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HEPATOPROTECTIVE EFFECT OF VITAMIN C ON D-GALACTOSAMINE INDUCED TOXICITY

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Abstract

Liver is the major organ for biotransformation of xenobiotics (detoxification) mechanism. It is responsible for removing the chemical toxins in the blood. The present study deals with the investigation of the effect of vitamin C on acute liver injury caused by D–Galactosamine (D–Galn). in albino rats. In this experiment 24 healthy male rats weighing 100-150 g were divided into 4 groups; Group 1 served as control, while Group 2 exposed with the D-Galn(400mg/kg), Group 3 only with vitamin C (100mg/kg) and Group 4 with pretreated vitamin C+ D-Galn. D-Galactosamine (400mg/kg) administration significantly increased the activities of marker enzymes such as Aspartate Transaminase (AST), Alanine Transaminase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP) of liver and serum and decreased the activity of antioxidant enzymes such as SOD and Catalase of liver and serum when compared to normal values. The remarkable changes in the enzyme activity were due to hepatotoxicity of D-Galn. The results showed that the liver injury induced by D-Galn was improved in the pretreated groups to variable extents. Vitamin C administration (100mg/kg) significantly ($p<0.05$) reduced the activity of marker enzymes and increased the level of antioxidant .Hence, it was concluded that vitamin C possess hepatoprotective activity and free radical scavenging activity.

Keywords: D-Galactosamine, Vitamin C, Marker enzymes, Hepatotoxicity, Oxidative stress.

INTRODUCTION

Liver injury can be caused by different agents, such as viruses, chemicals, alcohol, and auto-immune disease. It is also the central site for the biotransformation of xenobiotics and therefore it is involved in the detoxifying mechanism of the body. Liver is responsible for clearing the chemical toxins in the blood and in this process it is exposed to high concentration of toxicants and toxic metabolites making it very susceptible to injury(1). Hepatotoxin may cause liver cell necrosis or biochemical changes without necrosis, these changes may be manifested by impaired formation with fat accumulation or altered enzyme activity. Immunosuppressive agents and certain agents used in chemotherapy of cancer may be hepatotoxin (2). D-Galactosamine(D-Galn) is a well established hepatotoxicant, it induces a diffuse type of liver injury closely resembling human viral hepatitis(3) and acute self- limiting hepatitis with necrosis, inflammation and regeneration, resembling a drug induced disease in humans(4). Early biochemical studies postulated that

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D-Galactosamine caused liver injury by depleting the uridine pools in hepatocytes resulting in the inhibition of messenger RNA (mRNA) and protein synthesis, particularly coagulation factors, this leads to liver necrosis. Uridine prevents or reserves the inhibition, which indicates that the depression is the result of the UTP deficiency induced by D-Galactosamine (5).

Some ailments have been cured by the treatment of Drugs and chemical agents. Certain compounds of these drugs are known to have hepatotoxic effects in human and animals. Vitamin C has shown tremendous protective effect against drugs and chemical agents induced hepatotoxicity (6, 7). One of these reports was published by Awodele and Co-researchers. Similar finding was reported by Peterson and Knodell in (1984) (8). They observed that ascorbic acid ameliorated acetaminophen and cocaine induced hepatic damage in mice.

The hepatoprotective effect of vitamin C against drug induce hepatic damage was also reported by Remiao *et al*. (2004) (9) Pretreatment with vitamin C normalized aspartate aminotransferase and alkaline phosphatase in halothane induced hepato-toxicity (10). Research showed that pretreatment with 50, 100 and 200 mg/kg of vitamin C attenuated carbamazepine (50 mg/kg) provoked hepatotoxicity in rats (11).

Vitamin C is an antioxidant that scavenges reactive oxygen species there by reducing oxidative stress and related complications(12) Experimental evidence suggests that there are proofs on hepatoprotective effect of vitamin C against carbon tetrachloride, paracetamol, dichlorous and malathion induced hepatotoxicity(13). Vitamin C also acts as a good effective agent against radiation induced hepatotoxicity. It efficiently inhibits *in vitro* lipid interception and interaction with alpha-tocopherol as co-antioxidant Vitamin C restores the antioxidant abilities of Vitamin E, suggesting that a major function of ascorbic acid is to recycle the tocopheroxyl radical(14).Ascorbate behaves as a weak singlet oxygen quencher, it is a better one-electron reductant than tocopherol, as it recycles the tocopheroxyl radical invivo(15).The aim of the present study was to examine the hepatoprotective effect of Vitamin C against hepatotoxicity induced by D-Galactosamine.

MATERIALS AND METHODS

Experimental Animals

Wistar strain male albino rats weighing 120-150 g were used in this study and were fed with commercial pellete manufactured by Hindustan lever limited and marketed under the trade name **"GOLD MOHUR RAT FEED''** mixed of 0.55% carbohydrate , 24% protein and 5% lipids with wheat flour in the ratio of 1;1 (w/w) . This diet contained 3.7 Kcal Per gram which comprised of 0.55% carbohydrate ,24% protein and 5% lipids. The contents of Vitamin C and, D- Galactosamine in this diet were added respectively.

DOSE AND MODE OF ADMINISTRATION

The solution of vitamin C (100mg/kgbw) was administered orally to rats as suspension in water. D-Galactosamine(400mg/kgbw) suspension in water was used to induce hepatic injury by means of oral administration.

GROUPING EXPERIMENTAL ANIMALS

Albino wistar male rats were kept on fasting for over night before the experiment. The experiments were carried on four groups. Each group contains six animals.

GROUP 1:

Animals served as vehicle- administered only with water.

GROUP 2:

Galactosamine (400mg/Kg) was administered once in a day by orally for a period of 5days. On the 6th day the animals were killed and estimations were done.

GROUP 3:

Animals were administered only with Vitamin C(100mg/Kg) alone orally for 5 days and were bled on 6th day.

GROUP 4:

Animals were pretreated with Vitamin C (100mg/Kg) for 3 days by orally and then D- galactosamine (400mg/kg) was administered for next 2 days, on the 6th day, animals were sacrificed and estimations were performed.

TISSUE HOMOGENIZATION

Serum and tissues *viz* liver and kidney were quickly removed. The tissues were homogenized in Potter-Elvejhemhomogenizer in 5 ml of ice cold phosphate buffer, at pH 7.4 to give a 10% homogenate 2000 rpm for 10 minutes. The assay of enzyme activities was completed within 16 hrs of sacrifice in the liver respectively.

BIOCHEMICAL ANALYSIS

Serum and liver enzymes alanine amino-trasferase (ALT) and aspartate amino-trasferase (AST) were measured according to the method of Mohur and Cook method. Alkaline phosphatase was assayed by the method described by King and king (1965). Acid Phosphatase concentration was assayed following the procedure adopted by Moog et al (1946). Superoxide dismutase (SOD) activity was estimated by the method of Kakkar et al, (1924). Catalase was assayed according to the method of Sinha (1972).

STATISTICAL ANALYSIS

The results are expressed as mean ± SD .Statistical analysis was performed using one way analysis of variance [ANOVA] with statistical tests. $p<0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Table:I Activities of Aspartate transaminase (AST), Alanine transaminase(ALT),Acid Phosphatase (ACP) and alkaline phosphatase (ALP**)** in blood and liver tissue in normal and experimental group of rats.

Table: II

Levels of SOD and CAT in liver of normal and experimental rats. The values are expressed as mean ± S.D. for 6 animals in each group.

PARTICULARS	LIVER	
	SOD (Units/ mg of protein)	CAT (Units/ mg protein)
GROUP I (Normal)	6.58 ± 0.60	12.13 ± 1.20
GROUP II (D-Galactosamine)	4.23 ± 0.38	6.16 ± 0.58
GROUP III (Vitamin C)	6.50 ± 0.56	12.01 ± 1.06
GROUP IV (Vitamin C + D-Galactosamine)	5.87 ±0.48	10.98 ± 1.01

The values are expressed as mean ± S.D. for 6 animals in each group.

DISCUSSION

Hepatotoxicity induced by D-Galn are the best characterized system of xenobiotic – induced hepatotoxicity and commonly used models for the screening of anti-hepatotoxic and /or hepatoprotective activities of drugs (16).The metabolism of D-Galn may deplete several Uracil nucleotides including UDP-glucose, UDP-galactose and UTP, which trapped in the formation of uridine –diphosphogalactosamine. Accumaltion of UDP -sugar nucleotide may contribute to the change in the rough endoplasmic reticulum and to the disturbance of protein metabolism. Toxicity of galactosamine is thought to be responsible for loss in

PARTICULARS	AST		ALT		ACP		ALP	
	BLOOD	LIVER	BLOOD	LIVER	BLOOD	LIVER	BLOOD	LIVER
GROUP I (Normal)	22.48 ± 0.666	15.22 ± 0.057	22.48 ± 0.666	15.22 ± 0.057	0.145 ± 0.017	0.125 ± 0.017	124.2 ± 2.627	215.05 ± 1.509
GROUP II (D-Galactosamine)	53.17 ±0.078	45.46 ± 0.024	53.17 ±0.078	45.46 ± 0.024	0.251 ± 0.025	0.238 ± 0.024	640.3 ± 1.916	641.33 ± 2.070
GROUP III (Vitamin C)	21.02 ± 0.602	14.76 ± 0.034	20.02 ± 0.602	13.76 ± 0.034	0.121 ± 0.013	0.108 ± 0.013	120.5 ± 2.931	210.11 ± 1.705
GROUP IV (Vitamin C + D-Galactosamine)	36.23 ± 1.306	30.33 ± 0.022	36.23 ± 1.306	30.33 ± 0.022	0.211 ± 0.010	0.198 ± 0.017	438.42 ± 1.827	427.91 ± 1.592

The values are expressed as mean ± S.D. for six animals in each group

The values are expressed in i) µ moles liberated / mg protein in tissue. ii) In blood as IU / L

the activity of ionic pumps in the membrane structures. D-Galn intoxification is known to cause marked elevation in the activity of the enzymes (17).

In our study it has been observed that the activities of marker enzymes AST and ALT were prominently increased in the liver and blood samples of galactosamine toxicity. The levels were nearly reduced to the normal levels in the Vitamine C pretreated rats. Reduction in the activities of marker enzymes towards normal level and thus promising, the Vitamin C is a very good hepatoprotective agent. However, there was no significant changes observed in Group III rats compared to the control values. The Transaminases AST and ALT, are well known diagnostic indicators of liver disease .In our study we found that the activities of the marker enzymes were increased about twice the times that of normal values in the D-Galactosamine induced rats, indicating malignant infiltration, which is the pre-stage of cirrhosis,. and may be due to the increase of liver damage with hepatocellular lesions and parenchymal cell necrosis,the enzymes like AST, ALT are released from the damaged tissues in to the blood stream(18).

The enzymes acid phosphatase and alkaline phosphatase were increased in D-Galactosamine treated rats and were significantly reduced nearly to the normal level after the administration of Vitamin C. Decker et al (1993) showed that D-Galactosamine increased the activities of alkaline phosphatase and acid phosphatase. The increased activities of acid phosphatase and alkaline phosphatase in blood and liver could be due to the damage to cell membrane of tissues where these enzymes are firmly attached to cell membranes, the free radical formation mediated cell membrane damage releases the enzyme from the membrane joining the bilarycanaliculus and on the sinusoidal border of the parenchymal cell (19).

The increased activity of ACP and ALP in serum is known to result largely from an increased synthesis of the enzyme by hepatocyte. This endogenous parameter which is commonly used for the assessment of excretory liver function is alkaline phosphatase. It increases in cholestasis, biliary obstruction and hepatic necrosis. Since the sole excretory route of this enzyme is the liver, serum alkaline phosphatase activities are used to test the liver function in experimented animals. Kumar et al. also reported that the D-Galactosamine induced changes, in serum acid phosphatase was (80%) and alkaline phosphatase was only 110%.

The levels of SOD and CAT are shown in Table: II. In the present study the level of these enzymes were decreased by the administration of D-galactosamine in rats and the contents were significantly increased in the vitamin C pretreated rats, confirms the protective effect of vitamin C El-Sherbiny (2009) showed that oxidative stress has been reported as one of the major causes of D-Galn induced liver damage. The decreased activities of superoxide dismutase and catalase in liver could be due to mitochondrial dysfunction as mitochondria are the major target in drug induced liver injury and extent of lipid peroxidation which causes marked alteration in the structural integrity and function of cell membrane (20).

CONCLUSION

Hepatotoxin may cause liver cell necrosis or biochemical changes without necrosis, these changes may be manifested by impaired formation with fat accumulation or altered enzyme activity. In the present study pretreatment with Vitamin C showed significant protection against D-Galactosamine induced hepatotoxicity by ameliorate the activity of markers and thus oxidative stress through its free radical scavenging activity. This study highlights the need of Vitamin C in the treatment of D-Galactosamine induced hepatotoxicity.

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