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Silymarin Ameliorates Cisplatin-Induced Hepatotoxicity in Rats: Histopathological and Ultrastructural Studies

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Abstract: The benefit of silymarin, a plant extract with strong antioxidant activity against hepatotoxicity induced by cisplatin in rats was investigated in this study. Cisplatin is one of the most effective chemotherapeutic drugs, yet it alone does not achieve a satisfactory therapeutic outcome and at high doses it can produce undesirable side effects. Five equal-sized groups (18 rats each) of male Sprague Dawley rats [control, vehicle; cisplatin; silymarin 2 h after cisplatin injection; and silymarin 2 h before cisplatin injection] were used. Histopathological and ultrastructural observation of livers were carried out using light and electron microscopy. Results documented that cisplatin produced behavioral, external features animal changes, as well as hazard pathological picture changes in liver where most hepatocytes appeared diminutive with vacuolated cytoplasm, sinusoids dilated and organelle disorganized. These results revealed that cisplatin may be toxic and terminates in complex liver damage. Administrations of silymarin 2 h after cisplatin, significantly increase the body weight returning it to normal, yet it failed in complete protection against the pathological alteration caused by cisplatin. Pretreatment with silymarin 2 h before cisplatin significantly decreased the pathological changes induced by cisplatin and appeared highly protective. These results suggested that silymarin possess protective effects against cisplatin hepatotoxic action in animal models. Since, no significant toxicity of silymarin is reported in human studies, this plant extract can be used as a dietary supplement by patients taking anticancerous medications and might serve as a novel combination agent with cisplatin since it plays a significant role in reducing its toxicity.

Key words: Cisplatin, light microscopy, liver, silymarin, electron microscopy

INTRODUCTION

Chemotherapy has been one of the major therapeutic modalities commonly used for the treatment of a variety of cancer patients (Borek, 2004). However, in many cases, chemotherapy or radiotherapy alone cannot achieve a satisfactory therapeutic outcome, namely the complete remission of tumors and induces severe side effects at therapeutically effective doses (Park *et al.*, 2009).

Cisplatin [cis-diamminedichloroplatinum (II)] (CDDP), a platinum-containing anticancer drug, is one of the most commonly used potent antineoplastic agents for the treatment of a wide range of cancers (Wang *et al.*, 2004). Despite its excellent anticancer activity, the clinical use of cisplatin is often limited by its undesirable severe toxic side effects that interfere with its therapeutical efficacy (Aly *et al.*, 2003; Ajani, 2008; Dank *et al.*, 2008). Although,

the precise mechanism for the cisplatin-induced toxicity is not well understood, cisplatin is preferentially taken up and accumulated in the liver and kidney cells (Stewart *et al.*, 1982; Ravi *et al.*, 1995). While nephrotoxicity of CDDP has been recognized as the most important dose-limiting factor, little is known about CDDP induced liver injury (Zicca *et al.*, 2004). Extensive investigations have been conducted on the hepatotoxicity as well as general organ toxicity of this anticancer drug (Kim *et al.*, 2004; Pal *et al.*, 2008). These include light and electron microscopic studies of various organs and biochemical studies of liver enzymes (Kim *et al.*, 2005; Venkatesan *et al.*, 2008). There is very limited information concerned with the effects of these drugs on histopathology and ultrastructure of liver cells. Further, a variety of agents including antioxidants have been shown to attenuate the hepatotoxicity of cisplatin (Yuce *et al.*,

2007). Oxidative stress is one of the most important mechanisms involved in CDDP-induced toxicity resulting in the enhanced production of reactive oxygen species, reduction in the mitochondrial membrane potential (Saad *et al.*, 2004) and the decrease in antioxidant enzymes (Mora *et al.*, 2003). Therefore, antioxidants administered before cisplatin treatment to act against toxicity (Lee *et al.*, 2007).

Much attention has been given to the possible role of dietary antioxidants in protecting liver against cisplatin-induced toxicity (Behling *et al.*, 2006). Phytochemicals, including flavonoids, are naturally occurring antioxidants that possess various pharmacological actions and therapeutic applications (Hasanloo *et al.*, 2005; Karimi *et al.*, 2005). So, due to their phenolic structures they inhibit free radical-mediated processes (Singh *et al.*, 2005). Many antioxidant compounds have been studied as chemoprotective agents such as, curcumin, selenium and other dietary components that scavenge free radicals formed by exposure to CDDP (Antunes *et al.*, 2001; Silva *et al.*, 2001). Silymarin, an antioxidant flavonoid complex derived from herb milk thistle (*Silybum marianum*), has long been used as a dietary supplement for hepatoprotection (Khadr *et al.*, 2007; Laekeman *et al.*, 2003; Eminzade *et al.*, 2008). These properties seem to be due to their ability to scavenge free radicals and to chelate metal ions (Borsari *et al.*, 2001; Agarwal *et al.*, 2006). Silymarin has been shown to be safe in animal models and no significant adverse reactions were reported in human studies (Hogan *et al.*, 2007). Thus, the aim of the present research was to study the effect of cisplatin (CDDP) on liver tissues in rats and to investigate on the other hand, the role of silymarin in experimentally induced hepatotoxicity by CDDP.

MATERIALS AND METHODS

Animals and chemicals: Ninety adult healthy male Sprague Dawley rats (*Rattus norvegicus*), weighing approximately 150 g each, were used in this work. A stock of inbreeding rats was obtained by brother-sister mating in the animal house of Beirut Arab University during May 2008. Throughout the experiment, all rats were housed conventionally in cages, four rats per cage and were adapted to constant conventional and controlled conditions with temperature of $23\pm 2^{\circ}\text{C}$, relative humidity 60-70% and artificial regimen of light: dark-10: 14 h (light on 7:00 a.m.) at least one week prior to the experiment for acclimatization. Rats were provided with standard food diet and drinking tap water *ad libitum*.

Cisplatin [(CDDP) or cis-diamminedichloroplatinum (II)] was obtained as yellowish crystalline powder, soluble in physiological saline solution and purchased from Sigma-Aldrich, CAS Number 15663-27-1 (P4394 Sigma). Silymarin was purchased from Sigma-Aldrich Chemical Co. (S0292).

Experimental design and procedures: The animals were divided into five equal sized groups (18 rats/each). In the first group (GIa) animals received no chemical treatment. The second group of animals (GIb) served also, as controls and were dosed with vehicle solutions only (propylene glycol and saline; 75/25 v/v). In the third group (GIIa) animals were i.p injected with single dose of cisplatin dissolved in normal saline (5 mg kg^{-1}) at the beginning of the experiment (Mansour *et al.*, 2006). In the fourth group (GIIb) animals were i.p. injected with single dose of cisplatin (5 mg kg^{-1}), followed after 2 h by i.p., injection of silymarin (50 mg/kg/day) dissolved in vehicle solution as in the second group (Karimi *et al.*, 2005). In the fifth group (GIIc) animals were i.p., injected with silymarin (50 mg/kg/day) dissolved in vehicle solution as in the second group, 2 h before CDDP injection.

During the experimental period, animal behavior and body weight were recorded 4 times a week. Randomly selected animals of different groups were sacrificed by decapitation after 6 and 12 days. Liver tissues were isolated, weighed, cleaned from the adhering matter and washed with saline, cut into small pieces then fixed in 10% buffered formalin and dehydrated in ascending grades of ethanol concentration, cleared in xylene and embedded in paraffin wax. Sections ($5\text{--}6\text{ }\mu\text{m}$ thick) were cut and stained with hematoxylin and eosin method and examined with light microscope.

For electron microscopic study, anaesthetized rats from all groups were rapidly dissected and then perfusion fixation with formalin-glutaraldehyde fixative (F_1G) in phosphate buffer was performed. Liver tissues (1 mm^3) were removed and dropped as soon as possible into F_1G buffered with 0.1 M phosphate at 4°C . After rinsing in the buffer, samples were post-fixed in 2% OsO_4 for 2 h at 4°C in the same buffer. The specimens were washed and dehydrated at 4°C through a graded series of ethanol. Tissues were then treated with propylene oxide solution and embedded in a mixture of 1:1 of Epon-Araldite. Specimens were embedded for 1 h. Polymerization was done in the oven at 65°C for 24 h. Ultrathin sections ($50\text{ }\mu\text{m}$) were cut on LKB ultratome, then mounted on copper grids, double stained with uranyl acetate and lead citrate and investigated on a JEOL 100CX TEM.

All the data was expressed as Mean \pm SE. The statistical significance was evaluated by ANOVA using

SPSS Version 16 and the individual comparison were obtained by LSD method. Values were considered statistically significant when $p < 0.05$. In order to discern the possible interaction between cisplatin and silymarin, two-way analysis of variance was used.

RESULTS

Effects of chemical used on animal behavior: Animals of all groups showed no obvious symptoms or signs of toxicity throughout the experiment. Moreover, they did not exhibit any case of mortality or death. Rats of the control groups (GIa and Ib), suffered no serious illness and they remained bright, active and alert. Rats dosed with single injection of cisplatin (GIIa) (5 mg kg^{-1}) also, showed no adverse effects and remained alert until about the 10th day of the experiment and then they became slightly nervous and less active. Besides; minimal loss of furring of the skin was recorded in rats of this group. On the other hand, it was observed throughout the experiment, that rats treated with silymarin (GIIb) 2 h after cisplatin injection and those received the same dose of silymarin 2 h before cisplatin injection (GIIc); showed no changes in any specific behavioral or clinical symptoms apart from increasing activity especially after the 7th day of the experiment.

Effects of chemical used on body weight and liver weight:

It was noticed that in cisplatin treated rats, water and pellet diet consumption was decreased, if compared to normal. Also, a significant decrease in the body weight gain after 6 and 12 days as compared to controls was noticed. However, the body weight of rats exposed to cisplatin and silymarin (GIIb and GIIc) were similar to that of controls (Table 1). Two way analysis of variance indicated that cisplatin and silymarin showed no interaction between time and doses on body weight.

Moreover, a significant decrease in liver wet weight as well as hepatosomatic index was noticed in cisplatin-treated rats (GIIa) after 12 days. Administrations of cisplatin along with silymarin significantly increase liver wet weight and hepatosomatic index in both groups receiving silymarin (GIIb and GIIc) after 12 days. Two way of analysis of variance has shown that both cisplatin and silymarin had significant interaction between time and doses on liver wet weight (Table 2).

Histopathological and ultrastructural findings of liver:

Light microscopical examination of hematoxylin and eosin stained liver sections of control rats (GIa) after 6 and 12 days from the beginning of the experiment revealed normal basic structure (Fig. 1a, b). Liver sections showed hexagonal lobules with central vein and peripheral

Table 1: Body weight gain in control and experimental rats

Groups	Body weight (g)		
	0 ¹ days	6 days	12 days
Control GIa	151.85±4.29 ^a	218.95±4.44 ^a	299.02±6.68 ^a
Vehicle GIb	152.5±3.73 ^a	219.66±5.28 ^a	230.82±7.43 ^a
Cisplatin GIIa	152.57±3.73 ^a	189.6±7.86 ^b	186.08±5.08 ^b
Silymarin 2 h after cisplatin GIIb	153±2.58 ^a	214.83±3.083 ^a	219.3±3.47 ^a
Silymarin 2 h before cisplatin GIIc	153.14±3.33 ^a	212.66±7.46 ^a	218.86±4.88 ^a
Results of one way ANOVA			
F-value	1.1	4.39	1.59
p-value	>0.05	<0.05*	>0.05

¹Insignificant interaction was observed between time and dose among groups by overall 2-way ANOVA ($n = 6$); $F = 0.839$, $p = 0.57$. Treatment effect, $p < 0.05$, $F = 30.712$; time effect, $p < 0.05$, $F = 5.344$. Data are Mean±SE. Means in the same column with different superscript letters are significantly different, $p < 0.05$ (one-way ANOVA followed by post-hoc LSD)

Table 2: Effects of cisplatin and silymarin on liver wet weight and hepatosomatic index among control and experimental rats¹

Groups	Time					
	Wet liver weight			Hepatosomatic index		
	0 days ²	6 days	12 days	0 days ²	6 days	12 days
Control GIa	9.56±1.41 ^a	9.40±1.11 ^a	9.36±0.69 ^a	0.04±0.02 ^a	0.04±0.05 ^{a,b}	0.03±0.03 ^a
Vehicle GIb	9.40±2.06 ^a	8.86±0.36 ^a	9.67±0.30 ^a	0.04±0.04 ^a	0.03±0.01 ^{a,b}	0.03±0.01 ^a
Cisplatin GIIa	9.02±1.03 ^a	8.5±0.74 ^a	7.66±0.40 ^b	0.05±0.02 ^a	0.04±0.04 ^{a,b}	0.029±0.02 ^b
Silymarin 2 h after cisplatin GIIb	9.03±1.60 ^a	10.04±0.44 ^a	11.28±0.28 ^c	0.04±0.02 ^a	0.04±0.01 ^{a,b}	0.04±0.009 ^c
Silymarin 2 h before cisplatin GIIc	9.30±1.19 ^a	10.58±0.37 ^a	11.79±0.33 ^c	0.044±0.01 ^a	0.05±0.01 ^{a,c}	0.05±0.01 ^c
Results of one way ANOVA (Dose)						
F-value	1.46	1.5	14.5	1.46	1.5	14.5
p-value	>0.05	>0.05	<0.05	>0.05	>0.05	<0.05

¹Data are Mean±SE ($n = 6$). Means in the same column with different superscript letter(s) are significantly different, $p < 0.05$ (one-way ANOVA followed by post-hoc LSD). ²Significant interaction was observed between time and dose among groups by overall 2-way ANOVA; $F = 2.83$, $p < 0.05$. Treatment effect, $p < 0.05$, $F = 33.37$; time effect, $p = 0.21$, $F = 1.55$

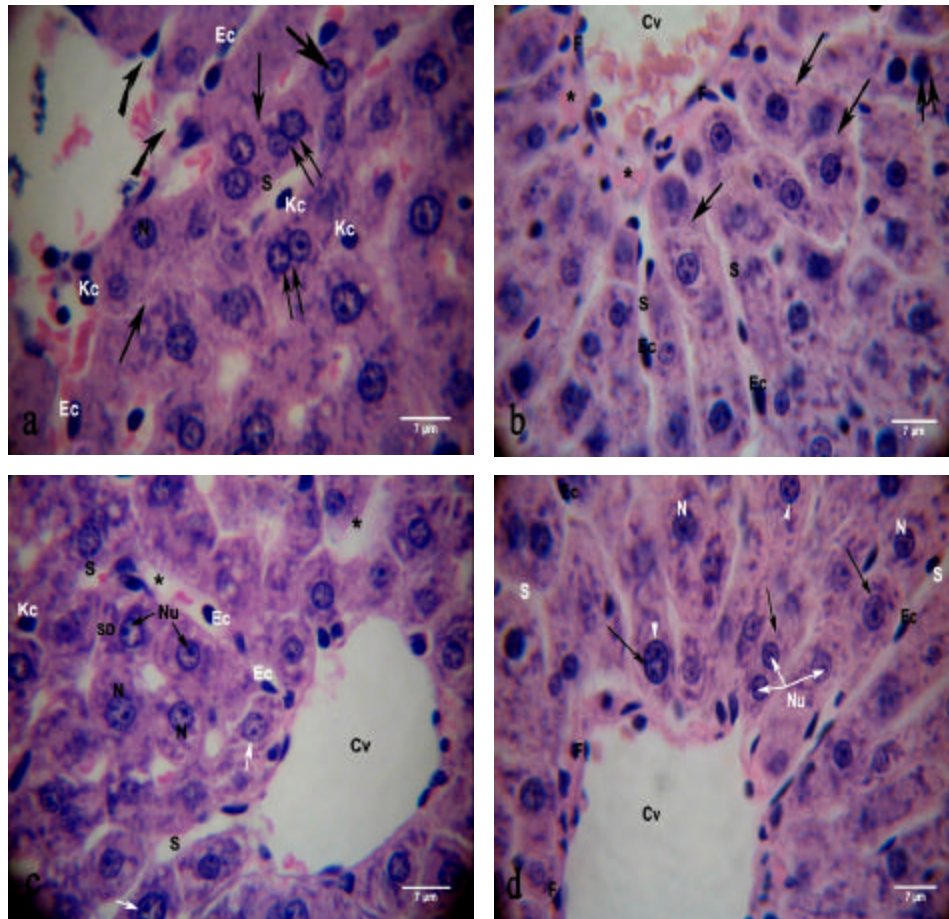


Fig. 1: Light micrographs of sections of rat livers in control and vehicle administrated groups. (a) Normal rat liver after 6 days, showing trabecular hepatocytes arrangement, Central vein (Cv) sinusoids (S) with Kupffer cells (Kc), Endothelial cells (Ec). Curved arrow: opening of sinusoids into central vein. Polygonal hepatocytes; spherical Nucleus (N), distinct nucleolus (thick arrow). Binucleated cells (double arrow), cytoplasm (arrow), (b) Normal rat liver after 12 days, Central vein (Cv), Fibroblast (F), Sinusoids (S), Endothelial cell (Ec), binucleated hepatocyte (double arrow), cytoplasm (arrow), erythrocytes (asterisk), (c) Vehicle administrated group after 6 days. Central vein (Cv), Sinusoids (S) with dilatations (asterisk), nucleus (Nu) of hepatocytes, eccentric nucleolus (Nu), peripheral chromatin material (arrow). Endothelial cell (Ec), Kupffer cell (Kc) and (d) Vehicle administrated group after 12 days. Hepatocytes: Nucleus (N), Nucleolus (Nu), nuclear envelope (arrow head), chromatin (thick arrow), Cytoplasm (arrow). Sinusoids (S), Kupffer cell (Kc), Endothelial cell (Ec), Fibroblast (F) (Hematoxylin and eosin stained section)

triads embedded in connective tissue. Hepatocytes were arranged in trabecules running radiantly from the central vein and were separated by sinusoids containing Kupffer cells. Also, hepatocytes of control exhibited heterogeneity in morphology within the pericentral and periportal areas of the hepatic acinus: the pericentral hepatocytes were larger than those of periportal area. In control preparations, hepatocytes appeared polygonal in

shape and mononucleated (Fig. 2a-c). Most of their nuclei appear centrally located with one prominent nucleolus. However, it is worthy to mention that two nucleoli were evident in the nuclei of some hepatocytes. The chromatin appears regularly distributed within the nucleoplasm. Moreover, many hepatocytes appeared binucleated.

In vehicle administered group (GIb) (Fig. 1c, d), liver sections did not show any alteration from normal

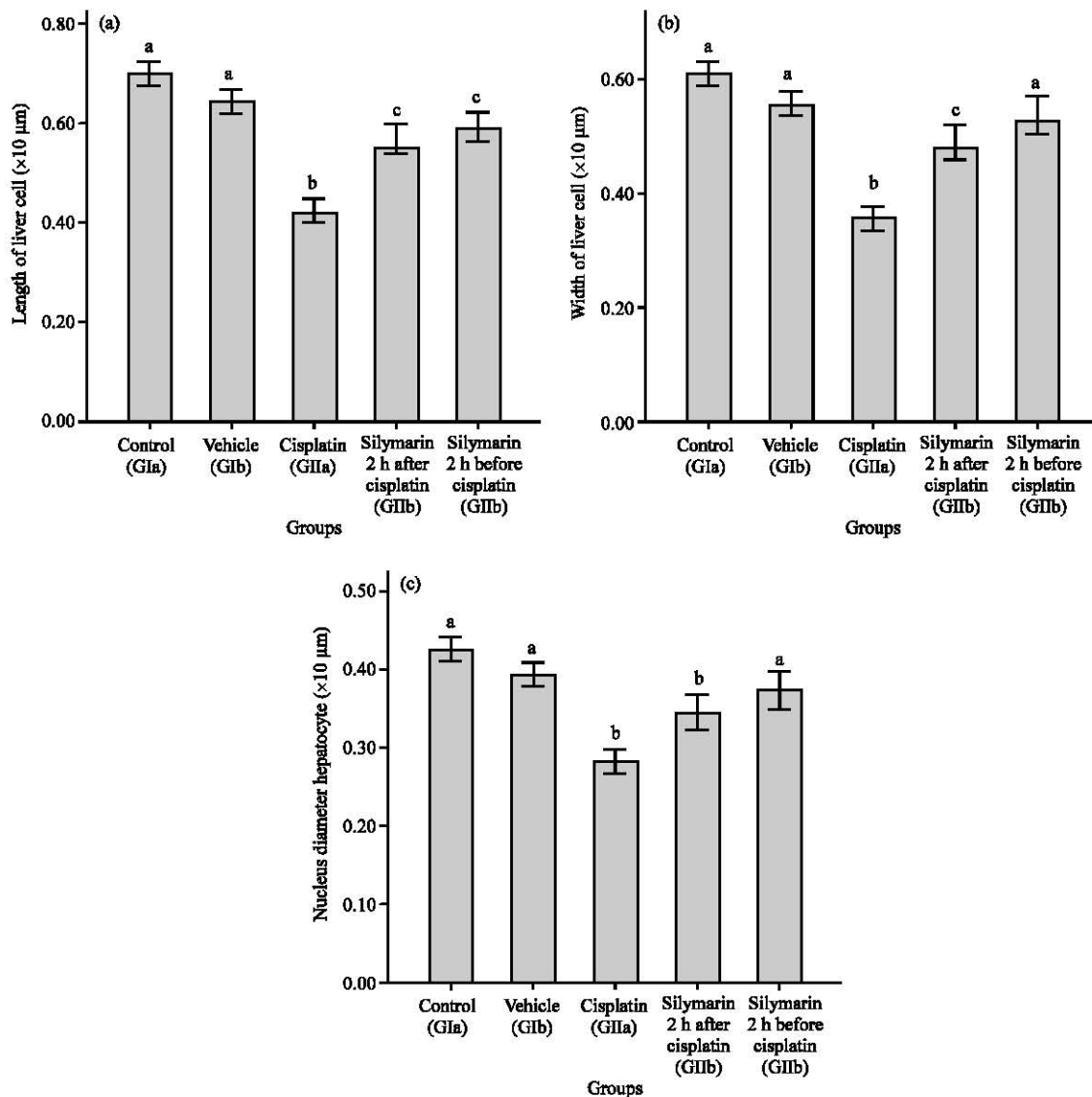


Fig. 2: (a) Illustrating changes in liver cellular length (μm), (b) illustrating changes in liver cellular width (μm) among control and experimental groups. Cisplatin administration; G1Ia shows the highest significant atrophy of liver cell dimensions when compared to control, G1a and vehicle, G1b. Note also, administration of silymarin 2 h after cisplatin and silymarin 2 h before cisplatin; G1Ic exhibit the lowest significant damage induced by cisplatin on liver cellular length as compared to controls and (c) showing changes in the diameter (μm) of the hepatocyte nucleus among control and experimental groups. Cisplatin, G1Ia shows significant pyknotic nuclei with the highest significant decrease in diameter and the administration of silymarin 2 h after cisplatin, G1Ib attains the lowest significant destructive effect induced by cisplatin on nuclei of liver cells as compared to controls. Data are Mean \pm SE, n = 6. Bars with different letters are significantly different, $p < 0.05$ (two-way analysis of variance ANOVA adjusted by post-hoc LSD)

architecture where pericentral and periportal hepatocytes revealed morphological features similar to those of control rats after 6 and 12 days (Fig. 2a-c).

In the present study, it was found that in cisplatin injected rats (G1Ia), the trabecular liver structure was

seriously affected and become highly blurred after 6 and 12 days (Fig. 3a, b). Cisplatin-induced degenerative changes in numerous hepatocytes; cells were significantly diminutive when compared to control (Fig. 2a, b). Morphometric measurements showed that

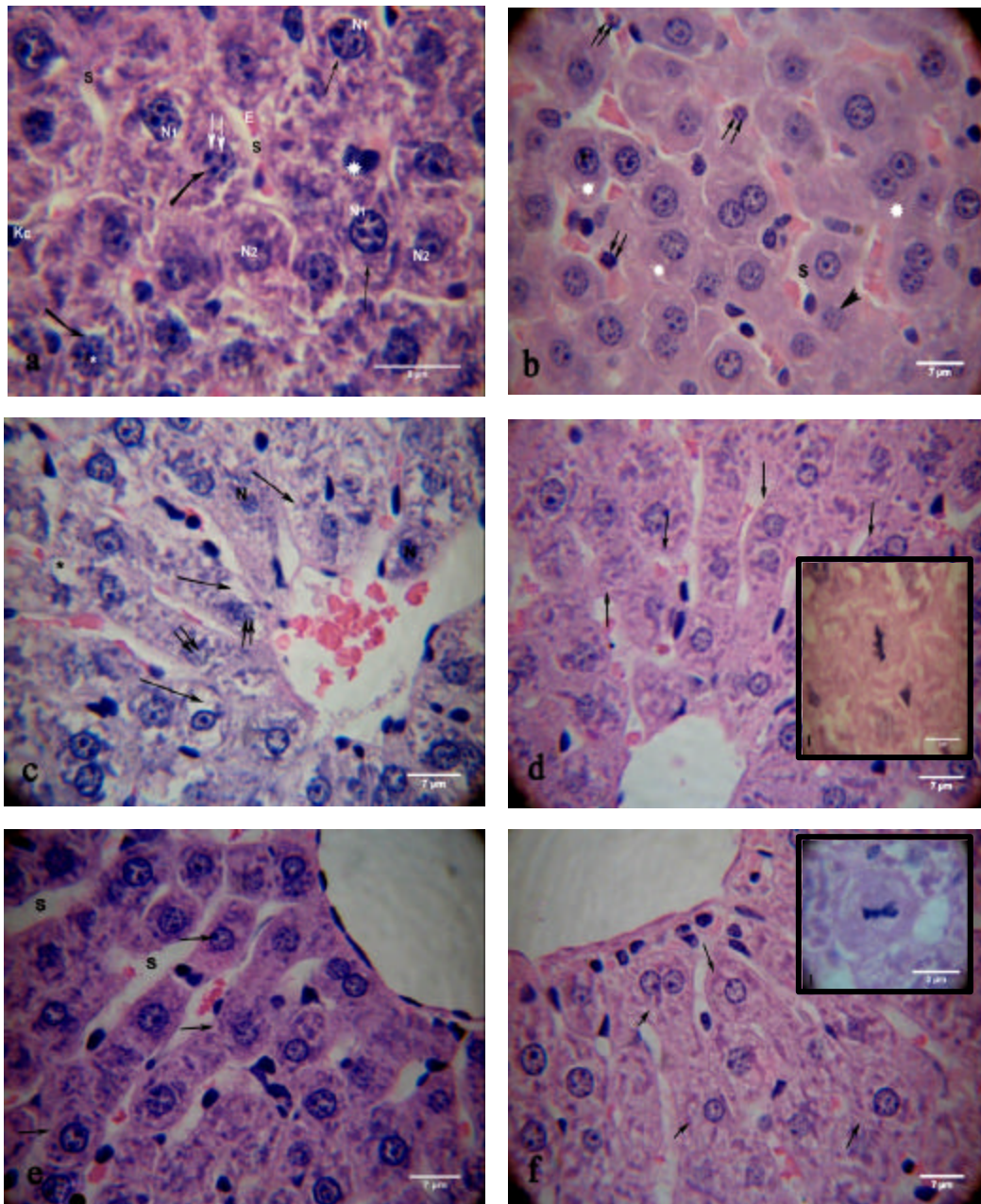


Fig. 3: Sections of rat livers in experimental groups. a) Cisplatin treated rat after 6 days, showing fragmented nuclear envelope (curved arrow), triozmy of nucleoli (double arrow), centric nucleolus (asterisk), cytoplasmic vacuoles (arrow), pyknotic nucleus (star). Large size nucleus (N1), small size nucleus (N2). Wide Sinusoids (S), Erythrocytes (E), Kupffer cell (Kc), (b) Cisplatin treated rat after 12 days; diminutive nucleus (arrow head), swollen cytoplasm (star), neutrophil infiltration (double arrow), (c) Silymarin 2 h after cisplatin treated rats after 6 days; hepatocyte Nucleus (N), karyoplasmic bodies (double arrow), few vacuoles (arrow), (d) Silymarin 2 h after cisplatin treated rats after 12 days, with normal hepatocytes (arrow). (1) Mitotic figure in pericentral hepatocytes, (e) Silymarin 2 h before cisplatin treated rats after 6 days; observe normal hepatococyte (arrow) with dilated sinusoid (S) and (f) Silymarin 2 h before cisplatin treated rats after 12 days; showing normal hepatocytes (arrow), numerous mitotic figure in pericentral (1) and periportal (2) hepatocytes (Hematoxylin and eosin stained sections)

most nuclei appeared significantly reduced in size (Fig. 2c) and showed uneven chromatin distribution (Fig. 3a, b). Besides, nuclear karyoplasmic bodies as well as nucleoli become significantly numerous. In addition, centric nucleoli and in some cases triozemy were evident among some nuclei. Moreover, fragmentation of the nuclear envelope was common in some nuclei. The cytoplasm of most hepatocytes was light and foamy filled with numerous vacuoles. The walls of the sinusoids become dilated and filled with RBCs and Kupffer cells. However, in few zones hepatocytes revealed necrotic changes; including pyknotic nuclei, lack of nucleoli and strongly acidophilic cytoplasm. Noteworthy, after 12 days cisplatin administration caused more serious and additional changes including tremendous cytoplasmic vacuolization, increased frequency of pyknotic nuclei and numerous Kupffer cells and blood infiltration overfilling sinusoids (Fig. 3b).

In rats exposed to silymarin 2 h after cisplatin injection (GIIb), 6 and 12 days of treatment revealed that the trabecular structure of the liver lobule was slightly blurred at certain foci; where hepatocytes appeared less organized improperly radiating away from a congested central vein (Fig. 3c, d). Moreover, post-treatment with silymarin increased significantly hepatocytes cellular and nuclear dimensions (Fig. 2a, b). Nuclei of hepatocytes of this groups appeared regular in their outlines, few possessed centric nucleoli, with almost evenly distributed chromatin. On the other hand, increased density of the nuclear chromatin and a very compact nuclear structure were rarely noted in the liver of all rats of this group. However, cytoplasmic vacuolization was noticed at certain sites but they become less prominent (compared to cisplatin alone). Sinusoidal walls were dilated to a lesser extent with a few numbers of Kupffer cells and RBCs. Remarkably, after 12 days silymarin post-treatment succeeded in maintaining organized hepatocytes trabecular arrangement. Mitotic figures (Fig. 3d-1) were commonly detected among pericentral hepatocytes.

Further examinations revealed that pretreatment of silymarin 2 h before cisplatin remarkably reduced the pathological lesions induced by cisplatin alone (Fig. 3e, f). Morphometric measurements of hepatocyte length and nuclear dimensions indicate significant increase when compared to those in cisplatin where values approached those in control (Fig. 2a-c). Rats of this group showed clear and organized trabecular arrangement of hepatocytes. Although, pericentral and periportal hepatocytes morphology and structures were, in many aspects similar to those in control animals; yet sinusoidal wall remained dilated at certain foci with a moderate number of Kupffer cells. After 12 days, GIIc exhibited additional features of recovery where both pericentral (Fig. 3f-1) and periportal hepatocytes (Fig. 3f-2) demonstrated increased frequency of mitotic figures.

At the ultrastructural level using electron microscopy, all general aspects of liver were observed in the control rats (GIa) after 6 and 12 days (Fig. 4a, b). The luminal surface of hepatocytes was fringed with numerous short and blunt microvilli protruding into the space of Disse. Lateral borders of adjacent cells were closely interdigitated where two adjacent hepatocytes delimited bile canaliculus occupied by few small microvilli (Fig. 4a). Sinusoids were lined by discontinuous oval shaped endothelial cells with spindle shape nuclei; besides, Kupffer cells with many processes and large triangular nuclei were found lining the sinusoids. Moreover, hepatocytes appeared with polygonal outline and central and large nuclei (Fig. 4b). The hepatocytes cytoplasm showed abundant round and oval-shaped mitochondria (Table 3). These mitochondria having tubular cristae and condensed matrices. Numerous RER aggregates dispersed throughout the cytoplasm, ensheathing numerous mitochondria (Fig. 4a, b). Moreover, hepatocyte cytoplasm contained rosettes glycogen, numerous ribosomes, few lipid droplets and peroxisomes. On the other hand, vehicle administration (GIb) (Fig. 4c, d) did not show great ultrastructural abnormalities from those of

Table 3: Changes on morphometric measurements of oval and rounded mitochondrial dimensions (μm) in liver cells among control and experimental groups¹

Experimental groups	Oval mitochondria ²				Rounded mitochondria ³	
	Length (μm)		Width (μm)		Diameter (μm)	
	6 days	12 days	6 days	12 days	6 days	12 days
Control GIa	1.31 \pm 0.2 ^a	1.30 \pm 0.05 ^a	0.41 \pm 0.07 ^a	0.42 \pm 0.01 ^a	0.59 \pm 0.09 ^a	0.58 \pm 0.02 ^a
Vehicle GIb	1.29 \pm 0.2 ^a	1.25 \pm 0.06 ^{a,d}	0.42 \pm 0.1 ^a	0.40 \pm 0.01 ^a	0.58 \pm 0.08 ^a	0.59 \pm 0.01 ^a
Cisplatin GIIa	1.27 \pm 0.3 ^a	1.07 \pm 0.04 ^{b,d}	0.29 \pm 0.02 ^b	0.34 \pm 0.01 ^b	0.42 \pm 0.12 ^b	0.41 \pm 0.01 ^b
Silymarin 2 h after cisplatin GIIb	2.00 \pm 0.5 ^b	1.35 \pm 0.06 ^a	0.34 \pm 0.04 ^b	0.29 \pm 0.008 ^c	0.49 \pm 0.05 ^c	0.56 \pm 0.01 ^c
Silymarin 2 h before cisplatin GIIc	2.22 \pm 0.41 ^b	2.00 \pm 0.12 ^c	0.38 \pm 0.1 ^c	0.34 \pm 0.01 ^c	0.53 \pm 0.12 ^c	0.60 \pm 0.03 ^c
Results of one way ANOVA						
F-value	28.06	23.76	9.9	12.77	9.8	13.14
p-value	<0.05*	<0.05*	<0.05*	<0.05*	<0.05*	<0.05*

¹Data are Mean \pm SE (n = 20). Means in the same column with different superscript letter(s) are significantly different, p<0.05 (one-way ANOVA followed by post-hoc LSD). ²Significant interaction was observed between time and dose among groups (length) by overall 2-way ANOVA; F = 5.57, p<0.05. Treatment effect, p<0.05, F = 46.7; time effect, p<0.05, F = 18.05. Insignificant interaction was observed between time and dose (width) among groups by overall 2-way ANOVA; F = 3.14, p = 0.16. Treatment effect, p<0.05, F = 19.05; time effect, p = 0.48, F = 0.49. ³Insignificant interaction was observed between time and dose among groups by overall 2-way ANOVA; F = 1.68, p = 0.15. Treatment effect, p<0.05, F = 21.12; time effect, p = 0.98, F = 2.76

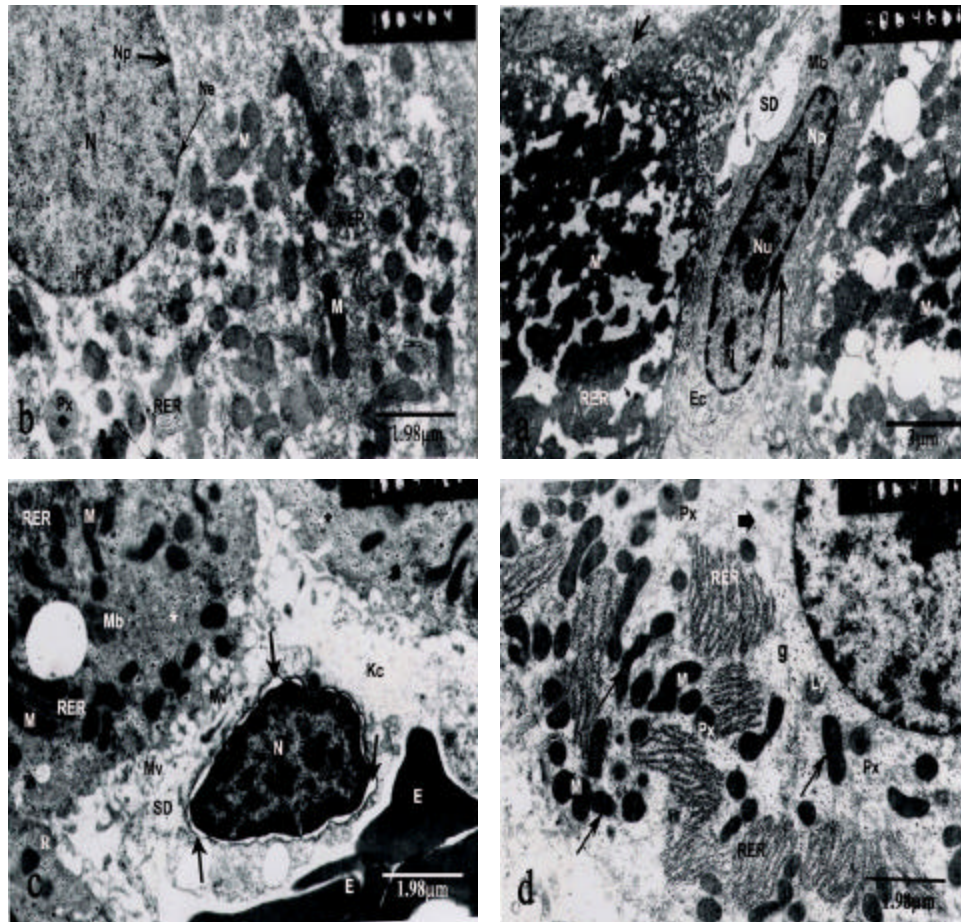


Fig. 4: Electron micrographs, sections of liver of male Sprague Dawley rats in control groups. (a) Control liver of rats after 6 days; showing numerous irregular Microvilli (Mv) projecting into Space of Disse (SD). Few spherical shaped Mitochondria (M) encircled with minimal RER. Arrow: bile canaliculus between hepatocytes. Notice; endothelial cell has spindle shape Nucleus (N) with clumps of heterochromatin along Nuclear envelope (Ne) and spherical shaped Nucleolus (Nu). Distinct membranes of Nuclear envelope (Ne) Nuclear pores (Np), electron-dense Microbodies (Mb), (b) Control liver of rats; showing, part of hepatocyte; large spherical shape nucleus (N), patches of Heterochromatin (He) along Nuclear envelope (Ne), Nuclear pores (Np). Observe: aggregates of rough endoplasmic reticulum (RER) partially encircling numerous oval-shaped dense mitochondria (M). Peroxisomes (Px), (c) Vehicle administrated rats' liver; revealing Kupffer cell (Kc), with triangular heterochromatic Nucleus (N); dilated perinuclear space (arrow). Numerous irregular Microvilli (Mv) extending into Space of Disse (SD). Rough Endoplasmic Reticulum (RER), Mitochondria (M) with dense matrix (arrow), microbodies (Mb); glycogen (asterisk) and Ribosome (R). Erythrocytes (E) within sinusoid, and (d) Vehicle administrated rats' liver; numerous oval-shaped Mitochondria (M), aggregates of Rough Endoplasmic Reticulum (RER). Rosettes of Glycogen (g), free ribosomes (arrow heads), Lysosomes (Ly), Peroxisomes (Px)

control group (G1a). However, few changes were detected including slightly irregular microvilli, mildly congested sinusoids. However, Kupffer cells were minimally hypertrophied with vacuolated cytoplasm and large heterochromatic nuclei exhibiting blebbing in the perinuclear space (Fig. 4c).

In cisplatin administrated group (G1Ia), paramount nuclear and cytoplasmic changes were greatly detected among liver structures (Fig. 5a-d). Dilated space of Disse, into which fragmented minute microvilli projected from the luminal surface of adjacent hepatocytes were observed. Besides, some microvilli were completely lost at certain

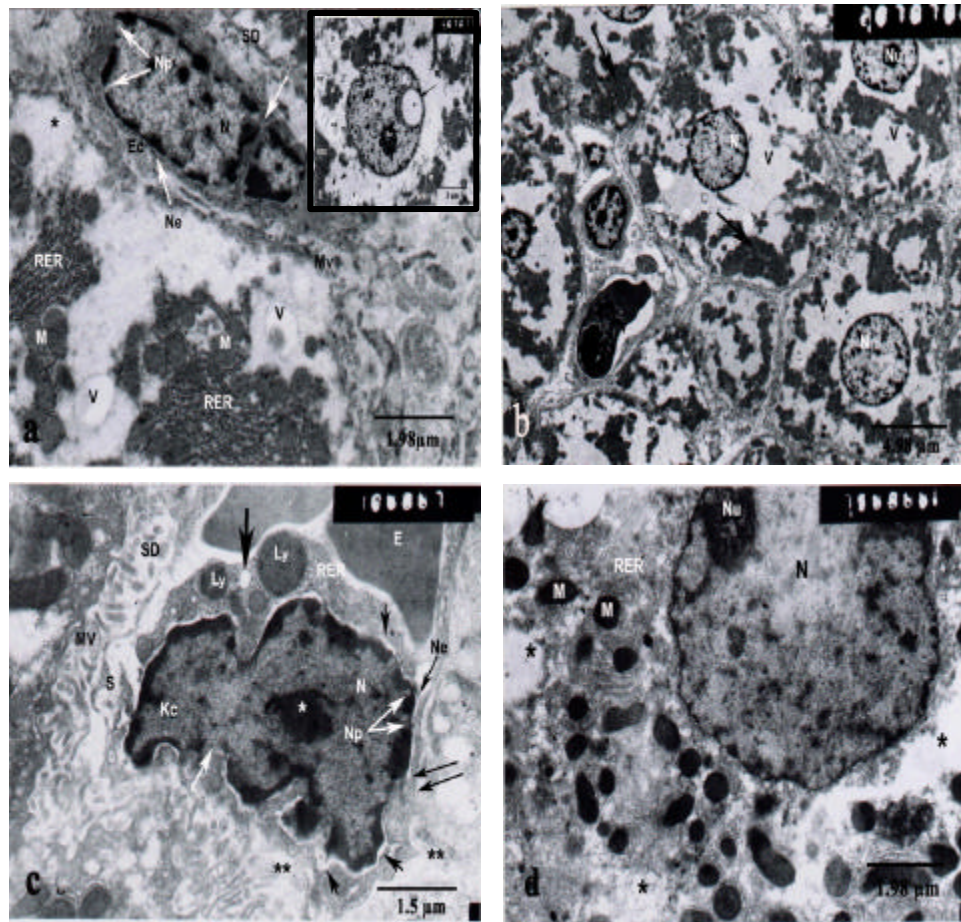


Fig. 5: Electron micrographs, sections of liver of cisplatin injected male Sprague Dawley rats. a) After 6 days; minimal reduced Microvilli (Mv) projecting toward Sinusoid (S); Space of Disse (SD), Vacuolated cytoplasm (V) with pale spaces (asterisk); minimal rough endoplasmic reticulum (RER) encircling small dense Mitochondria (M). Notice; Endothelial cell (Ec), Nucleus (N) cleaved (arrow), Nuclear pores (Np), Nuclear envelope (Ne), (b) After 6 days; diminutive hepatocytes; Nucleus (N), centric Nucleolus (Nu); great cytoplasmic Vacuoles (V), minimal small mitochondria (arrow). (1) Nuclear inclusion (arrow) enclosing lipid droplet (asterisk), (c) After 12 days; few irregular and detached Microvilli (Mv); extending into Space of Disse (SD). Observe hepatocytes with cytoplasmic vacuoles (double asterisk). Deformed Erythrocytes (E) within sinusoid (S). Distorted Kupffer cell (Kc) with corrugated nucleus (N); central clumps of heterochromatin (astrisk) and along nuclear envelope (Ne), numerous nuclear pores (Np). Disrupted nuclear envelope (arrow), dilation of the perinuclear space (arrow head); small profiles of Rough Endoplasmic Reticulum (RER), vacuolated mitochondria (thick arrow), primary lysosomes (L1) and (d) after 12 days; showing hepatocytes with corrugated nucleus (N), terminalized and segregated nucleolus (Nu); great cytoplasmic vacuolization (astrisk), few Rough Endoplasmic Reticulum (RER), small dense Mitochondria (M)

foci (Fig. 5a). Moreover, necrotic sinusoidal lining cells including endothelial cells were observed with irregular and distorted outlines and heterochromatic and cleaved nuclei with irregular nuclear envelope and dilated nuclear pores. In this group, hepatocytes appeared diminutive with significant reduction in their cellular and nuclear

dimensions. Also most nuclei were endowed with minimal heterochromatin content and thick and centric nucleoli (Fig. 5b). In severely affected hepatocytes, segregated nucleoli as well as pseudonuclear inclusions enclosing lipid droplets were detected among nuclei (Fig. 5b-1). Moreover, hepatocytes cytoplasm showed vacuolar

degeneration with regression and irregular distribution of RER and mitochondria. The RER ensheathing mitochondria were rarely pronounced (Fig. 5a, b), whereas mitochondria tended to be dense oval with significantly reduced mean width (Table 3), rounded with significantly reduced mean diameter, slipper or bizarre shape with fragmented disrupted cristae (Table 3). Glycogen rosettes in most hepatocytes were obscured within the cytoplasm and complete absent in few cells. On the other side, after 12 days and were completely absent microvilli appeared elongated and highly fragmented with hazardous orientation; Kupffer cells become significantly distorted

in their shape with corrugated nuclear envelope and numerous cytoplasmic processes (Fig. 5c). Hepatocytes showed many altered nuclei with segregated and marginated nucleoli (Fig. 5d). The RER and mitochondria reduced in number loss their normal association. Besides, mitochondria exhibited ruptured and obscure cristae and condensed matrices. Glycogen rosettes were rarely detected within the cytoplasm (Fig. 5d).

In group IIb receiving, silymarin treatment 2 h after cisplatin injection (Fig. 6a-d), space of Disse was minimally dilated and the microvilli were short and more prominent. Kupffer cells were regular in outlines; their

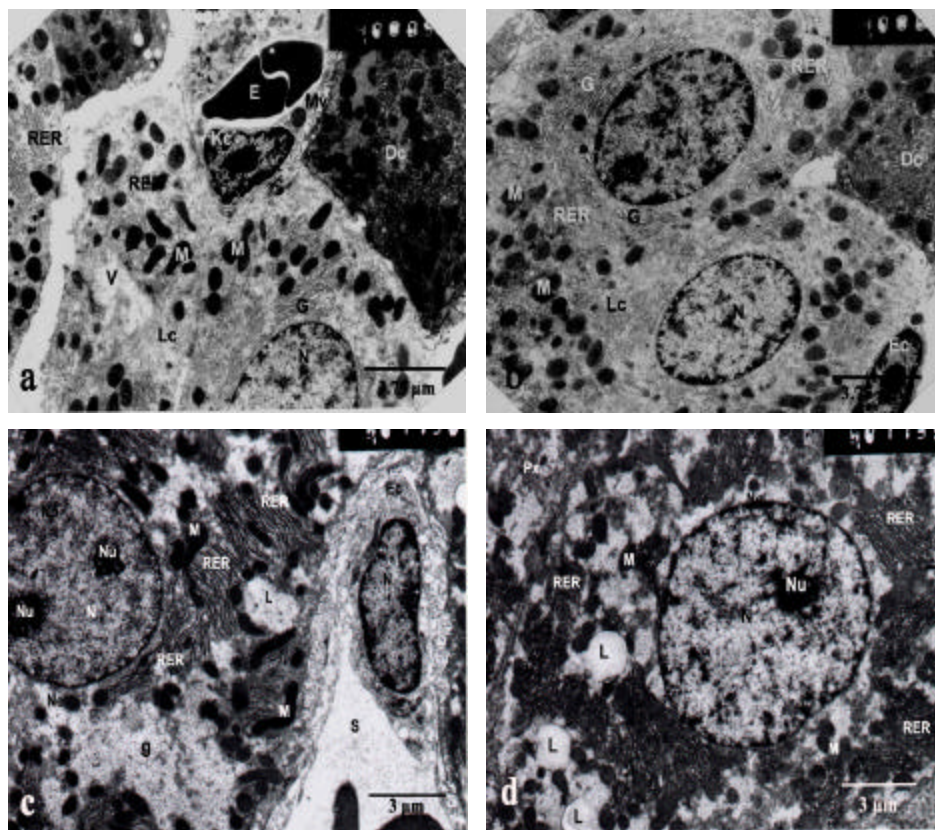


Fig. 6: Electron micrographs, sections of liver of Male Sprague Dawley rats treated with silymarin 2 h after cisplatin, (a) After 6 days; illustrating light (Lc) and Dark (Dc) hepatocytes; part of Nucleus (N). Rough Endoplasmic Reticulum (RER), moderately encircling dense Mitochondria (M), Golgi profile (G), small cytoplasmic vacuoles (V), numerous microvilli (Mv), congested sinusoid with deformed erythrocytes (E), Kupffer cell (Kc) with triangular nucleus, (b) After 6 days; oval nuclei (N) of light cell (Lc), aggregates rough endoplasmic reticulum (RER) with mitochondria (M), evident Golgi bodies (G), oval shape endothelial cell (Ec). Part of Dark hepatocyte (Dc), (c) After 12 days; endothelial cell (Ec) with oval Nucleus (N) lining Sinusoid (S). Notice, hepatocyte with rounded euchromatic Nucleus (N), two slightly segregated Nucleoli (Nu), Nuclear pores (Np). Numerous Rough Endoplasmic Reticulum (RER) ensheathing condensed Mitochondria (M), Lipid droplet (L), Glycogen rosette (g) and (d) After 12 days; nucleus of hepatocyte euchromatic with normal outlines, eccentric nucleolus (Nu), aggregates of Rough Endoplasmic Reticulum (RER) with Mitochondria (M), Lipid droplet (L)

nuclei showed corrugated triangular nuclei and predominant heterochromatin. However, endothelial cells attained spindle shape with oval nuclei and centric nucleoli (Fig. 6a). Hepatocytes were normally differentiated into dark and light hepatocytes. Large, oval and euchromatic nuclei with eccentric nucleolus, distinct nuclear membranes and moderate number of nuclear pore were frequent among most cells. In addition to the normal association between numerous RER and mitochondria, RER profiled were found minimally encircling mitochondria at certain foci. Although, normal shaped mitochondria with increased dimensions (Table 3) were common, a few numbers of them with obscure cristae and

dense matrices were observed (Fig. 6b). Prominent Golgi apparatus were perinuclear in position. Fluctuations in glycogen content were observed at different cytoplasmic sites. Cytoplasmic vacuolization significantly depressed when compared to cisplatin group. After 12 days, hepatocytes become highly regular with organized cytoplasmic organelles. Mitochondria with dense matrices were noticed surrounding mild number of lipid droplets (Fig. 6c, d). Numerous ribosomes and glycogen rosettes were more frequently detected. Cytoplasmic vacuolization were greatly reduced.

Pre-treatment with silymarin 2 h before cisplatin (GIIc) (Fig. 7a-d) revealed ultrastructural changes when

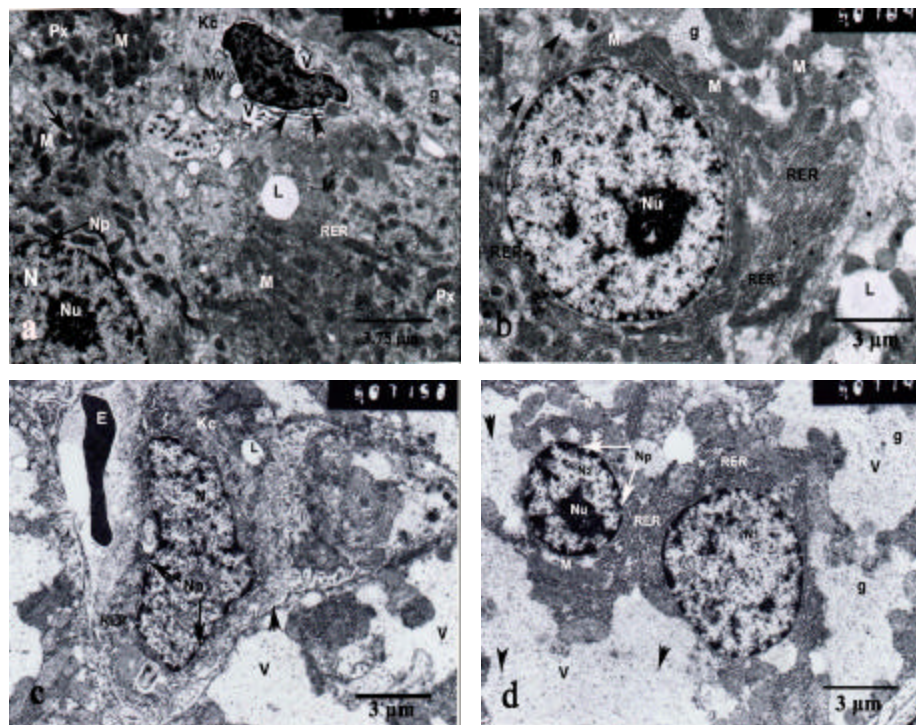


Fig. 7: Electron micrographs, sections of liver of male Sprague Dawley rats treated with silymarin 2 h before cisplatin, (a) After 6 days; long numerous Microvilli (Mv); Kupffer cell (Kc) with wide perinuclear space (arrow head) vacuolated cytoplasm (V). Nucleus (N) of hepatocyte, eccentric nucleolus (Nu), Nuclear pores (Np); Rough Endoplasmic Reticulum (RER), several mitochondria (M) around lipid droplet (L), numerous glycogen rosettes (g), (b) After 6 days; showing hepatocyte with large centric euchromatic nucleus (N), eccentric nucleolus (Nu), Rough Endoplasmic Reticulum (RER) highly encircling dense mitochondria (M). Lipid droplet (L), numerous rosettes of glycogen (g) and ribosomes (arrow head) within cytoplasm, (c) After 12 days; congested sinusoid with deformed Erythrocyte (E), numerous Microvilli (Mv); Kupffer cell (Kc) with triangular nucleus (N), Nuclear pores (Np), few Rough Endoplasmic Reticulum (RER), Lipid (L) droplet and cytoplasmic processes (arrow head). hepatocyte cytoplasm with vacuoles (V), myelin body (asterisk) and (d) After 12 days; showing binucleated hepatocyte with large centric euchromatic nucleus (N1), reduced nucleus (N2): with thick eccentric Nucleolus (Nu), numerous Nuclear pores (Np); Rough Endoplasmic Reticulum (RER) encircling dense Mitochondria (M); great cytoplasmic vacuolization (V), numerous rosettes of glycogen (g) and ribosomes (arrow head)

compared to those receiving post-treatment with silymarin (GIIb) including; elongated and detached microvilli exposed from the sinusoidal hepatocyte surface; Kupffer cells with irregular cellular and nuclear outlines with heterochromatic nuclei, dilated perinuclear space, membranes blebbing and increased cytoplasmic processes and vacuolization (Fig. 7a). Hepatocytes attained organized outlines with oval euchromatic nuclei and eccentric nucleoli. Moreover, cytoplasmic organelles become more organized. Numerous RER profiles encircling mitochondria exhibited regular orientation around the nucleus and within the cytoplasm. Oval and rounded mitochondria showed significant increase in their dimensions when compared to those found in cisplatin treated group (Table 3). Glycogen rosettes increased and were detected at high frequency at different cytoplasmic sides. Besides, moderate number of lipid droplets was surrounded by numerous electron dense mitochondria with transverse cristae. Cytoplasmic vacuolization were less prominent (Fig. 7b). After 12 days, further significant ultrastructural modifications were recorded, thus reflecting remarkable signs of tissue recovery (Fig. 7c, d). Microvilli regained their blunt short and organized arrangement within narrow space of disses. Endothelial cells as well as kupffer cells attained regular nuclear and cytoplasmic outlines (Fig. 7c, d). Hepatocytes showed organized nuclear profiles and organelles arrangement and increased glycogen content.

DISCUSSION

In the current study, the proposed plan aimed to assess and examine the possibility of silymarin both to protect and reduce the histopathological and ultrastructural alterations induced by cisplatin on normal liver tissues of male Sprague Dawley rats, which were used as biological test animals.

Cisplatin is an effective chemotherapeutic agent for a wide variety of tumors (Park *et al.*, 2009). Nevertheless, it has several toxicities and sides effects including hepatotoxicity (Mansour *et al.*, 2006; Pratibha *et al.*, 2006) and nephrotoxicity (Park *et al.*, 2009). 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 (Stewart *et al.*, 1982), resulting in the enhanced production of reactive oxygen species. In their study, Mora *et al.* (2003) 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 the protective effects against cisplatin induced tissue toxicities have been reported for extracts of natural products and dietary antioxidant (Behling *et al.*, 2006).

Silymarin, the root extract from *Silybum marianum*, is known to have hepatoprotective effect against numerous liver diseases (Victorrajmohan *et al.*, 2005; Eminzade *et al.*, 2008). Ahmed *et al.* (2003) reported that silymarin has antihepatotoxic activity against CCl_4 induced hepatotoxicity in albino rats. However, available study revealed that minimal studies have focused on the hepatoprotective effect of silymarin on cisplatin-induced toxicity at cellular levels.

In the present investigation, a single dose of cisplatin (5 mg kg^{-1}), in male Sprague Dawley rats resulted in significant body weight retardation and decreased food intake. This loss was most pronounced after 12 days. In accordance with present results, Chirino *et al.* (2004) suggested that after 3 days i.p. administration of a single dose of cisplatin to male Wistar rats (7.5 mg kg^{-1}) significantly depressed their body weight. Confirming our point of view, Shimeda *et al.* (2005) and Norrgren *et al.* (2006), stated that cisplatin has been shown to decrease total body weight in male Sprague Dawley rats and Wistar rats, respectively.

During the course of the present study, post-treatment and pre-treatment of silymarin (50 mg kg^{-1}) 2 h after and 2 h before cisplatin injection remarkably ameliorated the reduction in body weight induced by cisplatin, thus leading to a quicker recovery. Our results also were in agreement with Gaedeke *et al.* (1996), who noticed that daily i.v. injections of silibinin (active compound of silymarin) (200 mg kg^{-1}) to female Wistar rats succeeded in the complete recovery of the experimental animals' body weight over a period of 11 days. Contradicting our results, Shimeda *et al.* (2005) stated that daily oral administration of capsaicin antioxidant (10 mg kg^{-1}) for 6 consecutive days increased male Sprague Dawley body weight yet it failed in the complete recovery to normal.

In the present study, our results showed that cisplatin induced liver damage characterized by a significant decrease in the wet liver weight manifested by a significant depression in hepatosomatic index. This change was more pronounced after 12 days from the beginning of the experiment. Our results were in accordance with the experimental studies conducted by Lee *et al.* (2007), who indicated that cisplatin treatment for 15 days in BALB/C mice resulted in liver weight loss manifested by significant depression as a percentage of the total body weight.

In the present study, both post- and pre-treatments of silymarin significantly returned liver weight and hepatosomatic index values to normal after 6 days. However, after 12 days silymarin treatment resulted in higher significant elevation of these values compared to control and cisplatin treated groups. Results reported by Favari and Pérez-Alvarez (1997) were in agreement with our results; where rats, receiving chronic administration of CCl_4 followed by oral intake of silymarin, 50 mg kg^{-1} for 5 days, exhibited elevation in the liver weight and liver to body weight ratio and was more than twice that of the CCl_4 treated group. During the present work, it could be elucidated that alterations of organ-body weight ratio in cisplatin intoxicated rats could be due to tissue damage and reduction in their functions as reported by Lee *et al.* (2007) and Park *et al.* (2009).

It is well known that the liver is the center of detoxifying and eliminating the toxic substances that are carried to it via the blood. So, it is uniquely exposed to a wide variety of exogenous and endogenous products, which include environmental chemicals and toxins (Wight, 1982). In the present investigation, a single dose of cisplatin (5 mg kg^{-1}) in rats showed abnormalities in the liver tissues. Histological data showed that by day 6, cisplatin caused structural alteration indicated by blurred hepatocyte trabecular arrangement. Irregularities and reduction of hepatocytes cellular and nuclear dimensions were manifested among animals of this group. Enlargement, centric localization and triozomy of nucleoli as well as increased number of karyoplasmic bodies were commonly observed, an observation similar to that of malignancy (Ghadially, 1997). After 12 day of treatment, great disruption of hepatic cords and trabecules were greatly detected. The present findings were in general agreement with those stated by Murthy *et al.* (2002), who reported that male Wistar rats receiving single oral dose of CCl_4 (2 g kg^{-1}) for two weeks, exhibited total loss of hepatic architecture. Besides; the present study showed great cytoplasmic vacuolization and increased frequency of pyknotic nuclei. In agreement with present study, El-Sayyad *et al.* (2009) reported that i.p., administration of cisplatin (1 mg kg^{-1}) resulted in massive histopathological abnormalities where necrotic hepatocytes showed increased incidence of vacuolar degeneration and apoptotic cell death after 20 days showed.

The liver is known to accumulate significant amounts of cisplatin, second only to the kidney (Stewart *et al.*, 1982), thus hepatotoxicity can be associated with cisplatin treatment (Liao *et al.*, 2008). Cell death can result from naturally occurring apoptosis (physiological apoptosis) or from irreparable cell injury (pathological apoptosis) as described by Farber (1994). Apoptosis is a common feature of hepatotoxicity induced by many chemicals: it

may precede necrosis as in hepatotoxicity induced by thioacetamide (Ledda-Columbano *et al.*, 1991), or it may occur concurrently with necrosis as in hepatotoxicity associated with acetaminophen (Knight *et al.*, 2003). Cisplatin is thought to kill cells primarily by forming DNA adducts, causing G2 arrest in the cell cycle, triggering apoptosis (Kishimoto *et al.*, 2000).

Another major purpose of the present study was to assess the effect of silymarin, as a natural flavonoid with potent antioxidant properties on cisplatin induced liver injury, as well as the mechanism of the possible protection afforded by silymarin. Present results revealed that silymarin administrated 2 h after and 2 h before cisplatin administration has a marked partial and complete protective effect on liver cell toxicity, respectively. This observation confirmed data reported by Eminzade *et al.* (2008), who demonstrates that male Wistar albino rats injected i.p., with silymarin (200 mg kg^{-1}) over 14 days reduced liver injury.

During the present investigation, the protective effect of silymarin was confirmed when liver tissues were observed histologically. In rats exposed to post-treatment with silymarin (GIIB), the trabecular structure of the liver lobule appeared regular but slightly blurred at certain foci by day 6. Collectively, the present results resembled to a great extent those reported by Murthy *et al.* (2002), who reported that male Wistar rats receiving pretreatment with antioxidant (pomegranate peel extract) followed by exposure to CCl_4 (2 g kg^{-1}) for 14 day, resulted in retaining livers to normal hepatic architecture with few areas of hemorrhage between columns of hepatocytes.

In addition, the present observation revealed that hepatocytes in silymarin treated groups appeared less organized with increased cellular and nuclear dimensions. Regular nuclei with minimal nucleoli of centric localization and evenly distributed chromatin were frequently detected. In a few animals, necrosis of single cells was evident. Cytoplasmic vacuolization were less prominent (compared to cisplatin alone). Sinusoidal walls were slightly dilated with a few numbers of Kupffer cells and erythrocytes. These results revealed a sort of resemblance to those results suggested by Wu *et al.* (2008), who demonstrated that liver after 7 days of oral administration of silymarin (300 mg kg^{-1}) recovered to normal morphology, thus decreasing liver degeneration including fatty changes, pleomorphic and bizarre nuclei, ballooning of the hepatocytes and abnormal arrangement of the sinusoid were detectable in the HBx transgenic mice. In the present study, after 12 day post-treatment with silymarin resulted in reduced frequency of pyknotic nuclei. The most striking feature was the presence of mitotic figure in most pericentral hepatocytes indicating signs of liver cell proliferation and regeneration.

The protective effect of silymarin was further manifested when administrated 2 h before cisplatin (GIIc). In present experiment, silymarin administration, exhibited higher protective effect than those administrated 2 h after cisplatin. After 6 days, trabecular hepatocyte architecture was more organized, cellular and nuclear dimensions approached those in control, highly reduced sinusoidal wall dilation and pyknosis rarely detected among most hepatocytes. However, after 12 days, silymarin succeeded in the complete protection against cisplatin induced damage, where most of the pathological features were entirely attenuated. Besides, the majority of both pericentral and periportal hepatocytes exhibited the highest frequency of mitotic figures.

Present results were in general agreement with those reported by Ferri *et al.* (2005), who revealed that liver cells after 96 hrs from partial hepatectomy showed signs of proliferation and regeneration where pericentral and periportal cells exhibited mitotic figures. These structural attributes to proliferation, could be involved in the changes of metabolic and functional heterogeneity of the hepatocyte within the hepatic acinus during the regenerative process. Similar results were recorded by Wu *et al.* (2008), who demonstrated that silymarin stimulated hepatocyte cellular proliferation leading to cell replacement in HBx transgenic mice. These results suggested that the enhanced cell proliferation and liver regeneration induced by silymarin help to replace damaged cells in the HBx transgenic mice and this may contribute in part to the chemopreventive effect of silymarin on liver pathogenesis.

In the present study, silymarin administration significantly reduced pathological liver changes and the severity of changes when compared to the cisplatin group. Consistent with previous studies, Eminzade *et al.* (2008), reported similar results in the protective effect of silymarin against liver damage. Recently, several studies have been carried out to elucidate the mechanism of action of silymarin. Accumulated data show that this herbal drug inhibits several isoforms of CYT P450 enzymes (Vuilleumier *et al.*, 2006), potentiates the antioxidant capacity of the liver (Adhvaryu *et al.*, 2007), acts as a scavenger of oxygen free radicals (Meeran *et al.*, 2006), inhibits the synthesis of pro-inflammatory cytokines and enhances apoptosis.

Our electron micrographs demonstrated that after 6 and 12 days of cisplatin administration paramount nuclear and cytoplasmic changes among liver structures were observed. Dilated space of Disse occupied with fragmented and minute microvilli projected from the luminal surface of hepatocytes. Also, hepatocytes appeared diminutive with reduced cellular and nuclear

dimensions and minimal heterochromatin content and thick, centric and segregated nucleoli. Pseudonuclear inclusions as well as cytoplasmic vacuolar degeneration were detected among some cells, indicating signs of apoptosis. However, increased frequency of pyknotic nuclei and cytoplasmic vacuolization were highly pronounced after 12 days. El-Sayyad *et al.* (2009) recorded similar information, after 20 days of cisplatin (1 mg kg^{-1}) treatment were hepatocytes showed pyknotic nuclei with irregular nuclear membrane.

Present observation showed that after 6 and 12 days, mitochondria attained dense matrix, reduced dimensions and slipper or bizarre shape with fragmented disrupted cristae and complete loss of mitochondria-RER association. Our results were in agreement with those reported by El-Sayyad *et al.* (2009), who reported that the cytoplasm of cisplatin treated rats contained vesiculated RER and atrophied mitochondria with ill-differentiated cisternae. Besides, many studies indicated that the ER is a dynamic labile organelle which can readily undergo either hypertrophy or atrophy and these changes usually reflect a state of altered functional activity (Ghadially, 1997). On the other side, our results showed that glycogen rosettes were obscured within the cytoplasm of most hepatocytes and were complete absent in few cells.

On the other side, it was clearly observed that silymarin administration almost reduced the ultrastructural damage induced by cisplatin in liver cells. Treatment with silymarin 2 h after cisplatin depressed significantly damage induced by cisplatin after 6 days. Hepatocytes with prominent microvilli, with normal large euchromatic nuclei, normal association between numerous RER and mitochondria, prominent Golgi apparatus with perinuclear position, endothelial cells with regular outlines; cytoplasmic vacuolization minimally detected. By day 12 liver cells and structures restored their normal features with highly reduced cytoplasmic vacuoles, RER and mitochondrial association appeared almost normal, glycogen rosette content slightly increased. In accordance with our results, Wu *et al.* (2008) demonstrated that daily oral administration of silymarin (300 mg kg^{-1}) in HBx transgenic mice for 14 days depress severe ultrastructural alterations where hepatocytes appeared more organized including: normal organized RER and mitochondria.

The importance and the protective effect of silymarin were greatly detected when administrated 2 h before cisplatin. By day 6, silymarin pre-treatment attained similar ultrastructural features to those recorded in post-treatment with silymarin, yet it displayed additional and highly protective features manifested by higher cellular and organelles organization, reduced cytoplasmic

vacuolization, increased glycogen rosette content and the most striking feature was the great association between RER and mitochondria, where mitochondria were surrounding numerous lipid droplet. The number of lipid droplets increased by day 12 and hepatocytes appeared to restore their normal nuclear and cytoplasmic ultrastructure. Present results agree with the previous study reported by Van Noorden *et al.* (1994), who studied lipid accumulation in hepatocytes. It is conceivable that the excess of lipids might be used for the synthesis of new biological membranes during hepatocytes proliferation. These structural attributes to proliferation, could be involved in the changes of metabolic and functional heterogeneity of the hepatocyte within the hepatic acinus during the regenerative process (Ferri *et al.*, 2005).

It could be concluded that the alterations in the structure of the liver tissue in male rats treated with cisplatin may possibly affect other biological perturbations (feeding behavior, body weight, liver weight, liver tissue histology and ultrastructure). Present results confirmed that silymarin treatment effectively regressed morbid liver pathology and recovered hepatocytes ultrastructure. These results suggested that silymarin may be considered as a potentially useful candidate in dietary supplement by patients taking antineoplastic drugs like cisplatin. However, further studies are needed in order to explore the exact cellular mechanisms underlying the cytoprotective effect of silymarin.

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