Histological Study the Effect of Captopril on the Liver of Albino Mice

Manal H El kafrawy

Anatomy Department - Faculty of Medicine- El Azhar University, Cairo, Egypt
manal.kafrawy@gmail.com

Abstract: The present study has been designed to evaluate the possible hepatotoxicity of the angiotensin converting enzyme inhibitor, captopril on liver of adult of albino mice. The study included the effect of oral administration of captopril for two months in adult mice. The dose used in the present study represents the dose equivalent to the therapeutic daily dose taken by human. Ninety adult albino mice weighing (25±20) g were used in this study. They were divided into three main groups(n=30 each).Control group (G1),.Group (G2)&group (G3) treated with captopril (0.01mg& o.o2mg daily for two months).Light and electron microscopic examination of liver revealed pathological lesions in group.(G2) and group(G3).The liver of group(G2)showed necrosis and dilatation of sinusoids, necrosis of stage of karyolysis which remarkable changes appeared in the hepatocytes in group (G3)as compared with those in group(G2) . Ultrastructural examination of the liver of the treated group (G2) revealed the hepatocytes contain the distorted cytoplasmic organelles, the nucleus possessed condensed chromatin and dentate nuclear membrane .The sinusoidal lining revealed fragmented endothelial cell and distorted kupffer cell. The group (G3)revealed the hepatocytes contain the degenerated cytoplasmic organelles, with indented and fragmented nuclear membrane, clumped the chromatin .The hepatocytes showed cytoplasmic degeneration ,with scattered apoptotic bodies and distorted blood cells and hypertrophy kupffer cell in sinusoid.
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Keywords: Captopril; Liver; albino mice

1. Introduction

Stroke is a major cause of mortality and disability worldwide. The main cause of stroke is atherosclerosis, and the most common risk factor for atherosclerosis is hypertension. Therefore, antihypertensive treatments are recommended for the prevention of stroke[1].

Angiotensin-converting enzyme (ACE) inhibitors are considered to be effective clinical therapy for hypertension and heart failure. It has become obvious that ACE inhibitors reduce ischemic myocardial injury [2].

Captopril is an angiotensin-converting enzyme (ACE) inhibitor is widely used in the treatment of hypertension and congestive heart failure. It contains a sulphydryl (-SH) group. This sulphydryl group may have the ability to scavenge cytotoxic oxygen-derived free radicals, which play an important role in post ischemic contractile dysfunction [3]. It contains active sulphydryl group and shares other structural feature with cysteine, the main substrate of glutathione [4].

Captopril can also improve the severity of inflammation through the following pathways. First, it inhibits endothelial derived growth factor (EDGF)/nitric oxide (NO) degradation through eliminating oxygen free radicals, and reinforced EDGF/NO functions [5]. Secondly, it improves microcirculatory disturbance through promoting the synthesis of vascular endothelial cell and the release of prostacyclin [6]. Thirdly, it reduces endothelin release to attenuate tissue injury [7]. Finally, it reduces intracellular Ca2+ levels by decreasing Ca2+ passage and inhibiting the activity of Ca2+ in passing through cell membrane to avoid intracellular Ca2+ overloading, thus attenuating cell injury [8].

Angiotensin converting enzyme (ACE) inhibitors are standard therapy for cardiovascular diseases including congestive heart failure and hypertension. ACE inhibitors have been used worldwide and have been reported to cause relatively few side effects. The antihypertensive effects of these drugs are related to their ability to block the conversion of the decapeptide, angiotensin I, to the potent pressor octapeptide , angiotensin II. Thus cause vasodilatation and lowering of blood pressure[9].

Captopril (CP) is an angiotensin-converting enzyme inhibitor whose metabolism involves endogenous thiols which may be depleted at high doses of CP. Following intraperitoneal administration of CP (50-300 mg/kg), dose-dependent depletion of hepatic glutathione, increased serum transaminase (SGPT) levels and hepatic necrosis were observed. The hepatic necrosis observed was either subcapsular or parenchymal in distribution. Both types of necrosis showed a dose-dependent increase in severity but with a large inter-animal variation. Oral CP (300 mg/kg) caused parenchymal necrosis in only one animal. It is suggested that subcapsular necrosis may be due to the direct effect of i.p. captopril whereas

parenchymal necrosis may be a consequence of hepatic GSH depletion. [10].

Angiotensin-converting enzyme (ACE) inhibitors have been widely used to control hypertension, but their use during gestation may result in fetal death, intra-uterine growth retardation, oligoamnium sequence, hypotension, acute renal failure and ductus arteriosus patency in the newborn. The aim of this case report is to highlight the risks of using this drug during gestation. The authors present a case of captopril use during pregnancy, whose newborn developed acute renal failure and ductus-arteriosus patency early in the newborn period.[11]

Use of angiotensin-converting–enzyme (ACE) inhibitors during the second and third trimesters of pregnancy is contraindicated because of their association with an increased risk of fetopathy. In contrast, first-trimester use of ACE inhibitors has not been linked to adverse fetal outcomes. We conducted a study to assess the association between exposure to ACE inhibitors during the first trimester of pregnancy only and the risk of congenital malformations**[12].**

ACE inhibitors can induce angioedema. ACE inhibitors block the enzyme ACE so it can no longer degrade bradykinin; thus, bradykinin accumulates and causes angioedema **[13].** Angioedema is a well-recognized side effect of angiotensin-converting enzyme (ACE) inhibitor therapy. Angioedema can also be seen with angiotensin receptor blocker therapy but much less frequently than is the case with ACE inhibitors. For unclear reasons, ACE inhibitor-related angioedema occurs more commonly in black patients. Angioedema can be life threatening **[14].**

This study was designed to investigate the effect of captopril, on liver of mice.

2. Material and Methods

A total of 90 adult albino mice weighing (25±20) g were used in this study. They were divided into three main groups (n=30 each),. Captopril is commonly marketed by Bristol-Myers Squibb under the trade name Capoten in the form of tablets 25mg, 50mg.Captopril was used in daily oral dose which represents the equivalent of the daily human therapeutic dose Captopril was administered by oral gavage at a dose calculated according to Paget and Barnes **[15].**

The experimental groups were divided into three groups:

Group (G1): control group

Group (G2): mice were treated with captopril (0.01mg, daily for two months.

Group (G3):mice were treated with captopril (0.02mg,daily for two months.

The mice were maintained on distilled water and a standard mice diet for 14 days and kept in a well-ventilated room with a 12-hour dark=light cycle before the study. The mice were housed in individual metal wire metabolic cages with constant temperature (25 °C). A specific air ventilation system assured an efficient flow within the room to keep the level of humidity within the animals' immediate environment at an acceptable level; thus, the relative humidity was within the range of 50±5 %. The mice were exposed to a 12: 12-h light-dark cycle, were fed *ad libitum* with a standard diet and allowed free access of water and were acclimatized for at least one week under these conditions before the start of the study.

At the end of the experimental period, mice were sacrificed, the liver specimens were obtained and immediately processed for histopathological and ultrastructural studies.

Light microscopic (L/M) study:

The liver sections were fixed in a 10% solution of formaldehyde in 0.1 mol/L phosphate-buffered saline(pH 7.4), and embedded in paraffin. Five-micrometer slides were prepared. Heamtoxylin and eosin staining was used. **[16].**

Transmission electron microscopic (TEM) study: For ultrastructural examination, the liver samples were cut into small pieces of about 1-mm3 in size and immediately. fixed infixed in 2.5% glutaraldehyde and 0.25 M sodium cacodylate, post fixed in 1% osmium tetroxide and embedded in Spurr's epoxy. Ultrathin sections were picked up on nickel grids, stained with uranyl acetate/lead citrate[17]**.**

3. Results

Control group (G1)

Light microscopic (L/M) results:

At the light microscope level, H&E-stained sections and toluidine blue semithin sections of liver of the control mice is showing the hepatic lobules possess distinct outlines, a small **central vein** in the center of a hepatic lobule .The blood **sinusoids**, which run between plates of **hepatocytes**(Fig. 1a).The plates of hepatocytes are appearing polyhedral cells with round central nuclei(Fig. .1b).

Transmission electron microscopic (TEM) results:

Ultrastructural examination of the liver of the control group revealed normal structure of the hepatocytes contain the normal cytoplasmic organelles, including mitochondria, Golgi apparatus, rough and smooth endoplasmic reticula, few lysosomes. The nuclus is large prominent, spherical in shape and centrally located(Fig.1c).A specialized stellate macrophage (Kupffer) cell and fenestrated endothelial cell within the sinusoid lining(Fig. 1d).A bile canaliculus between the cells of the hepatocytes plates in the hepatic lobule (Fig. 1e).

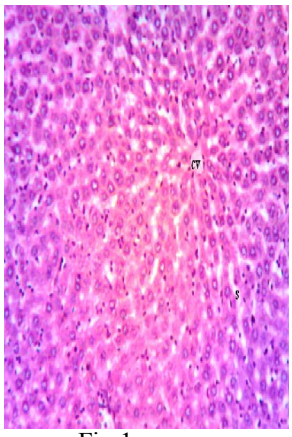

Fig 1 a

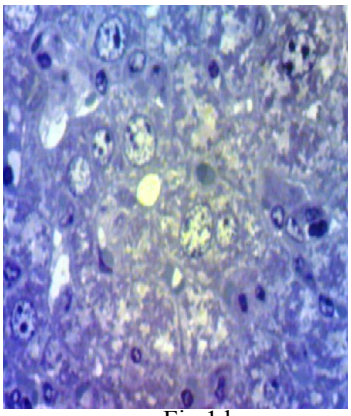

Fig 1 b

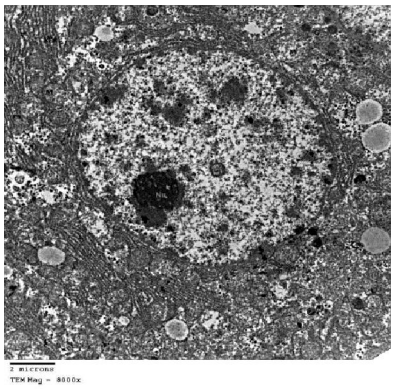

Fig 1 c

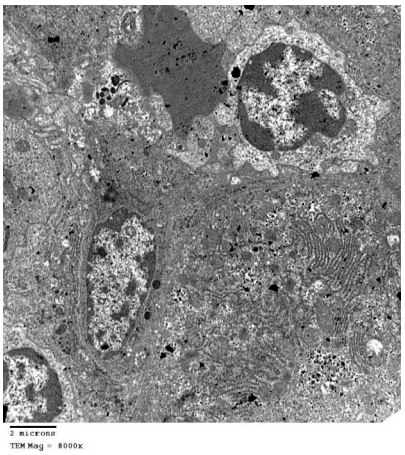

Fig 1d

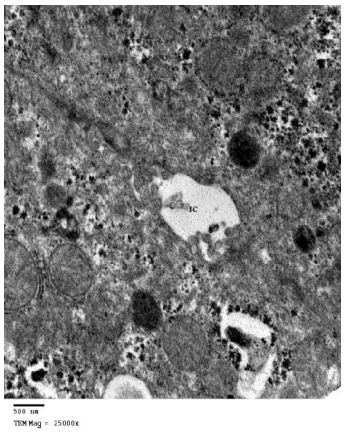

Fig 1e

Figs.1 a: A photomicrograph of a control mice liver group (G1) showing hepatic lobule, central vein (**Cv**), (H&E X100).
1b: A photomicrograph of semithin section of liver of the control mice is showing the polyhedral hepatocytes with round central nuclei. (Toluidine blue x400)
1c: TEMof the liver of the control group showing mitochondria, Golgi apparatus, rough and smooth endoplasmic reticula, few lysosomes. The nuclus is prominent, spherical in shape and centrally located (x8000)
1d: TEM of the liver of the control group showing stellate (Kupffer) cell and endothelial cell within the sinusoid lining (x8000)
1e: TEM of the liver of the control group showing bile canaliculus between hepatocytes (x25000)

Treated group (G2)

Light microscopic (L/M) results:

At the light microscope level, H&E-stained sections and toluidine blue semithin sections of liver of the treated mice with captopril group (G2) induced an obvious pathological changes in liver, including hydropic degeneration and necrosis and dilatation of sinusoids(Figs 2 a,b.).

Transmission electron microscopic (TEM) results:

Ultrastructural examination of the liver of the treated group (G2)revealed the hepatocytes contain the distorted cytoplasmic organelles, including mitochondria, which exhibited different signs of damage; some were condensed, others were irregular in shape with damaged cristae The nuclus possessed condensed chromatin and dentate nuclear membrane (Fig. 2. c.).The sinusoidal lining revealed fragmented endothelial cell and distorted kupffer cell (Fig. 2 d).

Treated group (G3)

Light microscopic (L/M) results:

At the light microscope level, H&E-stained sections and toluidine blue semithin sections of liver of the treated mice with captopril group (G3) induced an obvious damage in liver, including necrosis of stage of karyolysis which remarkable changes appeared in the hepatocytes in this group as

compared with those in group(G2) (Figs.3 a,b).

Transmission electron microscopic (TEM) results:

Ultrastructural examination of the liver of the treated group (G3)revealed the hepatocytes contain the degenerated cytoplasmic organelles, with indented and fragmented nuclear membrane, clumped the chromatin (Fig.3 c).The hepatocytes showed cytoplasmic degeneration ,with scattered apoptotic bodies and glycogen granules, also showed narrowed bile canaliculi and distorted blood cells and kupffer cell in sinusoid (Fig.3d).

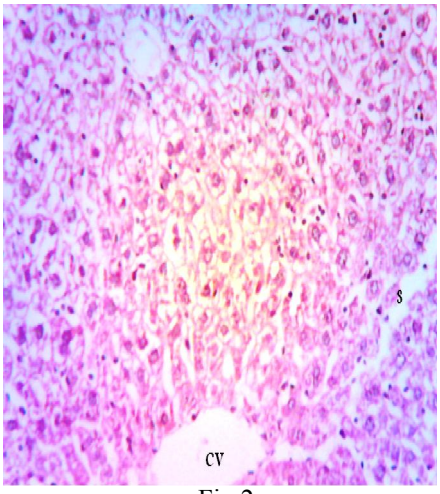

Fig 2a

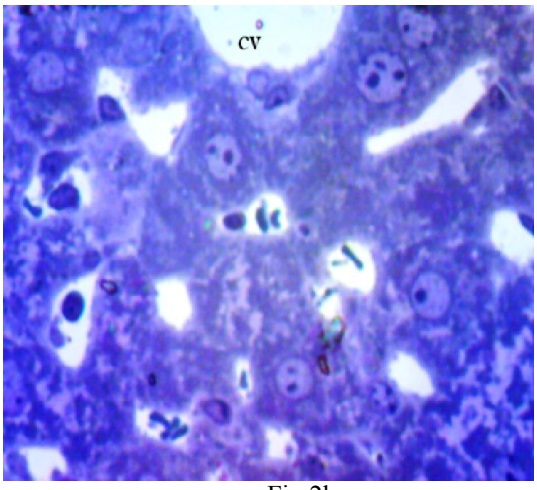

Fig 2b

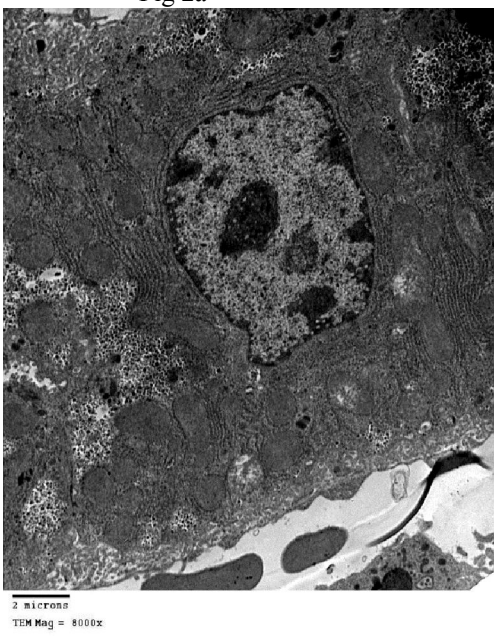

Fig 2 C

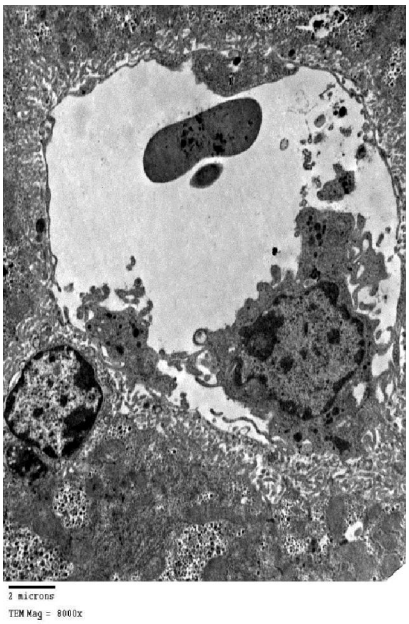

Fig 2 d

Figs.2 a:A photomicrograph of treated liver group (G2) showing including hydropic degeneration and necrosis and dilatation of sinusoids (H&E X100).

2b: A photomicrograph of semithin section of treated liver group (G2) showing cytoplasmic degeneration and necrotic nuclei. (Toluidine blue x400)

2c:TEMof the liver of group(G2) showing dentate nucleus ,distorted organelles ,mitochondria lost cristae (x8000)

2d: TEM of the liver of group(G2) showing fragmentd endotheial cell and hypertrophy distorted (Kupffer) cell (x8000)

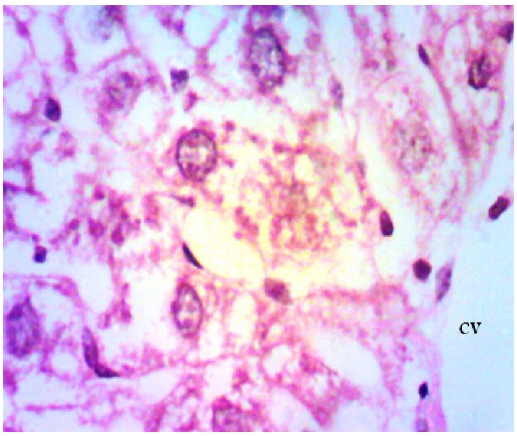

Fig 3a

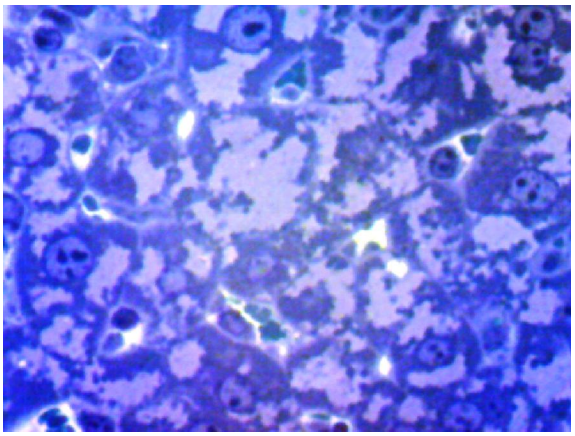

Fig 3b

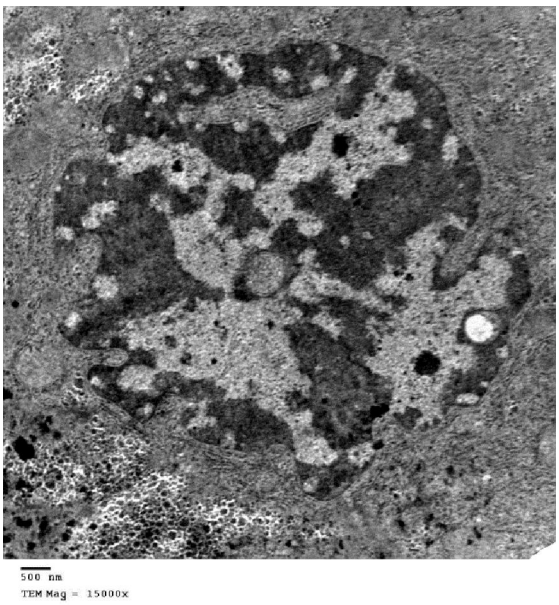

Fig 3c

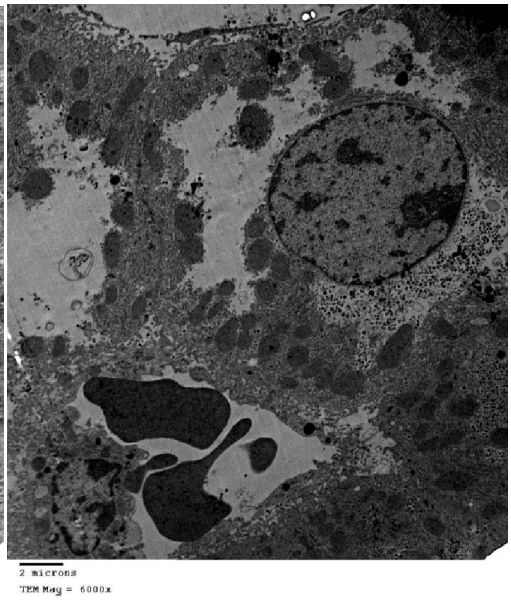

Fig 3d

Figs.3 a:A photomicrograph of treated liver group (G3) showing conspicuous degeneration and necrosis of hepatocytes (H&E X400).
3b: A photomicrograph of semithin section of treated liver group (G3) showing cytoplasmic degeneration and necrotic nuclei.(Toluidine blue x400)
3c:TEMof the liver of group(G3) showing degenerated cytoplasmic organelles, with indented and fragmented nuclear membrane, clumped the chromatin (x15000)
3d: TEM of the liver of group(G3) showing cytoplasmic degeneration ,with scattered apoptotic bodies, narrowed bile caniliculus and distorted blood cells and kupffer cell (x6000)

4.Discusion

Captopril is an angiotensin-converting enzyme (ACE) inhibitor is widely used in the treatment of hypertension and congestive heart failure[4].

The study finds obvious pathological changes in liver, including hydropic degeneration and necrosis and dilatation of sinusoids, necrosis of stage of karyolysis which remarkable changes appeared in the hepatocytes in group (G3)as compared with those in group(G2) . Ultrastructural examination of the liver of the treated group (G2) revealed the hepatocytes contain the distorted cytoplasmic organelles, including mitochondria, which exhibited different signs of damage; some were condensed, others were irregular in shape with damaged cristae The nuclus possessed condensed chromatin and dentate nuclear membrane .The sinusoidal lining

revealed fragmented endothelial cell and distorted kupffer cell.The group (G3) revealed the hepatocytes contain the degenerated cytoplasmic organelles, with indented and fragmented nuclear membrane, clumped the chromatin .The hepatocytes showed cytoplasmic degeneration ,with scattered apoptotic bodies and glycogen granules, also showed narrowed bile canaliculi and distorted blood cells and kupffer cell in sinusoid. These findings are in general agreement with the observations in ballooning (feathery) degeneration. Swelling of hepatocytes with increased and pale cytoplasm, In centrilobular necrosis, necrotic hepatocytes around central vein, usually due to ischemia, drugs or toxins[18].

A 50-year-old black female developed hepatic dysfunction secondary to captopril therapy, 25 mg tid for one month. Liver biopsy showed primarily cholestasis, with secondary hepatocellular elements. Symptoms consisted of jaundice, pruritus, anorexia and weight loss, hepatomegaly, and abdominal tenderness. Total bilirubin, alkaline phosphatase, lactate dehydrogenase (LDH), and serum glutamic-oxaloacetic transaminase (SGOT), reached highs of 506 mumol/L, 737 U/L, 319 U/L, and 100 U/L, respectively[19].

Captopril, the competitive inhibitor of angiotensin-converting enzyme, is of considerable benefit in difficult-to-manage forms of hypertension. Its use has been associated with various untoward effects, but hepatic injury has not been widely reported. We treated a patient with captopril-associated cholestatic jaundice; a review of cases reported to the drug manufacturer and a review of the literature showed 13 additional cases of hepatic injury associated with captopril. In 9 of these the jaundice was categorized as cholestatic, and in 4 of the remaining 5 as mixed cholestatic-hepatocellular. These findings show that jaundice may be an idiosyncratic side effect of captopril, and that captopril-associated jaundice characteristically has strongly cholestatic features[20].

Captopril, like other ACE inhibitors, has been associated with a low rate of serum aminotransferase elevations (<2%), The cause of the minor serum aminotransferase associated with captopril is not known. The clinically apparent acute liver injury from captopril is likely an idiosyncratic reaction to a metabolite. Captopril is hydrolyzed by the liver to its active metabolite captoprilat and has little further hepatic metabolism[21].Captopril has attained widespread use as an effective agent in the treatment of heart failure and hypertension. Dermatological, renal and haematological toxicity associated with its use has been widely described and is usually well recognized. There have been comparatively few reports implicating it as causing hepatic drug reactions. Most descriptions have emphasized strongly cholestatic features, although a mixed hepatocellular cholestatic picture and predominant hepatocellular reactions have been reportedCaptopril-induced liver disease, the jaundice may persist for many weeks after drug withdrawal[22].The liver lobule is formed by parenchymal cells, i.e., hepatocytes and nonparenchymal cells. In contrast to hepatocytes that occupy almost 80% of the total liver volume and perform the majority of numerous liver functions, nonparenchymal liver cells, which contribute only 6.5% to the liver volume, but 40% to the total number of liver cells, are localized in the sinusoidal compartment of the tissue. The walls of hepatic sinusoid are lined by three different cell types: sinusoidal endothelial cells (SEC), Kupffer cells (KC), and hepatic stellate cells (HSC, formerly known as fat-storing cells, Ito cells, lipocytes, perisinusoidal cells, Kupffer cells are intrasinusoidally located tissue macrophages with a pronounced endocytic and phagocytic capacity. They are in constant contact with gut-derived particulate materials and soluble bacterial products so that a subthreshold level of their activation in the normal liver may be anticipated. Hepatic macrophages secrete potent mediators of the inflammatory response (reactive oxygen species, eicosanoids, nitric oxide, carbon monoxide, TNF-alpha, and other cytokines), and thus control the early phase of liver inflammation, playing an important part in innate immune defense. High exposure of Kupffer cells to bacterial products, especially endotoxin (lipopolysaccharide, LPS), can lead to the intensive production of inflammatory mediators, and ultimately to liver injury. Besides typical macrophage activities, Kupffer cells play an important role in the clearance of senescent and damaged erythrocytes[23]. Disturbances in RNA and protein synthesis, mitochondrial function, or release of lysosomal enzymes do not play a primary causative role in cell death. Many previous studies have tended to implicate the plasma membrane and its presumed role in maintaining the proper Ca2+ balance as the primary site of the development of irreversible hepatocyte damage. These studies have generally faced a major difficulty in determining if the observed changes are the cause or an effect of cell death[24].

References

1-Kiyoshi Kikuchi , Salunya Tancharoen , Takashi Ito, Yoko Morimoto-Yamashita , Naoki Miura, Ko-ichi Kawahara , Ikuro Maruyama , Yoshinaka Murai and Eiichiro Tanaka(2013): Potential of the Angiotensin Receptor Blockers (ARBs)

Telmisartan, Irbesartan, and Candesartan for Inhibiting the HMGB1/RAGE Axis in Prevention and Acute Treatment of Stroke *Int. J. Mol. Sci. 14*(9), 18899-18924.

2-Grover, G.J., P.G. Sleph, S. Dzwonczyk, P. Wang, W. Fung, D. Tobias and D.W. Cushman, (1991:) Effects of different angiotensin-converting enzyme (ACE) inhibitors on ischemic isolated rat hearts: relationship between cardiac ACE inhibition and cardioprotection. J. Pharmacol. Exp. Ther., 257(3): 919-929**.**

3-Takeda, H., T. Hanedan and K. Kikuchi, 1997. Protective effect of the angiotensin-converting enzyme inhibitor captopril on post ischemic myocardial damage in perfused rat heart. Jpn. Circ. J., 61(8): 687-694.

4-Habior A (1992):Effect of captopril on glutathione level in the liver and paracetamol-induced liver damage in rats. Polskie Archiwum Medycyny Wewnetrznej, 87(6):332-340.

5-Kanno, S., Y.J. Wu, P.C. Lee, T.R. Billiar and C. Ho, (2001). Angiotensin-converting enzyme inhibitor preserves p21 and endothelial nitric oxide synthase expression in monocrotaline-induced pulmonary arterial hypertension in rats. Circulation, 104(8): 945-950.

6-Pawlak, R., E. Chabielska, T. Matys, I. Kucharewicz, R. Ró̜łkowski and W. Buczko, (2000.): Thiol repletion prevents venous thrombosis in rats by nitric oxide/prostacyclin-dependent mechanism: relation to the antithrombotic action of captopril. J. Cardiovasc. Pharmacol., 36(4): 503-509.

7-Plusczyk, T., B. Bersal, S. Westermann, M. Menger and G. Feifel, (1999). ET-1 induces pancreatitis-like microvascular deterioration and acinar cell injury. J. Surg. Res., 85(2): 301-310.

8-Krizanova, O., J. Orlicky, C. Masanova, M. Juhaszova and S. Hudecova, (1997). Angiotensin I modulates Ca transport systems in the rat heart through angiotensin II. J. Mol. Cell Cardiol., 29(6): 1739-1746.

9-Yoshida, H., Kondratenko, N., Green, S., Steinberg, D. and Quehenberger,O. (1998): Identification of the lectin-like receptor for oxidized low-density lipoprotein in human macrophages and its potential role as a scavengerreceptor. Biochem. J. 334, 9-13.

10-Helliwell TR , Yeung JH , Park BK (1985):Hepatic necrosis and glutathione depletion in captopril – treated mice.Br. J. exp. Path, 66(1):67-78 .

11-Sadeck LS, Fernandes M, Silva SM, Trindade AA, Chia CY, Ramos JL, Leone CR.(1997): [Captopril use in pregnancy and its effects on the fetus and the newborn: case report]. Rev Hosp Clin Fac Med Sao Paulo. ;52(6):328-32.

12-Cooper WO Hernandez-Diaz S Arbogast PG *et al.(2006):* Major congenital malformations after first-trimester exposure to ACE inhibitors. N Engl J Med; 8354:2443–51.

13- Sabroe RA; Black AK (February 1997). "Angiotensin-converting enzyme (ACE) inhibitors and angio-oedema". *British Journal of Dermatology* **136** (2): 153–8.

14-Sica DA, Black HR.(2002): Current concepts of pharmacotherapy in hypertension: ACE inhibitor-related angioedema: can angiotensin-receptor blockers be safely used? J Clin Hypertens (Greenwich).;4(5):375-80.

15-Paget, J.F. and Barnes, I.M.(1964): Toxicity testing. In evaluation of drug activities, Pharmocometrics, ed. D.R. Lawrence and A.L. Bacharach, Academic Press, London,161.

16-Bancroft JD, Gamble M (2002) :Theory and Practice Histological Techniques,5th ed., Churchill Livingstone. New York, Edinburgh and London, 126: 173-175.

17-Woods, A.E. and Stirling, J.W. (2002):Electron microscopy: The preparative techniques In: Theory and practice of histological teqniques. Ed. Bancroft, J. D. and Gamble, M. 5th ed. Harcourt Puplishers.

18-Komal Arora, M.D. (2013):, PathologyOutlines.com, Inc.

19-Parker WA.(1984): Captopril-induced cholestatic jaundice .Drug Intell Clin Pharm.;18(3):234-5.

20-Rahmat J, Gelfand RL, Gelfand MC, Winchester JF, Schreiner GE, Zimmerman HJ.(1985): Captopril-associated cholestatic jaundice. Ann Intern Med.;102(1):56-8.

21-Tabibian N, Alpert L, Alpert E.(1987): Captopril-induced liver dysfunction. South MedJ; 80: 1173-5.

22-Crantock L, Prentice R, Powell L.(1991): Cholestatic jaundice associated with captopril therapy. J Gastroenterol Hepatol.;6(5):528-30.

23-Kmieć Z.(2001):Cooperation of liver cells in health and disease. Adv Anat Embryol Cell Biol. ;161:III-XIII, 1-151.

24-Farber J Land . El-Mofty S. K.(1975); The biochemical pathology of liver cell necrosis.Am J Pathol. Oct 1975; 81(1): 237–250.

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