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Protective Effect of Cimetidine, Isosorbide Dinitrate & Vitamin C in Experimental Model of Acute Liver Injury

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Original Research Article

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Abstract: Acute liver injury is a serious state of severe extensive damage of liver tissue caused by various reasons. It is experimentally induced by CCl_4 the hepatotoxic agent. Forty healthy rabbits were involved in the present study. They were allocated in five groups. Each group was given one of the following drugs: Cimetidine, Isosorbide dinitrate, Vitamin C & distilled water two hours before administration of CCl_4, in addition to control group. The same doses of the tested drugs were continued for five days after CCl_4 administration. The effect of drugs was evaluated at two occasions 24 and 120 hours after ALI induction on the basis of biochemical analysis of the liver function tests as well as histopathological examination to liver of treated animals. The study showed that all the tested drugs produced significant reduction in SALT, SALP, SAST & TSB with a significant elevation of TSP levels as compared with treated control group. The histological examination showed clear improvement in the sections of liver tissue that supports the effect of these drugs on the liver. All tested drugs proved to have hepatoprotective effect of varying degree on ALI model in the rabbits.

Keywords: Cimetidine, Isosorbide dinitrate, Vitamin C, ALI, hepato-protection.

INTRODUCTION

Acute liver injury (ALI) is a clinical condition that results from extensive damage of the hepatocellular tissue with reduced cell mass & blood flow that occurs by different toxic agents. It is associated with increase in serum alanine aminotransferase (SALT), serum aspartate aminotransferase (SAST), serum alkaline phosphatase (SALP) & total serum bilirubin (TSB)[1].

Carbon tetrachloride (CCl_4) is a hepatotoxic agent used to induce ALI when administered orally to rabbit due to formation of free radicals mediated lipid peroxidation of the cytoplasmic membrane phospholipids that cause functional & morphological changes in the cell membrane [2]. The present study was performed to explore the possible hepatoprotective effect of Cimetidine, Isosorbide dinitrate, Vitamin C in experimental model of ALI induced by CCl_4.

MATERIALS & METHODS

Forty healthy rabbits weighing 650-750 gm were used in the present study. They were supplied by animal house of AL- Nahrain College of medicine. Animals were housed under good conditions in separated cages & were fed standard oxoid pellets and were given water ad libitum. The rabbits were randomly allocated to five groups (each group contained eight animals). Each tested drug was given orally to one group of animals at 10 am and two hours later at 12 am. CCl_4 was given orally at a dose of 1.25 ml /kg as a mixture with olive oil to animals. The tested drugs were given continually to animals at the same doses for five days. The effect of the tested drugs was evaluated at 24 and 120 hours after induction of ALI by CCl_4 on the basis of histopathological changes in the liver of treated animals by the tested drugs. The tested drugs were given by following schedule:

Group-1 (normal control) received two ml of distilled water orally as a single dose continued for 5 days without administration of CCl_4.

Group -2 (treated control) received two ml of distilled water as a single dose, two hours before induction of ALI by CCl_4 (Merck) in a dose of 1.5 ml/Kg orally and continued distilled water for 5 days after induction.

Group- 3 received cimetidine (tagadin) 40 mg/ Kg orally as a single dose started two hours before CCl_4 administration & continued for 5 days.

Group -4 received isosorbide dinitrate (Isocard) 1.42 mg/ Kg orally as a single dose started two hours before CCl_4 administration & continued for 5 days.

Group-5 received Vitamin C (cetavit) 250 mg/Kg orally as a single dose started two hours before CCl_4 administration & continued for 5 days.

Blood samples were taken directly from the marginal ear vein for biochemical analysis of the liver function tests at two occasions 24 &120 hours after ALI induction to determine the normal serum values of AST, ALT, ALP, TSB & TSP (total serum protein) levels after treatment with drugs by using spectrophotometer method [3]. The histopathological examination for the sections of liver which was conducted under chloroform anesthesia & after sacrificing the animals- to check the microscopic changes of the liver tissue by using polarized microscope [4]. The kits used for estimation of ALT, AST &ALP were purchased from Biomerieux- France and that for estimation of TSB & TSP were supplied from Randox-England.

Statistical analysis: all the results were expressed as mean ± SEM, the differences among mean was analyzed by student's test [5]. A probability value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Administration of CCl_4 to the rabbits resulted marked increase in SAST, SALT, SALP& TSB levels while TSP level decreased when compared with the control group. All the tested drugs revealed significant reduction in SALT, SALP, SAST & TSB levels with a significant elevation of the TSP level. $p < 0.05$ when compared with treated control group. Isosorbide dinitrate was the most potent drug in lowering significantly SAST level from 130± 2.22 U/I to 51.66± 0.88 U/I after 24 hours & from 103.8 ± 1.98 to 22.33± 1.14 after 120 hours at the same time. Isosorbide dinitrate also significantly reduced SALP level from 153± 7.92 to 56 ±0.57 U/I after 120 hours, whereas Vitamin C significantly decreased both SALP & SALT levels from 189± 2.61 & 92.5± 1.61 U/I to 110.5± 4.59 & 28.88± 0.47 U/I respectively after 24 hours to SALP & 120 hours to SALT. The tested drugs cimetidine, isosorbide dinitrate & vitamin C were significantly increased TSP level but cimetidine was the best drug in its action on both TSB & TSP. (Table-1).

The histopathological examination of the liver in all tested drugs showed clear improvement in the hepatocytes congestion, fatty changes, infiltration of lymphocytes, necrosis that occur with CCL_4. These improvements support the hepatoprotective effect of these drugs against CCL_4 induced ALI (Figure 1, 2, 3, 4, 5).

DISCUSSION

The hepatotoxic compound CCL_4 is well known to be used for induction of hepatotoxicity in experimental animal models. It is bio-transformed in cytochrome P450 system to its metabolite "trichloromethyl peroxyl free radical" (CCL_3) which in the presence of O_2 forms trichloromethyl- peroxyl free radical CCL_3O_2 that attacks lipids of endoplasmic reticulum eliciting lipid peroxidation with the leakage of hepatocellular enzymes like SALT, SALP, SAST causing an increase in serum TSP levels and decrease in serum (TSB) levels [6]. The same results were obtained by others [7] who used rat as a model of induction of ALI. The results of treated control (group-2) in the present study are compatible with results of others [8]. Administration of cimetidine which is histamine H_2 receptor blocking drug (group-3) showed hepatoprotective effect against CCl_4 induced ALI. These results are similar to the results of the others when they used cimetidine against acetaminophen induced hepatic necrosis in rat [9]. This protective effect of cimetidine may be related to its chemical structure & also independent of H2 receptor blocking action. Cimetidine is slowly bio activated by several important hepatic cytochromes P450 drug metabolism pathways these catalyzed by CYP1A2, CYP2D6, CYP3A4 & CYP2C9 the half-life of drugs metabolized by these pathways may be prolonged [10].

Isosorbide dinitrate which is the effective drug in treatment of angina pectoris (group-4) produced significant positive results in the present study against CCl_4 induced liver injury. These results were more evident with the therapeutic doses of drug [11]. Isosorbide dinitrate is a member of organic nitrate that causes the release of nitric oxide thus the hepatoprotective effect of the drug attributed to the effect of NO that activates guanylyl cyclase leading to synthesis of cGMP [12]. Nitric oxide proved to have antiapoptotic activity in hepatocytes [13], and may have a role in liver regeneration.

The protective effect of Vitamin C (group-5) can be interpreted on the basis of antioxidant activity which helps in mopping up of free radical oxidants and in suppression of lipid peroxidation of liver cells [14]. In addition, some researchers found that the liver of CCl_4 treated rats showed reduction in Vitamin C concentration after CCl_4 administration [15]. Thus administration of Vitamin C can raise its concentration potentiating the biodefense system activity. The hepatoprotective effect of Vitamin C is similar to protective effect of vitamin E when used against CCl_4 induced ALI in rabbits [16]. The results of the present study confirm the important role of oxidative stress in initiation of liver damage & also in agreement with possible role of antioxidants in prevention or attenuation of ALI. The histological architecture of the liver section of group 3, 4 & 5 showed a more or less

lobular pattern with a mild degree of fatty changes, lymphocyte infiltration & mild congestion with minimal or no necrosis (Figure 1, 2, 3, 4 & 5).

Table-1: the effect of tested drugs on CCl_4 induced ALI in rabbits

Group	Dose	SALP U/ L	SALT U/ L	SAST U/ L	TSB UMOL/ L	TSP g/ dl
1-Normal control		45.66 ± 6.53	26.33±3.33	25.33±4.5	11.33±0.76	55± 0.36
2- CCl_4	1.5 ml/kg	*189±2.61 after 24 hr *153± 7.92 after 120 hr	*120±1.8 after 24 hr *92.5 ± 1.61 after 120 hr	*130± 2.22 after 24 hr *103.8± 1.98 after 24 hr	*24.61±0.60 after 24 hr *15.66±0.33 after 120 hr	*48.66±0.44 after 24 hr *45 ± 1.81 after 120 hr
3-cimetidine	40 mg/kg	*121 ± 7.81 after 24 hr *70.5± 2.47 after 120 hr	*54.3±4.19 after 24 hr *30± 0.57 after 120 hr	*82.16±2.5 after 24 hr *42.66±0.9 after 120 hr	*12.66± 1.01 after 24 hr *11.5±0.84 after 120 hr	*58± 1.39 after 24 hr *53.5±0.43 after 120 hr
4-Isosorbide dinitrate	1.42 mg/kg	*123.5 ±0.83 after 24 hr *56± 0.57 after 120 hr	*61.16±6.27 after 24 hr *40.1± 0.51 after 120 hr	*51.66±0.88 after 24 hr *22.33±1.14 after 120 hr	*10.66±0.33 after 24 hr *9.33±0.80 after 120 hr	*55.16± 0.66 after 24 hr *51.±0.44 after 120 hr
5-Vitamin C	250 mg/kg	*110.5 ±4.59 after 24 hr *77± 0.85 after 120 hr	*65.83±1.05 after 24 hr *28.88± 0.4 after 120 hr	*73±1.59 after 24 hr *20.5±1.09 after 120 hr	*11± 0.89 after 24 hr *9.5±0.42 after 120 hr	*56.5± 1.61 after 24 hr *51.46±0.66 after 120 hr

*Significant lowering effect at P < 0.05 for (ALP, AST, ALT& TSB) after 24 hours & 120 hours post induction.
*significant rising effect at P < 0.05 for TSP after 24 hours & 120 hours post induction

Fig-1: Normal rabbit liver section shows hepatocytes architecture with normal lobular appearance stain (H & E stain, X10)

Fig-2: Rabbit liver section after CCl_4 administration only showing massive necrosis, fatty changes, lymphocytes infiltration and congestion (H & E stain, X 10)

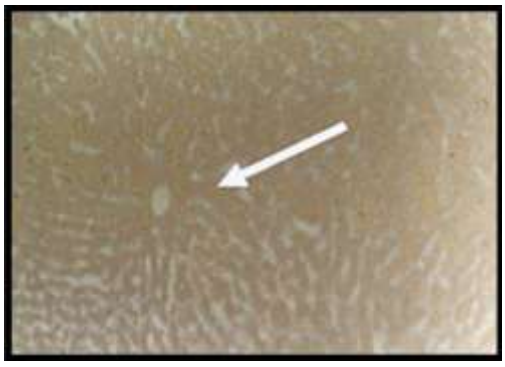

Fig-3: Rabbit liver section after CCI_4 and Vit. C administration showing no necrosis, fatty changes and mild congestion (H & E stain, X 10)

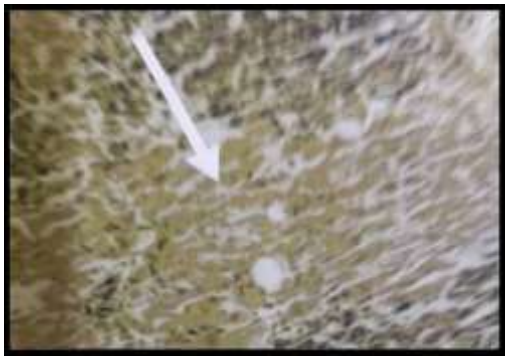

Fig-4: Rabbit liver section after CCI_4 and cimetidine showing mild necrosis and mild fatty changes (H & E stain, X 10)

Fig-5: Rabbit liver section after CCl_4, and Isosorbide dinitrate showing mild necrosis, mild fatty changes and congestion (H & E stain, X 10)

CONCLUSION

All the tested drugs have hepatoprotective effect restoring the normal hepatic functions; enhance bio-defense of the liver against oxidative damage produced by CCl_4 administration with possibility to be used for patients with hepatic toxicity after clinical trials.

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