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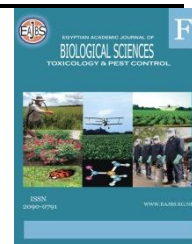
Study of the Antifibrotic Effect of Olmesartan on the Carbon Tetrachloride-Induced liver Toxicity in Rats

Sobhy Hassan Ewis[1]; Ali Abd El Salaam Ahmed[2]; and Hassan Awwad[3]

Departments of Anatomy[1],Pharmacology[2] and Rheumatology, Physical Medicine and Rehabilitation[3] Faculty of Medicine, Al-Azhar University

ABSTRACT
Hepatic fibrosis is a major medical problem in which excessive connective tissue accumulates in the liver; this tissue represents scarring in response to chronic, repeated liver cell injury. Commonly, fibrosis progresses, disrupting hepatic architecture and eventually function, as regenerating hepatocytes attempt to replace and repair damaged tissue. When such disruption is widespread, cirrhosis is occurred. Olmesartan medoxomil is an antihypertensive agent, which has a hepatoprotective effect in man and the administration of olmesartan to patients with mild alcoholic liver disease improved the abnormal liver function tests more rapidly. Fifty adult male albino rats were used in this study. The rats were divided into 5 groups each group 10 rats. The animal groups received carbon tetrachloride CCL4 except the first group (served as control group) to perform hepatic fibrosis, also the treated groups three, fourth and fifth received olmesartan with different doses in addition to CCL4. Finally, the rats were anesthetized with ether and their abdomens were opened and the livers were dissected and prepared for histological examination by light and electron microscopes. Also the blood samples were withdrawn to evaluate the liver functions. The results of the present study revealed that administration of CCL4 to the rats produced liver fibrosis and these effects were relatively improved by administration of olmesartan.

INTRODUCTION

Liver is composed of a parenchyma and stroma. The parenchyma is formed of multiple similar subunits called liver lobules. Each lobule is composed of central vein and cords of hepatocytes that radiate from the central vein towards the corners of the hexagonal lobule. At this corner, there are the portal triads; each tract is a small triangular area containing a bile duct, hepatic artery and portal vein that are enclosed by connective tissue (Sage *et al.,* 2007). Between the hepatic cords which are formed by hepatocytes, there are irregular slit-like spaces called hepatic sinusoids which are lined by endothelial cells and Kupffer cells (Fawcett and Jensh, 1997).

The liver contains different cells that include hepatocytes, pre-sinusoidal cells, endotheliocytes, macrophages «Kupffer cells», lymphocytes «pit cells», the cells of the biliary tree «cuboidal to the columnar epitheliocytes » and connective tissue cells of the capsule and portal tracts (Cormack, 2001).

Hepatocytes have rounded central nuclei and many cells in the adult liver are binucleated. Nuclear chromatin is present as scattered clumps in nucleoplasm and as distinct bands under nuclear envelop, and two or more well developed nucleoli are present inside the nucleus (Ross and Pawlina, 2006).

Adams *et al.* (1952) stated that carbon tetrachloride was found to cause hepatic injury as demonstrated b y histopathological and biochemical studies. Histopathological study showed increased lipid content of the liver and centrilobular fatty degeneration of the liver. While biochemical studies showed increased levels of serum alkaline phosphatase and plasma prothrombin clotting time.

It has already been suggested that the plasma liver cell membrane, rather than the microsome or mitochondria, is the initial site for development of liver injury produced by carbon tetrachloride administration (Yano *et al.,* 1973).

Olmesartan is an antihypertensive agent, which belongs to the class of medications called angiotensin-II receptor blockers. It is indicated for the treatment of high blood pressure (Tombolini and Cingolani, 1996).

Olmesartan is also contraindicated in diabetes mellitus patients taking aliskiren (Sanzgiri *et al.*, 1997). Administration of Olmesartan medoxomil prevented the increase in liver peroxidation caused by carbon tetrachloride induced liver damage as lipid peroxidation is one of the primary events of carbon tetrachloride (Recknagel, 1976). Olmesartan medoxomil has been shown to have a hepatoprotective effect in man. The administration of olmesartan medoxomil to patients with mild alcoholic liver disease improved the abnormal liver function tests more rapidly than in patients receiving a placebo (Salmi and Sarna, 1982).

MATERIAL AND METHODS

The study was conducted on fifty adult male albino rats with body weight ranging between 180-200g. All animals were housed in a well-ventilated room with free access to food and water. The rats had been familiarized with the environment for one week before the study.

Drug and Chemical:

Olmesartan medoxomil is a product of Sankyo Company Limited, in the form of tablets, which were crushed and suspended uniformly in 1% solution of carboxymethyl-cellulose (CMC). Carbon tetrachloride (CCl4) was purchased from El-Nasr Chemical Industries Company, in the form of liquid; it was dissolved in olive oil at a ratio of 1:1, then 1ml of this mixture was used as a dose (1ml/kg of rats).

Experimental Design:

Fifty rats were divided into 5 groups each consisted of 10 rats and distributed as follows:

Group I: *(control group)* where the animals received no treatment.

Group II: Each animal received CCl4 subcutaneously in a dose of 1 ml/kg twice weekly for 8 weeks to show the hepatic fibrosis of CCl4 (Zhang *et al*., 2001).

Group III: **Low dose olmesartan-treated group** Each animal received CCl4 (i.e. olive oil) by subcutaneous route in a dose of 1ml/kg twice weekly for 8 weeks and simultaneously the animals administered olmesartan medoxomil (i.e. CMC solution) orally by gavage in a daily dose of 0.6 mg/kg for the same period.

Group IV: **Ordinary dose olmesartan-treated group**, each animal received 1ml/kg CCl4 subcutaneously twice weekly concomitant with olmesartan medoxomil in a dose of 1 mg/kg/day orally by gavage for 8 weeks (Mizuno *et al*., 2002).

Group V: **High dose Olmesartan - treated group**, each animal received 1 ml/kg CCl4 subcutaneously twice weekly concomitant with Olmesartan medoxomil in a dose of 6 mg/kg/day orally by gavage for 8 weeks (Mizuno *et al.*, 2002).

Twenty four hours after the end of the experimental period, the rats were anesthetized with ether; the blood samples were taken to evaluate the liver functions tests in EL.HUSSEIN Hospital and their abdomens were opened through incision and liver was excised and prepared for histological examination by light and electron microscopes (Banchroft *et al.,* 1996).

Biochemical Analyses:

At the end of the experiment, after the rats were subjected to a 12 h fasting, rats were sacrificed and immediately the blood samples were withdrawn. Approximately 1.0 mL of the blood was placed in Eppendorf tubes and centrifuged at 1000xg at 4° C for 15 mins to obtain the serum that was frozen for further analysis. After samples preparation, total bilirubin was estimated according to Steven (1996). Alanine Aminotransferase (SGPT) and serum Aspartate Aminotransferase (SGOT) activities were determined according to Schumann and Klauke (2003) and Schumann *et al*. (2002) respectively. Determination of GGT activity was carried out according to Onyema *et al.* (2006). The serum proteins and serum albumin levels of the rats groups were estimated according to Burtis (1999) and Rodkey (1964) respectively. Serum Alkaline Phosphatase was determined according to Fischbach and Zawta (1992).

Statistical Analysis:

The analysis of variance (ANOVA) was performed Using Minitab-18 software, followed by the Tukey test for comparison between the means. Data were presented in the form of mean ± standard error. Values of $P <$ 0.05 were used as the limit for statistical significance.

RESULTS

The light microscopic examination of the rat's liver of the control group (group I) showed arrangement of hepatocytes in cords radiating from the central vein, separated by hepatic blood sinusoids which were lined by Kupffer cells. The hepatocytes had granular cytoplasm and vesicular nuclei, some hepatocytes contain double nuclei. The portal triad which contained bile ductule, hepatic arteriole and portal venule was seen at the periphery of the hepatic lobule (Figures 1, 2, 3, 4 and 5).

Electron microscope examination of the control group (group I) showed that the hepatocytes were surrounded by intact cell membrane (Figure 6). Each hepatocyte contained homogeneous cytoplasm with well-defined rounded mitochondria. The rough endoplasmic reticulum was well organized with partially attached ribosomes and smooth endoplasmic reticulum (Figure7). It was also contained normal lysosomes. The nucleus of hepatocyte appeared spherical in shape, two types of chromatin materials were found within each nucleus, heterochromatin, appeared attached to the inner side of the nuclear envelope and euchromatin, appeared distributed throughout the nucleus.

Sections of the rat's liver treated by CCl4 only (group II) for 8 weeks showed that the hepatocytes lost their normal arrangement in cords compared to the control group. The cords of the hepatocytes were separated by dilated blood sinusoids. Some hepatocytes showed vacuolation of their cytoplasm with pykno or karyolytic nuclei and presence of necrotic area. The portal triad showed increased number of bile ductules where some of them dilated. There was cellular infiltration of the pericentral vein by phagocytic cells).

Also there was cellular infiltration in the portal tracts and triad. Multiple Lipid droplets were found all over the hepatic lobules (Figures 8, 9, 10 and 11).

Electron micrograph of the group II showed that the hepatocytes had foam-like cytoplasm which contained karyolitic or pyknotic nuclei. Some nuclei transformed into apoptotic bodies. The cytoplasm contained red blood cells in area of hemorrhage. The mitochondria appeared to be abnormal with destructed cristae. Lipid droplets were seen inside the hepatocyte. The blood sinusoids contained collagen bundles (Figures 12, 13 and 14).

Sections of rat's liver of the group III showed that nearly normal pattern in arrangement of hepatocytes in cords radiating from the central vein. The cords were separated by hepatic sinusoids where some of them were less dilated and lined by kupffer cells. The cytoplasm appeared granular and the nuclei were vesicular. The portal tirade was nearly normal (Figure 15, 16 and 17).

Electron micrographs showed that the hepatocytes had vacuolated cytoplasm contained more or less normal mitochondria, well organized rough endoplasmic reticulum, normal smooth endoplasmic reticulum and euchromatic nucleus which contained condensed chromatin materials (Figure 18 and 19). The hepatocyte was surrounded by intact cell membrane. The lipid droplets were seen within the hepatocyte.

Sections of rat's liver (group IV) showed the hepatocytes remained nearly arranged in cords radiating from the central vein in some hepatic lobules and disarrangement of cords in other hepatic lobules comparing to the control group. There is vacuolation of cytoplasm in some hepatocytes. The hepatocytes were separated by dilated blood sinusoids. The wall of central vein in some hepatic lobule was intact but irregular with cellular infiltrations of the pericentral vein. Lipid droplets were increased from the periphery toward the central vein. The portal triad which contains portal venule, hepatic arteriole and bile ductule showed increased number of bile ductules (Figures 20, 21 and 22).

Electron microscope showed that the hepatocyte had rounded heterochromatic nuclei and other contained irregular and shrinked nuclei with condensed chromatin at the nuclear membrane. The hepatocyte showed vacuolated cytoplasm which contained swollen mitochondria. The rough endoplasmic reticulum was destructed with increased of ribosomes. The cell membrane was disorganized (Fig. 31). Bile canaliculus was seen between the hepatocytes. Lipid droplets can be seen within the hepatocytes. The blood sinusoids appeared normal (Figures 23, 24, 25, 26 and 27).

Sections of rat's liver of group V showed that the hepatocytes showed granular cytoplasm and vesicular nuclei and they were nearly normal arranged in cords radiating from the central vein, separated by hepatic blood sinusoids where some of them were less dilated and lined by Kupffer cells. The portal tirade which contained portal venule, hepatic arteriole and bile ductule was nearly normal (Figures 28, 29 and 30).

Electron microscope showed that the hepatocytes had vacuolated cytoplasm contained more or less normal mitochondria, well organized rough endoplasmic reticulum and nearly normal euchromatic nucleus which contained its nucleolus. The blood sinusoid was lined by endothelial cell and Kupffer cells and contained red blood cells (Figures 31 and 32).

The liver functions of Group (1) were the mean ± SE level of the serum glutamic pyruvic transaminase (SGPT) 36.2±1.874 U/ML., serum glutamic oxaloacetic transaminase (SGOT) 36.2±2.098 U/ML. and Gamma Glutamyl Transpeptidase (GGT) 35.4

±3.2 I.U. Alkaline phosphatase (ALP) 107.5 ± 10.07 I.U., Serum protein (T.P) 7.1±0.2582g/dL, Serum Albumin 4.36±0.32g/dL and Total Bilirubin 0.61±0.1792 mg/dL. Table (1) and Figures 33-39.

Table 1: Showing means ± SE values of liver function tests

Groups	Mean values						
	GPT (U/l)	GOT (U/l)	GGT (U/l)	ALP (U/l)	T. Protein (mg/dL)	T. Bilirubin (mg/dL)	Albumin (mg/dL)
Group I	36.2 ± 1.874[D]	36.2 ± 2.098[D]	35.4 ± 3.2[D]	107.5 ± 10.07[D]	7.1 ± 0.2582[A]	0.61 ± 0.1792[D]	4.36 ± 0.32[A]
Group II	109.5 ± 9.41[A]	109.2 ± 10.77[A]	119.6 ± 7.88[A]	240.5 ± 15.34[A]	4.14 ± 0.0966[C]	2.54 ± 0.1506[A]	2.422 ± 0.1191[D]
Group III	84.2 ± 6.99[B]	83.2 ± 5.65[B]	87.6 ± 6.17[B]	192.9 ± 9.22[B]	4.82 ± 0.2781[B]	1.849 ± 0.1483[B]	3.088 ± 0.0596[C]
Group IV	53 ± 3.8[C]	54.1 ± 6.05[C]	58 ± 4.92[C]	124.1 ± 13.15[C]	7.1 ± 0.2582[A]	0.96 ± 0.1897[C]	3.69 ± 0.677[B]
Group V	39.8 ± 2.974[D]	36.9 ± 4.53[D]	36.9 ± 5.04[D]	107.9 ± 6.74[D]	7.2 ± 0.34[A]	0.55 ± 0.1841[D]	4.22 ± 0.575[AB]

The liver functions of Group 2 were markedly affected. The mean level of the serum SGPT 109.5±9.41 U/ML., SGOT 109.2 ± 10.77 U/ML. and GGT 119.6±7.88 I.U., ALP 240.5±15.34 I.U, T.P 4.14±0.0966 g/dL, Serum Albumin 2.422 ± 0.1191 g/dL, and Total Bilirubin 2.54 ± 0.1506 mg/dL. Table (1) and Figures 33-39.
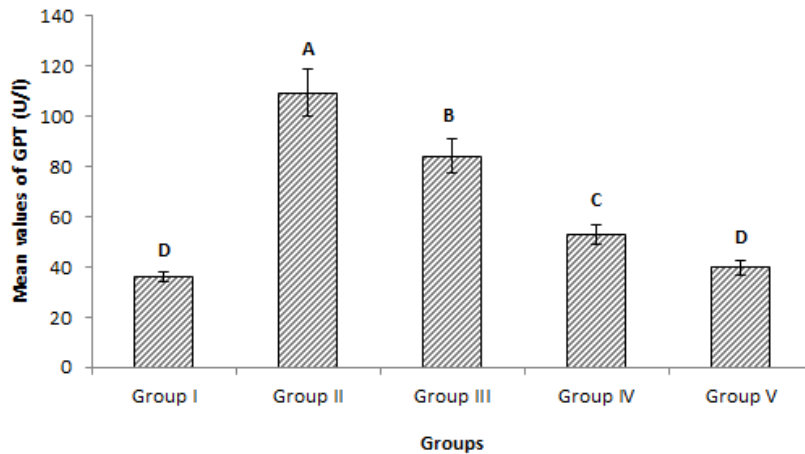
Fig. 33: Showing mean ± SE values of SGPT (Means that do not share a letter are significantly different, p<0.001).

The liver functions of Group 3 were declined. The mean level of the serum SGPT 84.2 ± 6.99 U/ML., SGOT 83.2 ± 5.65 U/ML. and GGT 87.6 ± 6.17 I.U. ALP 192.9 ± 9.22 I.U., T.P 4.82 ± 0.2781g/dL, Serum Albumin 3.088 ± 0.0596 g/dL, and Total Bilirubin 1.849 ± 0.1483 mg/dL. Table (1) and Figures 33-39.

The liver functions of Group 4 showed a marked improvement. The mean level ± SE of the serum SGPT

53 ± 3.8U/L., SGOT 54.1 ± 6.05U/L. and GGT 58±4.92I.U. ALP 124.1±13.15 IU, T.P 7.1 ± 0.2582g/dL, Serum Albumin 3.69 ± 0.677g/dL, and Total Bilirubin 0.96 ± 0.1897mg/dL Table (1) and Figures 33-39.
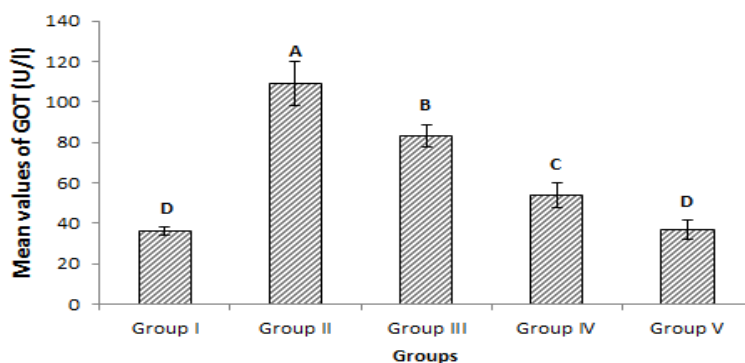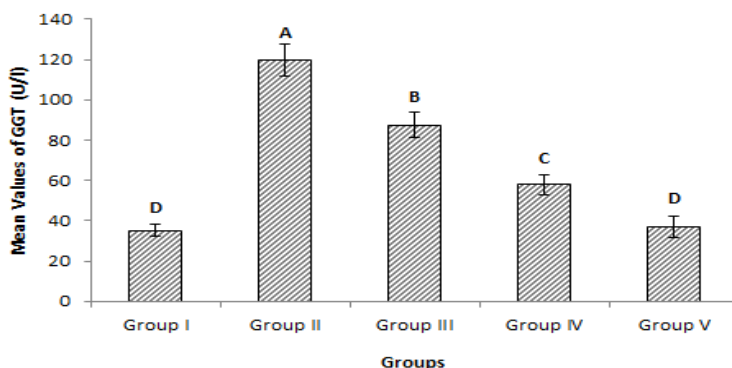

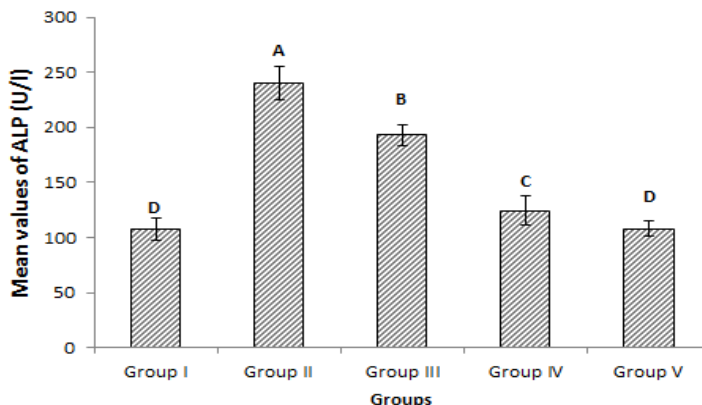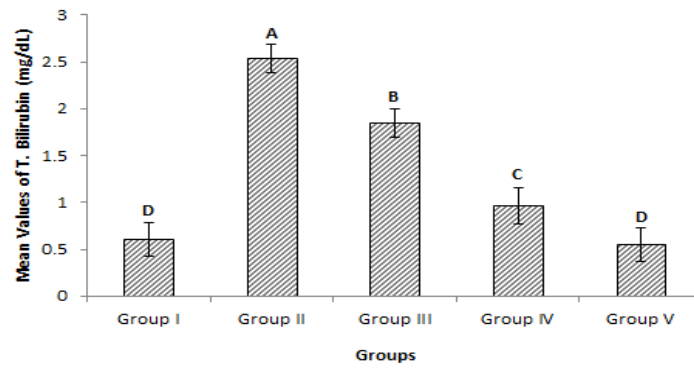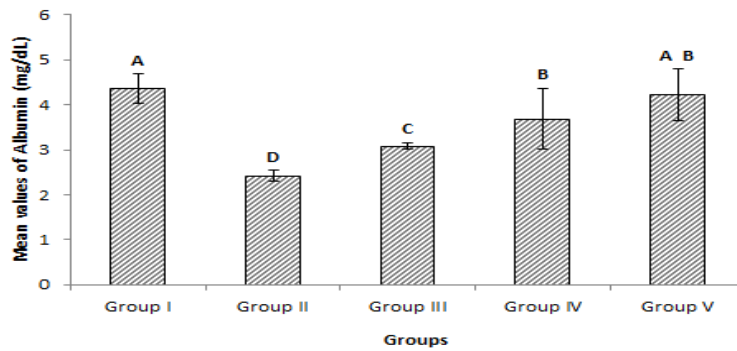Fig. 34: Showing mean ± SE values of SGOT (Means that do not share a letter are significantly different, p<0.001)

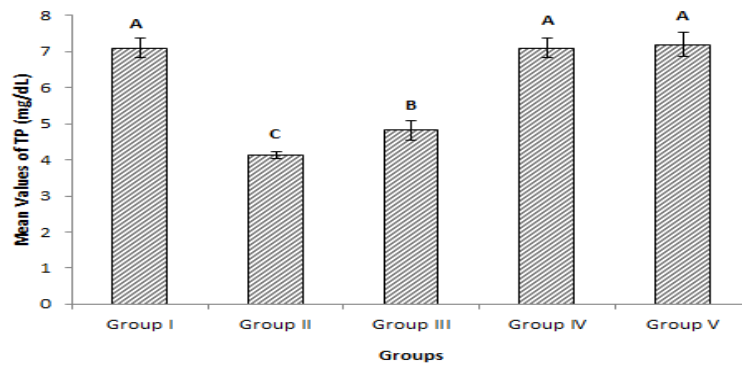Fig. 35: Showing mean values of GGT (Means that do not share a letter are significantly different, p<0.001)

The liver functions of Group 5 were showed an improvement. The mean level ± SE of the serum SGPT 39.8 ± 2.974 U/L., SGOT 36.9 ± 4.53U/L. and GGT 36.9 ± 5.04I. U. ALP 107.9 ± 6.74 I.U., T.P 7.2 ± 0.34g/dL, Serum Albumin 4.22 ± 0.575 g/dL, and Total Bilirubin 0.55±0.1841 mg/dL Table (1) and Figures 33-39.

Fig. 36: Showing mean ± SE values of Alkaline Phosphatase (Means that do not share a letter are significantly different, p<0.001).

Fig. 37: Showing mean ± SE values of Total Bilirubin (Means that do not share a letter are significantly different, p<0.001)

Fig. 38: Showing mean values of Albumin (Means that do not share a letter are significantly different, p<0.001).

Fig. 39: Showing mean values of Total Protein (Means that do not share a letter are significantly different, p<0.001).

DISCUSSION

Administration of olmesartan medoxomil prevented the increase in the liver peroxidation caused by carbon tetrachloride induced liver damage as lipid peroxidation is one of the primary events of carbon tetrachloride (Recknagel, 1976). Olmesartan medoxomil has been shown to have a hepatoprotective effect in man (Salmi and Sarna, 1982).

In the present study, the possible antifibrotic effects of low or high doses of olmesartan medoxomil, a new AT1 receptor antagonist was investigated, in a chronic model of liver fibrosis experimentally-induced by CCL4. CCL4-induced fibrosis shares several

characteristics with human fibrosis of different etiologies; thus, it is an adequate model of human fibrosis (Liu *et al.*, 2000). The hepatotoxicity of CCL4 depends on its metabolism in hepatocytes by cytochrome P450 2E1 (CYP 2E1), which generates highly reactive trichloromethyl free radicals, leading to lipid peroxidation and membrane damage (Shi *et al.*, 1998). Kupffer cells (KCs) are activated by free radicals and produce proinflammatory mediators, resulting in the triggering of an inflammatory cascade. The use of CCL4 to induce experimental oxidative stress and liver damage is quite common (Khan and Youns, 2011). It is also well known that CCl4 rapidly metabolizes to free radical products in the hepatic tissue with subsequent initiation of lipid peroxidation (Boll *et al.,* 2001). Repeated exposure with this hepatotoxin is known to exhaust the endogenous antioxidant defense pool of biological systems. To correct this oxidative imbalance, antioxidative principles derived from varied sources are utilized Rajesh and Ramtej (2014).

Antioxidants are important endogenous defense mechanism against injury caused by lipid peroxidation and harmful reactions induced by reactive oxygen species, which are constantly produced in the body during normal metabolic processes (Krensky, 1992). Olmesartan offers good protection in various toxic models of experimental liver diseases in laboratory animals. It acts as an antioxidative, anti-lipid peroxidation, antifibrotic, anti-inflammatory, membrane stabilizing and immuno- modulatory (Pradhan and Girish, 2006).

The present study showed that the light microscopic examination of the liver rats treated by CCL4 only for 8 weeks were affected as it showed disarrangement of liver cords, vacuolation of cytoplasm of hepatocytes, presence of some karyolitic and other pyknotic nuclei, necrotic area and dilatation of blood sinusoids. These results go in agreement with the results by George *et al.* (2001) which revealed parenchymal disarrangement, swelling of hepatocyte and cytoplasm degeneration. Also it showed necrotic hepatocyte in the liver sections of mice treated by CCL4. Also, the results of the present study are in parallel with results of Gi-Ppeum *et al.* (2005) who found that CCL4 induced centrilobular necrosis in liver section of CCL4 treated group.

Also in liver of rats received CCL4 alone for 8 weeks of the present study, examination of rat's liver by light microscope showed increased of bile ductules where some of them were dilated, congestion of the central vein, shedding of the wall of the central vein with hemorrhage, presence of cellular infiltration around the central vein and in the portal tract. Lipid droplets were also seen in these groups. These results go in agreement with results by (George *et al.* 2001), who found marked macro and microvesicular fatty changes of hepatocytes around the central vein and sever centrilobular congestion and intense neutrophilic infiltration. Also, with results by Gi-Ppeum *et al.* (2005) who found fatty changes and collagen fibers separating the central veins in rat liver treated by CCL4.

The results of the present study are in agreement with results in a study by Abdel Salam *et al.* (2007) who found in CCL4 treated group cellular infiltration and damaged bile ductule and with results by Padhy *et al.* (2007) which revealed leukocytic infiltration, in CCL4 treated rats. It also in agreement with the results found by Das *et al.* (2008) who fund that the liver of rat affected by CCL4 revealed fatty degeneration.

The present work shows that serum SGPT, SGOT, GGT, ALP, albumin

were increased in the CCl4- treated rats in comparison with the normal untreated rats (group 1). Hepatocellular damage causes release of these enzymes into circulation. Increase in serum levels of SGOT shows hepatic injuries similar to viral hepatitis, infarction, and muscular damages. SGPT, which mediates conversion of alanine to pyruvate and glutamate, is specific for liver and is a suitable indicator of hepatic injuries. Increased levels of these enzymes are an indicator of cellular infiltration and functional disturbance of liver cell membranes Drotman and Lawhorn (1978).

GGT, bilirubin is part of fibrotest which is a good indicator of fibrosis is corrected by high and ordinary doses of olmesartan denoting antifibrotic effect. The antifibrotic effect is evident to this class of drugs and human clinical trials to explore its efficacy is underway for candesartan, losartan, irbesartan, ambrisartan in various models of liver diseases as virus C hepatits (HCV), nonalcoholic steatohepatitis (NASH), liver cirrhosis, hepatic fibrosis with HCV. (Naftaly and Friedman 2011)

AT1a receptor knockout mice have reduced lipid peroxidation products, inflammation and fibrosis following bile duct legation (Yang *et al*. 2005).

Consequently, blocking the renin angiotensin system (RAS) by ACE inhibitors or AT1 receptor blockers (ARBs) may be an effective strategy in the treatment of liver fibrosis, and is already undergoing testing in human trials. Long-term administration of losartan to patients with chronic HCV leads to decreased NADPH oxidase, decreased inflammation, and reduced fibrogenesis. (Ghany *et al*. 2003).

A beneficial role of Ang II blockade in HCV-related fibrosis has been reported from a randomized control trial comparing three groups; HCV patients with hypertension treated with ARB compared with non-ARB treatment, and compared with non-hypertensive patients (Corey *et al*. 2009).

Administration of an ARB, compared with other antihypertensive drugs in patients with recurrent HCV after liver transplantation, was associated with less progression in inflammation but not in fibrosis (Cholongitas *et al*. 2010).

With the discovery that Ang II has two major receptor subtypes, the Ang II type 1A and 1B receptors (AT1AR and AT1BR) and Ang II type 2 receptor (AT2R), with AT2R has anti-proliferative, anti-inflammatory and anti-fibrotic effects. It has been reported that the increased stimulation of AT2R may be responsible for some of the therapeutic effects observed during AT1R blockade. Moreover, AT1R antagonists are less effective in AT2R-deficient mice, again confirming that AT2R play a pivotal role in the effect of AT1R antagonists (Kanno *et al.*, 2003). So the present results may be explained by upregulation of AT2R activity secondary to AT1R blockade which may need another study to elucidate role of AT2R action.
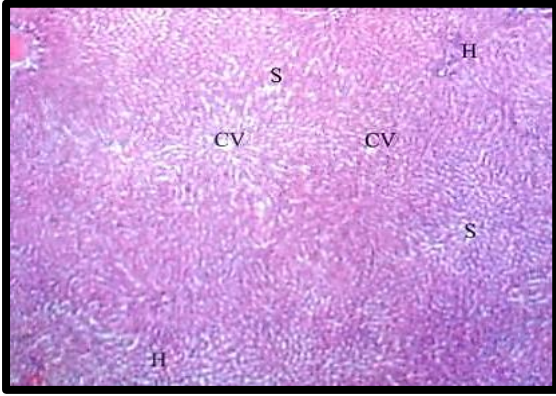
Fig. 1: Photomicrograph of a section of control liver rat showing; hepatocytes (H) arranged in cords radiating from the central vein (Cv) which had regular intact wall. The hepatocytes were separated by blood sinusoids (S). H & E (X-100).
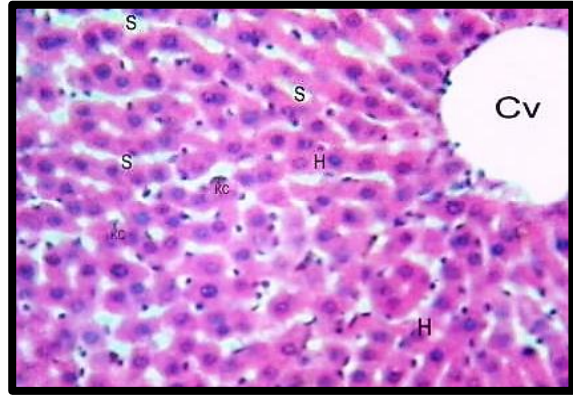

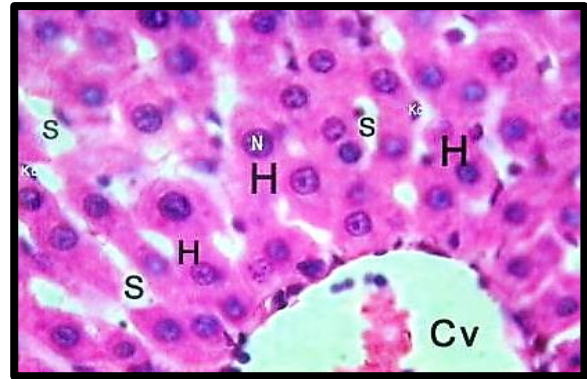Fig. 2: Photomicrograph of a section of control liver rat showing; hepatocytes (H) arranged in cords radiating from the central vein (Cv) which had regular intact wall. The hepatocytes were separated by blood sinusoids (S) which were lined by kupffer cells (Kc). H & E (X-200).
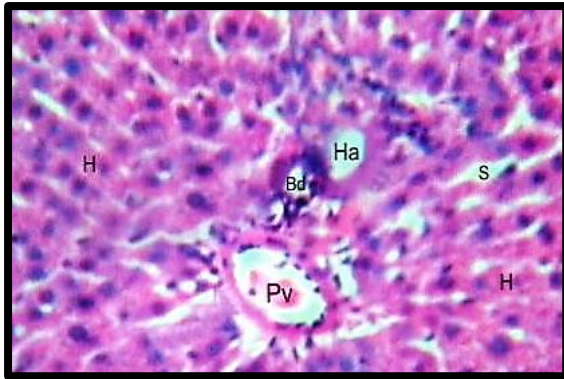
Fig. 3: Photomicrograph of a section of control liver rat showing; hepatocytes (H) arranged in cords and separated by blood sinusoids (S). The portal triad was found at the periphery of the lobule which contained bile ductule (Bd), hepatic arteriole (Ha) and portal venule (Pv). H & E (X-200).

Fig. 4: Photomicrograph of a section of control liver rat showing; hepatocytes (H) arranged in cords radiating from the central vein (Cv) separated by blood sinusoids (S) which lined by kupffer cells (Kc). H & E (X-1000)
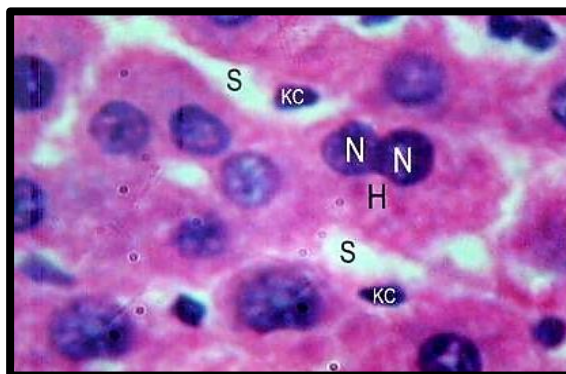
Fig. 5: Photomicrograph of a section of control liver rat showing; hepatocytes (H) separated by blood sinusoids (S) which were lined by kupffer cells (Kc). Some hepatocytes contain double nuclei (N). H & E (X-1000)
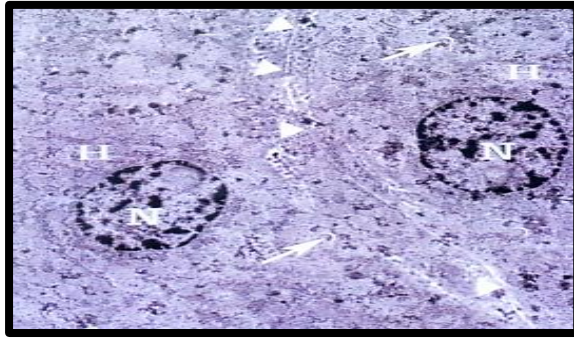
Fig. 6: Electron micrograph of a hepatocytes of control rat liver showing two normal hepatocytes (H) with cytoplasm contained normal lysosomes (arrow) and rounded euchromatic nuclei (N) with condensed chromatin. The two hepatocytes were separated by cell membrane (arrow head). (X-6000)
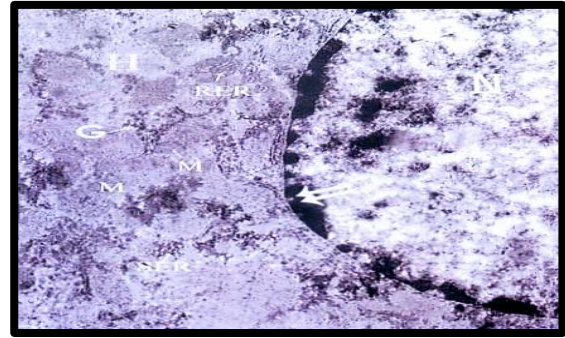

Fig. 7: Electron micrograph of a hepatocyte of control rat liver showing the cytoplasm of the hepatocyte (H) contained well defined rounded mitochondria (M), rough endoplasmic reticulum (RER), smooth endoplasmic reticulum (SER) and rounded euchromatic nucleus (N) with condensed peripheral chromatin (double arrow). (X-20000)
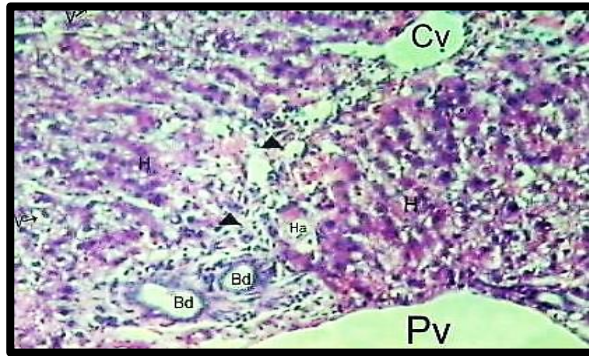
Fig. 8: Photomicrograph of a section of treated rat liver by CCL4 for 8 weeks showing; the hepatocytes (H) lost their normal arrangement in cords radiating from the central vein (Cv) and vacuolation (V) of cytoplasm of hepatocytes. Branch of portal vein (Pv) with hepatic arteriole (Ha) and bile ductile (Bd) which was increased in the portal triad were seen surrounded by increased of cellular infiltration (arrow head). H & E (X-200).
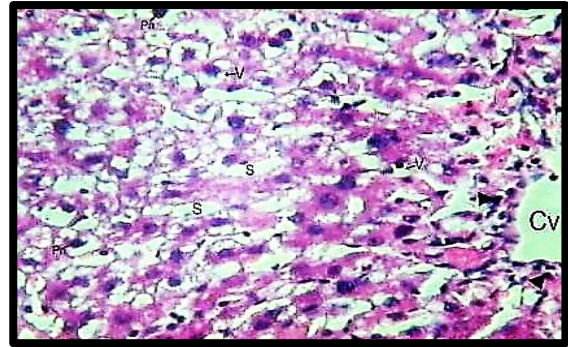
Fig. 9: Photomicrograph of a section of the liver of rat received CCL4 for 8 weeks showing; the hepatocytes (H) lost their normal arrangement in cords radiating fromthe central vein (Cv) and are separated by dilated blood sinusoids (S). Some hepatocytes showed vacuolation (V) of their cytoplasm and pyknosis of some nuclei (Pn). There is also cellular infiltration (arrow head) around the central vein. H & E (X-200).
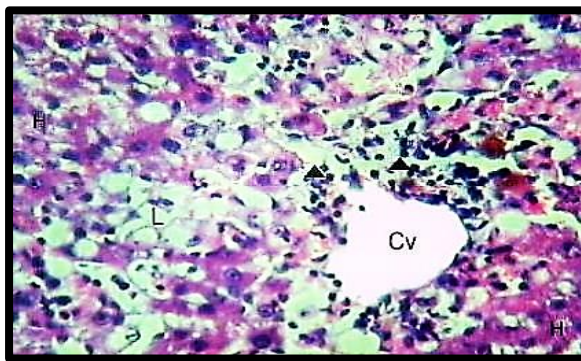

Fig. 10: Photomicrograph of a section of treated rat liver by CCL4 for 8 weeks showing; the hepatocytes (H) lost their normal arrangement in cords radiating from the central vein (Cv). Cellular infiltration (arrow head) was found around the central vein. Multiple lipid droplets (L) scattered all over the hepatic lobule. The wall of the central vein (Cv) is intact but irregular. (H & E, X-200)
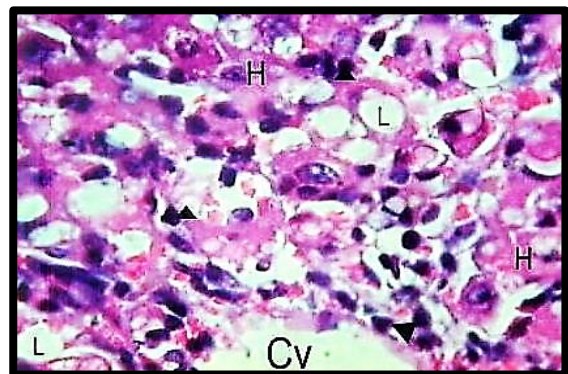

Fig. 11: Photomicrograph of a section of treated rat liver by CCL4 for 8 weeks showing; the hepatocytes (H) lost their normal arrangement in cords radiating from the central vein (Cv) with cellular infiltration (arrow head) around the central vein. Multiple lipid droplets (L) are scattered all over the hepatic lobule. H & E (X-400).
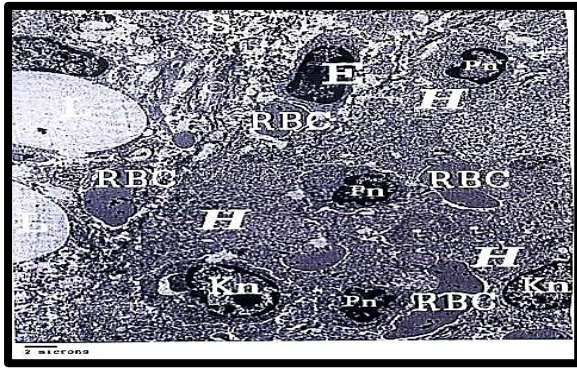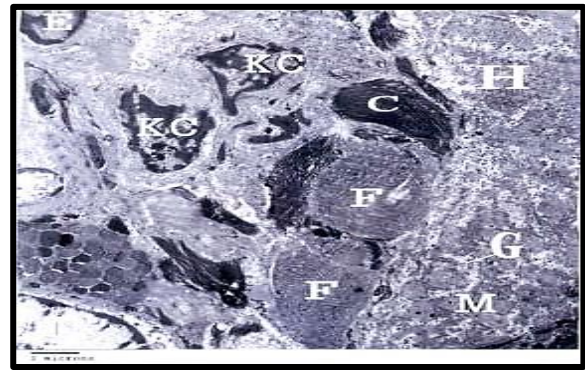
Fig. 12: Electron micrograph of a hepatocyte of treated rat liver by CCL4 for 8 weeks showing; foam-like cytoplasm of hepatocyte (H) contained red blood cells (RBC) in hemorrhagic area and some pyknotic nuclei (Pn) and other karyolitic nuclei (Kn). There were two large lipid droplets (L). Blood s i n u s o i d (S) l i n e d by endothelial cells (E) and contained collagen fibers (C) was also seen (X-4000).

Fig. 13: Electron micrograph of a hepatocyte of treated rat liver by CCL4 for 8 weeks showing; foam-like cytoplasm of hepatocyte (H) contained overcrowded mitochondria (M) and a little of glycogen granules (G). The blood sinusoid (S) was lined by Kupffer cells (Kc) and endothelial cells (E) and contained collagen bundles (C). The fibroblasts (F) were clearly seen. (X-6000).
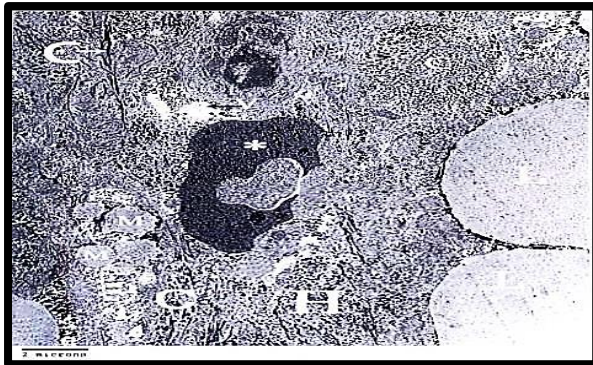

Fig. 14: Electron micrograph of a hepatocyte of treated rat liver by CCL4 for 8 weeks showing; vacuolated (V) cytoplas of hepatocyte (H), abnormal mitochondria (M) with destructed cristae and apoptotic bodies (asterisk). Multiple lipid droplets (L) and collagen bundle**S** (C) can be seen. (X-8000).
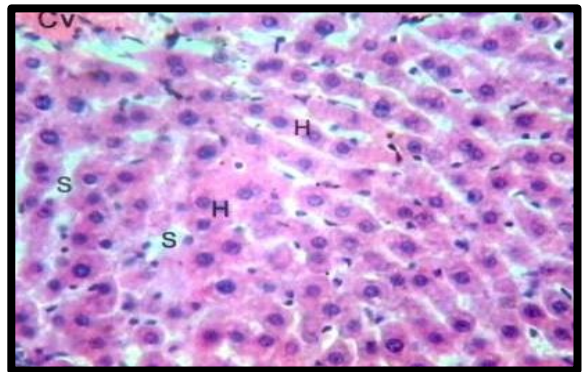

Fig. 15: Photomicrograph of a section of the liver of rat treated by CCL4 Simultaneously with Olmesartan for 8 weeks showing; hepat-ocytes (H) nearly normally arranged in cords radiating from the central vein (Cv) and are separated by less dilated blood sinusoids (S). H & E (X-200).
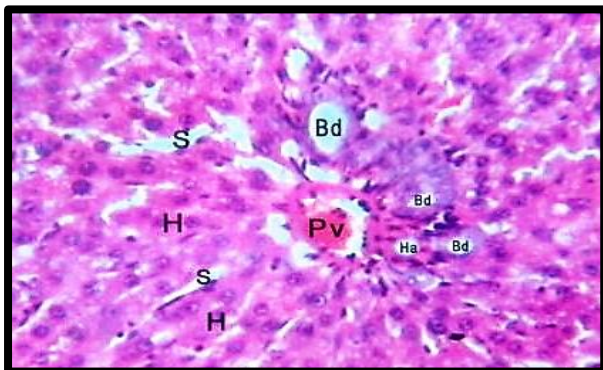

Fig. 16: Photomicrograph of a section of the liver of rat treated by CCL4 Simultaneously with Olmesartan for 8 weeks showing; the hepatocytes (H) are normally arranged in cords and are separated by less dilated blood sinusoids (S). The portal triad is normal where it contains portal venule (Pv), hepatic arteriole (Ha) and bile ductule (Bd). H & E (X-200).
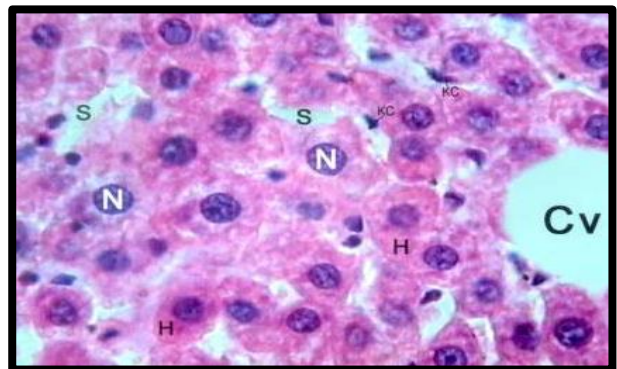

Fig. 17: Photomicrograph of a section of the liver of rat treated by CCL4 Simultaneously with Olmesartan for 8 weeks showing; hepatocytes (H) nearly normal arranged in cords radiating from the central vein (Cv) which has intact and regular wall. The hepatocytes have normal nuclei (N) and they are separated by less dilated blood sinusoids (S) which are lined by kupffer cell (Kc). H & (X 1000)
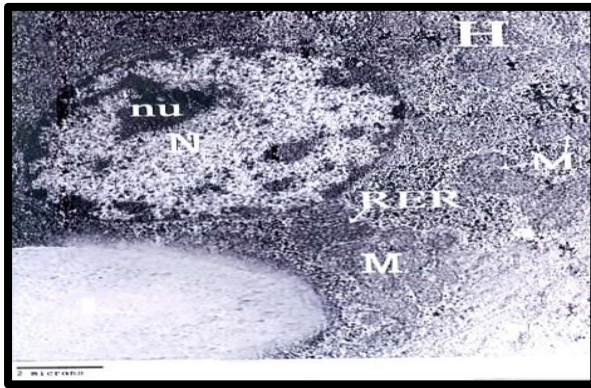
Fig. 18: Electron micrograph of a hepatocyte of treated rat liver by CCL4 8 weeks simultaneously with Olmesartan for 8 weeks showing; cytoplasm of hepatocyte (H) contained more or less normal mitochondria (M) and rough endoplasmic reticulum (RER). It also contained normal smooth endoplasmic reticulum (SER) with euchromatic nucleus (N). Large lipid droplet (L) can be seen. (X-10000).
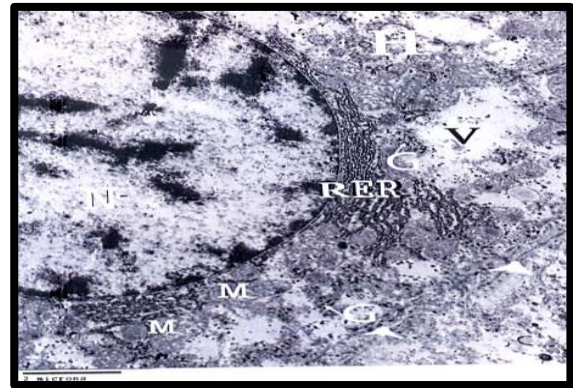

Fig. 19: Electron micrograph of a hepatocyte of treated rat liver by CCL4 8 weeks simultaneously with Olmesartan for 8 weeks showing; vacuolated (V) cytoplasm of hepatocyte (H) contained nearly normal mitochondria (M), disturbed glycogen granules (G) and well organized rough endoplasmic reticulum (RER). The cell membrane (arrow head) appears intact. Part of normal euchromatic nucleus (N) with its condensed chromatin can be seen. (X-12000).
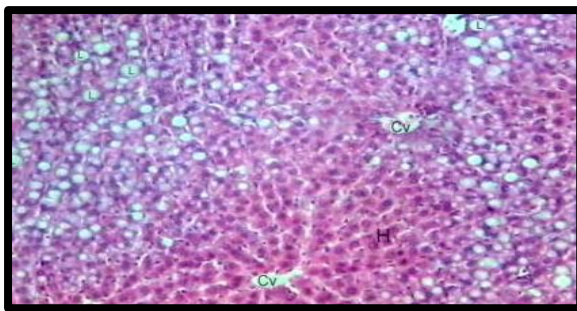

Fig. 20: Photomicrograph of a section of treated rat liver by CCL4 for 8 weeks Concomitant with Olmesartan in Low dose for 8 weeks showing; hepatocytes (H) nearly normally arranged in cords radiating from the central vein (Cv) with multiple lipid droplets (L) increasing from the periphery towards the central vein. H & E (X-200)
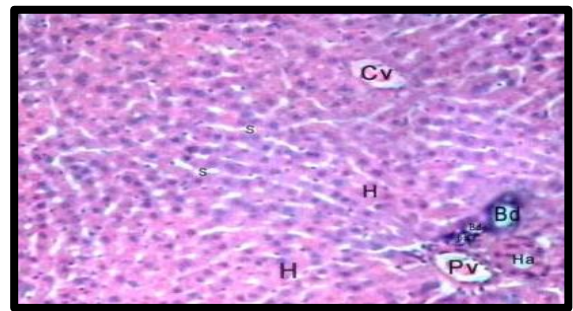

Fig. 21: Photomicrograph of a section of treated rat liver by CCL4 for 8 weeks Concomitant with Olmesartan in Low dose for 8 weeks showing; hepatocytes (H) lost their normally arrangement in cords radiating from the central vein (Cv) and are separated by blood sinusoids (S). The portal triad which contains portal venule (Pv), hepatic arteriole (Ha) and bile ductule (Bd) showing increased number of bile ductules. H & E (X-200).
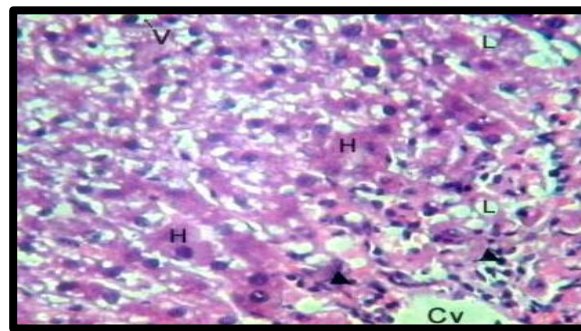

Fig. 22: Photomicrograph of a section of treated rat liver by CCL4 for 8 weeks Concomitant with Olmesartan in Low dose for 8 weeks showing; the hepatocytes (H) lost their normal arrangement in cords radiating from the central vein (Cv) and vacuolation (V) of cytoplasm. Cellular infiltration (arrow head) around the central vein with lipid droplets (L) are also found. H & E (X-200).
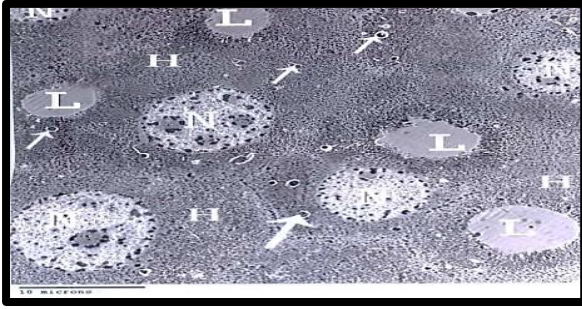
Fig. 23: Electron micrograph of hepatocytes of treated rat liver by CCL4 for 8 weeks concomitant with Olmesartan in low dose for other 8 weeks showing; hepatocytes (H) which contained rounded heterochromatic nuclei (N) with normal lysosomes (arrow). Multiple lipid droplets (L) can be seen clearly within the hepatocytes. (X-6000).
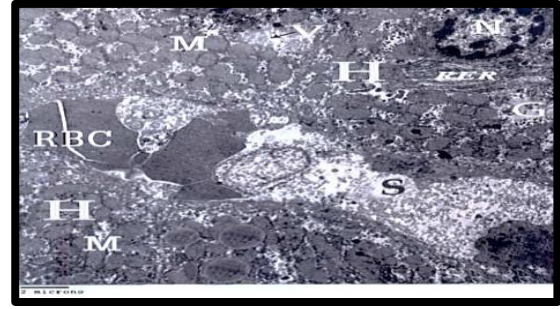

Fig. 24: Electron micrograph of a hepatocyte of treated rat liver by CCL4 8 weeks concomitant with Olmesartan in low dose for other 8 weeks showing; the cytoplasm of the hepatocyte (H) was vacuolated (V) with swollen and crowded mitochondria (M). The rough endoplasmic reticulum (RER) was destructed and the nucleus (N) was small with condensed chromatin. Red blood cell (RBC) can be seen clearly inside the lumen of the sinusoids (S). (X-6000)

Fig. 25: Electron micrograph of a hepatocyte of treated rat liver by CCL4 8 weeks concomitant with Olmesartan in low dose for other 8 weeks showing; a hepatocyte (H) contained swollen mitochondria (M). The nucleus (N) was irregular and shrinked with condensed chromatin at its membrane (double arrow). (X-10000)
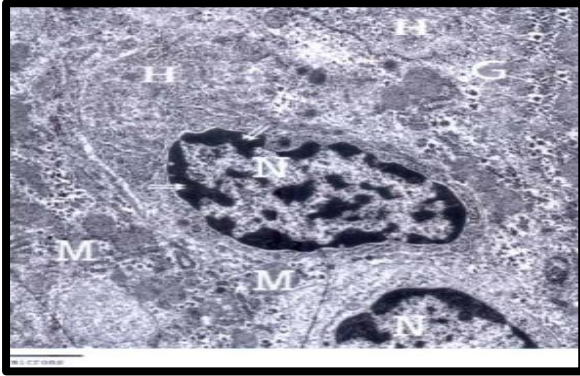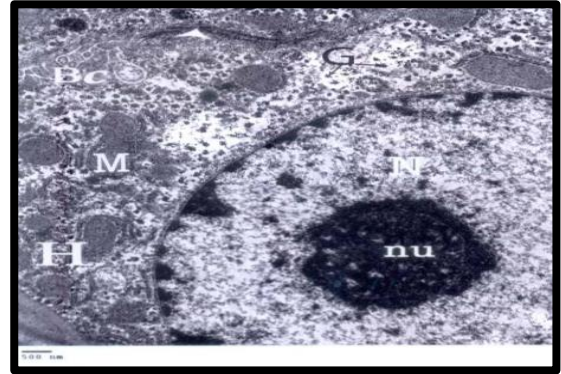

Fig. 26: Electron micrograph of a hepatocyte of treated rat liver by CCL4 8 weeks concomitant with Olmesartan in low dose for other 8 weeks showing; a hepatocyte (H) with swollen mitochondria (M). It had intact cell membrane (arrow head). The nucleus (N) appeared normal with nucleolus (nu). Bile canaliculus (Bc) was also seen at the periphery of hepatocyte. (X-15000)

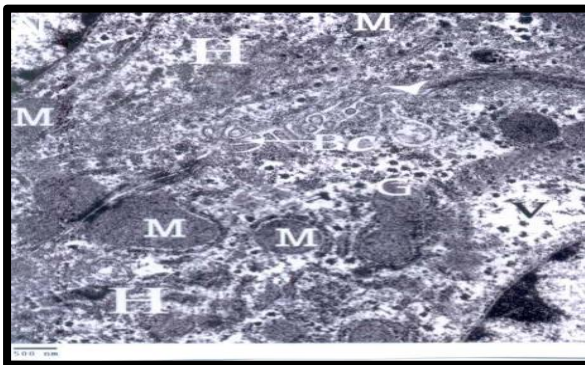Fig. 27: Electron micrograph of a hepatocyte of treated rat liver by CCL4 for 8 weeks concomitant with Olmesartan in low dose for other 8 weeks showing; two hepatocytes (H) with vacuolated (V) cytoplasm contained swollen mitochondria (M) and part of their nuclei (N). The two hepatocytes were separated by disorganized cell membrane (arrow head). Bile canaliculus (Bc) was seen between the hepatocytes. (X-10000)
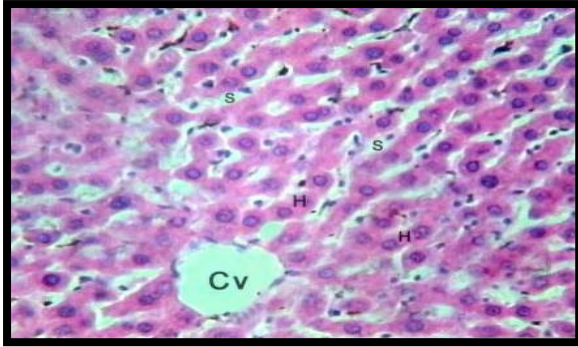
Fig. 28: Photomicrograph of a section of the liver of rat received by CCL4 for 8 weeks Concomitant with olmesartan in high dose for 8 weeks showing; hepatocytes (H) are normally arranged in cords radiating from the central vein (Cv) and are separated by less dilated blood sinusoids (S). H & E (X-200).
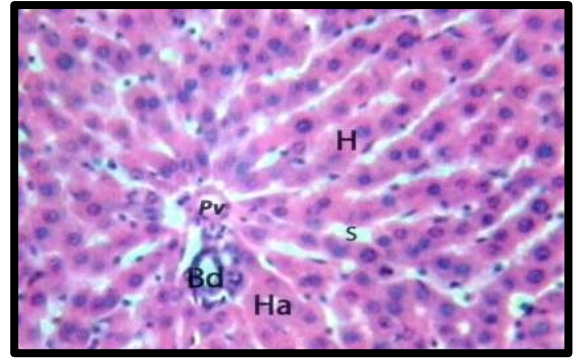

Fig. 29: Photomicrograph of a section of treated rat liver by CCL4 for 8 weeks Concomitant with olmesartan in high dose for 8 weeks showing; hepatocytes (H) are normally arranged in cords and are separated by less dilated blood sinusoids (S). The portal triad which contains bile ductule (Bd), hepatic arteriole (Ha) and portal venule (Pv) is normal. H & E (X-200).
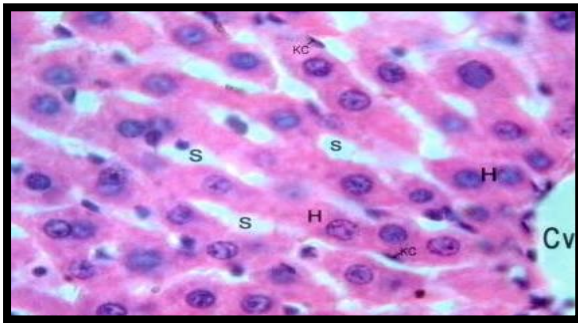

Fig. 30: Photomicrograph of a section of the liver of rat received CCL4 for 8 weeks Concomitant with olmesartan in high dose for 8 weeks showing; hepatocytes (H) are normally arranged in cords radiating from the central vein (Cv) and are separated by less dilated blood sinusoids (S) which are lined by kupffer cells (Kc). H & E (X-1000).

Fig. 31: Electron micrograph of a hepatocyte of treated rat liver by CCL4 for 8 weeks concomitant with Olmesartan in high dose for other 8 weeks showing; vacuolated (V) cytoplasm of hepatocyte (H) contained more or less normal rough endoplasmic (RER). Normal euchromatic nucleus (N) can be seen. The blood sinusoid (S) contained red blood cell (RBC) and lined by endothelial cell (E) was also seen. (X-5000).
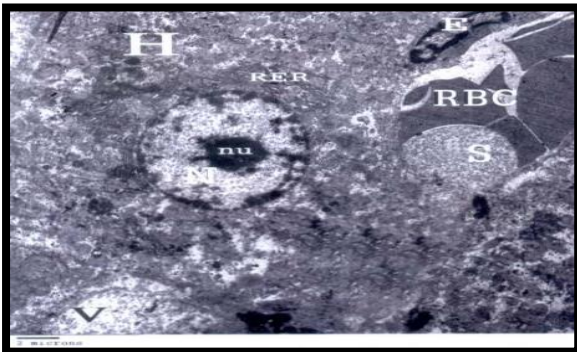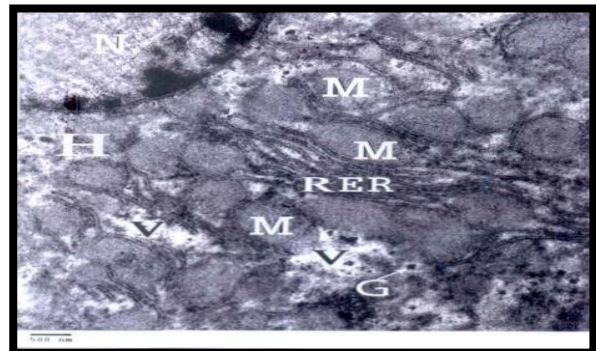

Fig. 32: Electron micrograph of a hepatocyte of treated rat liver by CCL4 for 8 weeks concomitant with Olmesartan in high dose for other 8 weeks showing; vacuolated (V) cytoplasm of hepatocyte (H) contained more or less normal mitochondria (M), dispersed glycogen granules (G) and normal rough endoplasmic reticulum (RER). (X-20000).

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الملخص العربي

دراسة تأثير الأولميسارتان المضاد للتليف المحدث بسمية رابع كلوريد الكربون في اكباد الجرذان

صبحي حسن عويس١ ـــ علي عبد السلام احمد٢ـ حسن محمد محمد عواد٣
١- اقسام التشريح
٢- الأدوية طب بنين اسيوط، جامعة الازهر
٣- الروماتيزم والطب الطبيعي والتاهيل   طب بنين القاهرة

يعد التليف الكبدي مشكلة طبية رئيسية يتراكم فيها النسيج الضام المفرط في الكبد. هذا النسيج يمثل تندبا ينشأ عن إصابة الخلية الكبدية المزمنة والمتكررة. عادة، يتطور التليف ، ويعطل العمارة الكبدية في نهاية المطاف ، حيث أن خلايا الكبد المتجددة تحاول استبدال وإصلاح الأنسجة التالفة. عندما ينتشر هذا الاضطراب ، يحدث تليف الكبد.

اولميسارتان عقار يعطي للمرضى كدواء خافض لضغط الدم وله تأثير مضاد للالتهاب في الانسان . كما ان اعطاؤه للمرضى الذين يعانون من امراض الكبد الكحولية قد حسن من وظائف الكبد غير الطبيعية بدرجة اكبر

تم استخدام خمسون من ذكور الجرذان البيضاء في هذه الدراسة. تم تقسيم الجرذان إلى ٥ مجموعات كل مجموعة ١٠جرذان. تلقت مجموعات الحيوانات رابع كلوريد الكربون CCL4 باستثناء المجموعة الأولى (خدمت كمجموعة مراقبة) لاحداث التليف الكبدي، كما تلقت المجموعات المعالجة الثالثة والرابعة والخامسة أولميسارتان بجرعات مختلفة بالإضافة إلى رابع كلوريد الكربون .

وبعد انتهاء مدة العلاج تم سحب عينات الدم لتقييم وظائف الكبد ثم تخدير الجرذان باستخدام الاثير وتم استئصال الكبد وتشريحه وتجهيز انسجة الكبد للفحص بواسطة الميكروسكوب الضوئي والالكتروني

اظهرت نتائج الدراسة الحالية ان اعطاء رابع كلوريد الكربون للجرذان ادى الي تليف في الكبد وأن العلاج بعقار اولميسارتان ادي الى تحسن نسبي في تليف الكبد