Histological Study of the Effect of Cisplatin on the Liver of Adult Male Albino Rat

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ABSTRACT

Objective: This study was designed to assess the toxic effect of Cisplatin drug on the histological structure of the liver of adult male rats and the degree of improvement after abstinence of the drug for 2 months for recovery as well as its effect on the body weight.

Methods: Thirty adult male albino rats were divided into 3 groups 10 rats each. Animals of the control group (G1) received normal saline 0.9% intra-peritoneally, while those of the treated group (G2) received intra-peritoneal cisplatin in a dose of 0.2 mg/kg body weight (low dose) twice weekly for two months. Rats of recovery group (G3) received the same treatment as those of (G2) for two months and then were left for two months without treatment for recovery. The animals of (G1) and (G2) were sacrificed after two months from the start of the experiment while those of (G3) were left for two months more without treatment then sacrificed.

Results: The body weights of cisplatin treated rats were significantly decreased in comparison to controls (P<0.001) Two months after withdrawal of the drug, there was a significant increase in body weights of the rats in the recovery group (P<0.01). Both light and electron microscopic studies revealed that low doses of cisplatin caused hepatotoxicity manifested by cytoplasmic vacuolization of hepatocytes, congested blood sinusoids, apparent increase in the number of Kupffer cells and focal mononuclear cellular infiltration. However, some specimens showed distortion of hepatocytes with rarified cytoplasm and focal necrosis while others revealed severely congested blood sinusoids. At ultrastructural level, hepatocytes showed cytoplasmic vacuoles (dilated cisternae of endoplasmic reticulum), irregular nuclei and many small mitochondria with ill distinct cristea. In some cells, the observed cytoplasmic vacuoles were remarkably large. Apparent increase in the number of Kupffer cells with many secondary lysosomes and lipofuscin deposits were noticed. Two months after withdrawal of the drug, lesser and smaller cytoplasmic vacuoles in the hepatocytes were observed, while others appeared more or less normal.

Conclusion: Our results may provide histological evidence of hepatotoxicity caused by cisplatin and, on the other hand, the possibility of recovery. This can be used to consider administration of the lowest possible dose of this drug in order to ameliorate its hepatotoxic effects.

Keywords: Cisplatin, Hepatotoxicity, Rat liver, Chemotherapy, Recovery.

I. INTRODUCTION

Cisplatin, cisplatinum, or *cis*-diamminedichloroplatinum (II) (CDDP) (trade names Platinol and Platinol-AQ) is a chemotherapy drug. It is used to treat various types of cancers, including sarcomas, some carcinomas (e.g. small cell lung cancer, and ovarian cancer), lymphomas, and germ cell tumors [1]. It was the first member of a class of platinum-containing anti-cancer drugs, which now also includes carboplatin and oxaliplatin. These platinum complexes react in vivo, binding to and causing crosslinking of DNA, which ultimately triggers apoptosis (programmed cell death) [2].

The nephrotoxicity of cisplatin is recognized as the most important dose-limiting factor, but high doses of cisplatin also produce hepatotoxicity. Many efforts have been made to improve the therapeutic index of cisplatin using pharmacological strategies, such as the administration of chemoprotectors [3].

Cisplatin is a coordination compound containing two chloro and two ammine ligands. Many other amine complexes of the platinum group metals have been evaluated for this application. Cisplatin is crosslinks DNA in several different ways, interfering with cell division by mitosis. The damaged DNA elicits DNA repair mechanisms, which in turn activate apoptosis when repair proves impossible. Recently it was shown that the apoptosis induced by

cisplatin on human colon cancer cells depends on the mitochondrial serine-protease Omi/Htra2. Since this was only demonstrated for colon carcinoma cells, it remains an open question if the Omi/Htra2 protein participates in the cisplatin-induced apoptosis in carcinomas from other tissues [4].

Sheets of connective tissue divide the liver into thousands of small units called lobules. A lobule is roughly hexagonal in shape, with portal triads at the vertices and a central vein in the middle. The lobule is the structural unit of the liver and rather easy to observe. In contrast, the hepatic acinus is more difficult to visualize, but represents a unit that is of more relevance to hepatic function because it is oriented around the afferent vascular system. In the case of the liver, the roads are connective tissue septae which convey vascular and biliary traffic, and the clusters of houses are cord-like arrangements of hepatocytes, the parenchymal cell of the liver [5].

Hepatocytes are polygonal cells joined to one another in anastomosing plates, with borders that face either the sinusoids or adjacent hepatocytes. The ultrastructure appearance of hepatocytes reflects their function as metabolic superstars, with abundant rough and smooth endoplasmic reticulum, and Golgi membranes. Glycogen granules and vesicles containing very low density lipoproteins are readily observed. Hepatocytes make contact with blood in sinusoids, which are distensible vascular channels lined with highly fenestrated endothelial cells and populated with phagocytic Kupffer cells. The space between endothelium and hepatocytes is called the Space of Disse which collects lymph for delivery to lymphatic capillaries [6]**.**

The aim of this work was to evaluate the toxic effect of cisplatin on the body weight and the liver of the adult male rats and the degree of improvement after abstinence of the drug for 2 months for recovery.

II. MATERIALS ASND METHODS

Cisplatin was obtained as yellowish crystalline powder, soluble in physiological saline solution and purchased from Sigma-Aldrich. The dose of cisplatin administered in this research was 0.2 mg/kg body weight (low dose) [7]. Thirty adult male albino rats of Sprague-Dawley strain weighing (200-220g.) were divided into 3 groups:

- *The first (control) group (G1):* consisted of 10 rats and received normal saline 0.9% serving as control.
- *The second (treated) group (G2):* ten rats comprised the treated group and each of them received cisplatin o.2 mg/kg B.WT. (Low dose) intraperitoneally, twice weekly for two months.
- *The third (recovery) group (G3):* 10 rats received the same treatment as those of (G2) for two months and then were left for two months after withdrawal of the drug.

The animals of (G1) and (G2) were sacrificed after two months from the start of the experiment while those of (G3) were left for two month more without treatment then sacrificed. Body weights of the rats were measured initially and after treatment in all groups, using an electronic analytic and precision balance.

At the appropriate time, the animals were sacrificed and their livers were carefully dissected out and cut into small pieces, about 1mm^3 each. The specimens were immediately fixed in 5% phosphate buffered gluteraldehyde (Ph 7.4) for 2 hours at 4°C, post fixed in 1% osmium tetroxide for 2 hours, dehydrated in ascending grades of ethyl alcohol and then embedded in Epoxy resin. Semithin sections (1μm) were cut, stained with toludine blue and examined by light microscope. Ultrathin sections of 60 nm were cut with an LKB ultramicrotome using a diamond Knife, mounted on copper grids and stained with uranyl acetate followed by lead citrate [8]. Examination and photography were carried out using JEOL transmission electron microscope (JEM-1200 EXII) in faculty of science, Ain shams University.

III. RESULTS

The mean body weight of the rats in treated and recovery groups showed very highly significant decrease at the end of cisplatin treatment compared to controls (P<0.001), with highly significant increase in the recovery group following 2 months of cessation of treatment (P<0.01). The weight changes are clarified in the following table:

Table: Mean± SD of body weights (grams) of all groups n=10.

	Control (saline) (G1)		Treated group (G2)	Recovery groups (G3)
	Initial Wt (g)	Wt (g) After 2 months	Wt (g) After 2 months ***	Wt (g) After 2 months **
Mean± SD	210.6±1.798	216.1±1.798	195.0 ±2.608	206.9±2.387

Mean± SD of body weights (grams) of all groups n=10. There was very highly significant difference ***at P<0.001 when comparing both the treated group to control group. Also there was a highly significant difference ** at P<0.01 when comparing the treated group to recovery group. On the other hand, there was significant difference between control and recovery groups.

Light microscopic examination of toludine blue stained sections revealed that the control hepatic tissue showed normal large polygonal cells with prominent rounded nuclei and few spaced hepatic sinusoids arranged in between the hepatic cords with fine arrangement of Kupffer cells (Fig. 1).

In contrast, the group receiving cisplatin showed hepatotoxicity manifested by cytoplasmic vacuolization of hepatocytes (v), inflammatory cells (arrow) and congested blood sinusoids (S) (Fig. 2), apparent increase in the number of Kupffer cells (Fig. 3), and focal mononuclear cellular infiltration (Fig. 4). However, some specimens showed distortion of hepatocytes with rarified cytoplasm and focal necrosis (Fig. 5) while other areas revealed severely congested blood sinusoids (Fig. 6). Two months after withdrawal of the drug, signs of recovery appeared in the form of lesser and smaller cytoplasmic vacuoles in hepatocytes while others appeared normal (Fig. 7).

Ultrastructural study revealed that control hepatocytes were normal polygonal cells with oval-shaped nuclei, cytoplasm crowded with organelles, particularly rough endoplasmic reticulum, smooth endoplasmic reticulum, ribosomes, mitochondria and glycogen particles (Figs. 8).

However, electron microscopic examination of ultrathin sections of liver of treated rats showed hepatocytes with cytoplasmic vacuoles (dilated cisternae of ER), irregular nuclei and many small mitochondria with ill distinct cristea (Fig. 9). Apparent increase in the presence of Kupffer cells with many secondary lysosomes and lipofuscin deposits was noticed (Fig.10). In some cells the observed cytoplasmic vacuoles were remarkably large (Fig.11). Two months after stoppage of treatment, the hepatocytes showed lesser and smaller cytoplasmic vacuoles than the treated ones and the nuclei appeared more or less normal (Fig.12).
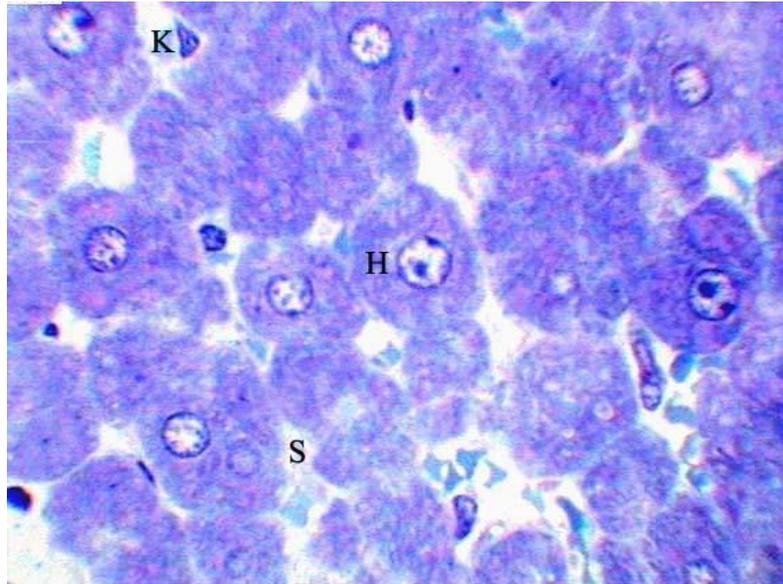
Fig. 1: A photomicrograph of a section in the liver of a control rat showing normal polygonal hepatocytes (H), blood sinusoids (S) in between the cords of cells and few Kupffer cells (K). (Toludine blue X1000).
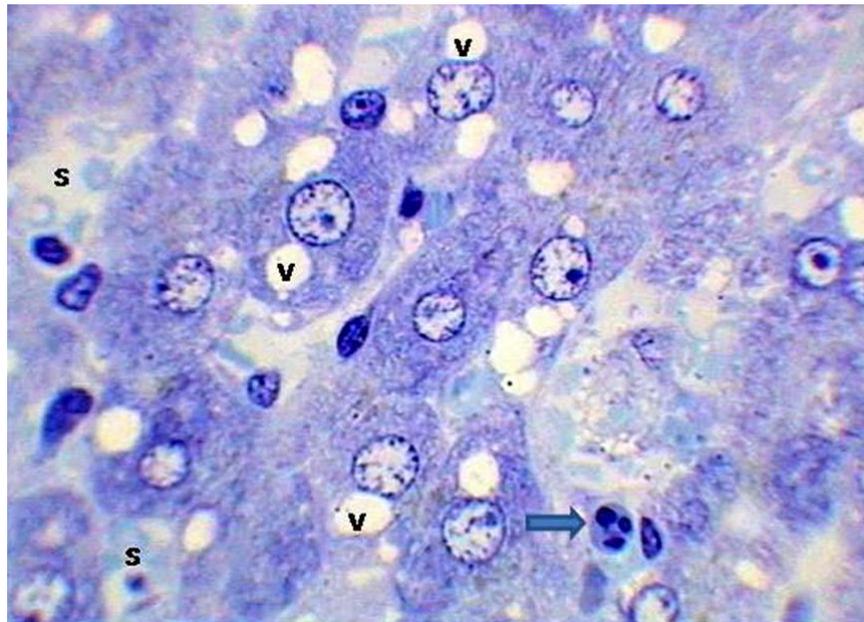

Fig. 2: A photomicrograph of a section in the liver of treated rat showing hepatocytes with cytoplasmic vacuoles (v), inflammatory cells (arrow) and congested blood sinusoids (s). (Toludine blueX 1000).
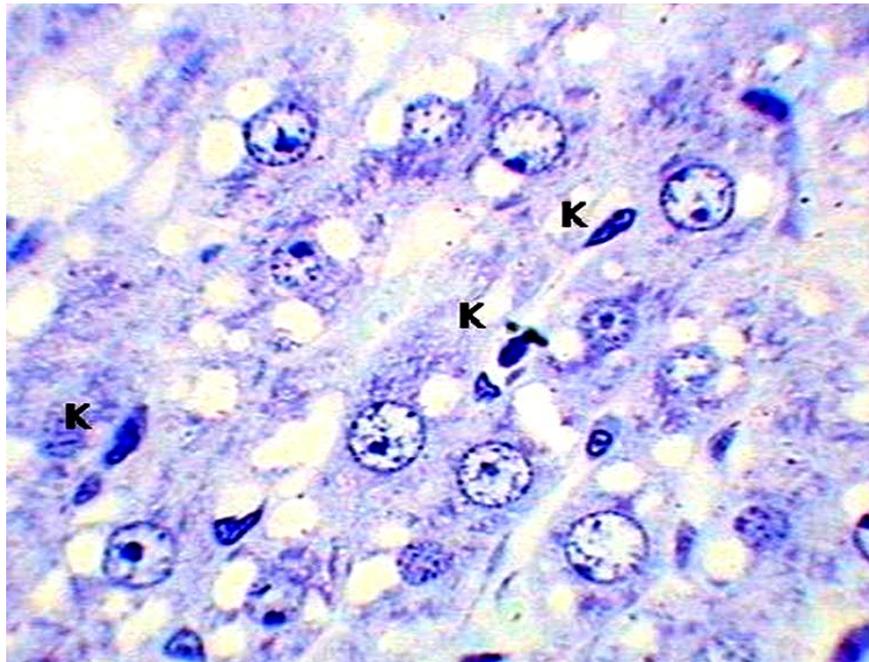
Fig. 3: A photomicrograph of a section in the liver of a treated rat showing a focal area of more cytoplasmic vacuolation than the previous figure and many Kupffer cells (k). (Toludine blueX1000).
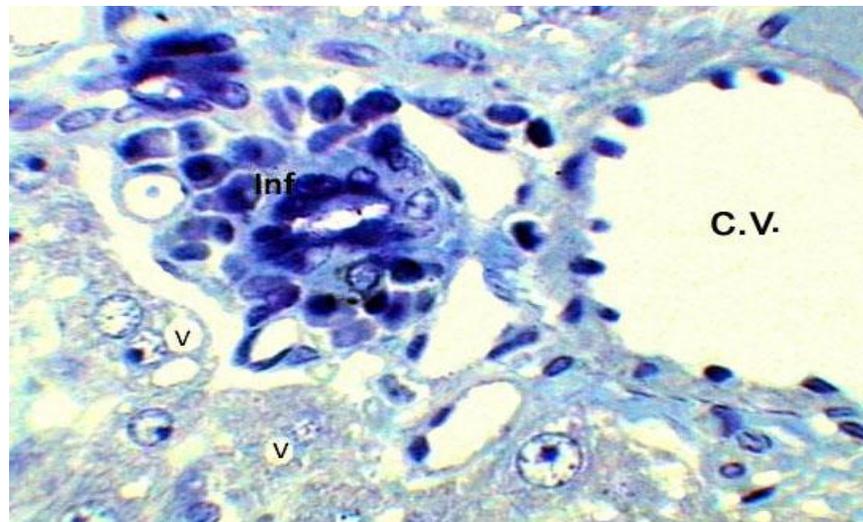

Fig. 4: A photomicrograph of a section in the liver of treated rat showing focal mononuclear cellular infiltration (Inf), central vein (C.V.), cytoplasmic vacuoles (v). (Toludine blue X1000).
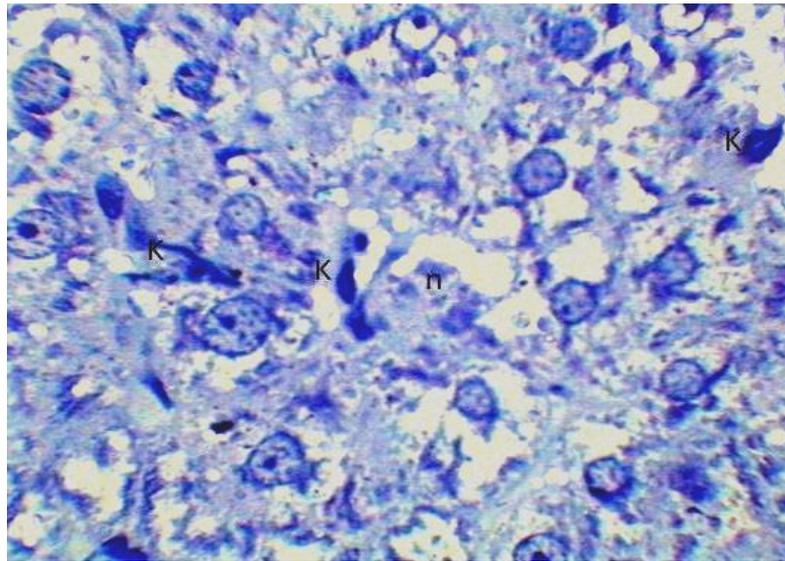
Fig. 5: A photomicrograph of a section in the liver of treated rat showing a focal group of hepatocytes with distorted and severely vacuolated cytoplasm , and some necrotic cells (n). Kuffer cells (K) were also seen. (Toludine blue X 1000).
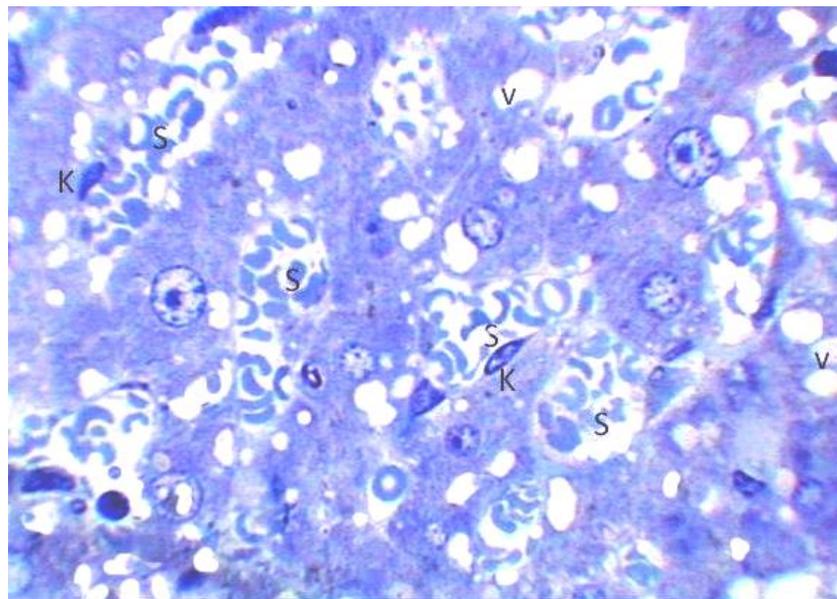

Fig. 6: A photomicrograph of a section in the liver of treated rat showing severely congested blood sinusoids (S) with many kuffer cells (K) (Toludine blue X 1000).
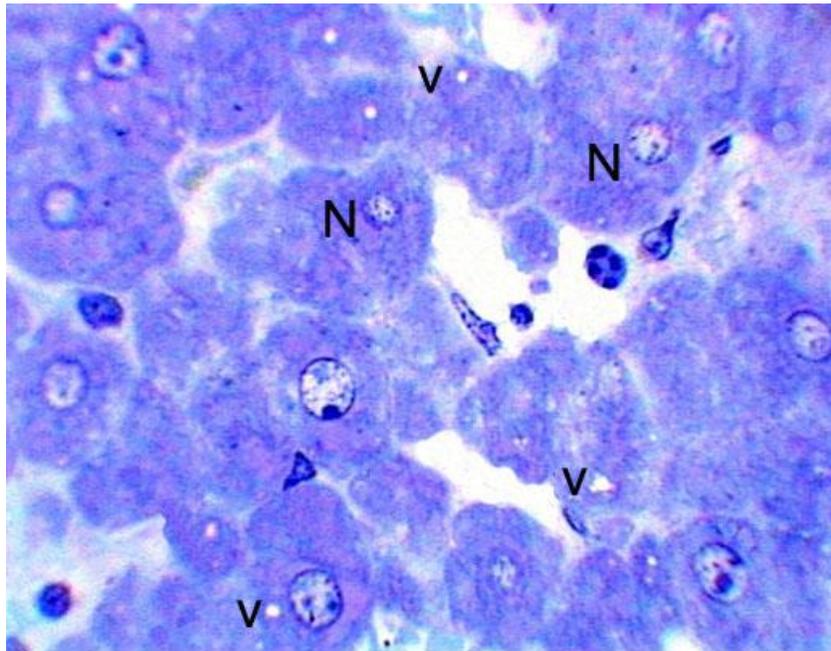
Fig. 7: A photomicrograph of a section in the liver of experimental rat two months after stoppage of treatment showing hepatocytes with lesser and smaller cytoplasmic vacuoles (v) and others apparently normal ones (N) (Toludine blue X 1000).
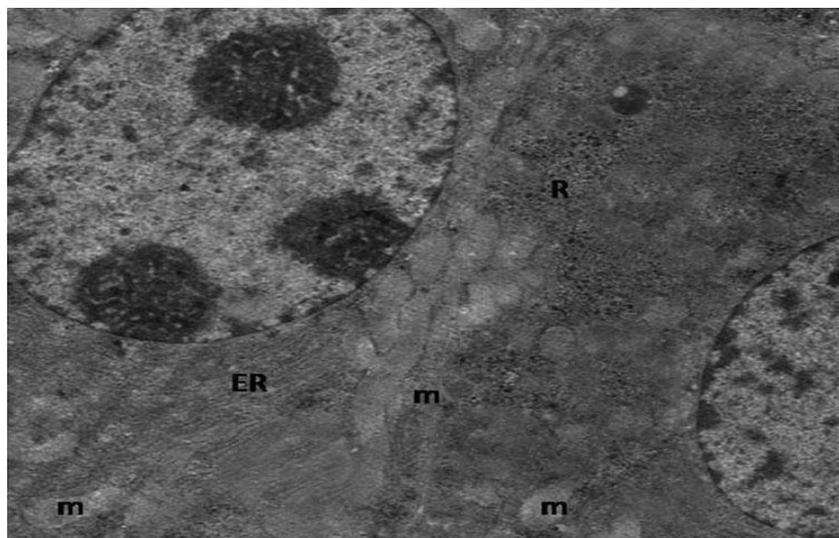

Fig. 8: An electron micrograph of ultrathin section of liver control rat showing normal hepatocyte with oval nuclei and cytoplasm crowded with organelles particularly endoplasmic reticulum (ER), glycogen particles (R), mitochondria (m). (Uranyl acetate and Lead citrate X 2000).
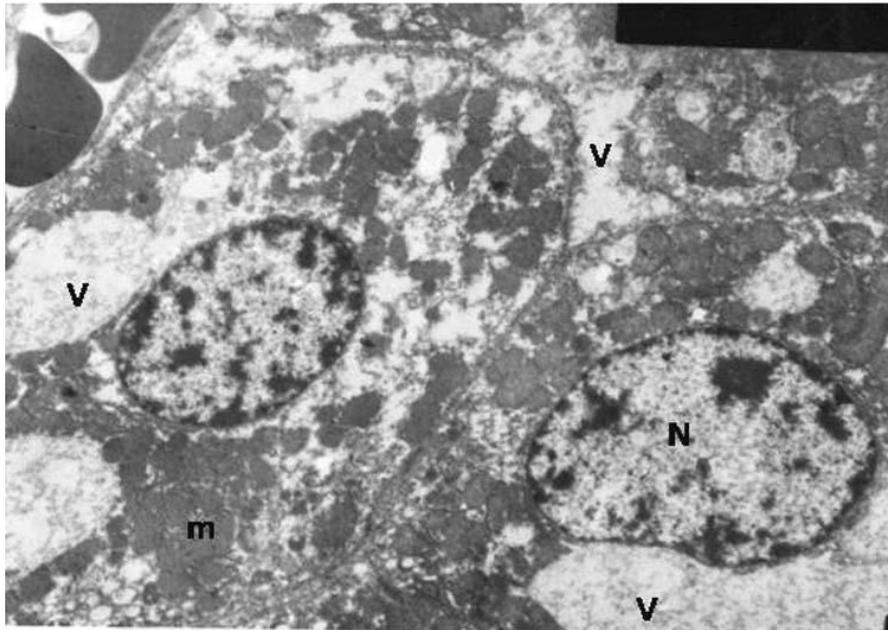
Fig. 9: An electron micrograph of ultrathin section of liver of treated rat showing hepatocytes with cytoplasmic vacuoles (dilated cisternae of rER) (V), irregular nucleus (N) and many small mitochondria with ill distinct cristea (m). (Uranyl acetate and Lead citrate X 5000).
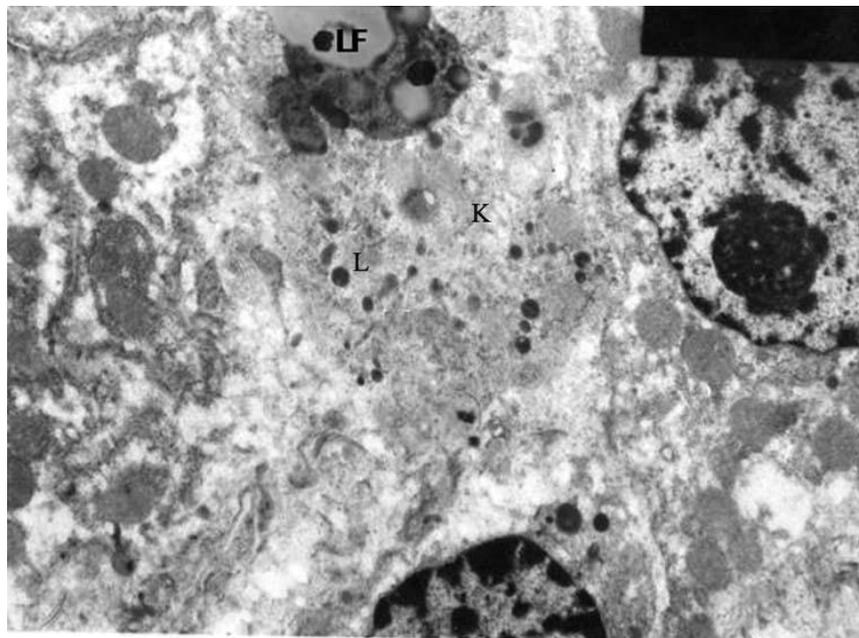

Fig. 10: An electron micrograph of ultrathin section of liver of treated rat showing hepatocytes with cytoplasmic vacuoles and many small mitochondria with ill distinct cristea. A Kupffer cell (K) with many secondary lysosomes (L) and lipofuscin deposits (LF) is evident. (Uranyl acetate and Lead citrate X 5000).
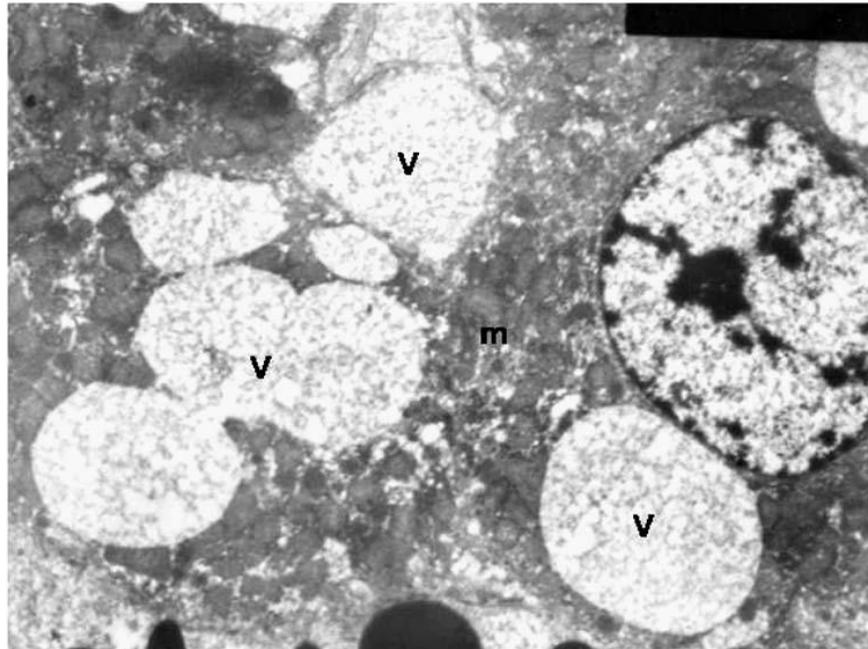
Fig. 11: An electron micrograph of ultrathin section of liver of treated rat showing hepatocytes with many large cytoplasmic vacuoles (V) and many small mitochondria with ill distinct cristea (m). (Uranyl acetate and Lead citrate X 7000).
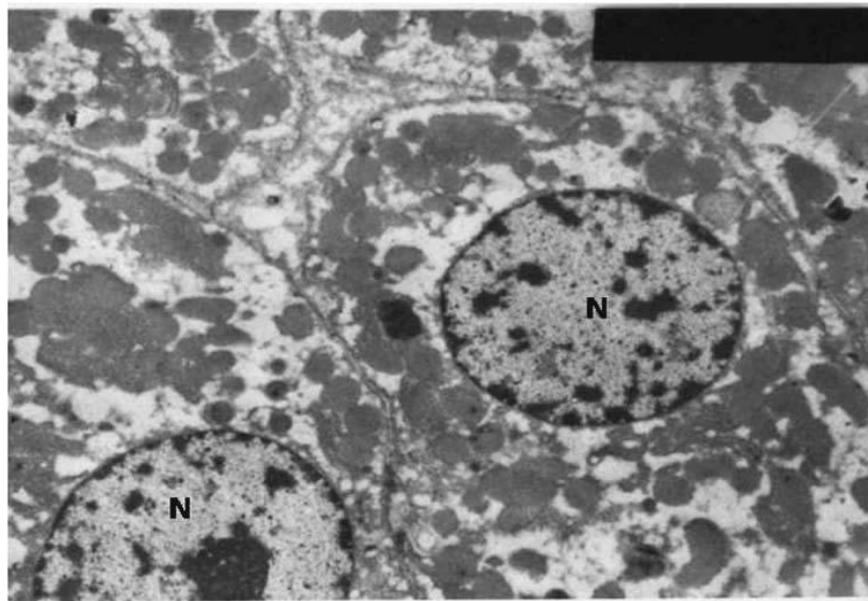

Fig. 12: An electron micrograph of ultrathin section of the liver of experimental rat one months after stoppage of treatment showing hepatocytes with lesser and smaller cytoplasmic vacuoles than the treated ones and regular nulei (N). (Uranyl acetate and Lead citrate X 5000).

IV. DISCUSSION

Cisplatin is one of the most commonly used potent antineoplastic agents for treatment of a wide range of cancers ([9], [10]). Despite its excellent anticancer activity, the clinical use of cisplatin is often limited by its undesirable sever toxic side effects that interfere with therapeutic efficacy ([11], [12], [13]).

The several toxicities and sides effects of cisplatin included hepatotoxicity ([14], [15]). and nephrotoxicity [10]. Although, the precise mechanism for the cisplatin- induced toxicity is not well understood, many studies documented that cisplatin is preferentially taken up and accumulated in the liver and kidney cells, resulting in the enhancing production of reactive oxygen species ([16], [17]).. In their study, Mora et al., (2003) [18] added that a decrease in antioxidant enzymes resulted from cisplatin induced tissue toxicity.

Moreover, the development of therapies to prevent the appearance of cisplatin- induced tissue toxicities has focused on administration of antioxidants along with cisplatin treatment. Thus, many studies for protective effects against cisplatin induced tissue toxicities have been reported for extracts of natural products and dietary antioxidant [19].

The current study was designed to evaluate the toxic effects of the low dose of cisplatin for two month on the adult male rats, then after cutting of the treatment for two months. We found that cisplatin significantly decreased the body weights in the treated rats compared to the controls in agreement with King and Berry, (2001) [20]**,** who suggested that hepatotoxicity might have contributed to this loss. Also Leite et al., (2009) [21] stated that mice treated with high doses of free cisplatin showed a greater loss of body weight and more delayed recovery time. Dissection of these animals indicated that the loss of the body weights was mostly due to loss of the mass of skeletal muscles and adipose tissue as previously suggested by Devlin et al., (1997) [22]. Moreover, the reduction in body weight of the animals in this study can be correlated with the decreased food intake by the animals of treated group observed during the experimental period. However, in the current study the mean body weight was significantly increased in the recovery group indicating evidence of the effect of abstinence of the drug.

In the present study the histological findings of the treated liver illustrated hepatotoxicity manifested by cytoplasmic vacuolization of hepatocytes, inflammatory cells and congested blood sinusoids, apparent increase in the number of kuffer cells and focal mononuclear infiltration.

El-Sayyad et al. (2009) [7] and Abdelmeguid et al., (2010) [23]Supporting our results also observed histological abnormalities in the liver including inflammatory infiltration, dissolution of hepatic cords which appeared as empty vacuoles and dilated blood sinusoids. In agreement with our study, Liao et al., (2008) [24] mentioned that the liver is known to accumulate significant amounts of cisplatin, thus hepatotoxicity and its histological abnormalities were associated with cisplatin treatment. In contrast, Leite et al., (2009) [21] stated that, concerning hepatotoxicity, no histopathological alteration was observed after treatment by cisplatin.

In this work we found some specimens showing distortion of hepatocytes with rarified cytoplasm and focal necrosis while other areas revealed severely congested blood sinusoids. El-Sayyad et al., (2009) [7] also revealed that many hepatocytes showed marked degeneration in hepatic cords in addition to karyomegally and pyknotic nuclei indicating apoptosis.

In the present work, ultrastructure examination showed hepatocytes with cytoplasmic vacuoles (dilated cisternae of endoplasmic reticulum, irregular nuclei and many small mitochondria with ill distinct cristae. Apparent increase in the presence of kuffer cells with many secondary lysosomes and lipofuscin deposits were noticed. In some cells the observed cytoplasmic vacuoles were remarkably large. Stewart et al., (1982) [14] and El-Sayyad et al., (2009) [7] demonstrated that the ultrastructure of liver sections showed dense collection of inflammatory cells including macrophages and fibrocytes forming pattern of cirrhotic liver. Also they supported our study mentioning

that the cytoplasm contained atrophied mitochondria with ill defined cisternae and vesiculated rough endoplasmic reticulum.

Hae et al., (2009) [25]claimed that cisplatin accumulation shows toxicity to normal tissues and this is the cause of hepatotoxicity. Nuria et al., (2008) [26] Moreover said that, cisplatin toxic side effects seemed to be associated with mitochondrial injury both with in vivo treatment with the drug and in vitro exposure to it. They also showed that cisplatin caused a direct and significant impairment of mitochondrial DNA and RNA synthesis.

In the current study, signs of recovery appeared in the form of lesser and smaller cytoplasmic vacuoles in the hepatocytes while others appeared normal and the nuclei appeared more or less similar to those of the control. In contrast, Herrera et al., (1998) [27]deducted that cisplatin inhibits liver regeneration.

V. CONCLUSION

In conclusion, our results may provide histological evidence of hepatotoxicity caused by cisplatin and, on the other hand, the possibility of recovery. This can be used as the basis for determining the appropriate dose of this drug in order to ameliorate its hepatotoxic effects. Administration of the lowest therapeutic dose of cisplatin with some protective measures (antioxidants and others) can be recommended.

REFERENCES

[1] Ivana, S. T.; Branka,I. O.; Nataza, Z.; Ordevici, S.; Markovici, A.; Štajni, G.; Zorica, S. and Saicic, C. (2010): Effects of cisplatin on lipid peroxidation and the glutathione redoxstatus in the liver of male rats.The protective role of selenium. *Arch. Biol. Sci.*, Belgrade, 62 (1), 75-82.

[2] Lippard, S. J. and. Berg, J. M. (1994): "Principles of Bioinorganic Chemistry" University Science Books: Mill Valley, CA; ISBN 0-935702-73-3.

[3] Liu, J.; Liu, Y.; Habeebu, S.S. and Klaassen, C.D. (1998): Metallothionein (MT)-null mice are sensitive to cisplatin-induced hepatotoxicity. Toxicol Appl Pharmacol; 149: 24-31.

[4] Pruefer, F.G.; Lizarraga, F.; Maldonado, V. and Melendez,-Z. J. (June 2008): "Participation of Omi Htra2 serine-protease activity in the apoptosis induced by cisplatin on SW480 colon cancer cells". *J Chemother* 20 (3): 348–54.

[5] Fawcet, D.W. and Jensh, R.P. (2001): Bloom& Fawcett: Concise Histology, Liver and Gall bladder. Chapter 18, 212 – 217.

[6] Ross, M.H. and Pawlina, W. (2005): Histology with correlated cell and molecular biology, Digestive System III, Liver. Chapter 18, 576- 589.

[7] El- Sayyad, H.I.; Ismail, M.F.; Shalaby,F.M.; Abou-El-Magd, R.F. and Gaur,R.L. (2009): Histopathological effects of Cisplatin, Doxorubicin and 5- flurouracil (5-FU) on the liver of male albino rats.Int. J.Biol.Sci., 5: 466- 473.

[8] Hayat, M., (1986): Basic Techniques for Transmission Electron Microscopy. 2nd Edn. Academic Press, Baltimore.

[9] Wang, G. Reed, E. and Li, Q.Q. (2004): Molecular basis of cellular response to cisplatin chemotherapy in non- small cell lung cancer (Review). Oncol. Rep., 12: 955- 965.

[10] Park, H.R.; Ju, E.J.; Jo, S.K.; Kim,S.H. and Yee, S.T. (2009): Enhanced antitumour efficacy of cisplatin in combination with hemo HIM in tumour- bearing mice. BMC Cancer, 9: 85- 85.

[11] Aly, M.S.; Ashour, M.B.; El-Nahas and Abo-Zeid, (2003): Genotoxicity and cytotoxicity of the anticancer drugs gemcitabine and cisplatin, separately and in combination: In vivo studies. J. Biol. Sci., 3: 961- 972.

[12] Ajani, J.A. (2008): Optimizing docetaxel chemotherapy in patients with cancer of the gastric and gastroesophageal junction: Evolution of the docetaxel, Cisplatin and 5-fluorouracil regimen. Cancer, 113: 945- 955.

[13] Dank, M. ; Zaluski, J.; Barone. C.; Valvere, V. and Yalcin, S. (2008): Randomized phase III study comparing irinotecan combined with 5-fluorouracil and folinic acid to cisplatin combined with 5- fluorouracil in chemotheraoy naïve patients with advanced adenocarcinoma of the stomach oresophagogastric junction Ann. Oncol., 19: 1450- 1457.

[14] Mansour, H.H.; Hafez, H.F. and Fahmy, N.M. (2006): Silymarin modulates Cisplatin 10mg Kg^{-1}platin induced oxidative stress and hepatotoxicity in rats. J.Biochem. Mol. Biol., 39: 656- 661.

[15] Pratibha, R.; Sameer, R..; Rataboli, P.V; Bhiwgade, D.A. and Dhume, C.Y. (2006): Enzymatic studies of cisplatin induced oxidative stress in hepatic tissue of rats. Eur. J. Pharmacol., 532: 290- 293.

[16] H. El-Beshbishy, Bahashwan, S, Ali, H, and H. Fakher, "Abrogation of cisplatin-induced nephrotoxicity in mice by alpha lipoic acid through ameliorating oxidative stress and enhancing gene expression of antioxidant enzymes", European Journal of Pharmacology, vol. 668, pp. 278-284, 2011.

[17] Stewart, D.J.; Bemjamin, R.S.; Luna, M.; Feun, L.; Caprioli, R.; Seifert, W. and Loo, T.L. (1982): Human tissue distribution of platinum after cis-diamminedichloroplatinum. Cancer Chemother. Pharmacol. 10: 51- 54.

[18] Mora, L.O.; Antunes, L.M.; Francescato, H.D. and Bianchi, M. (2003): The effects of oral glutamine on cisplatin-induced nephrotoxicityin rats. Pharmacol. Res., 47:517- 522.

[19] Behling, E.B.; Sendao, M.C.; Francescato, H.D.; Antunes, L.M.; Costa,R.S. and Bianchi- Mde, L. (2006): Comparative study of multiple dosage of quercetin against cisplatin- induced nephrotoxicity and oxidative stress in rat kidneys. Pharmacol. Rep., 58:526- 532.

[20] King, P.D.and Berry, M.C. (2001): Hepatotoxicity of chemotherapy. Oncologist; 6:162-76.

[21] Leite, E.A.; Giuberti, C. S.; Wainstein, A.J.; Wainstein, A.P.; Coelho, L.G.; Lana, A.M.; Savassi, R. R. and De Oliveira, M.C. (2009): Acute toxicity of long-circulating and pH-sensitive liposomes containing cisplatin in mice after intraperitoneal administration. Life Sci. May 8;84(19-20):641-9.

[22] Devlin, T.M. (1997): Text book of biochemistry: with clinical correlation, 4th ed. New York: John Wiley and Sons Inc; 553.

[23] Abdelmeguid, N.E.; Chmaisse, H.N.; Abdou Zeinab, N.S. and Moharram,B. (2010): Silymarin ameliorates Cisplatin-induced hepatotoxicity in rats: Histopathological and Ultrastructure Studies. Pakistan journal of Biological Sciences, 13(10): 463- 479.

[24] Liao, Y.; Lu, X.; Lu, C.;Li, G.; Jin, Y. and Tang, H. (2008): Selection of agents for prevention of cisplatin-induced hepatotoxicity. Pharmacol Res; 57:125-31.

[25] Hae, R. P.; Eun, J. J.; Sung, K. o.; Uhee, J.; Sung, H. K. and Sung, T. Y. (2009): Enhanced antitumor efficacy of cisplatin in combination with HemoHIM in tumor-bearing mice. BMC- Cancer; 17 March.

[26] Nuria, G.; Acisclo, P.M.; Mercedes, F.; Jose, M.L.; Patricio, F. S. and Jose, A. E. (2008): Cisplatin- mediated impairment of mitochondrial DNA metabolism inversely correlates with glutathione levels. Biochem. J.; 414, 93- 102.

[27] Herrera, M.G.; Palomero, M.F.; Macias, R.I.; Serrano, M.A. and Marin. J.J. (1998): Comparison of the effects of bischolyl glycinatecholoro-platinum (II) versus cisplatin on liver regeneration after partial hepatoctomy. Anticancer Res. Sep.- oct.; 18(5A): 3555-63.