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Role of Amlodipine Besylate in Prevention or Attenuation of Hepatic Centrilobular Necrosis Induced by CCl_4 in Rabbit Models

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Abstract

Centrilobular necrosis of hepatic tissue is a major cause of acute liver injury associated with cellular death and impairment of liver functions. It was experimentally induced by hepatotoxic substances called carbon tetrachloride. Eighteen healthy domestic rabbits were used to evaluate the hepatoprotective activity of amlodipine besylate, they were allocated to three groups; G1 (negative control), GII (positive control) and GIII (treatment group). G1 received only distilled water 3 ml orally, GII was given single dose of carbon tetrachloride (1.5 ml/kg body weight) orally as a mixture with olive oil to induce liver damage. Whereas GIII was given amlodipine besylate (1.25 mg/body weight) orally one hour before induction of hepatic centrilobular necrosis by carbon tetrachloride, then the same dose of tested agent continued daily for two successive days after the administration of carbon tetrachloride. The duration of experiment was 3 days i.e. 72 hours.Blood samples were taken for biochemical analysis of liver functions at 2 occasions, 24 and 72 hour after induction of hepatic centrilobular necrosis to determine the values of serum alanine aminotansferase (SALT), serum aspartate aminotansferase (SAST), serum alkaline phosphatase (SALP), total serm bilirubin (TSB) and total serum protein (TSP) of the tested animals. The histopathological examination of liver samples was then done after 72 hours to check the microscopic changes of the liver tissue. Amlodipine besylate with this daily dose showed a significant reduction ($p<0.05$) in the levels of serum ALT, AST , ALP, total bilirubin and significant elevation ($p<0.05$) in the level of total serum protein in comparison to GII, both after 24 hour and 72 hour of carbon tetrachloride administration. Histologically, liver sections showed that amlodipine produced no necrosis, mild fatty change, mild inflammatory infiltration and mild congestion when compared with that of GII. As conclusion, Amlodipine besylate was proved to have a significant hepatoprotective activity on hepatic centrilobular necrosis model .

Key words: Amlodipine Besylate, centrilobular necrosis, carbon tetrachloride, hepatoprotective

Introduction

The most severe clinical consequence of liver disease is liver failure . This may be the result from sudden and extensive damage of the hepatocellular tissue, with reduced cell mass and blood flow (1). Acute liver failure is asymptomatic in a large majority of affected individuals; however , when symptoms are present, they bring the patient to medical attention (2).Cell death (including that of hepatocytes) has been described, mainly on morphological appearances, as occurring by apoptosis or necrosis (3). Apoptosis is characterized by cell shrinkage, nuclear fragmentation, formation of apoptotic bodies and lack of inflammation. It is more difficult to detect histologically because of the rapid removal of the affected cells (4). On the other hand, massive liver necrosis is characterized by cellular swelling with loss of membrane integrity. As the cell dies it

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releases its contents, which evoke an inflammatory response, that causes further cell injury from cytokines and toxic oxygen species (5,6). In the setting of ischemia and a number of drugs immediately around the central vein causing centrilobular necrosis. Pure midzonal and periportal necrosis is rare (1,7).Amlodipine is one of long-acting third-generation di-hydropyridine calcium channel blockers which act via inhibition of calcium influx into myocardial cells and vascular smooth muscle cells, resulting in reduced peripheral vascular resistance (8).Amlodipine is generally well tolerated drug with side effects which are due to its action as a vasodilator. These include, dizziness, fatigue, nausea, diarrhea, palpitations, peripheral edema, flushing and rash (9).Therefore, it is interesting to evaluate the potential hepatoprotective effects of amlodipine besylate in experimental rabbit model of acute hepatic centrilobular necrosis induced by a hepatotoxic agent (carbon tetrachloride).

Materials and Methods

Chemicals

1- **Carbon tetrachloride:** It was used for induction of hepatic centrilobular necrosis in a rabbit model. CCl_4, pure liquid: one liter volume, Mol. mass: 153.82 gm/mol, MERCK- Germany.
2- **Amlodipine besylate (AMADAY-10):** 10 mg tablet, Ajanta-India.
3- **The kits:** kits used for the estimation of SALT, SAST and TSP were from Dialab, Austria. Kits for TSB and SALP were from Biolabo SA, Maizy, France.

Animals:

Eighteen healthy, local, domestic rabbits weighing 750-1500 g of both sexes were used in this study. They were supplied by the animal house at College of Vet. Medicine, University of Al-Qadisiyah, and housed two per cage, which was provided with a wire mesh floor and at a controlled temperature of 28 °C with a 12-hour. light/dark cycle. All rabbits were fed standard oxoid pellets and were given water *ad libitum*. Animals were randomly divided into three groups of six animals each:

GI (negative control) : received only distilled water 3 ml orally.

GII (positive control): The control group received 3 ml of distilled water orally one hour before induction of hepatic centrilobular necrosis by administration of $CC1_4$ as a single dose of 1.5 ml/kg body weight orally (The dose of CCl_4 had been chosen through different trials by using 0.5, 1, 1.5 ml/kg) mixed with olive oil 1:1 (v/v) in order to increase its absorption via gastric mucosa, then the same dose of distilled water continued for two successive days after $CC1_4$ administration.

GIII (treatment group): It was given amlodipine besylate 1.25 mg/kg body weight as a single daily dose orally started one hour before induction of hepatic centrilobular necrosis by administration of $CC1_4$ and continued for two successive days after $CC1_4$ administration. (dose of amlodipine was calculated on the basis of ideal human body weight (70 kg) divided on weight of one tablet)

- The duration of experiment was 3 days i.e. 72 hours.

Effect of amlodipine besylate was studied after 24 and 72 hour from induction of hepatic centrilobular necrosis by $CC1_4$. Blood samples were obtained from the heart of all groups, 5 ml of blood could be aspirated in each occasion to determine the values of serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total serum bilirubin and total protein of the tested animals using spectrophotometer method for comparison with the normal values of the collected samples. At the end of experiment, all rabbits were sacrificed under chloroform anaesthesia and subjected to liver resection. The liver was mobilized and the medial lobe was ligated and removed, it was placed in formaline 10% at room temperature for 2 hours to be ready for sectioning and staining with hematoxylin and eosin dye. The

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histopathological examination of sections was then done to check the microscopic change of the liver tissue by using of polarized microscopy (10).

Ethical approval:

The researchers obtained ethical approval from the research Ethical Approval Committee of the College of Veterinary Medicine, University of Al-Qadisiyah.

Statistical analysis

Data were expressed as mean ± standard deviation (S.D.), statistical significance among groups was determined by using of unpaired student's *t*-test, using SPSS (Version 12). P<0.05 was used as a criterion for significance.

Results

The GII group had significant elevation (p<0.05) in the levels of serum ALT, AST, ALP, total bilirubin and significant reduction (p<0.05) in the level of total serum protein, that were more evident and reached the maximum after 24 hour of CCl_4 administration, these levels were; 120±1.80 U/l, 130±2.22 U/l, 389±2.61 U/l 24.16 ±0.60 µmol/l, 48.66 ± 0.49 gm/l respectively, then they declined but still abnormal after 72 hour of CCl_4 administration to; 92.5 ± 1.61 U/l, 103.8 ± 1.99 U/l, 173 ± 7.99 U/l, 15.66 ± 0.33 µmol/l, 45 ± 1.81 gm/l respectively, whereas the normal values were 26.33 ± 3.33 U/l, 25.33 ± 4.5 U/l, 45.66 ± 7.53 U/l, 11.33 ± 0.76 µ mol/l , 55 ± 0.36 gm/l (table 1,2). Histopathological studies of the liver sections of control group showed very clear histological changes, that were massive centrilobular necrosis, fatty change, inflammatory infiltration of the portal area, and congestion of sinusoids (figure 2). In GIII, amlodipine besylate, which is a calcium channel blocker was used at a dose of 1.25 mg/kg body weight given orally one hour before induction and repeated as a single daily dose for 2 successive days after induction. This drug with this daily dose showed a significant reduction (p<0.05) in the levels of serum ALT, AST , ALP, total bilirubin and significant elevation (p<0.05) in the level of total serum protein in comparison to the GII group, both after 24 hour; 58.16 ± 1.49 versus 120 ± 1.80 U/1, 70.5 ± 1.54 versus 130 ± 2.22 U/l, 64.33 ± 0.66 versus 389 ± 2.61 U/l, 11.33± 1.08 versus 24.16 ± 0.60 µmol/l ,55.66 ± 0.49 versus 48.66 ± 0.49 gm/1 respectively, and after 72 hour; 64± 1.24 versus 92.5± 1.61 U/l, 27.5± 3.07 versus 103.8± 1.99 U/l ,70.33 ± 2.19 versus 173 ± 7.99 U/l ,9.66± 0.49 versus 15.66 ± 0.33 µmol/l , 51.83 ± 0.47 versus 45± 1.81 gm /1 respectively (table 1,2). Histologically, liver sections showed that amlodipine produced no necrosis, mild fatty change, mild inflammatory infiltration and mild sinusoidal congestion when compared with that of the GII group which denoted massive centrilobular necrosis, fatty change, inflammatory infiltration of the portal area and sinusoidal congestion. (figure 3).

Table (1): The serum ALT, AST, ALP, total bilirubin and total protein levels of the studied groups measured 24 hr after induction of hepatocellular necrosis by CCl_4

Groups	SALT (U/L)	SAST (U/L)	SALP (U/L)	TSB (µmol/L)	TSP (g/dI)
GI	26.33±3.33	25.33±4.5	45.66±7.53	11.33±0.76	55±0.36
GII	120±1.80*	130±2.22*	389±2.61*	24.16±0.60*	48.66±0.49*
GIII	58.16±1.49**	70.5±1.54**	64.33±0.66**	11.33±1.08**	55.66±0.49**

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*** Significant effect at P<0.05. compared with Group I**
**** Significant effect at P<0.05. compared with Group II**
mean ± S.D., n=6
SALT- serum alanine aminotansferase, SAST- serum aspartate aminotansferase, SALP- alkaline phosphatase, TSB- total serum bilirubin, TSP- total serum protein.

Table (2): The serum ALT, AST, ALP, total bilirubin and total protein levels of the studied groups measured 72 hr after induction of hepatocellular necrosis by CCl_4

Groups	SALT (U/L)	SAST (U/L)	SALP (U/L)	TSB (µmol/L)	TSP (g/dl)
GI	26.33±3.33	25.33±4.5	45.66±7.53	11.33±0.76	55±0.36
GII	92.5±1.61*	103.8±1.99*	173±7.99*	15.66±0.33*	45±1.81*
GIII	64±1.24**	27.5±3.07**	70.33±2.19**	9.66±0.49**	51.83±0.47**

*** Significant effect at P<0.05. compared with Group I**
**** Significant effect at P<0.05. compared with Group II**
mean ± S.D., n=6
SALT- serum alanine aminotansferase, SAST- serum aspartate aminotansferase, SALP- alkaline phosphatase, TSB- total serum bilirubin, TSP- total serum protein.

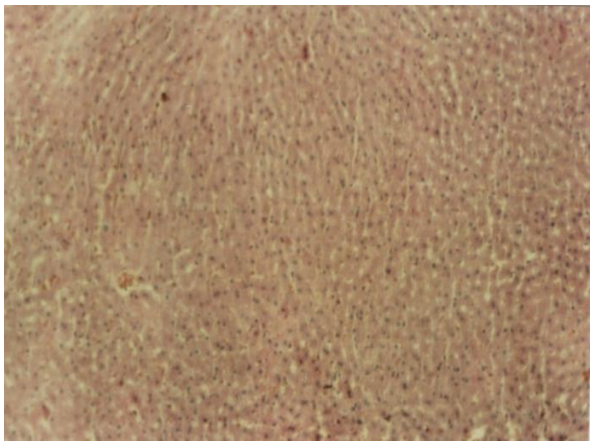

Figure (1): Normal rabbit liver of GI group, showing normal hepatocytes with normal lobular appearance.

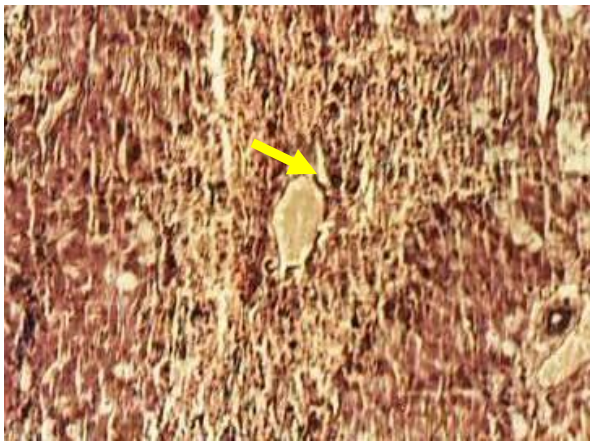

Figure (2): Rabbit liver of GII group after 72 hours from administration of CCl_4 as a single oral dose, showing massive centrilobular necrosis, fatty change, inflammatory infiltration of the portal area and sinusoidal congestion.

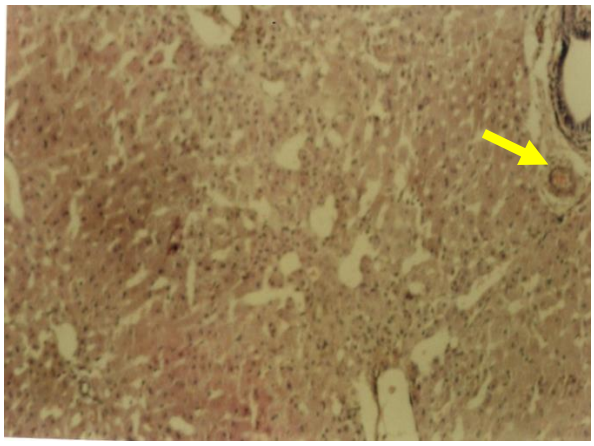

Figure (3): Liver of rabbit treated with amlodipine besylate (GII) one hour prior and repeated for two days after CCl_4 administration, showing no necrosis, mild fatty change, mild inflammatory infiltration and mild sinusoidal congestion.

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Discussion

Carbon tetrachloride is a potent hepatotoxic agent, that it is widely used in experimental model of severe liver failure (11). CCl_4 can rapidly produce acute hepatocellular injury and massive centrilobular necrosis by lipid peroxidation of the hepatocyte membranes, which caused by the action of free radicals. The dose of CCl_4 had been chosen through different trials by using 0.5, 1, 1.5 ml/kg. 1.5 ml/kg body weight of CCl_4 as a single dose was found to be the most effective dose in producing severe liver injury and significant elevation of the liver enzymes. CCl_4 was given orally by using of gastric tube to avoid aspiration of this agent. 2-5 minutes after administration of CCl_4, the animals were presented with signs of excitement, shouting, jumping, depression, and then drowsiness due to the anaesthetic and irritant effect of CCl_4, also there was some reduction in the animals' weights due to the loss of appetite of the GII group. Sometimes one or two per 6 animals of the GII group were die several hours after administration of CCl_4, this may be due to severe renal failure accompanied by severe liver failure (12). Liver enzymes (alanine aminotransferase, aspartate aminotransferase) were measured in the serum of each group (GII and GIII groups) 24 and 72 hour after CCl_4 administration. These enzymes are highly sensitive to liver injury and they are released in significant quantities in the serum after any oxidative stress performed on the liver. Serum alkaline phosphatase was also measured to assess the hepatocellular injury and cholestasis, in which large quantities of this enzyme is released. Total serum bilirubin (both conjugated and unconjugated) was measured as an index for bilirubin metabolism and excretion. In addition to total protein measurement in order to evaluate the liver functions and its capacity to synthesize proteins. The results of the GII group were demonstrated by Taira *et al*., 2004 (13) who found that the levels of liver enzymes (specific pictures of liver injury) rose to a maximum level within a day after CCl_4 administration then decreased after 3 days. In addition, he concluded that the change at day 1 corresponds to the acute action of CCl_4 intoxication, and that the change at day 3 is the effect of physiologically reduced liver function due to the liver regeneration for tissue repair after the CCl_4 hepatic injury.On the other hand, There is no available data about the hepatoprotective role of amlodipine, but the present study suggested that amlodipine (which has a greater affinity for vascular calcium channels) can attenuate hepatic centrilobular necrosis, in which there was a large increase in intrahepatic portal-systemic shunting that may be a major mechanism whereby vasoactive agent reach the systemic circulation (14). This observation was also demonstrated by Hung *et al*., 2002 (15) who found that CCl_4 - induced hepatic centrilobular necrosis produced not only acute hepatoceullar injury with centrilobular necrosis , but also result in stenosis of the portal vein ; therefore, the local circulation of blood is bound to be upset, and this will tend to increase the damage which will spread in an increasingly haphazard manner (7).Thus administration of vasoative drugs, which markedly affect intrahepatic hemodynamics and decrease intrahepatic portal-systemic shunting, could correct the impaired hepatocyte function and other abnormalities characteristic of liver damage. Whereas, Varghese *et al*., 2020 (16) concluded that amlodipine, though not well known to be hepatotoxic agent, can induce elevations of liver enzymes in an idiosyncratic manner. Chronic therapy with amlodipine is associated with a low rate of serum enzyme increment at rates that are similar to matched control populations. The enzyme elevations are usually transient, mild, and asymptomatic and may resolve even during continued therapy. The exact mechanism by which amlodipine causes hepatic injury is unknown yet (17).

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Conclusion Our data recorded that Amlodipine besylate showed a significant effect in adjustment of liver functions as well as restoring hepatic tissue in models of hepatic centrilobular necrosis when the drug was administered in single dose per day of 1.25 mg/kg body weight.

Conflict of Interest: there is no conflict of interest.

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