Mansoura Journal of Forensic Medicine and Clinical Toxicology

Nigella Sativa oil Alleviates Cadmium-Induced Hepatic Insult in Adult Male Albino Rats

Amal S. A. F. Hafez, Ehab M. Hantash, Essam M. Hafez

KEYWORDS
Cadmium toxicity
Nigella sativa oil
Liver

ABSTRACT

Cadmium (Cd) is a toxic heavy metal and an environmental pollutant. This study aimed to investigate the potential protective effect of Nigella sativa oil (NSO) on Cd-induced liver damage in adult male rats. Forty adult male albino rats were allocated into four equal groups. Group I served as a control. Group II received intraperitoneal injections of 2 mg/kg of cadmium chloride. Group III received NSO 1 ml/kg orally. Group IV were given NSO prior to Cd in the same doses and routes described above. All animals were treated for 8 days, and then were euthanized and their livers were dissected and examined histologically by light and electron microscope. Group II demonstrated hepatic structural injury in the form of dilated central veins with distorted walls, dilated congested sinusoids, pyknotic nuclei, vacuolated cytoplasm, depletion of glycogen granules and lipid droplets, swollen mitochondria, areas of focal hepatic necrosis and infiltration with inflammatory cells. Group IV showed marked improvement of the hepatic damage induced by Cd exposure. Groups I and III showed comparatively normal liver structure with no obvious damage. In conclusion, Nigella sativa oil could be used as a safe and effective plant product to mitigate hepatic insult associated with Cd toxicity.

Introduction

Cadmium (Cd) is a toxic heavy metal, and it is considered as an environmental and industrial pollutant. Occupational exposure to Cd occurs during mining activities and in industries such as metal plating and production of pigments, plastics, glass, fertilizers and batteries. Sources of non-occupational exposure to Cd include tobacco smoking, air pollution and consumption of Cd-contaminated drinking water (Waisberg et al., 2003; Liao et al., 2005; Lawal and Ellis, 2010).

Cadmium has a long half-life and accumulates in the biological system (Järup, 2002), causing severe toxicity in various organs including kidney, bone and testis (Arita and Costa, 2009; Zhang et al., 2010). Several studies demonstrated that Cd toxicity can lead to hepatocellular injury and compromised liver function (Wen et al., 2010; Prabu et al., 2011; Rogalska et al., 2011). The high susceptibility of liver damage is explained by its ability to produce large amounts of metallothionein, a metal-binding protein with high affinity for Cd (Waalkes et al., 1984). The mechanisms of Cd-induced toxicity may be related to its inhibition of liver metabolic enzyme systems containing sulfhydryl groups and/or uncoupling of oxidative phosphorylation in the mitochondria (Williams et al., 1999). This results in

Amal S. A. F. Hafez
Forensic Medicine and Clinical Toxicology Department Faculty of Medicine, Tanta University, Tanta, Egypt
Ehab M. Hantash
Anatomy Department, Faculty of Medicine, Tanta University, Tanta, Egypt
Essam M. Hafez
Forensic Medicine and Toxicology Department, Faculty of Medicine, El-Minia University- El-Minia-Egypt

depletion of sulfhydryls, increased lipid peroxidation, DNA damage and altered calcium homeostasis. These changes may end in hepatic congestion, ischemia and hypoxia (Kennedy et al., 2008; Ognjanovic et al., 2008; Bharavi et al., 2010).

Several studies demonstrated the effectiveness of some antioxidants and anti-inflammatory agents, such as zinc, curcumin and thymoquinone (TQ), against cadmium-induced liver injury (Massadeh et al., 2007; Tarasub et al., 2012; Zafeer et al., 2012; Abd El-Hamid et al., 2014). Nigella sativa (NS) (Family Ranunculaceae) is a herbal plant commonly known as the black seed (Habatul-barakah in Arabic countries). It is an annual flowering plant, which grows in many countries including the Middle East region, South Europe, India, Pakistan, Syria, Turkey and Saudi Arabia (Ahmad et al., 2013).

The major active chemical compound of the Nigella Sativa Oil (NSO) is TQ, which have a prominent antioxidant effect. Other active compounds such as dithymoquinone and thymol are present in lesser amounts. However, the seeds contain isoquinoline and pyrazol alkaloids (Al-Jassir, 1992; Malik et al., 1995), besides some vitamins and minerals such as copper, zinc, phosphorus and carotene (Malik et al., 1995; Nickavar et al., 2003; Gali-Muhtasib et al., 2006; Nehar and Rani, 2011). Nigella sativa exhibits a wide spectrum of pharmacologic activities and potential therapeutic uses as a diuretic, antihypertensive (Zaoui et al., 1999), antidiabetic, anticancer, immunomodulatory, analgesic, anti-inflammatory (Al-Ghamdi, 2001), gastro-protective, renal protective, hepatoprotective and antioxidant (Assayed, 2010; Boskabady et al., 2010; Abdel-Zaher et al., 2011; Abel-Salam, 2012).

Therefore, the aim of this study was to investigate the potential protective effect of NSO on Cd-induced liver damage in adult male albino rats.

Material And Methods

Chemicals:

Cadmium chloride ($CdCl_2$ analytical grade) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used in the study were of the highest purity available, and were obtained from local commercial sources.

Preparation of Nigella sativa (NS) oil:

The dried seeds of NS were purchased from the local market and were identified and authenticated in the Department of Pharmacognosy, Faculty of Pharmacy, Tanta University, Tanta, Egypt. Nigella sativa seeds were crushed and cold macerated in petroleum ether (40-60 °C) for three days. Nigella sativa oil (NSO) was obtained by filtration of the collected extract after evaporation of petroleum ether. Nigella sativa oil yield was 17.5 % v/w with reference to dried seeds. The extracted NSO was preserved in screw-capped dark tubes at −20 °C until used (Mohamadin et al., 2010).

Animals:

Forty adult male Wistar rats weighing 240-280 g were used in this study. Animals were obtained from the animal house of the Faculty of Medicine, Tanta University, Tanta, Egypt. They were housed under standard conditions of temperature (23 ± 2 °C) and lighting (12 h light/dark cycles) and were allowed free access to food and drinking water.

Experimental design:

The study protocol was approved by the research ethics committee of the Faculty of Medicine, Tanta University. Animals were randomly divided into 4 equal groups (10 rats in each group). Group I rats served as a control and received 1ml of isotonic saline i.p. Rats of group II received 2mg/kg of $CdCl_2$ dissolved in isotonic saline i.p. The administered dose of Cd was determined according to the absorption

percent of LD50 dose in rats by oral route (LD50 110 mg/kg $CdCl_2$) (Bolkent et al., 2007; Koyuturk et al., 2007). Group III rats were given NSO (1ml/kg) by gastric gavage (Mohamadin et al., 2010). Group IV rats received NSO an hour prior to $CdCl_2$ administration via the same routes and doses as previously described. All animals were treated for 8 days.

Preparation of the specimens:

At the end of the treatment period, the overnight-fasted rats were anaesthetized by diethyl ether. Animals were then sacrificed by cervical dislocation, dissected and their livers were removed. Specimens were taken from the liver of each animal and divided into two pieces. One piece was fixed in 10% formol saline, paraffin-embedded, sectioned at 5 µm and prepared for light microscopic study (Olympus BX-50) using hematoxylin and eosin (H&E) stain (Stevens and Bancroft, 1996). The other one was immersed in phosphate-buffered gluteraldehyde solution and stained with toluidine blue stain for semi-thin sections or uranyl acetate and lead citrate stains for ultra-structural examination using JEOL JEM electron microscope at 80 kilo volt (Hayat, 1978).

Results

Light microscopy:

1- Group-I (Control rats):

Each hepatic lobule appears formed of many cords of hepatocytes radiating from a central vein towards the periphery of the lobule. The cords are separated from each other by blood sinusoids. Each cord is formed of one or two rows of adjacent hepatocytes. Portal tracts or triads are present at the corners of the lobule. Each tract contains a bile ductule, hepatic arteriole and portal venule. The cells lining the bile ductules have round and central nuclei. Hepatic sinusoids appear as irregular slit-like, tortuous spaces with very thin walls. Irregular shaped Kupffer cells with oval nuclei are seen on the luminal side of the sinusoids. Hepatocytes appear polygonal in shape, having acidophilic granular cytoplasm and rounded vesicular nuclei with prominent nucleoli. The nuclei are of variable sizes and had scattered chromatin granules. Some hepatocytes appear binucleated (Figures 1, 2 and 3).

2- Group-II (CdCl2-treated rats):

Administration of $CdCl_2$ produced hepatic damage. The central veins appear markedly dilated with distorted walls, and many of the sinusoids are dilated and congested. Hepatocytes show small, dark, pyknotic nuclei, and the cytoplasm of many hepatocytes appears rarefied and contains many vacuoles. Areas of focal hepatic necrosis are seen. Some of these necrotic areas are infiltrated with many inflammatory cells (Figures 4, 5 and 6).

3- Group-III (NSO-treated rats):

Treatment with NSO produced no changes in the hepatic parenchyma. The liver structure of the Nigella sativa treated rats showed preserved normal lobular architecture similar to group I.

4- Group-IV (rats treated with CdCl2 and NSO):

The damaging effect induced by $CdCl_2$ appears markedly reduced by administration of NSO. The central veins appear normal and surrounded by radiating hepatic cords with blood sinusoids in between the cords. The cords are formed of rows of many normal hepatocytes. Most of the hepatocytes appear normal. However, few hepatocytes show slight cytoplasmic vacuolation. Some of the blood sinusoids are apparently slightly dilated (Figures 7 and 8).

Electron microscopy:

1- Group-I (Control rats):

Hepatocytes appear normal with central, large, spherical nuclei, scattered heterochromatin and one or two nucleoli. The cytoplasm has abundant rough endoplasmic reticulum (RER), lipid droplets, glycogen granules as well as many mitochondria. Each hepatocyte appears with three functional surfaces. The first is facing other parenchymal cells without special features. The second surface is facing a bile canaliculus and is studded with many microvilli. The third one is facing the perisinusoidal space of Disse and is also studded with microvilli. Bile canaliculi are seen in between the adjoining membranes of the hepatocytes. The canaliculi are closed peripherally by the occluding junctions. These junctions appear as barriers separating the canaliculi from the remaining extracellular space. Short, blunt microvilli are seen projecting from the hepatocyte plasmalemma into the bile canaliculi. The narrow perisinusoidal space is seen between the endothelial lining of the hepatic sinusoids and the hepatocytes. Many short microvilli are seen projecting from the membrane of hepatocytes into this space. The slit-like hepatic sinusoids are seen in between the hepatocytes and their lumens contain lymphocytes and RBCs. The walls of the sinusoids are lined by flattened endothelial cells and irregularly shaped Kupffer cells. These cells are directly related to the hepatocytes without intervening basement membrane or connective tissue. Kupffer cells have spherical or oval nuclei and their cytoplasm show a lot of endocytic vesicles. Their surfaces have also numerous projections. Endothelial cells appear as small flattened cells with elongated or ovoid nuclei and their cytoplasm contain many pinocytic vesicles (Figures 9, 10 and 11).

2- Group-II (CdCl2-treated rats):

Treatment of this group with $CdCl_2$ induced structural changes in the hepatic parenchyma. The cytoplasm of the hepatocytes shows many vacuoles together with depletion of its glycogen granules and lipid droplets. Many mitochondria appear swollen with damaged cristae. Many of the nuclei appear small, dark and pyknotic. The bile canaliculus, the hepatocytes plasmalemma and the perisinusoidal space appear normal. On the other hand, the blood sinusoids appear congested and infiltrated with eosinophilic granulocytes (Figures 12, 13 and 14).

3- Group-III (NSO-treated rats):

Rats treated with NSO showed no apparent differences in the structure of the liver compared with the control rats.

4- Group-IV (rats treated with CdCl2 and NSO):

Administration of NSO was associated with significant reduction in the structural changes induced by CdCl2. Most of the hepatocytes appear normal. They have large spherical nuclei and the cytoplasm shows plenty of normal mitochondria, RER, lipid droplets and glycogen granules. Some cells have few cytoplasmic vacuoles. The blood sinusoids and the surrounding spaces of Disse appear normal. Similarly, the bile canaliculi and the nearby plasmalemma of the hepatocytes appear normal (Figures 15 and 16).

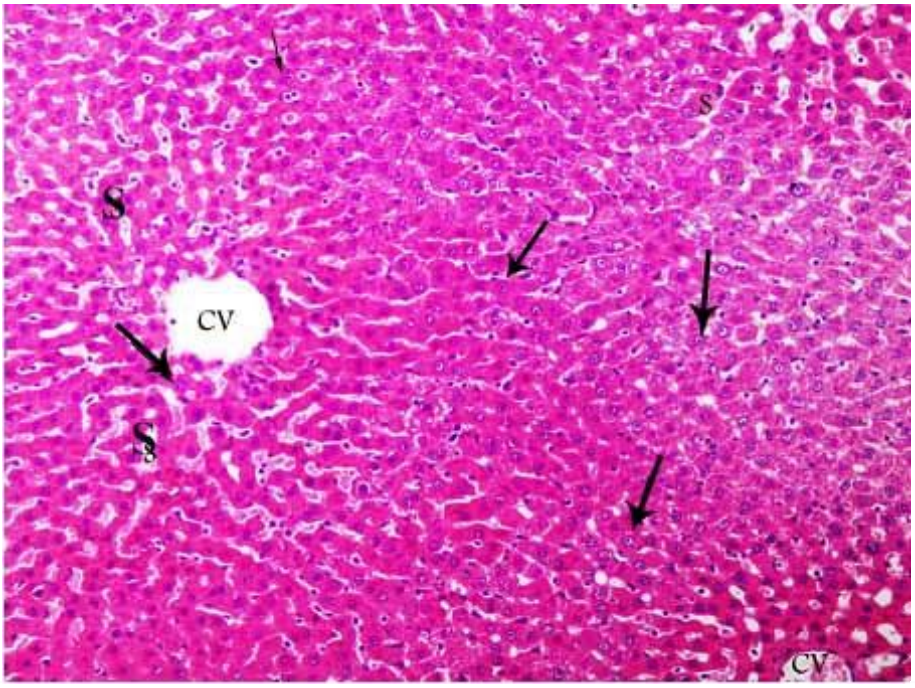

Fig. (1): A photomicrograph of a section in the liver of a control rat showing many hepatic cords (arrow) radiating from a central vein (CV) and separated by blood sinusoids (S) (H&E X 200).

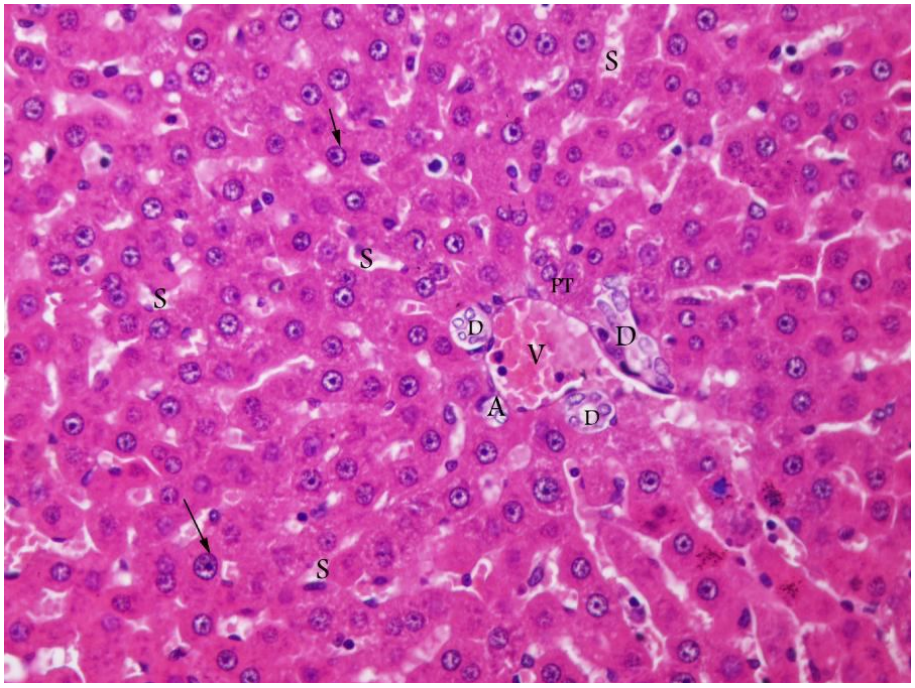

Fig. (2): A photomicrograph of a section in the liver of a control rat showing many hepatic cords radiating towards a portal triad (PT) and separated by blood sinusoids (S). The cords are formed of one or two rows of hepatocytes (↑). The portal triad region contains a large venule with thin irregular wall (V), a much smaller arteriole (A) and three bile ductules (D) lined with cells having centrally placed, round nuclei (H&E X 400).

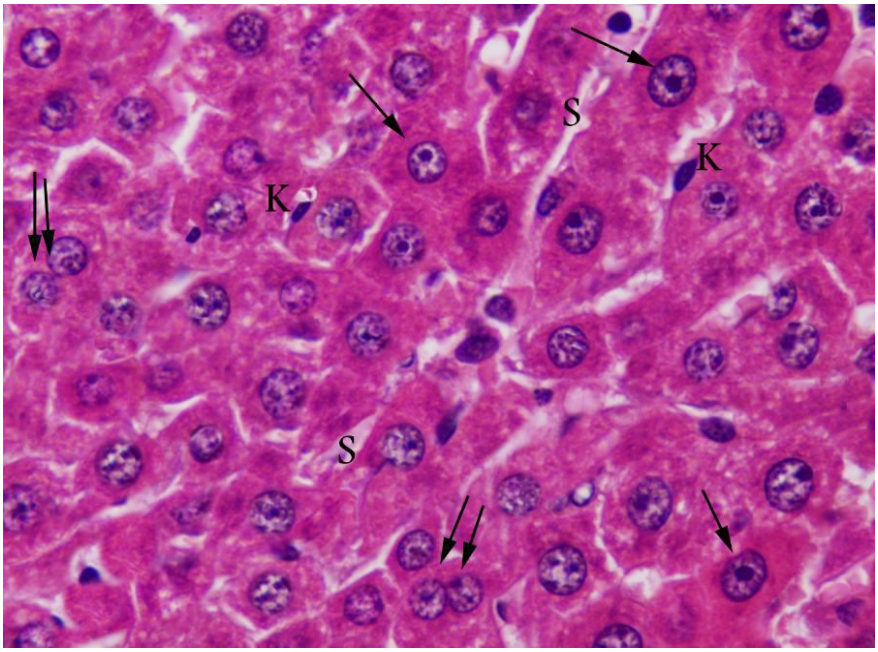

Fig. (3): A photomicrograph of a section in the liver of a control rat showing normal polygonal hepatocytes (↑) having deeply acidophilic cytoplasm and rounded vesicular nuclei with prominent nucleoli. The nuclei vary in size and has scattered chromatin granules. Some cells are binucleated (double arrow). The blood sinusoids (S) are slit-like, tortuous spaces with thin wall. Kupffer cells (K) with their oval nuclei are also seen (H&E X 1000).

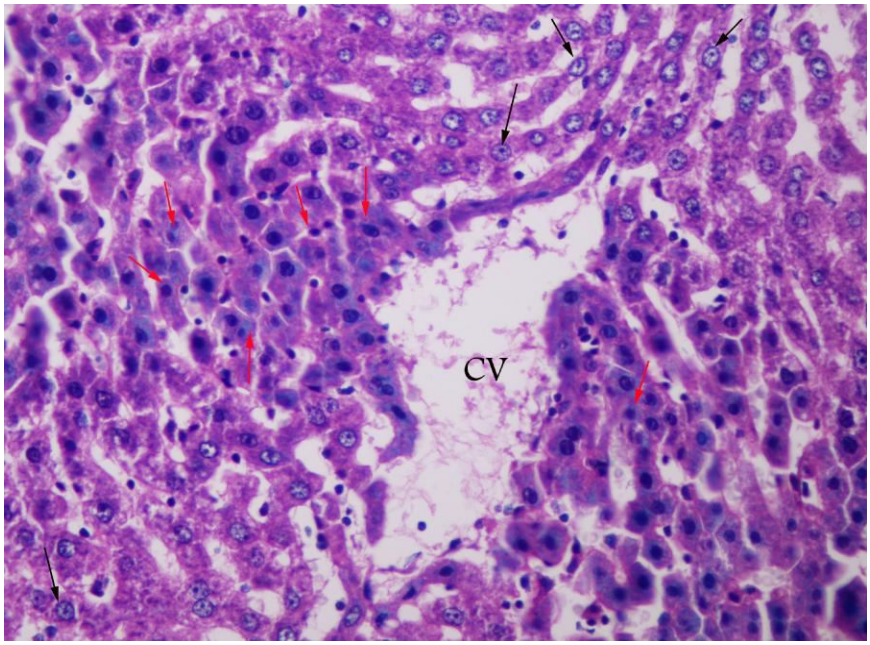

Fig. (4): A photomicrograph of a section in the liver of a group II rat (treated with $CdCl_2$) showing a distorted and dilated central vein (CV). Many of the surrounding hepatocytes have small, dark pyknotic nuclei (red arrow) while others show normal large, vesicular nuclei with prominent nucleoli (black arrow) (H&E X 400).

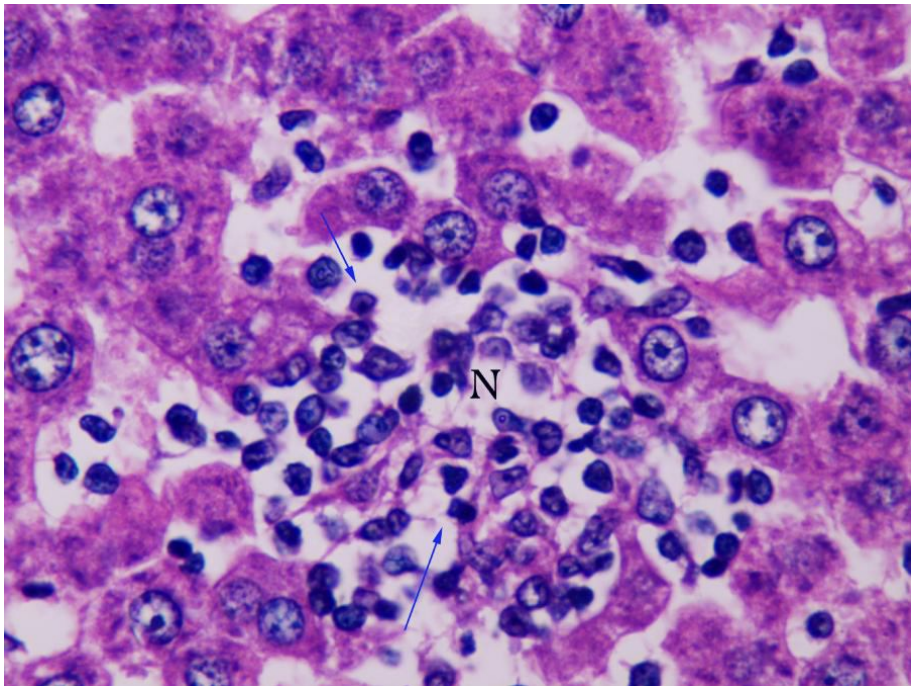

Fig. (5): A photomicrograph of a section in the liver of a group II rat (treated with $CdCl_2$) showing focal necrotic area (N) infiltrated with many inflammatory cells (↑) (H&E X 1000).

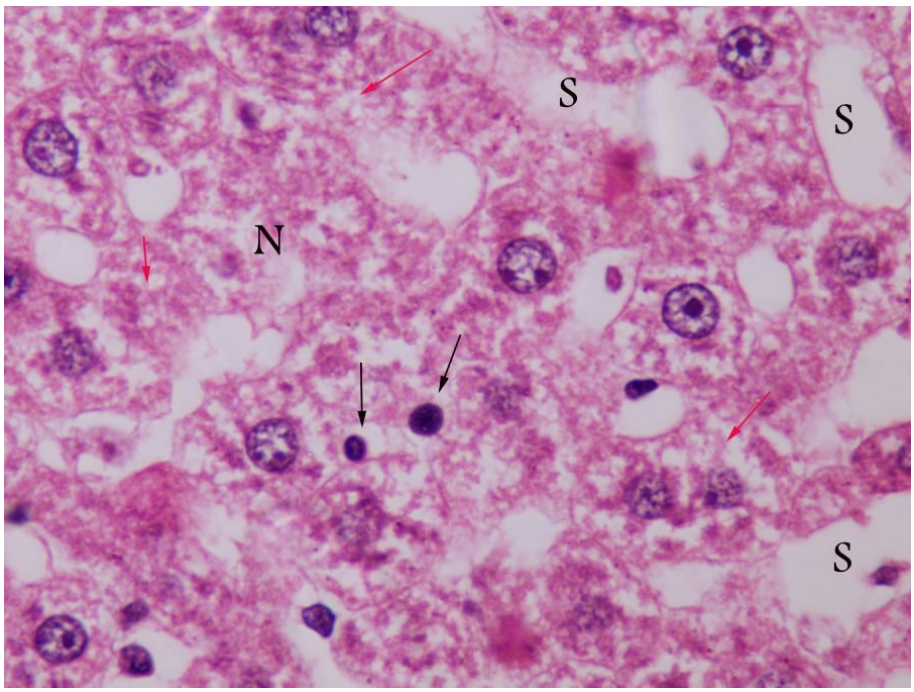

Fig. (6): A photomicrograph of a section in the liver of a group II rat (treated with $CdCl_2$) showing structural changes in the hepatocytes in the form of rarified and vacuolated cytoplasm (red arrow), pyknotic nuclei (black arrow), karyolysis of some nuclei (N) and dilated blood sinusoids (S) (H&E X 1000).

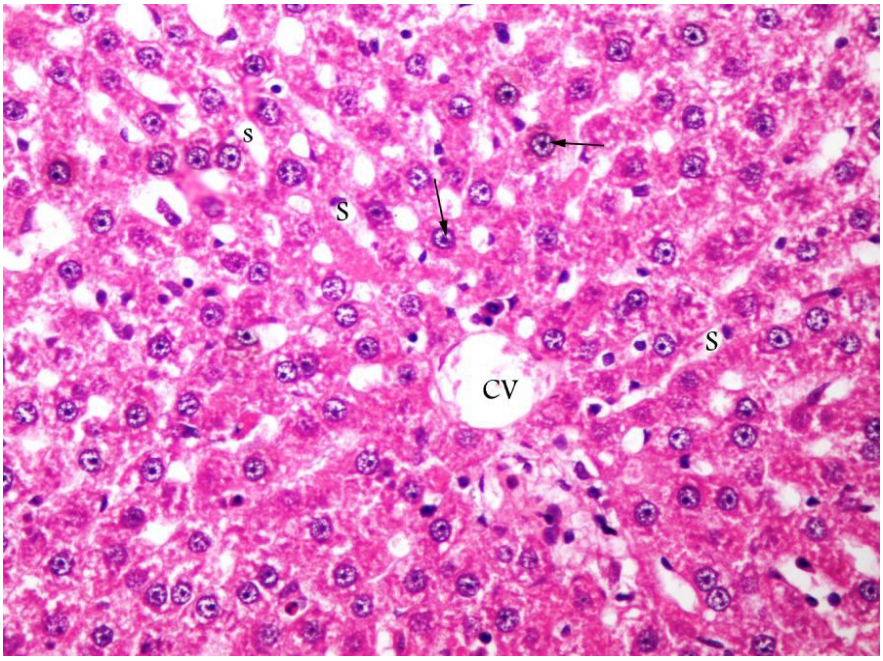

Fig. (7): A photomicrograph of a section in the liver of a group IV rat (treated with $CdCl_2$ and NSO) showing normal hepatocytes (↑) arranged in cords radiating from a central vein (CV) and separated by a slightly dilated blood sinusoids (S) (H&E X 400).

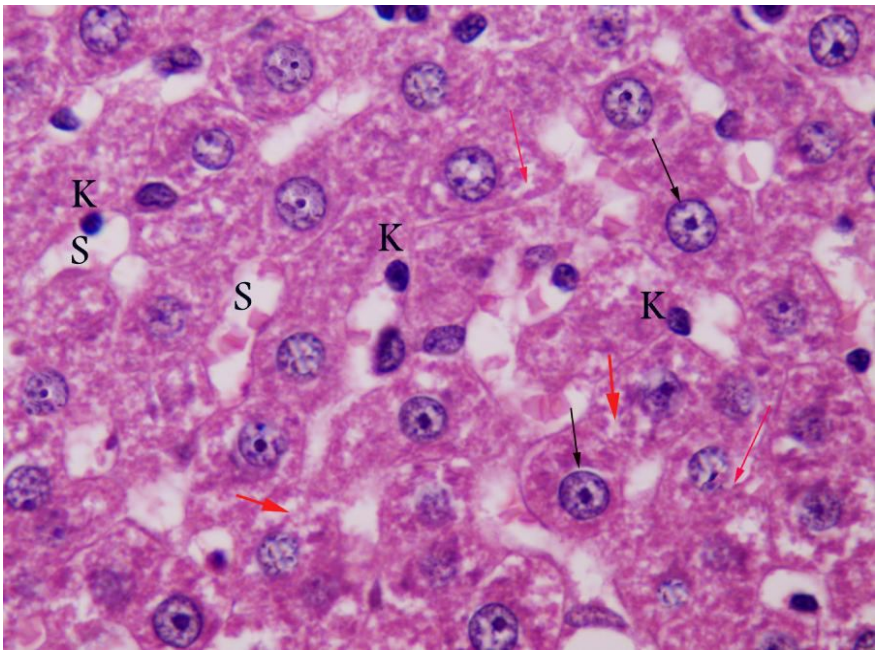

Fig. (8): A photomicrograph of a section in the liver of a group IV rat (treated with $CdCl_2$ and NSO) showing many hepatocytes with normal acidophilic cytoplasm and spherical, vesicular nuclei (black arrow) with prominent nucleoli. The cytoplasm of some hepatocytes appears vacuolated (red arrow). The blood sinusoids (S) appear slightly dilated and lined by normal Kupffer cells (K) (H&E X 1000).

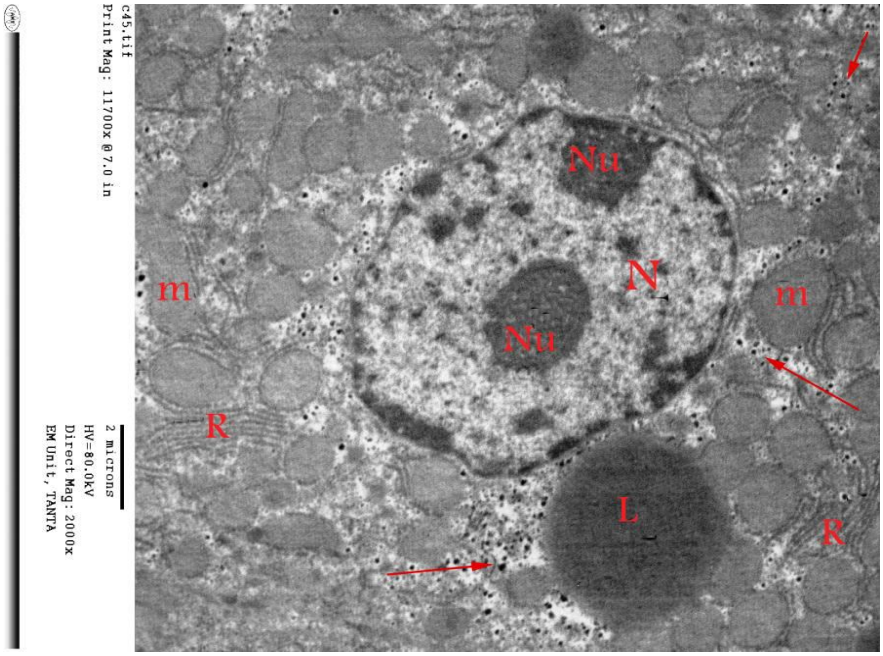

Fig. (9): An electron micrograph of an ultra-thin section from the liver of a control rat showing one normal hepatocyte. The nucleus (N) is central, large and spherical with scattered heterochromatin and two nucleoli (Nu). The cytoplasm contains many glycogen granules (arrow), many oval mitochondria (m), well-developed RER (R) and some lipid droplets (L) (EM X 2000).

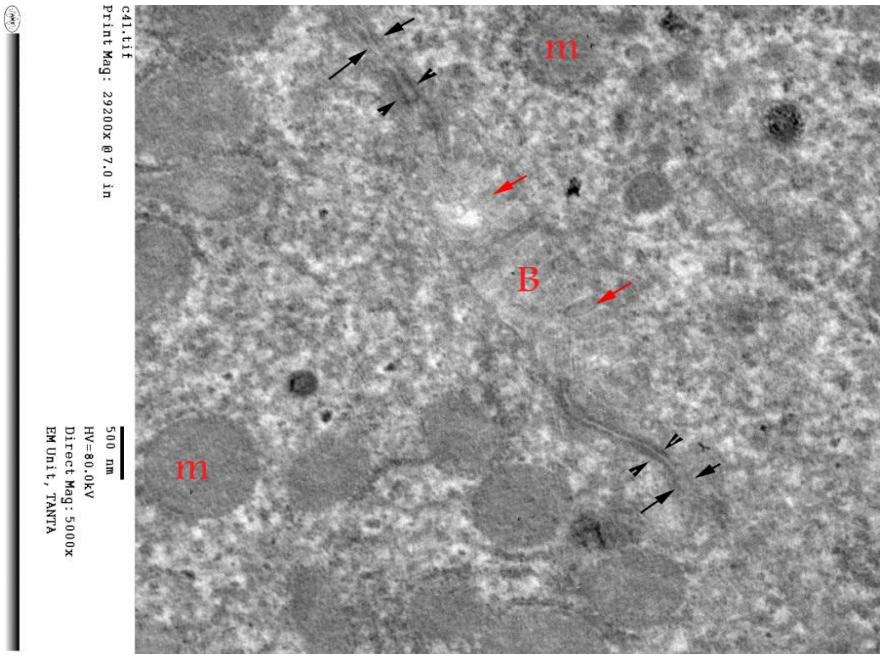

Fig. (10): An electron micrograph of an ultra-thin section from the liver of a control rat showing two of the three functional surfaces of two adjoining hepatocytes. The 1st surface (black arrow) is where the cell membranes of the two cells are in close contact. It shows no special characters. The 2nd surface is the one facing the bile canaliculus (B). This surface is studded with many short microvilli (red arrow) from the hepatocytes plasmalemma. The occluding junctions (black arrow head) close the peripheries of the canaliculus. Many mitochondria (m) are seen near the canaliculus (EM X 5000).

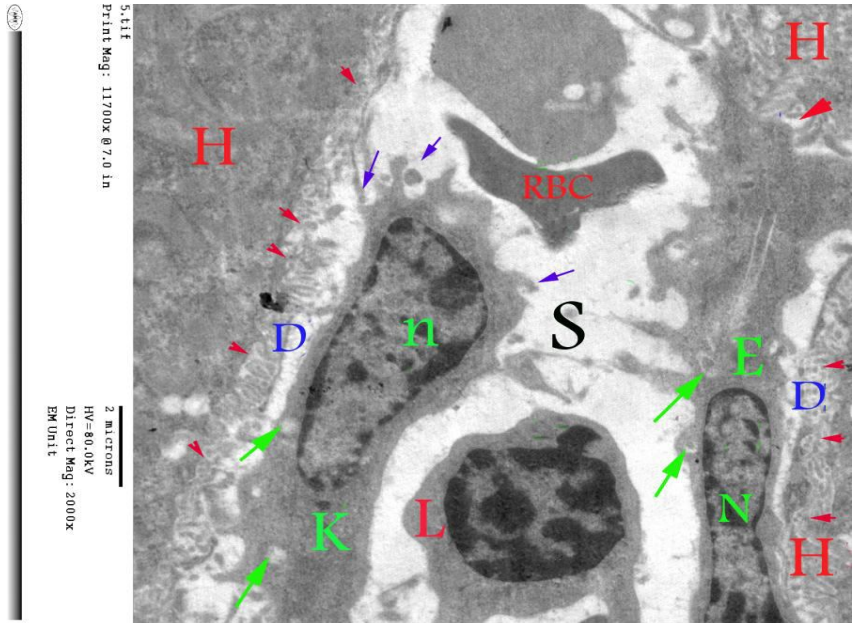

Fig. (11): An electron micrograph of an ultra-thin section from the liver of a control rat showing a blood sinusoid (S) with a perisinusoidal space of Disse (D). The surface of the hepatocyte (H) shows many short microvilli (red arrow head) projecting into the space of Disse. The sinusoid is lined with flat endothelial cell (E) and irregular Kupffer cell (K). The latter projects into the lumen of the sinusoid and has oval nucleus (n), many cytoplasmic vesicles (green arrows) and many short pseudopodia (blue arrows). Endothelial cell has elongated nucleus (N) and show many cytoplasmic vesicles (green arrow). The lumen of the sinusoid contains lymphocyte (L) and RBCs (EM X 2000).

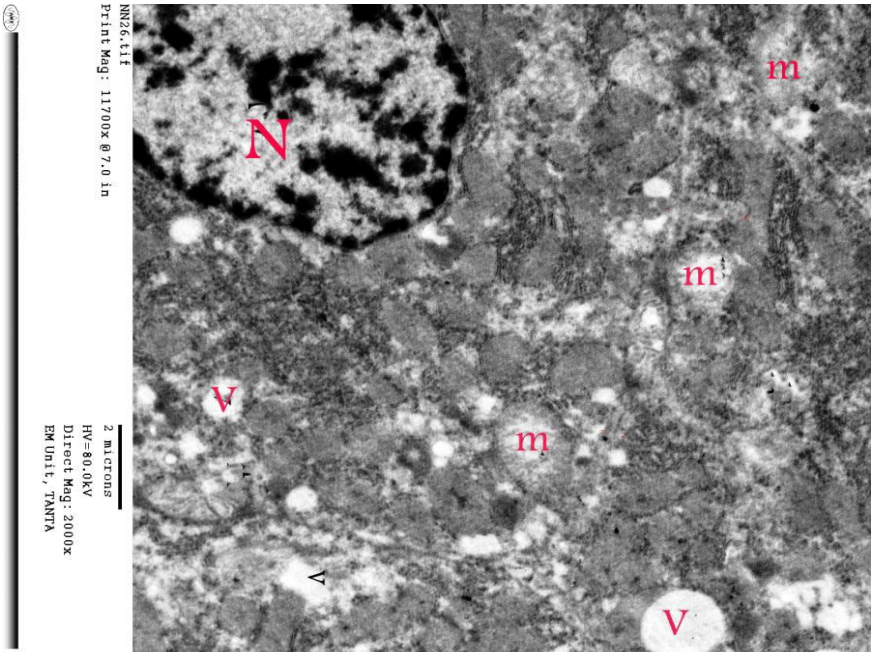

Fig. (12): An electron micrograph of an ultra-thin section from the liver of a group II rat (treated with $CdCl_2$) showing hepatocytes with degenerating and swollen mitochondria (m), cytoplasmic vacuolation (V) and disappearance of the glycogen granules and lipid droplets (EM X 2000).

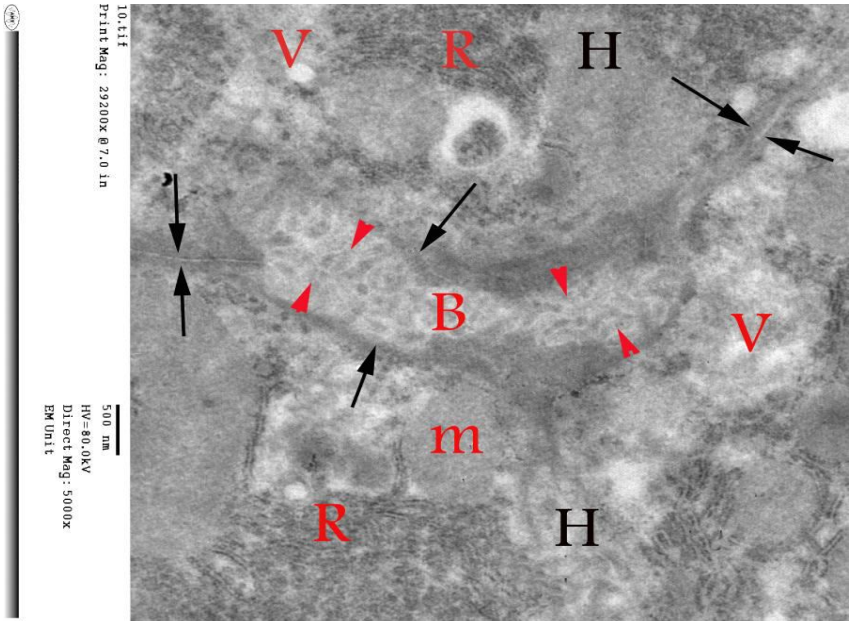

Fig. (13): An electron micrograph of an ultra-thin section from the liver of a group II rat (treated with $CdCl_2$) showing adjoining parts from two hepatocytes (H). The cell membranes (arrows) as well as the bile canaliculus (B) with its microvilli (arrow heads) appear normal. The cytoplasm is vacuolated (V) and contains few swollen mitochondria (m) and intact RER (R) (EM X 5000).

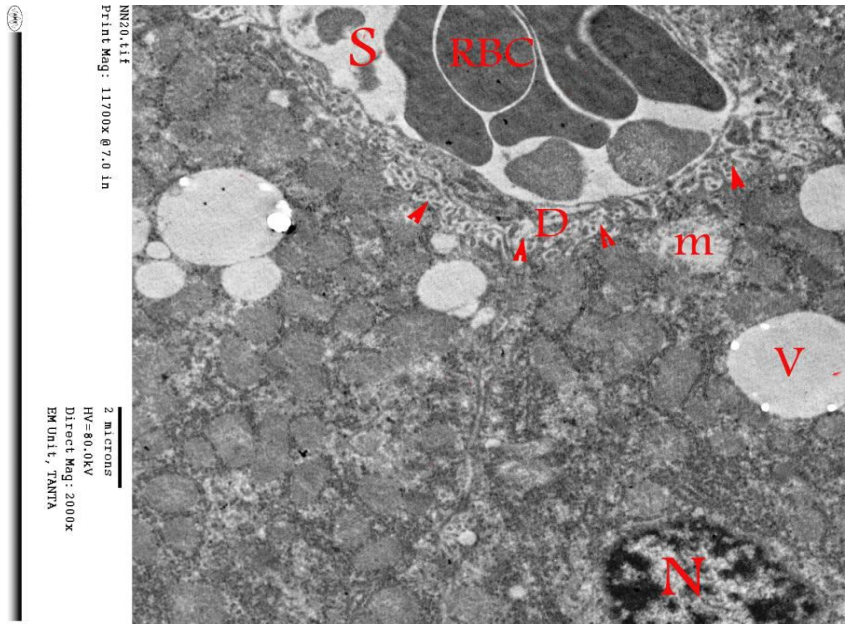

Fig. (14): An electron micrograph of an ultra-thin section from the liver of a group II rat (treated with $CdCl_2$) showing an apparently congested sinusoid (S) filled with RBCs. The perisinusoidal space of Disse (D) appears normal and is filled with microvilli (arrow heads). The hepatocytes show cytoplasmic vacuolations (V), lipid and glycogen depletion, degenerated mitochondria (m) and small, dark, pyknotic nucleus (N) (EM X 2000).

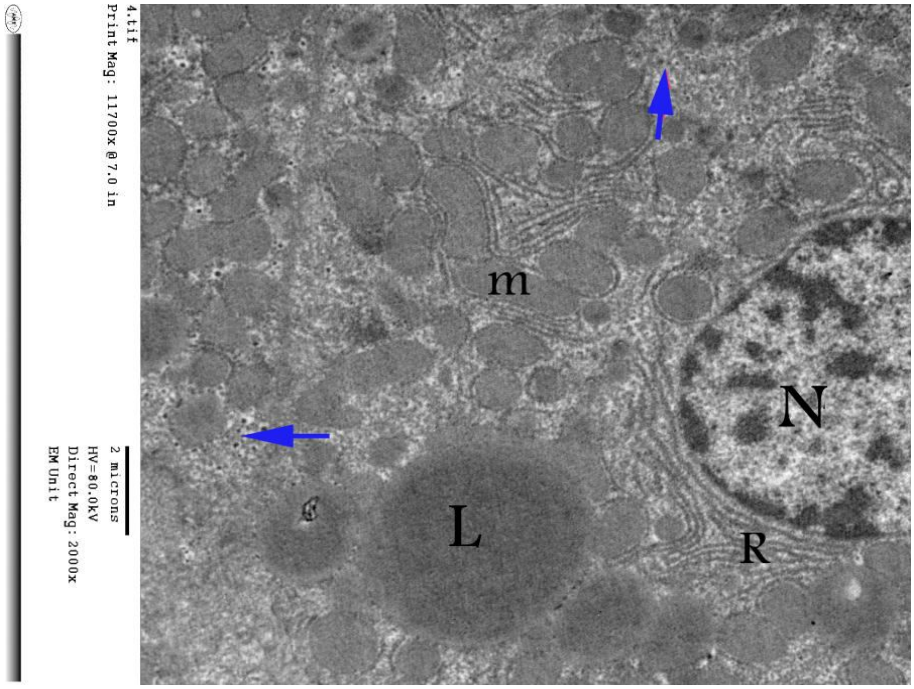

Fig. (15): An electron micrograph of an ultra-thin section from the liver of a group IV rat (treated with $CdCl_2$ and NSO) showing one hepatocyte with its normal spherical nucleus (N), many normal mitochondria (m), well developed RER (R), lipid droplets (L) and glycogen granules (arrow) (EM X 2000).

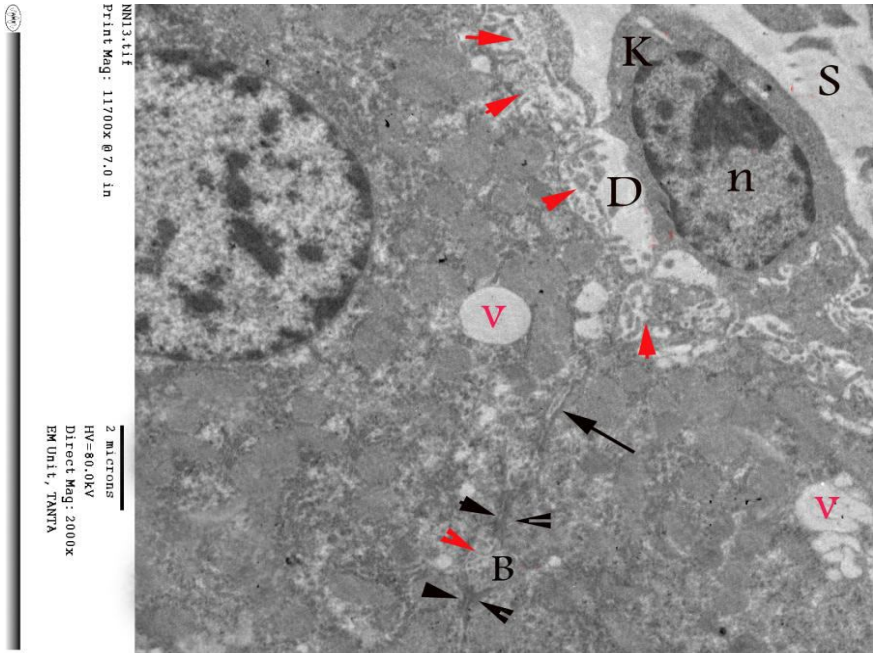

Fig. (16): An electron micrograph of an ultra-thin section from the liver of a group IV rat (treated with $CdCl_2$ and NSO) showing normal blood sinusoid (S), space of Disse (D) and bile canaliculus (B). Similarly, the hepatocyte cell membrane (black arrow), with its occluding junctions (black arrow head) and microvilli (red arrow head) appears normal. Kupffer cell (K) with its oval nucleus (n) is seen. On the other hand, the cytoplasm of the hepatocytes shows few vacuolations (V) (EM X 2000).

Discussion

Cadmium is an environmental pollutant that was found to produce deleterious effects on many body organs, particularly the liver (Waisberg et al., 2003; Arita and Costa, 2009; Wen et al., 2010; Zhang et al., 2010; Prabu et al., 2011; Rogalska et al., 2011). The toxic effects of Cd are presumed to be exerted through uncoupling of oxidative phosphorylation, excessive formation of free radicals and lipid peroxidation leading to affection of cellular proteins and DNA (Stohs et al., 2001; Santos et al., 2005; Prabu et al., 2007).

In this study, rats treated with $CdCl_2$ demonstrated hepatic damage in the form of dilated central veins with distorted walls, dilated congested sinusoids, pyknosis of hepatocytes nuclei, vacuolation of the cytoplasm, depletion of glycogen granules and lipid droplets, besides areas of focal hepatic necrosis. Some of these necrotic areas were infiltrated with many inflammatory cells. Many mitochondria appeared swollen with damaged cristae.

The rarefaction and vacuolation of the cytoplasm observed in the liver cells could be explained by the damage of lysosomal membrane by free radicals causing an increased lysosomal fragility and release of lysosomal hydrolases into the cystosol. Subsequently, uncontrolled extralysosomal proteolysis, enhanced autophagocytosis in the cells and tissue destruction occurs (Skrzydlewska et al., 2001). Furthermore, vacuoles may arise from dilated or destroyed cell organelles such as Golgi bodies and mitochondria due to ionic and osmotic imbalance leading to imbibition of water causing cellular vacuolation, which is a known sort of cell degeneration (El-Sayed et al., 2002). The infiltration of necrotic areas with inflammatory cells could be explained by oxidative stress activating nuclear factor-jB signaling pathway, which is crucial for regulation of many genes involved in inflammatory responses, as tumour necrosis factor-a, inducible nitric oxide synthase, cyclooxygenase-2 and caspase family of proteases leading eventually to cell death (Tugcu et al., 2010; Chen et al., 2012).

The shoots, roots and seeds of NS exhibit strong antioxidant activity. The LD_{50} of TQ in rats was found to be 57.5 mg/kg and 794.3 mg/kg after i.p. injection and oral ingestion respectively (Zaoui et al., 2002; Bourgou et al., 2012). These findings suggest a safe therapeutic margin for NS (Al-Ali et al., 2008). Thymoquinone was found to protect the liver from cyclophosphamide (Alenzi et al., 2010), acetaminophen (Nagi et al., 2010) and aflatoxin B1 induced hepatotoxicity (Nili-Ahmadabadi et al., 2011).

The results of this study are in agreement with (Tarasub et al., 2012) who found that Cd treatment resulted in extensive degeneration of hepatocytes with focal necrosis, vacuolated cytoplasms, inflammatory cell infiltrations and damaged central veins. They found that pretreatment with antioxidants like curcumin and vitamin C could recover these alterations.

In the present study, administration of NSO to rats ameliorated the toxic effect induced by $CdCl_2$, where central veins, most of blood sinusoids and hepatocytes appeared normal. Only few hepatocytes and some blood sinusoids showed some changes. Comparable research work by Abd El-Hamid et al., (2014) has studied the protective effect of some antioxidants, including NS, against Cd-induced hepatotoxicty. Cadmium exposure was associated with marked derangement of the liver structure. The study concluded that oral administration of NS in combination with Cd alleviated its toxic effect on the liver.

The hepatoprotective effect of NSO against Cd-induced toxicity could be attributed

mainly to TQ through its antioxidant and anti-inflammatory properties (Kruk et al., 2000; Nagi and Mansour, 2000; Badary et al., 2003). Studies on rodent models have effectively shown that TQ has played a pivotal role as an antioxidant and protective agent against chemically induced oxidative stress leading to hepatic injury (Mansour et al., 2001). Thymoquinone exerts its antioxidant effects through inhibition of iron-dependent lipid peroxidation, expression of inducible nitric oxide synthetase and reduction of lipogenesis in the hepatocytes (Fouda et al., 2014), In addition, it acts as a direct free radical scavenger, increases total thiol content and glutathione level (Badary et al., 2003; Mohamed et al., 2005; El-Tawil and Moussa, 2006). Also, it enhances the activities of antioxidant enzymes catalase, superoxide dismutase and glutathione transferase (Yildiz et al., 2008; Sayed-Ahmed et al., 2010; Awad et al., 2011).

Thymoquinone exhibits anti-inflammatory effect by inhibition of both cyclooxygenase and lipoxygenase (El-Tawil and Moussa, 2006), inhibition of nuclear factor-Kβ, reduction of cytochrome c production (Badary et al., 2000) and inhibition of prostaglandin E2 formation (Mahgoub, 2003). Inflammatory responses and activated neutrophils can increase myeloperoxidase activity in the liver tissue. Myeloperoxidase increases lipid peroxidation and free radicals formation, which could worsen the liver injury (Cetinkaya et al., 2006). Moreover, it induces cell proliferation leading to enhanced regeneration after tissue damage (Ince et al., 2012). Zafeer et al. (2012) reported that pretreatment with TQ (10 μM) showed a significant protection as manifested by noticed attenuation of protein oxidation and rejuvenation of the depleted antioxidants of the cellular fraction prepared from liver.

On top, NS could have a potential role in enhancing the elimination of Cd. Massadeh et al. (2007) found that NS reduced Cd levels in mice exposed to it by 75.5%, 83.3%, 47.0%, 95.3%, and 100% in the liver, kidney, heart, spleen, and blood, respectively. Blood Cd concentrations were lowered to below the detection limit. However, the mechanism behind this effect is unknown.

The results of the present study concluded that NSO may be used as a safe plant product to mitigate hepatic damage associated with Cd toxicity. However, further experimental and clinical studies are required to establish the exact mechanisms of its favorable effects and assert its clinical application.

References

Abd El-Hamid, A.A.; Badr, M.; Helal, A.F.; et al. (2014): "Protective effect of some medicinal plants and zinc on Some Serum Parameters and Histopathological features of liver, kidney and testis in rats treated with cadmium". Middle East J. Appl. Sci., 4 (3): 539-554.

Abdel-Zaher, A.O.; Abdel-Rahman, M.S.; ELwasei, F.M. (2011): "Protective effect of Nigella sativa oil against tramadol-induced tolerance and dependence in mice: role of nitric oxide and oxidative stress". Neurotoxicology, 32 (6): 725-733.

Abel-Salam, B.K. (2012): "Immuno-modulatory effects of black seeds and garlic on alloxan-induced diabetes in albino rat". Allergol. Immunopathol. (Madr). 40 (6): 336-340.

Ahmad, A.; Husain, A.; Mujeeb, M.; et al. (2013): "A review on therapeutic potential of Nigella sativa: A miracle herb". Asian Pac. J. Trop. Biomed., 3 (5): 337-352.

Al-Ali, A.; Alkhawajah, A.A.; Randhawa, M.A.; et al. (2008): "**Oral and** intraperitoneal LD50 of thymoquinone,

an active principle of Nigella sativa, in mice and rats". J. Ayub. Med. Coll. Abbottabad., 20 (2): 25-27.

Al-Ghamdi, M.S. (2001): "Anti-inflammatory, analgesic and anti-pyretic activity of Nigella sativa.". J. Ethnopharmacol., 76: 45-48.

Al-Jassir, M.S. (1992): "Chemical composition and microflora of black cumin (Nigella sativa L.) seeds growing in Saudi Arabia". Food Chem., 45 (4): 239-242.

Alenzi, F.Q.; El-Bolkiny Yel, S. and Salem, M.L. (2010): "Protective effects of Nigella sativa oil and thymoquinone against toxicity induced by the anticancer drug cyclophos-phamide". Br. J. Biomed. Sci., 67 (1): 20-28.

Arita, A.; Costa, M. (2009): "Epigenetics in metal carcinogenesis: nickel, arsenic, chromium and cadmium". Metallomics, 1 (3): 222-228.

Assayed, M.E. (2010): "Radioprotective effects of black seed (Nigella sativa) oil against hemopoietic damage and immunosuppression in gamma-irradiated rats". Immunopharmacol. Immuno-toxicol., 32 (2): 284-296.

Awad, A.S.; Kamel, R.; Sherief, M.A. (2011): "Effect of thymoquinone on hepatorenal dysfunction and alteration of CYP3A1 and spermidine/spermine N-1-acetyl-transferase gene expression induced by renal ischaemia-reperfusion in rats". J. Pharm. Pharmacol., 63 (8): 1037-1042.

Badary, O.A.; Abdel-Naim, A.B.; Abdel-Wahab, M.H.; et al. (2000): "The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats". Toxicology, 143 (3): 219-226.

Badary, O.A.; Taha, R.A.; Gamal el-Din, A.M.; et al. (2003): "Thymoquinone is a potent superoxide anion scavenger". Drug Chem. Toxicol., 26 (2): 87-98.

Bharavi, K.; Gopala Reddy, A.; Rao, G.; et al. (2010): "Reversal of cadmium induced oxidative stress and its bioaccumulation by culinary herbs Murraya koenigii and Allium sativum". Res. J. Pharmacol., 4 (3): 60-65.

Bolkent, S.; Koyuturk, M.; Bulan, O.K.; et al. (2007): "The effects of combined α-tocopherol, ascorbic acid, and selenium against cadmium toxicity in rat intestine". J. Environ. Pathol. Toxicol. Oncol., 26 (1): 21 - 27.

Boskabady, M.; Mohsenpoor, N.; Takaloo, L. (2010): "Antiasthmatic effect of Nigella sativa in airways of asthmatic patients". Phytomedicine, 17 (10): 707-713.

Bourgou, S.; Pichette, A.; Marzouk, B.; et al. (2012): "Antioxidant, anti-inflammatory, anticancer and anti-bacterial activities of extracts from Nigella sativa (black cumin) plant parts". J. Food Biochem., 36 (5): 539-546.

Cetinkaya, A.; Bulbuloglu, E.; Kurutas, E.B.; et al. (2006): "N-acetylcysteine ameliorates methotrexate-induced oxidative liver damage in rats". Med. Sci. Monit., 12 (8): Br274-Br278.

Chen, J.; Fok, K.L.; Chen, H.; et al. (2012): "Cryptorchidism-induced CFTR down-regulation results in disruption of testicular tight junctions through up-regulation of NF-κB/COX-2/PGE2". Hum. Reprod., 27 (9): 2585-2597.

El-Sayed, N.K.; Gaafar, K.M.; El-Ansary, A.K.; et al. (2002): "The protective potency of vitamins E and C in methanol-induced oxidative stress and retinotoxicity". J. Toxicol. Cutaneous Ocul. Toxicol., 21 (4): 307-327.

El-Tawil, O.; Moussa, S.Z. (2006): "Antioxidant and hepatoprotective effects of thymoquinone against carbon

tetrachloride-induced hepatotoxicity in isolated rat hepatocyte". J Egypt Soc. Toxicol., 34**:** 33-41.

Fouda, A.M.M.; Daba, M.H.Y.; Yousef Ahmed, A.R. (2014): "Antigenotoxic effects of thymoquinone against benzo[a]pyrene and mitomycin C - induced genotoxicity in cultured human lymphocytes". Res. Immunol., 2014**:** 1 - 13.

Gali-Muhtasib, H.; El-Najjar, N.; Schneider-Stock, R. (2006): "The medicinal potential of black seed (Nigella sativa) and its components". Adv. Phytomed. , 2**:** 133-153.

Hayat, M.A. (1978): Principles and techniques of scanning electron microscopy. Van Nostrand-Reinhold, New Jersey.

Ince, S.; Kucukkurt, I.; Huseyin Demirel, H.; et al. (2012): "Thymoquinone attenuates cypermethrin induced oxidative stress in Swiss albino mice". Pestic. Biochem. Phys., 104 (3)**:** 229-235.

Järup, L. (2002): "Cadmium overload and toxicity". Nephrol. Dial. Transplant., 17 (suppl 2)**:** 35-39.

Kennedy, P.J.; Vashisht, A.A.; Hoe, K.L.; et al. (2008): "A genome-wide screen of genes involved in cadmium tolerance in Schizosaccharomyces pombe". Toxicol. Sci., 106 (1)**:** 124-139.

Koyuturk, M.; Yanardag, R.; Bolkent, S.; et al. (2007): "The potential role of combined anti-oxidants against cadmium toxicity on liver of rats". Toxicol. Ind. Health, 23 (7)**:** 393-401.

Kruk, I.; Michalska, T.; Lichszteld, K.; et al. (2000): "The effect of thymol and its derivatives on reactions generating reactive oxygen species". Chemosphere, 41 (7)**:** 1059-1064.

Lawal, A.O.; Ellis, E. (2010): "Differential sensitivity and responsiveness of three human cell lines HepG2, 1321N1 and HEK 293 to cadmium". J. Toxicol. Sci., 35 (4)**:** 465-478.

Liao, X.-Y.; Chen, T.-B.; Xie, H.; et al. (2005): "Soil as contamination and its risk assessment in areas near the industrial districts of Chenzhou City, Southern China". Environ. Int., 31 (6)**:** 791-798.

Mahgoub, A.A. (2003): "Thymoquinone protects against experimental colitis in rats". Toxicol. Lett., 143 (2)**:** 133-143.

Malik, S.; Hasan, S.S.; Choudhary, M.I.; et al. (1995): "Nigellidine—a new indazole alkaloid from the seeds of Nigella sativa". Tetrahedron. Lett., 36 (12)**:** 1993-1996.

Mansour, M.A.; Ginawi, O.T.; El-Hadiyah, T.; et al. (2001): "Effects of volatile oil constituents of Nigella sativa on carbon tetrachloride-induced hepatotoxicity in mice: evidence for antioxidant effects of thymoquinone". Res. Commun. Mol. Pathol. Pharmacol., 110 (3-4)**:** 239-251.

Massadeh, A.M.; Al-Safi, S.A.; Momani, I.F.; et al. (2007): "Analysis of cadmium and lead in mice organs". Biol. Trace Elem. Res., 115 (2)**:** 157-167.

Mohamadin, A.M.; Sheikh, B.; Abd El-Aal, A.A.; et al. (2010): "Protective effects of Nigella Sativa oil on propoxur-induced toxicity and oxidative stress in rat brain regions.". Pestic. Biochem. Physiol., 98**:** 128-134.

Mohamed, A.; Afridi, D.M.; Garani, O.; et al. (2005): "Thymoquinone inhibits the activation of NF-kappaB in the brain and spinal cord of experimental autoimmune encephalomyelitis". Biomed. Sci. Instrum., 41**:** 388-393.

Nagi, M.N.; Almakki, H.A.; Sayed-Ahmed, M.M.; et al. (2010): "Thymoquinone supplementation reverses acetaminophen -induced oxidative stress, nitricoxide production and energy decline in mice

liver". Food Chem. Toxicol., 48 (8-9): 2361-2365.

Nagi, M.N.; Mansour, M.A. (2000): "Protective effect of thymoquinone against doxorubicin-induced cardiotoxicity in rats: a possible mechanism of protection". Pharmacol. Res., 41 (3): 283-289.

Nehar, S.; Rani, P. (2011): "HPTLC studies on ethanolic extract of Nigella sativa Linn. seeds and its phytochemical standardization.". The Ecoscan, 1: 105 - 108.

Nickavar, B.; Mojab, F.; Javidnia, K.; et al. (2003): "Chemical composition of the fixed and volatile oils of Nigella sativa L. from Iran". Z. Naturforsch C., 58 (9-10): 629-631.

Nili-Ahmadabadi, A.; Tavakoli, F.; Hasanzadeh, G.R.; et al. (2011): "Protective effect of pretreatment with thymoquinone against AflatoxinB1 induced liver toxicity in mice". DARU 19 (4): 282-287.

Ognjanovic, B.I.; Markovic, S.D.; Pavlovic, S.Z.; et al. (2008): "Effect of chronic cadmium exposure on antioxidant defense system in some tissues of rats: protective effect of selenium". Physiol. Res., 57 (3): 403-411.

Prabu, S.M.; Renugadevi, J.; Kumar, T.R. (2007): "Ameliorative effect of selenium on cadmium induced biochemical alterations in Cirrhinus mrigala (Ham.)". Asian J. Bio. Sci., 2 (1-2): 143-148.

Prabu, S.M.; Shagirtha, K.; Renugadevi, J. (2011): "Naringenin in combination with vitamins C and E potentially protects oxidative stress-mediated hepatic injury in cadmium-intoxicated rats". J. Nutr. Sci. Vitaminol. (Tokyo). 57 (2): 177-185.

Rogalska, J.; Pilat-Marcinkiewicz, B.; Brzoska, M.M. (2011): "Protective effect of zinc against cadmium hepatotoxicity depends on this bioelement intake and level of cadmium exposure: a study in a rat model". Chem. Biol. Interact., 193 (3): 191-203.

Santos, F.W.; Zeni, G.; Rocha, J.B.; et al. (2005): "Diphenyl diselenide reverses cadmium-induced oxidative damage on mice tissues". Chem. Biol. Interact., 151 (3): 159-165.

Sayed-Ahmed, M.M.; Aleisa, A.M.; Al-Rejaie, S.S.; et al. (2010): "Thymoquinone attenuates diethyl-nitrosamine induction of hepatic carcinogenesis through antioxidant signaling". Oxid. Med. Cell Longev., 3 (4): 254-261.

Skrzydlewska, E.; Dobrzynska, I.; Kasacka, I.; et al. (2001): "Effect of vitamin e derivative (u83836e) on membranes of rat liver cells after methanol intoxication". Pol. J. Environ. Stud., 10 (2): 95-100.

Stevens, A.M.; Bancroft, J.D. (1996): Theory and practice of histological techniques. 4th Ed. Churchill Livingstone, New York.

Stohs, S.J.; Bagchi, D.; Hassoun, E.; et al. (2001): "Oxidative mechanisms in the toxicity of chromium and cadmium ions". J. Environ. Pathol. Toxicol. Oncol., 20 (2): 77 - 88.

Tarasub, N.; Junseecha, T.; Tarasub, C.; et al. (2012): "Protective effects of curcumin, vitamin C, or their combination on cadmium-induced hepatotoxicity". J. Basic Clin. Pharm., 3 (2): 273 - 281.

Tugcu, V.; Gedikbasi, A.; Mutlu, B.; et al. (2010): "Increased testicular 8-hydroxy-2'-deoxyguanosine (8-OHdG) and inducible nitric oxide synthetase (iNOS) and nuclear factor kappaB (NF-kappaB) expressions in experimental rat varicocele". Arch. Ital. Urol. Androl., 82 (4): 148-153.

Waalkes, M.P.; Harvey, M.J.. Klaassen, C.D. (1984): "Relative in vitro affinity of hepatic metallothionein for metals". Toxicol. Lett., 20 (1)**:** 33-39.

Waisberg, M.; Joseph, P.; Hale, B.; et al. (2003): "Molecular and cellular mechanisms of cadmium carcino-genesis". Toxicol. Appl. Pharmacol., 192 (2-3)**:** 95-117.

Wen, Y.F.; Zhao, J.Q.; Bhadauria, M.; et al. (2010): "Pyridoxine mitigates cadmium induced hepatic cytotoxicity and oxidative stress". Environ. Toxicol. Pharmacol., 30 (2)**:** 169-174.

Williams, D.F.; Robertson, R.; Roworth, M. (1999): Detailed profiles of 25 major organic and inorganic substances. Glasgow: Scottish Centre for Infection and Environmental Health. P.P. 12 - 19.

Yildiz, F.; Coban, S.; Terzi, A.; et al. (2008): "Nigella sativa relieves the deleterious effects of ischemia reperfusion injury on liver". World J. Gastroenterol., 14 (33)**:** 5204-5209.

Zafeer, M.F.; Waseem, M.; Chaudhary, S.; et al. (2012): "Cadmium-induced hepatotoxicity and its abrogation by thymoquinone". J. Biochem. Mol. Toxicol., 26 (5)**:** 199-205.

Zaoui, A.; Cherrah, Y.; Lacaille-Dubois, M.; et al. (1999): "Diuretic and hypotensive effects of Nigella sativa in the spontaneously hypertensive rat". Therapie., 55 (3)**:** 379-382.

Zaoui, A.; Cherrah, Y.; Mahassini, N.; et al. (2002): "Acute and chronic toxicity of Nigella sativa fixed oil". Phytomedicine, 9 (1)**:** 69-74.

Zhang, M.; He, Z.; Wen, L.; et al. (2010): "Cadmium suppresses the proliferation of piglet Sertoli cells and causes their DNA damage, cell apoptosis and aberrant ultrastructure". Reprod Biol Endocrinol, 8**:** 97.

زيت الحبة السوداء يخفف اصابة الكبد الناشئة عن الكادميوم في الجرذان البيضاء الذكور البالغة

امل سعيد احمد فتحي حافظ و ايهاب محمد هنطش* و عصام محمود حافظ**

أقسام الطب الشرعي والسموم الإكلينيكية و التشريح* – كلية الطب- جامعة طنطا و فسم الطب الشرعي والسموم- كلية الطب- جامعة المنيا**

الكادميوم هو معدن ثقيل سام و ملوث للبيئة. يسبب الكادميوم سمية شديدة في اعضاء متعددة منها الكبد و الكلية و العظام و الخصيتين. يهدف هذا البحث إلي دراسة التأثير الوقائي المحتمل لزيت الحبة السوداء علي اصابة الكبد الناشئة عن الكادميوم. تم تقسيم اربعين جرذ ابيض ذكر بالغ الي اربع مجموعات متساوية. المجموعة (I) كانت المجموعة الضابطة. المجموعة (II) تلقت حقنا داخل البريتون بكلوريد الكادميوم ٢ مجم/كجم من وزن الجسم بينما المجموعة (III) تلقت زيت الحبة السوداء واحد ملليلتر/كجم بالفم. الجرذان في المجموعة (IV) تلقوا زيت الحبة السوداء قبل كلوريد الكادميوم بساعة بنفس الجرعات و الطرق الموصوفة عاليه. جميع الحيوانات عوملت لثمانية أيام ثم خضعت للقتل الرحيم و تم استخلاص اكبادها و فحصها هيستولوجيا بالميكروسكوب الضوئي و الالكتروني. وجد في جرذان المجموعة II اصابة في البنيان الكبدي علي هيئة اتساع في الاوردة المركزية الكبدية مع تغير في جدرانها و اتساع و احتقان الأوعية الجيبية. بالإضافة إلي ذلك، تم ملاحظة تغلظ أنوية الخلايا الكبدية و فجوات بالسيتوبلازم و استنفاد حبيبات الجليكوجين و قطيرات الدهن ، انتفاخ الميتوكوندريا و بؤر من النخر الكبدي و تغلغل الخلايا الالتهابية. واظهر جرذان المجموعة IV تحسنا ملحوظا للاصابة الكبدية الناشئة عن التعرض لكلوريد الكادميوم. و الحيوانات بالمجموعة I و المجموعة III اظهرت بنيانا عاديا نسبيا للكبد بدون اصابة واضحة. ويمكن أن نخلص إلى أن زيت الحبة السوداء من الممكن استخدامه كمنتج نباتي امن و فعال لتخفيف الاصابة الكبدية المقترنة بتسمم الكادميوم.