the walls of blood sinusoids. It contains microvilli projecting from the surface of hepatocytes, plasma,

reticular fibers and Stellate-shaped lipocytes, which store vitamin A **(Kuehnel, 2003; Young *et al.*, 2006; Mescher, 2010)**.

Chemotherapy involves the use of chemical agents to stop the growth and eliminate cancer cells even at distant sites from the origin of primary tumor. However, it does not distinguish between a cancer and normal cells, and eliminates not only the fast-growing cancer cells but also other fast-growing cells in the body, including, hair and blood cells. More than half of all people diagnosed with cancer receive chemotherapy regimen, that usually include drugs to treat cancer as well as drugs to help support the completion of the cancer treatment at the full dose on schedule, **(Pal *et al.*, 2007)**.

Silymarin is an active extract from *Silybum marianum* (seeds of the plant milk thistle; a medicinal plant). It contains approximately 65-80% silymarin flavonolignans (silymarin complex) with small amounts of flavonoids and approximately 20-35% fatty acids and other polyphenolic compounds. The major component of the silymarin complex is silybin that is synonymous with silibinin. Silymarin has been extensively studied and has shown anticancer efficacy against various cancer sites. This has been attributed to that silymarin interferes with the expression of cell cycle regulators and proteins involved in apoptosis. Additionally, silymarin has antioxidant properties and anti-metastatic activity, **(Deep & Agarwal, 2007, Pradeep *et al.*, 2007, Wu *et al.*, 2008, Shaarawy *et al.*, 2009)**.

The current comparative prospective study aimed to determine the structural and ultrastructural changes in adult rat liver induced by cisplatin and to evaluate the impact of drug withdrawal on liver and the effect of silymarin on such heptotoxic effects.

2. Material & Methods

Animals

The study comprised 40 normal healthy growing adult male albino rats, weighing 200-250 gm. Rats were purchased from the laboratories of Ministry of Agriculture, and kept under standard conditions, temperature 20^{o}C, humidity 60% and 12-hrs day/night cycle, and maintained on standard diet and free water supply till the start of study regimens.

Drugs

- Cisplatin supplied as 10mg/20ml vial (Sigma–Aldrich Co.) and was injected intraperitoneally in a dose of 1mg/kg which is documented to induce hepatotoxicity in rats without lethality **(El-Sayyed *et al.*, 2009)**.
- Silymarin supplied as 140mg packets (Sedico Co.) and was given orally in a dose of 16 mg/kg dissolved in 2 ml distilled water and given orally by gastric tube **(Patel *et al.*, 2010)**.

Study Protocol

The animals were divided into the following study groups (each in a separate cage) according to medication used:

- Control group included 10 rats kept on normal diet without medications.
- Positive control group (Cis-1 group) included 10 rats received cisplatin every other day for 10 injections on 20 days.
- Withdrawal group (Cis-2 group) included 10 rats received cisplatin every other day for 10 injections on 20 days followed by withdrawal of the drug for 4 weeks from the last injection.
- Study group (Cis-3 group) included 10 rats received cisplatin every other day for 10 injections on 20 days plus a daily oral dose of silymarin starting with cisplatin injection

After 20 days from the beginning of experiment, both control and study groups were sacrificed by inhalation of high dose of ether. The abdomen was explored and the liver was extracted and dissected. After 4 weeks, the withdrawal groups were also sacrificed and the liver was extracted for light and electron microscopic study.

For light microscopic examination, liver specimen were fixed in 10% buffered formalin, (pH 7.8) and, then thin sections (4 μm) were stained with hematoxilin-eosin (HE) for general histological features determination **(Bancroft & Gamble, 2002)**, Periodic acid Schiff (PAS) stain to demonstrate mucopolysaccharides as PAS positive materials **(Horbin & Bancroft, 1998)** and Masson's trichrome stain **(Leong, 1996)** for connective tissue staining. Specimens for electron microscopic examination were immersed in 2.5% gluteraldehyde buffered with 0.1 M phosphate buffer for 2 hours at room temperature, then post-fixed in 1% osmium tetroxide for 2 hours at 4^{o}C. After fixation, dehydration with ascending grades of ethanol was performed; specimens were cleared in propylin oxide, embedded in epoxy resin and sectioned with ultramicrotome **(Hayat, 1989)**.

3. Results

Light microscopic findings

Negative control group

Control rat showed normal hepatic architecture where hepatocytes were arranged in cords radiating from the central vein and separated by blood sinusoids lined by flat endothelial cells and few Kupffer cells. Hepatocytes showed an acidophilic cytoplasm and rounded vesicular nuclei with prominent nucleoli. Portal tract showed the branches of the portal vein, hepatic artery and bile ductule and surrounded by mild amount of connective tissue (Fig. 1, 2). The central vein was lined by flat endothelial cells and surrounded by scanty blue-stained perivascular connective tissue

(Fig. 3). In PAS stained sections, the cytoplasm of hepatocytes shows a positive PAS reaction for glycogen granules (Fig. 4). In semithin section, the hepatocytes have large, rounded nuclei with prominent one or two nucleoli.

Positive Control group (Cis-1 group)

Liver sections showed loss of normal hepatic architecture with diffuse cytoplasmic vacuolations of most hepatocytes. Some hepatic nuclei are vacuolated and become eccentric, while other nuclei were small pyknotic with areas of focal necrosis of hepatocytes. Marked cellular infiltration was observed around the dilated, congested central vein and in portal area around the dilated proliferated bile ductules and the hepatic artery branches. Focal hemorrhage among the hepatocyte and small areas of degeneration can be seen (Fig. 5). Connective tissue is increased around the dilated central vein and the portal tract with marked vacuolations of the hepatocytes (Fig. 6). In PAS staining, the hepatocytes are vacuolated with absence of glycogen granules in most of the hepatocytes and distortion of the cell membranes (Fig. 7). Semithin section revealed intranuclear and intracytoplasmic vacuolations. Some nuclei appear shrinked and become pyknotic.

Withdrawal group (Cis-2 group)

Liver sections showed restored hepatic cords with congestion of the central vein and sinusoids. Only some hepatocytes contain vacuolated nuclei (Fig. 8). Connective tissue is increased around the central vein and extended to nearby blood sinusoids (Fig. 9). In PAS staining, most of hepatocytes have good positive PAS for glycogen granules (Fig 10). In semithin section, hepatocytes appear normal with intact nuclei, some nuclei appeared small and become pyknotic.

Study group (Cis-3 group)

Silymarin administration allowed restoration of hepatic architecture around the central vein and preservation of most of blood sinusoids. Only few hepatocytes were vacuolated and appeared degenerated. Portal tract appeared normal with normal branches of hepatic artery, portal vein and bile ductile and little connective tissue (Fig. 11, 12). In PAS staining, hepatocyte cytoplasm showed good positive PAS reaction for glycogen granules (Fig. 13). Semithin section showed intact hepatocytes and the hepatic cords are separated by normal blood sinusoids.

Electron microscopic examination

Negative control group

The nucleus of hepatocytes appears rounded. The cytoplasm is crowded with cell organelles; the most numerous are mitochondria which appear variable in sizes and shapes, with apparent cristae. The rough endoplasmic reticulum (rER) is usually located near the nucleus and their cisternae are flattened, parallel to each other and studded with ribosomes. Golgi complex (GC) appeared close to the nucleus, and presents in stacks parallel to each other with concave and convex borders. The cytoplasm contains glycogen granules and lysosomes. Peroxisomes and multivesicular bodies were evident. The bile canaliculi were found in between adjacent hepatocytes and contained microvilli. Blood sinusoids are lined by endothelium which has elongated, heterochromatic nucleus. On the opposite side of endothelial cell, the space of Disse was seen and contained many microvilli of hepatocytes (Fig. 14).

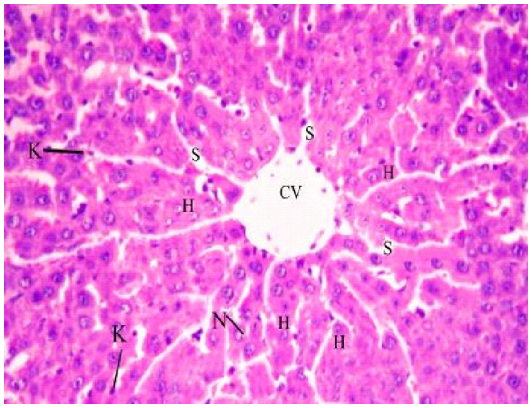


Fig (1): A photomicrograph of liver section of an adult male control rat showing central vein (CV) at the centre of hepatic lobule and cords of hepatocytes (H) with rounded vesicular nuclei (N) radiating from it. The hepatic cords are separated by blood sinusoids (S), containing Kupffer cells (K). [Hx&E200].

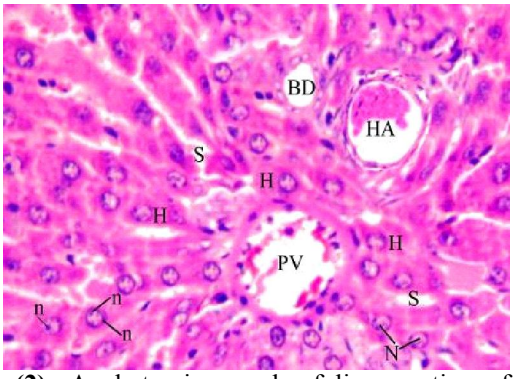


Fig (2): A photomicrograph of liver section of an adult male control rat showing portal tract containing a branch of hepatic artery (HA), a branch of portal vein (PV) and bile ductule (BD). Hepatic cords (H) and blood sinusoids (S) around the portal tract. Hepatocyte nuclei (N) appear rounded, vesicular, with one or more nucleoli (n). [Hx&E400]

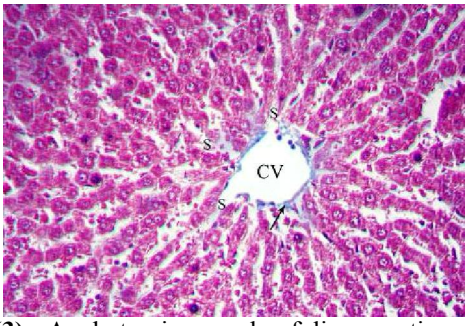


Fig (3): A photomicrograph of liver section of an adult male control rat showing normal scanty perivascular connective tissue (arrow) around the central vein (CV) and blood sinusoids (S) open in the CV. [Masson's Trichrome 200]

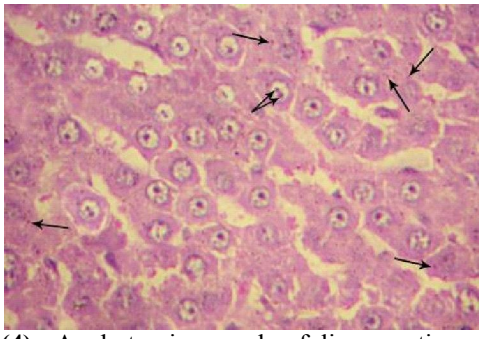


Fig (4): A photomicrograph of liver section of an adult male control rat showing positive PAS reaction for glycogen granules which are red in color (arrows) in the cytoplasm of hepatocytes. Many nuclei of the hepatocytes contain double nucleoli (double arrows). [PAS 400]

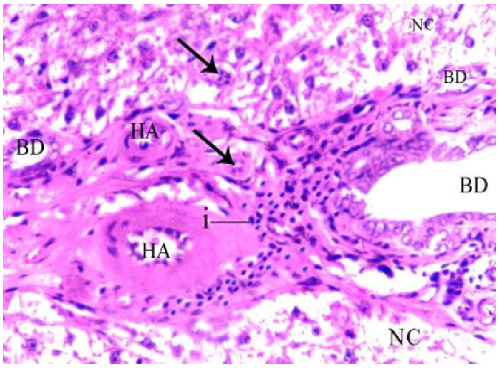


Fig (5): A photomicrograph of liver section of an adult male rat of Cis-1 group showing areas of hepatic necrosis (NC) and marked cellular infiltration (i) in the portal tracts round the proliferated dilated bile ductules (BD) and hepatic artery (HA). Multinucleted macrophage infiltration is also seen (arrows). [Hx & E 1000].

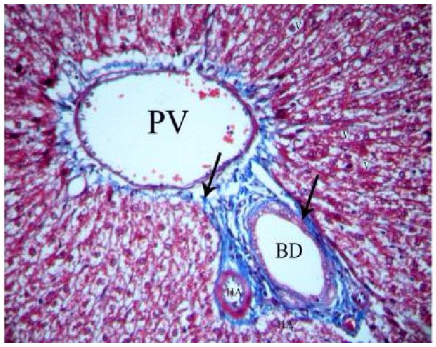


Fig (6): A photomicrograph of liver section of an adult male rat of Cis-1 group showing increased connective tissue (arrows) in portal tract around the dilated portal vein branch (PV), hepatic artery branch (HA) and bile ductule (BD). Marked vacuolations (V) of hepatocytes can be seen. [Masson's Trichrome 200]

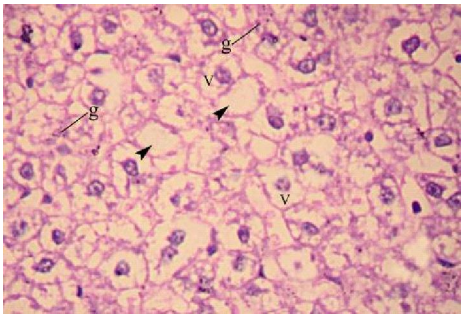


Fig (7): A photomicrograph of liver section of an adult male rat of Cis-1 group showing vacuolations of hepatocytes (V) with few glycogen granules (g). Notice some hepatocytes have no nuclei (arrow head) with distortion of their cell membrane. [PAS 400].

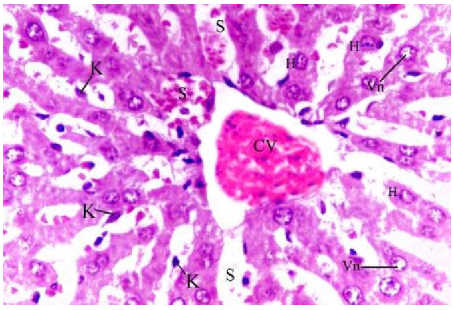


Fig (8): A photomicrograph of liver section of an adult male rat of Cis-2 group showing preserved hepatic architecture, congested central vein (CV) and its associated sinusoids (S). Most of hepatocytes (H) have acidophilic cytoplasm and intact nuclei (N); some hepatocytes contain vacuolated nuclei (vn). There is marked increase in Kupffer cells (K). [Hx & E 400]

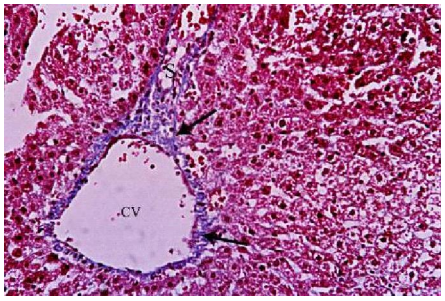


Fig (9): A photomicrograph of liver section of an adult male rat of Cis-1 group showing increased the perivascular connective tissue (arrows) around the central vein (CV) and extends to nearby blood sinusoids (S). [Masson's Trichrome 200]

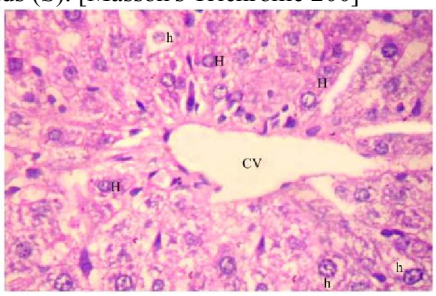


Fig (10): A photomicrograph of liver section of an adult male rat of Cis-2 group showing most of hepatocytes (H) have good positive PAS for glycogen granules. Few hepatocytes (h) have poor PAS reaction. Notice the central vein (CV). [PAS 400]

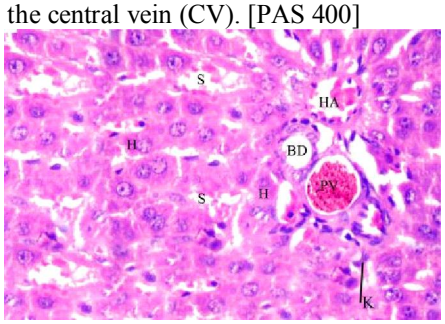


Fig (11): A photomicrograph of liver section of an adult male rat of Cis-3 group showing intact hepatocytes (H) around the portal tract which contains portal vein branch (PV), hepatic artery branch (HA) and bile ductule (BD). Notice the blood sinusoids (S) in between the hepatic cords and Kupffer cells (K). [Hx & E 400].

Positive Control group (Cis-1 group)

Liver sections showed marked intranuclear and intracytoplasmic vacuolations. The vacuoles are variable in sizes and shapes and coalesce with each other. The cytoplasm of most of the hepatocytes shows decrease in the number of organelles, the mitochondria appear electron dense arranged in clumps around the nucleus. Some hepatocytes show many lipid droplets, lysosomes, multivesicular bodies and peroxisomes. The nuclei of some hepatocytes are shrinked with irregular outlines, other nuclei have multiple nucleoli. The bile canaliculi lie between the cellular walls with evident junctional complexes (Fig 15, 16).

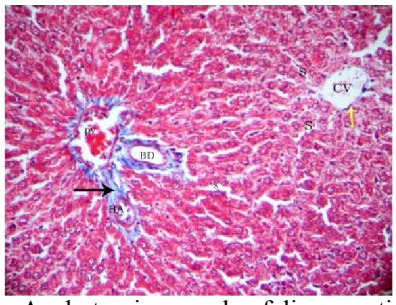


Fig (12): A photomicrograph of liver section of an adult male rat of Cis-3 group showing mild amount of perivascular connective tissue (yellow arrow) around the central vein (CV) and in portal area (Black arrow) around the branches of portal vein (PV), hepatic artery (HA) and bile ductule (BD). [Masson's Trichrome 200].

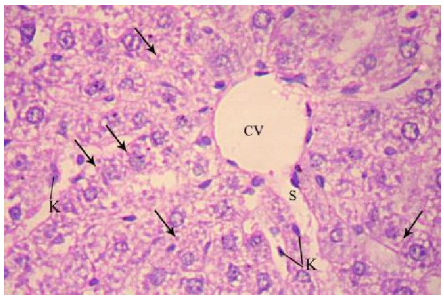


Fig (13): A photomicrograph of liver section of an adult male rat of Cis-1 group showing good positive PAS reaction for the glycogen granules (arrows) in the hepatocytes. Notice the blood sinusoids (S) opening in the central vein (CV), and Kupffer cells (K). [PAS 400]

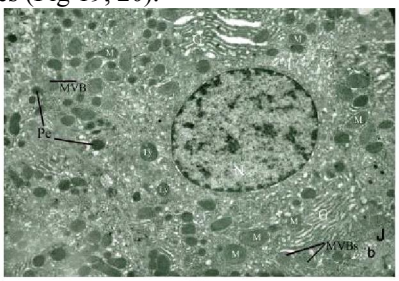
Withdrawal group (Cis-2 group)

Ultrastructural examination showed restored hepatocytes architecture with normal rounded nuclei containing normal nucleoli. The cytoplasm contains numerous intact mitochondria, lysosomes, peroxisomes with dense core, GC beside the nucleus, multivesicular bodies, rough and smooth ER. Most of hepatocytes contain few vacuoles; however some hepatocytes show many vacuoles. The blood sinusoids appeared between the hepatocytes and contain red blood cells (Fig 17, 18).

Study group (Cis-3 group)

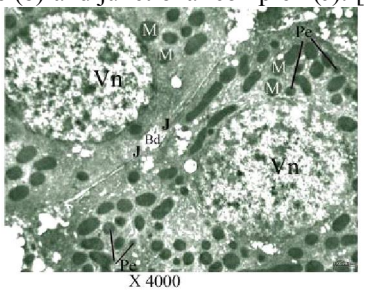
Hepatocytes appeared normal with rounded nucleus and nucleolus. The cytoplasm contains numerous organelles. Mitochondria are numerous and intact, with apparent cristae. Rough and smooth ER are preserved. Lipid droplets, glycogen granules, multivesicular bodies, some peroxisomes and few

vacuoles are also appeared. The bile canaliculi are found in between the adjacent hepatocytes, containing microvilli of hepatocytes with evident junctional complexes (Fig 19, 20).



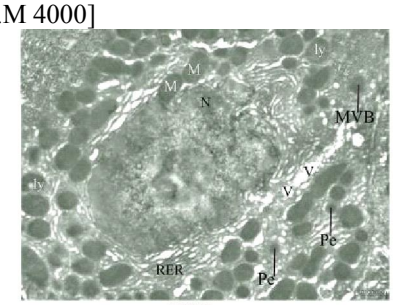
X4000

Fig (14): An electron photomicrograph of liver section of an adult male control rat showing hepatocyte with rounded nucleus (N). The cytoplasm contains mitochondria (M), Golgi apparatus (G), multivesicular bodies (MVBs), peroxisomes (Pe) and lysosomes (Ly). Notice the microvilli in the bile canaliculus (b) and junctional complex (J). [E.M 4000]



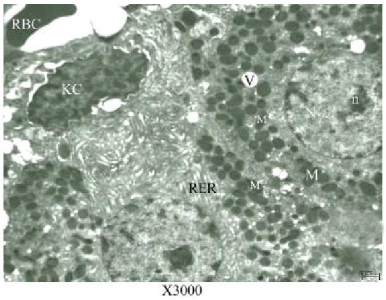
X 4000

Fig (15): An electron photomicrograph of liver section of an adult male rat of Cis-1 group showing hepatocyte with marked nuclear vacuolations (Vn), mitochondria increased around the nuclei (M). Notice bile canaliculi (BD) between the cellular walls, with the junctional complexes (J) and multiple peroxisomes (Pe). [E.M 4000]



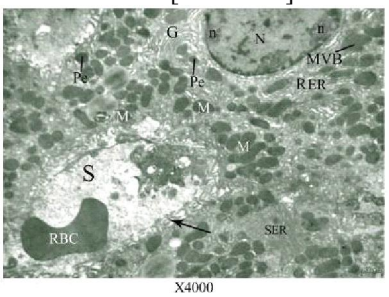
X6000

Fig (16): An electron photomicrograph of liver section of an adult male rat of Cis-1 group showing hepatocyte with electron dense mitochondria (M) arranged around the nucleus, rough endoplasmic reticulum (RER), lysosomes (Ly), Peroxisome (Pe) and multivesicular bodies (MVBs). Notice the shrinked nucleus (N) with irregular outline. [E.M 6000]



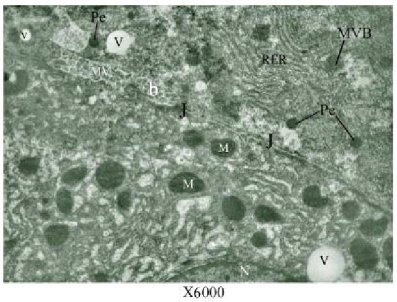
X3000

Fig (17): An electron photomicrograph of liver section of an adult male rat of Cis-2 group showing normal rounded nucleus (N) with prominent nucleolus (n). The cytoplasm contains numerous mitochondria (M), rough endoplasmic reticulum (RER) and few vacuoles (V). Notice Kupffer cell (KC) and blood sinusoid contains RBCs. [E.M 3000]



X4000

Fig (18): An electron photomicrograph of liver section of an adult male rat of Cis-1 group showing hepatocytes containing many intact mitochondria (M), rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER), Peroxisomes (Pe), multivesicular bodies (MVBs) and Golgi (G) beside the nucleus (N). Notice blood sinusoid (S) containing RBC and microvilli (arrow). [E.M 4000]



X6000

Fig (19): An electron photomicrograph of liver section of an adult male rat of Cis-3 group showing hepatocytes with mild vacuolations (V), intact rough enoplasmic reticulum (RER), intact mitochondria (M), Peroxisomes (Pe) and multivesicular bodies (MVBs). Notice the bile canaliculus (b) between two cells containing microvilli (MV). Notice also part of the hepatocyte nucleus (N) and junctional complexes (J). [E. M 6000].

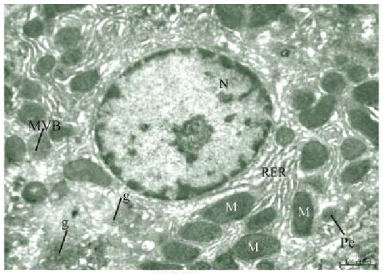


Fig (20): An electron photomicrograph of liver section of an adult male rat of Cis-3 group showing hepatocyte with intact rounded nucleus (N) and nucleolus (n). The cytoplasm contains mitochondria (M), rough endoplasmic reticulum (RER) studded with ribosomes, peroxisomes (Pe), multivesicular bodies (MVBs) and glycogen granules (g). [E.M 10000]

4. Discussion

Light microscopic examination of Cis-1 group specimens showed diffuse vacuolations of hepatocyte cytoplasm, marked perivascular cellular infiltration around the dilated and congested central vein, areas of necrosis and hemorrhage among the hepatocytes and depletion of glycogen granules. Moreover, there were periportal fibrosis, highly vacuolation of the hepatocytes cytoplasm, the central vein showed dilatation and congestion. Also, proliferation and dilatation of bile ductules, small areas of necrosis, and nuclear changes in the form of pyknotic nuclei and margination of chromatin inside nuclei were observed.

Ultrastructural findings observed in Cis-1 group included marked decrease in cell organelles, highly vacuolated hepatocyte cytoplasm, electron dense atrophied mitochondria, peroxisomes, lipid droplets and multivesicular bodies increase in number and the nuclei of most of the hepatocytes were shrinked with irregular outlines. These findings go in hand with that previously reported in literature; as regards proliferation of bile ducts; **Sanjiv (2002)** suggested that the observed proliferation of bile ducts in the portal tracts is partially caused by active proliferation of ductular cells and partly by tubular transformation of peripheral liver cell plates. As another attribution, **Sarah *et al.* (2005)** attributed proliferation of bile ducts and dilatation of bile canaliculi to a response of cholestasis which caused by continuous increase of the intrahepatic duct pressure causing dilatation of bile ducts in large portal areas.

Koc *et al.* (2005) found congestion of the central vein and the hepatic sinusoids in rats treated with cisplatin and with long duration of exposure to the drug the congestion became sever with subsequent extravasation of the blood and appearance of Kupffer cells loaded with hemosiderin granules. Presence of congestion in the central vein reported in the current study was in agreement with **Koc *et al.* (2005)** who found congestion of the central vein and the hepatic sinusoids in rats treated with cisplatin. **Lu & Arthur (2006)** stated that liver sections from mice treated with cisplatin showed degeneration and vacuolations of the hepatocytes but no necrosis was observed.

The obtained results supported that previously reported in literature concerning cytotoxic drugs used separately or in combination; **Yagmurca *et al.* (2007)** detected histopathological changes such as necrosis, hepatocyte degeneration, sinusoidal dilatation, hemorrhage and vascular congestion and dilatation in animals received doxorubicin. **El-sayyed *et al.* (2009)** who reported that light microscopic observations revealed that higher doses of cisplatin and doxorubicin caused massive hepatotoxicity including dissolution of hepatic cords, focal inflammation and necrotic tissues, and interestingly, low doses also exhibited abnormal changes, including periportal fibrosis, degeneration of hepatic cords and increased apoptosis. **Patel *et al.* (2010)**, added that the morphology of the livers in mice treated with doxorubicin reflected swelling, hemorrhage, heavy centrilobular spotting and extensive depletion of glycogen granules in hepatocytes.

However, these deleterious effects were found to be regressive as evidenced in withdrawal group wherein most hepatocytes in Cis-2 group showed normal acidophilic cytoplasm, normal vesicular nuclei and mild cellular infiltration with increased cell organelles, increased number of mitochondria, mild dilatation of space of Disse and the nuclei were apparently normal. These data indicated the temporary regressive effect of cisplatin on hepatic structure and strengthen the idea for preventive therapy which is assured by light and electron microscopic findings of Cis-3 group. Light and electron microscopic examination of Cis-3 group showed marked improvement in histological structure with minimal pathological changes. Ultrastructural changes showed increased number of organelles, mild vacuolation of the cytoplasm and most nuclei were normal with normal nucleoli.

These findings go in hand with previous literature concerning hepatic protective effects of silymarin; **Abdel Salam *et al.* (2007)** in a liver injury model induced in rats with carbon tetrachloride found that silymarin reduced the elevated serum activities of liver enzymes, morphometric analysis indicated significant reduction in the area of necrosis and fibrosis, decreased intracellular protein content in hepatocytes was improved and proliferating cell nuclear antigen was reduced in nuclei of hepatocytes. **Wu *et al.* (2008)**, who reported that the liver after 7

days of oral administration of silymarin recovered to normal morphology, with normal hepatocytes and normal blood vessels of liver. **Abdelmeguid *et al.* (2010)**, who found that silymarin treatment with cisplatin showed minimal dilatation of space of Disse, the hepatocytes appeared with normal large nuclei, numerous rough endoplasmic reticulum and mitochondria, prominent Golgi apparatus, higher cellular and organelles organization, and marked reduction in cytoplasmic vacuolations. **Patel *et al.* (2010)** found that silymarin prevent doxorubicin-induced liver injury, maintaining normal glycogen content and most cells appear normal with no cytoplasmic or nuclear anomalies. **Vitcheva *et al.* (2012)**, in hepatic toxicity models found that pre-treatment with silymarin resulted in significantly increased activities of ethylmorphine-N-demethylase and aniline 4-hydroxylase activity and cytochrome P450, compared to the CCl(4) only group.

In support of the hepatic protective effect of silymarin, multiple recent studies tried other medicinal plants for prophylactic liver protection and considered animals received silymarin as the standard group for comparison of protective effect **(Noorani & Kale, 2012; Girish *et al.*, 2012; Huang *et al.*, 2012)**.

The obtained results and review of literature allowed concluding that oral administration of silymarin ameliorated the deleterious hepatic structural and ultrastructural effects of cisplatin. Cytotoxic drug withdrawal allowed partial restoration of hepatic architecture so silymarin administration was recommended till complete hepatic convalescence to augment the effect of drug withdrawal.

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