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Research Article

Hepatoprotective and Antioxidant Effects of Commiphora Against CCl(4) -Induced Liver Injury in Adult Male Albino Rats

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ABSTRACT

Carbon tetrachloride (CCl4) is a well-known hepatocyte-destructive agent. Commiphora mukul is a medicinal plant found to be effective in the treatment of a variety of disorders. Aim of the work is to study the effect of Commiphora extract on liver injury induced by the administration of CCl4 in rats. Forty adult male albino rats were divided randomly into four groups. Group I (control group), group II (received CCl4 for 2 weeks), and group III and group IV, which received 250 and 500 mg/kg, respectively, of Commiphora extract orally before the administration of CCl4. Two weeks after the administrations of CCl4, animals were killed, and the livers were removed and processed for histological and electron microscopic examination. Liver functions were measured. The results revealed that a low dose of Commiphora extract did not lead to any improvement; while a high dose of Commiphora showed its potential to protect against CCI4 induced hepatotoxicity by controlling the serum alanine aminotransferase and aspartate amino transferase (ALT and AST) levels and alkaline phosphatase enzymes (ALP) and also hepatic lobules regenerate to their normal architecture with proliferating bile ductules in the portal tract. Some hepatic lobules still showed vacuolation and necrosis of their hepatocytes. It could be concluded that, Higher doses of Commiphora extract protects against CCl4-induced liver injury.

Keywords: Carbon Tetrachloride, Cirrhosis, Commiphora Mukul, Extracellular Matrix Proteins

INTRODUCTION

Liver cirrhosis is the terminal stage of various chronic liver diseases. Even mild but continuous injury in the liver soon results in excessive production of extracellular matrix components, with subsequent deposition in the space of Disse, which will lead to alterations in liver functions [1]. This fibrotic stage ultimately progresses to cirrhosis, which is characterized by nodule formation and distortions in liver architecture. Overproduction and accumulation of extracellular matrix proteins in the liver usually start after hepatocyte injury, which initiates a series of complicated cell-to-cell and cell-to-matrix interactions, eventually leading to activation of hepatic Ito cells, which are the main producers of excessive collagen during the cirrhosis process [2]. Commiphora mukul (Hook ex stock synBalsamodendron Mukul) is a shrub resembling a small tree of the Burseraceae family. Commiphora mukul is reported to a highly valuable medicinal plant and has been used in traditional ayurvedic medicine for centuries in the treatment of a variety of disorders [3]. Several pharmacologically active components have been identified in this plant, including guggulsterones and guggulipid [4]. It is known that a decoction or tincture of Commiphora can be used by local traditional healers for the treatment of chest, stomach, and kidney complaints; to promote digestion; and to relieve rheumatism, scurvy, and jaundice [5]. Also, antihypertensive activity of an aqueous extract of this plant in rats has been reported [6]. It was found that this plant can inhibit vitamin C/NADPHinduced lipid peroxidation in rat liver microsomes and has beneficial effects in various physiological and pathological conditions, mainly by reducing the production of reactive oxygen species concentrations [7]. As hepatocyte injury seems to be the first and the main fibrogenic stimulus in the liver, healing of these cells could be a desirable goal in preventing progression of fibrosis and finally cirrhosis.

Aim of the work: To study the effect of Commiphora extract on liver injury induced by the administration of CCl4 in rats.

Materials and methods

Animal grouping: Forty male albino rats weighing 150–200 g were obtained from the animal house, Moshtohor Faculty of Veterinary Medicine, Benha University, and received a balanced diet, with free access to water. All animal procedures were performed according to approved protocols and in accordance with the recommendations for the proper care and use of laboratory animals. Animals were divided into equally (n=10 rats) to four groups: group I seved as control group, , group II received I.P CCI4, and group III and group IV received combination therapy (oral Commiphora extract at doses of 250 and 500 mg/kg, respectively and CCl4), 3 weeks before the administration of CCl4 [8,9].

Drugs: Carbon tetrachloride solution (CCl4) was obtained from El Nasr Pharmaceutical & Chemical Company (Cairo, Egypt). CCl4 was injected intraperitoneally at a

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Table 1: Ultrastructural scoring system used in experimental liver fibrosis[11.12,13].

Ultrastructure Assesment		Score
Mitochondrie		
	Normal	0
	Prominent cristae	1
	Edematous mitochondrion	2
	Collection of amorphous material	3
rER		
	Normal	0
	Dilatation	1
	Irregular lamellar organization	2
	Presence of focal breaks	3
sER		
	Normal	0
	Vacuolization	2
	Presence of large degenerated areas	3
Nucleus		
	Normal	0
	Irregular chromatin distribution	1
	(margination, clumping)	2
	Increased heterochromatin	3
	Degenerated nucleus	

Table 2: Effect of *Commiphora* extract on serum enzymes and bilirubin level in rats with CCI_4- induced liver damage

Treatment [n=10]	AST IU/L	ALT IU/L	ALP IU/L	Bilirubin mg/dl
Control (Saline)	50.00 ± 9.75	64.8±3.93	128.19±46.60	0.71±0.025
CCI_4 (25mg/ kg)	591.95[a]±49.35	473.91[a]±35.57	1.05±0.03	1.68[a]±0.12
Commiphora (250mg/ kg)+CCI_4	473.91[b]±13.4	122.86[b]±6.0	509.33[b]±23.6	1.45±0.003
Commiphora (500mg/ kg)+CCI_4	200.06[b]±30.80	114.59±9.99	489.00[b]±24.5	1.006[b]±0.03

$p<0.001$ student's t-test; [a]as compared with the control [normal saline]group:[b]as compared with the CCI_4 group AST = Aspartate transaminase; ALT= Alanine Transaminase; ALP = alkaline ghosphatase

dose of 0.2 ml/100 g body weight in a 1:1 ratio with olive oil twice weekly for 2 weeks [8]; animals received 250 and 500 mg/kg, respectively, of Commiphora extract orally (LOSEROL 100 mg; El Phoronia Pharmaceutical & Chemical Company, Cairo Egypt) for 3 weeks before the administration of CCl4. Two weeks after the administration of CCl4, animals were anesthetized and killed using ether anesthesia. Blood and Liver samples were obtained and sections were prepared for histological study (H&E [10] and toluidine blue [11] stains) study, and electron microscopic examination.

Experimental parameters: The livers were immediately removed. Blocks were prepared for semithin and ultrathin sectioning. Semithin 1 µm sections were stained with 0.5% toluidine blue in borax and examined under a light microscope. For electron microscopic examination, specimens were immersed in 2.5% phosphate-buffered gluteraldehyde (pH 7.3) for 24 h, postfixed in 1% OSO4 for 2 h, and dehydrated in graded concentrations of alcohols. After immersion in propylene oxide, the specimens were embedded in epon resin (Embes 812; Electron Microscopy Sciences, Washington, Pennsylvania, USA). Cells of each specimen were examined for mitochondria, nuclei, rough endoplasmic reticulum (rER), and smooth endoplasmic reticulum (sER) of hepatocytes [12]. Histological evaluation was performed in four sections per slide for all groups (Table 1) [13–15].

Blood was collected by heart puncture and the serum was separated by centrifugation. Assay of serum liver transaminases, Aspartate transaminases (AST), Alanine transaminases(ALT), alkaline phosphatase (ALP) activities, total bilirubin, total lipids, total cholesterol, and triglyceride was performed. HDL, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) were also calculated.

STATISTICS

Data were expressed as mean ± SEM. Statistical analysis was performed by using Student's t-test. For ultrastructure scores data were expressed as mean± SD to find out the significance of differences between the tested groups.

Table 3- Effect of *Commiphora* extract on hepatic lipids and lipoproteins indices in rats with CCI4- induced liver damage:

Treatment[n=10]	Total lipids (mg/dl)	Total cholestelol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control	160.8±1.24	75.8±1.74	72.2±2.11	49.5±0.98	64.2±1.02	35.92± 0.78
CCI_4	250.4±1.74	85.6±1.95	98.2±3.41	92.8±0.88	102.3±2.02	85.82± 0.79
Commiphora+CCI_4	148.4±2.01**	68.8±0.86*	69.6±2.89 ns	64.4±1.66**	61.2±1.02 ns	30.72±0.61**
Commiphora+CCI_4	144.4±1.60***	64.4±2.72**	62.6±2.80**	69.2±1.15***	59.8±0.37**	27.84±0.64***

*Results were expressed as mean ±S.E.**As compared with the control [normal saline] group; b as compared with the control [CCI_4] $^{**}p$ <0.01; $^{***}p$< 0.001 by Student's t-test.*

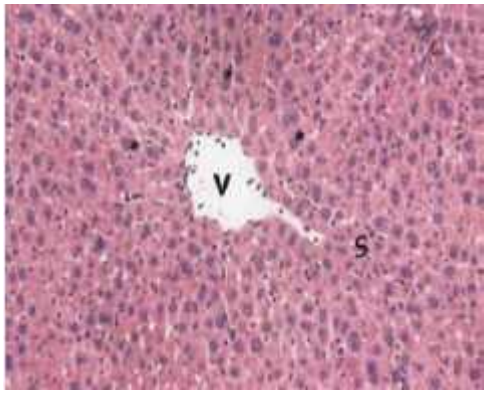

Fig. (1) A photomicrograph of a liver section from Group I showing hepatocytes with central vesicular nuclei and granular acidiophlic cytoplasm radiating from central vein (V). Notice the presence of many binucleated cell(*) around blood sinusoids (s). [H&E X 400]

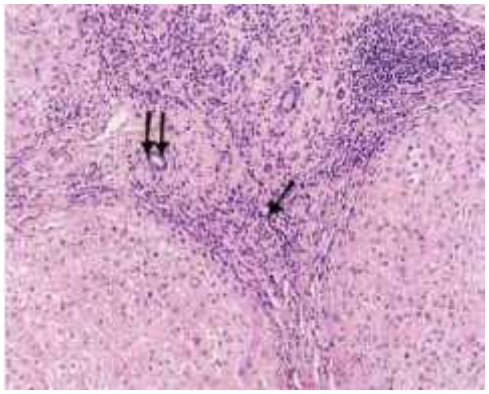

Fig. (2) A photomicrograph of a liver section from adult male rat from Group II: (treated with CCI4),showing connective tissue septa around hepatic lobule .The septa contain mononuclear inflammatory cells(↑) , preexisting portal tracts and proliferated bile ductules(↑↑). Notice loss of architecture.
[H&E X 400]

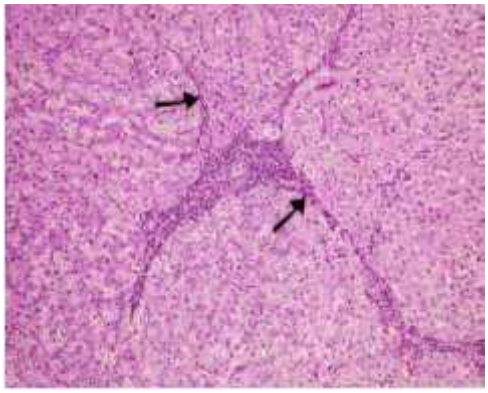

Fig. (3) A photomicrograph of a liver section from Group IIIa , showing apparent decreased fibrous tissue and cellular infiltration around the bile ductule in preexisting portal tract . Notice distortion of hepatic architecture (arrows).[H&E X 400]

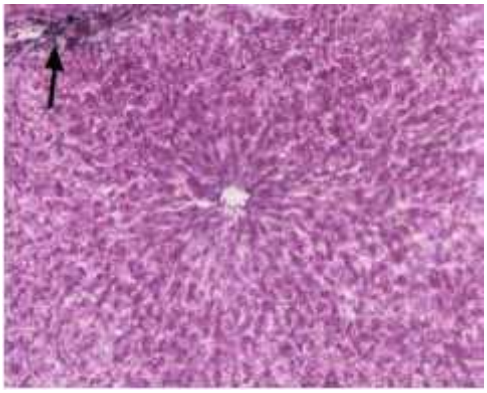

Fig.(4) A photomicrograph of a liver section from Group IIIb, showing hepatic lobules with central vein. Notice portal tract (arrow).
[H&E X 100]

RESULTS

Effect of Commiphora extract on CCl4-induced modulation of liver function parameters and lipid profile: Table(2) and table (3)explain that rats subjected to the CCI4- regimen alone developed significant hepatocellular damage as evidenced by a significant elevation in serum activities of AST, ALT, ALP activities and bilirubin concentration in addition to hepatic lipids and lipoproteins indices compared tothe corresponding control values. Oral administration of Commiphora extract [250 and 500 mg/kg] exhibited significant reductions in CCI4- induced increased levels of AST,ALT, ALP, serum bilirubin concentrations and hepatic lipids and lipoproteins indices .

Histological study

Light microscopic

H&E stain: Group I: livers of control rats showed normal sheets or cords of hepatocytes radiating from a central vein, each a single cell thick, which bifurcated and fused to form a network (Fig. 1).

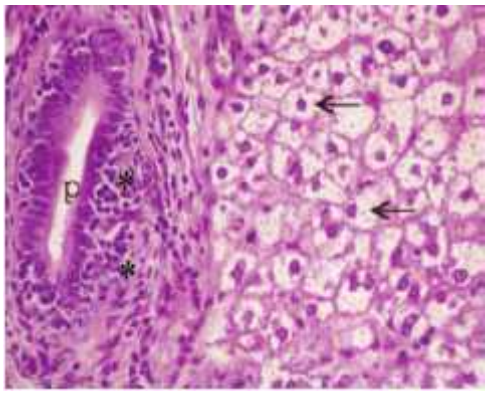

Fig. (5) A photomicrograph of a liver section from Group IIIb , showing portal trac(p)t containing proliferated bile ductules (*)surrounded by inflammatory cells Notice vacuolated hepatocytes(arrow). [H&E X 400]

Group II (treated with CCl4 0.25 mg/kg body weight, intraperitoneally): hepatocytes showed necrosis, inflammation lymphocytic infiltration, and fatty accumulation. The cytoplasm of hepatocytes was vacuolated and contained lipid droplets. Liver cords and cells were widely separated from each other with loss of the hepatic architecture and dilatation of the blood sinusoids (Fig. 2).

Group IIIa (treated with 250 mg/kg of Commiphora extract): showed a decrease in fibrous tissue and cellular infiltration around the bile ductule in preexisting portal tract with loss of hepatic architecture. Hepatocytes also had vacuolated cytoplasm (Fig. 3).

Group IIIb (treated with CCl4 and 500 mg/kg of Commiphora extract): the blood sinusoids appeared narrower, and a decrease in fibrous tissue with proliferated bile duct inflammatory cells with vacuolated hepatocyte was observed (Figs 4, 5).

Toluidine blue: Group I: livers of control rats showed normal hepatocytes with rounded vesicular nuclei and homogenous cytoplasm with no collagen fibers around the central vein (Fig. 6).

Group II (treated with CCl4 0.25 mg/kg body weight, intraperitoneally): livers of this group of rats showed necrosis and vacuolar degeneration of hepatocytes with pyknotic nucleus, large, and small cytoplasmic vacuoles. Fibrous capsule could be detected. Many mononuclear cells invaded the area and there was accumulation of collagen fibers around the central vein (Fig. 7).

Group IIIa (treated with 250 mg/kg of Commiphora extract): livers of this group of rates showed hepatocytes with vacuolated cytoplasm, dilated blood sinusoids, and macrovesicular steatosis of some hepatocytes (Fig. 8).

Group IIIb (treated with CCl4 and 500 mg/kg of Commiphora extract): livers of this group showed normal cords of hepatocytes with smaller cytoplasmic vacuoles separated by blood sinusoids (Fig. 9).

Electron microscopic examination

Group I: liver sections showed normal ultrastructure. The hepatocytes contained centrally located rounded nuclei, surrounded by the ground cytoplasm. rER and ribosomes were detected. Mitochondria were rounded or oval and uniformly distributed Ito cells with many fat globules (Figs 10 and 11).

Group II (treated with CCl4 0.25 mg/kg body weight): showed clumped cellular organelles around the nucleus and were mainly formed of mitochondria rER with fewer ribosomes. Affected hepatocytes were separated by large rarified areas indicating extensive necrotic areas (Fig. 12).

Group IIIa (treated with 250 mg/kg of Commiphora extract): it was found that Ito cells had transformed into myofibroblasts with collagen fiber, and there was decreased organelle injury with numerous mitochondria, vacuolated cytoplasm, and lipid droplets. Slight focal dilatations and breaks were observed in rERs (Figs 13 and 14) (Table 4).

Group IIIb (treated with 500 mg/kg of Commiphora extract): showed decreased organelle injury. rERs appeared to be normal with a normal nucleus and mitochondrial cristae were much more visible, with less vacuolation of hepatocyte cytoplasm. Ito cells with many fat globules.

DISCUSSION

The efficacy of any hepatoprotective drug is essentially dependent on its capability to either reduce harmful effects or to maintain the normal hepatic physiological mechanisms that have been unbalanced by the hepatotoxin [8]. CCl4 is a well-known hepatodestructive agent that is widely used to induce acute toxic liver injury in a large range of laboratory animals [16] and was used in this study as an experimental model to study liver necrosis and fibrosis.

It was found that the administration of CCl4 resulted in a rapid increase in serum AST, ALT, and ALP levels [17]. AST can be found in the liver, cardiac muscle, kidney, brain, pancreas, lungs, skeletal muscle, leukocytes, and erythrocytes in decreasing concentrations [18], whereas the highest concentration of ALT is found in the liver.

In tissues, ALT occurs in two locations: the cytosol and mitochondria. Serum ALT appears to be a more sensitive

Table 4: Organelle injury scores. (mean ± SD)

	Group 1	Group II	Group III	Group IV	P
Organelle injury scores					
Mitochondrion	33.7 ± 1.2	98.9 ± 2.1	53.6± 1.2	45.7 ± 1.3	< 0.001
Rough ER	20 ± 0.8	41.6 ± 2.1	29 ± 0.8	22 ± 0.8	<0.001
Smooth ER	15.3 ± 0.8	40.3 ± 1.6	28.6± 0.8	20.4± 0.8	<0.001
Nucleus	15.6 ± 1.2	33.3 ± 0.9	21.8 ± 1.2	17.9 ± 1.2	< 0.001
Total	84.6 ± 4	217 ± 6	135±4	106 ± 4	< 0.001

Mann-Whitney U test; Student's t-test. ER: Endoplasmic reticulum.

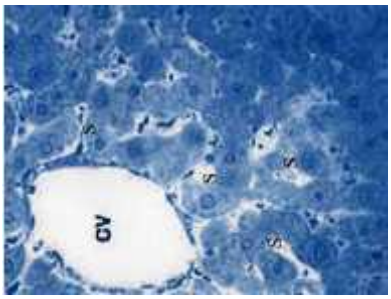

Fig.(6): A photomicrograph of a semithin section of control group , showing central vein(cv) ,cords of hepatocytes with vesicular nuclei and granular cytoplasm separated by blood sinusoids (s). (Toluidine blue stain. X400).

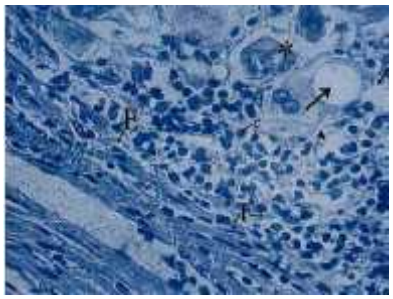

Fig.(7): : A photomicrograph of a semithin section from Group II showing capsule with fibrous tissue septa (f),mononuclear cellular infiltration (c) hepatocytes with fat globule (arrow,arrow head) and hepatocyte with abnormal metaphase chromatin (*). (Toludine blue stain.X1000).

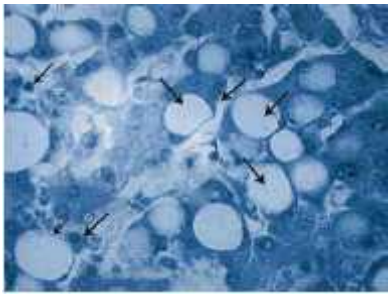

Fig.(8): A photomicrograph of a semithin section from Group IIIa showing macrovesicular steatosis of some hepatocytes (arrows). (Toludine blue stain X1000).

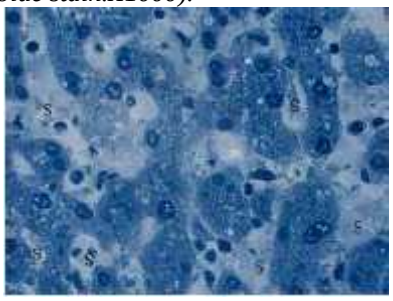

Fig.(9): A photomicrograph of a semithin section from Group IIIb showing normal cords of hepatocytes separated by blood sinusoids (s). (Toludine blue stain X1000).

and specific test of acute hepatocellular damage than serum AST [17]. Triglyceride (TG) and VLDL contents decreased in the liver after the oral administration of a gum extract of Commiphora mukul and, because of hormone-sensitive lipase, caused rapid breakdown of TG and mobilization of fatty acids. TG and VLDL also correlated with decreased cholesterol and LDL in the liver [19].

It has been observed that CCl4 is biotransformed by the cytochrome P-450 system into a trichioromethyl-free radical. This free radical may react again with oxygen to form a trichloromethylperoxyl radical, which may attack lipids on the membrane of endoplasmic reticulum. The trichloromethylperoxyl-free radical leads to lipid peroxidation, disruption of Ca2+ homeostasis, and finally results in cell death. Therefore, leakage of large quantities of enzymes into the blood stream is often associated with massive necrosis of the liver [20].

Furthermore, ALP is the prototype of these enzymes that reflects the alteration in biliary flow. CCl4-induced increase in this enzyme in serum is in line with the high level of serum bilirubin content [21]. The extractmediated suppression of the increased ALP activity with the concurrent depletion of increased bilirubin levels suggests that the extract may potentially stabilize biliary dysfunction in the rat liver, thereby indicating its effectiveness in maintaining the normal functional status of the liver [17]. In the present study, hepatocytes showed necrosis, mononuclear cell infiltration, prominent Kupffer cells, sinusoidal dilatation, and fatty accumulation. This is in agreement with the findings of some authors who reported that necrosis of liver cells was found following a single injection of CCl4 and centrilobular necrosis of liver cells and accumulation of fibers in the necrotic area was found following a single injection of CCl4.

Pretreatment with Commiphora extract had a protective dose-dependent effect in cases of hepatocyte injury, fibrosis, and the resulting cirrhosis [22]. This was in agreement with the findings of other authors [23] who reported that treatment with Commiphora extract inhibited the production of excess extracellular matrix in experimentally induced liver fibrosis. Therefore, the possible hepatoprotective mechanism of Commiphora extract CCl4-induced liver injuries may be because of inhibition of cytochrome P-450 activity, prevention of lipid peroxidation (stabilization of the hepatocellular membrane), and enhancement of protein synthesis [24]. Mitochondria, rER, sER, and nuclei were studied according to an ultrastructural scoring table [13–15]. Mitochondria are the energy source of the cell, as was specifically found in hepatocytes; it has two membranes: the outer one limits the organelle and the inner one is thrown into folds that project inward in a tubular manner called cristae. The molecules in the electron transport chain, which play a central role in ATP synthesis, are found in the cristae. It is also known that mitochondria are a major source of endogenous production of reactive oxygen species [25]. The major mitochondrial pathology in this work was ballooning, the major ultrastructural sign of cellular injury, resulting in swelling of the organelle and structural damage to cristae. This was still seen in the Commiphora-pretreated (250 ml/kg) group, and was much less in Commiphora extract (500 mg/kg)-pretreated

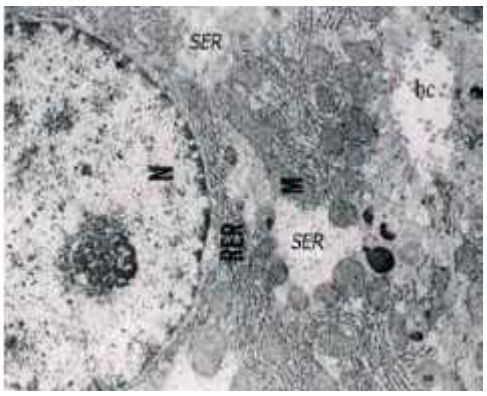

Fig (10): An electron micrograph of a section from control Group showing hepatocytes with euchromatic nucleus (N) containing nucleolus. The cytoplasm of hepatocytes contains numerous mitochondria (M), rough (rER)and smooth endoplasmic reticulum (SER) .Notice that presence of bile canaliculi(bc) containing microvili in between. (X5000).

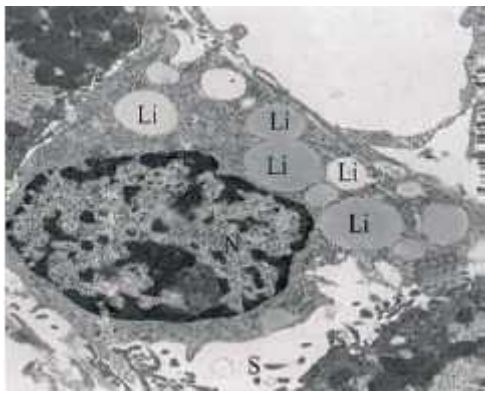

Fig (11): An electron micrograph of liver control showing Ito cell(N) with many fat globules(Li) lying inside space of Disse.(S) (X12000).

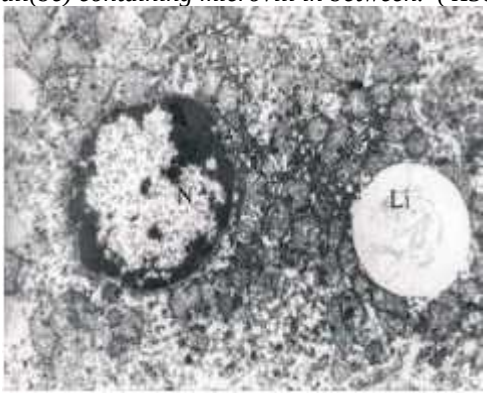

Fig (12): An electron micrograph of liver Group II showing hepatocytes with nucleus containing periphrall clumps of heterochromatin(N) and lipid droplet in cytoplasm(Li). (X12000)

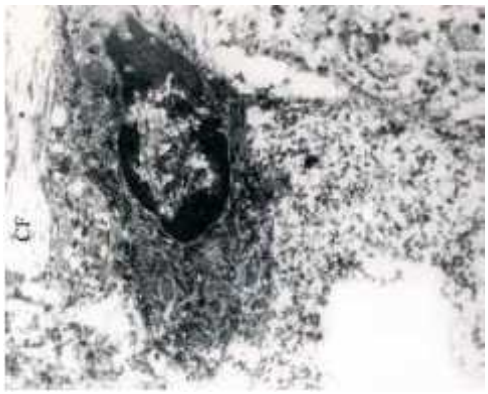

Fig (13): An electron micrograph of Group IIIa showing Ito cell (N) changed to myofibroblast containing dense body .Notice presence of collagen fiber near Ito cell(CF). (X12000)

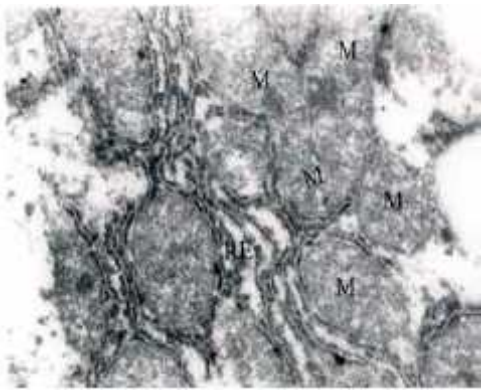

Fig (14): An electron micrograph of a section in liver after from Group IIIa showing hepatocytes with REr, degenerated mitochondria (M),and vacuolation. (X17000)

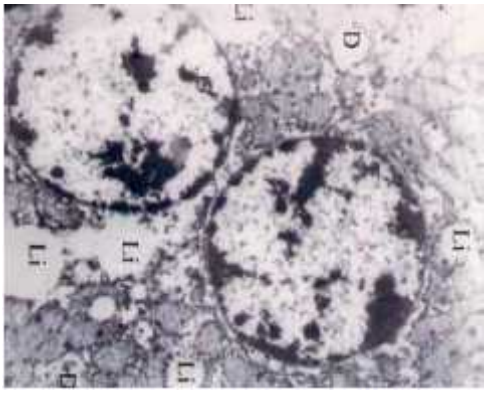

Fig (15): An electron micrograph of a section in liver from Group IIIb showing binucleated hepatocyte with lipid droplets(Li) and degenerated organelles (D). (X4600)

animals. In addition, there was minimal disruption in cristae that were preserved quite well in the fourth group. Swelling and loss of regular cristae structure, which are characteristics of deteriorated function of mitochondria, were also detected by Vanhorebeek and colleagues [26–28], and this decreased with Commiphora extract treatment.

Endoplasmic reticulum is normally composed of tubules, vesicles, and sacs forming a like shape [29]. Dilated ER is representative of severely damaged hepatocyte and other organelles [28]. rER is the place of action and stores important cellular enzymes [30]. The proteins synthesized in rER are stored in cisternae whereas glycogen synthesis and detoxification of drugs are performed in sER in the liver. In the present work, dilated rER in the rats treated with CCl4 were detected. Considerable damage such as irregular lamellar organization and focal breaks in rERs was detected. However, only some focal and slight breaks

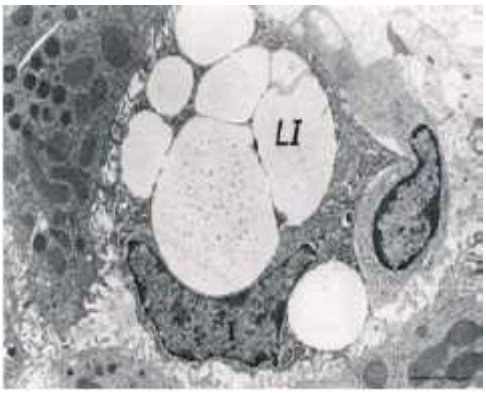

Fig (16): An electron micrograph of a section in liver from Group IIIb showing Ito cell in the space of Disse fat droplets (Li).Notice part of cytoplasm of hepatocyte with microvilli.(X12000)

were observed in Comiphora extract-treated animals [31] that were dose dependent, with significant improvement after 500 mg/kg body weight.

Nuclei, the hallmark of eukaryotic, contain DNA with its associated proteins. In the present work, alterations were detected in chromatin distribution of the nuclei of hepatocytes in CCl4-treated animals. Nuclear chromatin was almost normal in appearance and organization with Commiphora extract treatment, which was reported previously to prevent DNA damage [13].

CONCLUSION

This study showed changes in the morphology of hepatocyte organelles after induction of liver cell injury to induce fibrosis resulting in cirrhosis. Pretreatment with Commiphora extract maintained the morphology of major organelles of hepatocytes and delayed the development of fibrosis, which was dose dependent, and showed marked improvement at a dose of 500 mg/kg body weight. Structural changes in hepatocyte organelles observed in this study were likely the cause of significant histological improvements.

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