BIOCHEMICAL ACTIVITY OF SELENIUM AND GLUTATHIONE ON COUNTRY MADE LIQUOR (CML) INDUCED HEPATIC DAMAGE IN RATS

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ABSTRACT

The serum and hepatic enzymes of rats were studied after exposed to country made liquor (CML) along with two chelating agents (glutathione and Selenium). There was a significant increase in several serum enzyme levels (viz., aspartate transaminase, alanine transaminase, alkaline phosphatase, sorbitol dehydrogenase, glutamate dehydrogenase, bilirubin) and decrease in various hepatic enzymes (Succinic dehydrogenase, Glucose 6-phosphatase, 5' Nucleotidase, Acid phosphatase, Acid ribonuclease, Cytochrome P-450) due to repeated administration of CML (2ml/ 100g of body weight). Results of this study revealed that the GSH and Se could give a significant protective action in serum and hepatic enzymes of CML exposed rats.

KEY WORDS

Serum and Hepatic enzymes, CML, Chelating agents, Hepatoprotection.

INTRODUCTION

Alcoholic liver disease continues to be the most serious liver disorders throughout the world. In India, alcohol is mainly consumed in the form of country made liquor (CML). Severity of liver damage is often correlated with the amount of alcohol consumption in patients with history of heavy alcohol abuse (1). Alcoholism also leads to the fat accumulation in the liver, hyperlipidemia and ultimately cirrhosis (2). However, alcoholic liver disease not only depends on the total amount of alcohol consumed, drinking patterns and type of alcoholic beverage ingested are also playing important role in the development of alcoholic liver disease. (3).

Alteration in liver functions due to alcohol consumption may range from fatty liver to Hepatitis and Cirrhosis. Fatty liver is chosen as criteria for making of liver damage in this model as other changes cannot be produced in rats because of their

short life span. In the liver, the acetaldehyde produced by oxidation of ethanol interacts with lipids and proteins, generating free radicals and impairing protein function (4).

CML used in this study was very common brand 'Amutham' and was found to contain 28.5% v/v alcohol. It also consists of some unreported ingredients supposed to be the flavouring agents in alcohol. It was that a CML model of hepatic damage shall be more realistic to clinical setting (5). In this context, changes in the enzyme levels have been studied in the serum and liver of rats administered with CML. Along with this, the capacities of two chelating agents, which reduces or eliminate the toxicity, in relation to enzymes recovery have also been studied.

MATERIALS AND METHODS

Twenty-four young healthy, sexually mature in bred Wistar male albino Rats (180±5g) were used in this investigation. The individuals were kept in highly hygienic conditions, using polypropylene cages, maintained at 28 ± 2°C. They were fed with balanced food pellets and water ad libitum. Each study was conducted in accordance with the National Institute of Health guide (6). Rats were divided into four groups. Every treatment were given by intraperitoneal route (i.p)

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- Group I - Received only the vehicle (corn oil 1ml/100g bw day)
 Group II - vehicle with CML 2 ml/100 g daily for 10 days
 Group III - Received CML 2ml/100g followed by sodium selenite (Na_2SeO_3)- 0.25mg/kg with vehicle once daily for 10 days in succession
 Group IV - Received CML 2 ml/100g followed by Reduced glutathione (GSH)- 40mg/kg with vehicle once daily for 10 days in succession

Rats were sacrificed on 11th day by decapitation without using any anesthesia. Blood was collected and allowed to clot for 20-30min. Serum was separated by centrifugation at 37°C and used for estimations of various biochemical enzyme parameters. Liver of each animal was excised and placed in chilled saline (4°C) to remove excess of blood and adhering fibrous tissues. The weight of livers was noted. The livers were homogenized and centrifuged at 3000 rpm for 10 min. The supernatant was used for the biochemical estimations.

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated by the method of Reitman and Frankel (7), Serum alkaline phosphatase levels were determined by the method of Kind and King (8). Activities of sorbitol dehydrogenase and glutamate dehydrogenase were determined by the methods of Gerlach and Hiby (9); Bilirubin levels of serum were estimated by the method of Malloy and Evelyn (10). Hepatic enzyme activities of acid phosphatase was determined by Kind and King (8); Succinic dehydrogenase by Green et al (11); Glucose-6-phosphatase by Baginski et al (12); 5' Nucleotidase by Aronson and Touster (13); acid Ribonuclease by De Duve et al (14); were measured in the liver homogenate. A liver microsomal fraction was prepared Schneider and Hogeboom (15) and liver cytochrome P-450 content in this fraction was measured from a reduced carbon monoxide difference spectrum Omura and Sato (16). All chemicals used were of analytical grade.

Results of the biochemical estimations are reported as mean $\pm$ S.D. Statistical significance was determined by student's 't' test for unpaired data followed by one way analysis of variance (ANOVA). The difference were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Repeated administration of CML to rats revealed that a significant hepatic damage as observed from elevated serum levels of hepatospecific enzymes as well as severe alterations in different liver parameters (Table 1, 2). Activities of ALT, AST, ALP, sorbitol dehydrogenase, glutamate dehydrogenase and bilirubin in serum were increased in CML intoxicated control rats (Table-1). Treatment with the Se & GSH afforded a significant protection against CML induced increased in these serum enzymes levels.

Table-2 depicts that treatment with CML elevated hepatic enzymes of acid phosphatase, 5' nucleotidase, ribonuclease, succinic dehydrogenase and glucose-6-phosphatase and concurrent decline of a hepatic cytochrome P-450 level. These altered biochemical features were significantly brought towards normalization by treatment with Se & GSH (Table 2).

Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (17). Damage to liver cells cause leakage of cellular enzymes in to serum. A significant rise in ALT, AST could be taken as an index of liver damage. The reversal of increased transaminases returned to normal by Se & GSH supplementation with healing of hepatic parenchyma and regeneration of hepatocytes.

ALP and Bilirubin concentration have been used to evaluate chemically induced hepatic injury. Se and GSH prevented the CML effect on ALP activity in serum. It is reasonable to suggest that chelating agents limited the severity of liver injury. The

Table-1 : Effect of Se & GSH on serum biochemical parameters in CML induced hepatic damage in rats

Group	AST (IU/l)	ALT (IU/l)	ALP (U/l)	Sorbitol dehydrogenase (U/l)	Glutamate dehydrogenase (U/l)	Bilirubin (mg/dl)
I	42.5 $\pm$ 4.8	25.6 $\pm$ 2.8	50.5 $\pm$ 4.6	39.5 $\pm$ 5.5	6.5 $\pm$ 2.2	1.1 $\pm$ 0.5
II	120.5 $\pm$ 9.5 ^c	80.8 $\pm$ 5.8 ^c	136.5 $\pm$ 8.2 ^c	112.5 $\pm$ 8.9 ^c	13.8 $\pm$ 2.9 ^c	8.9 $\pm$ 3.2 ^c
III	65.5 $\pm$ 5.2 ^b	45.5 $\pm$ 4.3 ^b	85.8 $\pm$ 8.6 ^b	49.6 $\pm$ 5.2 ^b	8.6 $\pm$ 2.8 ^b	4.2 $\pm$ 2.9 ^b
IV	58.2 $\pm$ 6.9 ^b	30.1 $\pm$ 4.8 ^b	60.7 $\pm$ 6.3 ^b	43.6 $\pm$ 5.8 ^b	7.3 $\pm$ 2.6 ^b	3.1 $\pm$ 1.9 ^b

Values are Mean $\pm$ SEM from 6 animals in each group.

P values: a<0.05; b< 0.01; c<0.001 as compared with group I animals by ANOVA test

Table-2 : Effect of Se & GSH on liver biochemical parameters in CML induced hepatic damage in rats

Group	Succinic dehydrogenase (μmol indophenol reduced /min/mg protein)	Glucose 6-phosphatase (μmol inorganic phosphate released /min/mg protein)	5' Nucleotidase (μmol inorganic phosphate released /min/mg protein)	Acid phosphatase (μmol indophenol formed /min/mg protein)	Acid ribonuclease (U/mg protein)	Cytochrome P-450 (nmol /mg protein)
I	0.62 ± 0.15	7.35 ± 0.72	2.35 ± 0.28	5.15 ± 0.35	12.57 ± 2.2	3.5 ± 1.5
II	0.35 ± 0.09^c	4.64 ± 1.15^c	3.75 ± 0.32^c	9.32 ± 0.48^c	20.86 ± 4.3^c	1.5 ± 0.8^c
III	0.58 ± 0.13^b	6.75 ± 2.02^b	2.85 ± 0.26^b	7.32 ± 0.45^b	16.56 ± 3.2^b	2.5 ± 0.5^b
IV	0.64 ± 0.17^c	6.65 ± 2.23^b	2.43 ± 0.32^b	6.67 ± 0.43^c	14.34 ± 3.6^c	2.2 ± 0.6^b

Values are Mean $\pm$ SEM from 6 animals in each group.

P values: a<0.05; b< 0.01; c<0.001 as compared with group II animals by ANOVA test.

various forms of liver disorder showed hyperbilirubinemia. In the present study, there is seven times increase in bilirubin level in CML treated animals. Gadgoli et al (18) also found elevated serum bilirubin level in toxicants treated animals. Stabilization of serum bilirubin levels through the administration of Se & GSH is further a clear indication of the improvement of the functions of the liver cells.

The protective role of Se and GSH has also been reflected in the normal levels of succinic dehydrogenase, glucose 6 phosphatase and 5' nucleotidase activities that were severely altered in the CML intoxicated rat liver. Again acid phosphatase and acid ribonuclease are frequently employed as the marker enzymes to assess the lysosomal changes in vivo because it is localized almost exclusively in the particle and it is released parallels that of lysosomal hydrolase (19). A significant lower level of these enzymes, as a consequence of Se & GSH against CML induced marked elevation, indicates a protection against the rupture of lysosomes and leakage of these enzymes in situ resulting from damage to the integrity of liver call lysosomes by CML.

The inhibitory effect of CML on cytochrome P-450 level was also compensated by the Se & GSH through maintenance of its normal level. The role of Se & GSH in the protection of CML mediated loss in cytochrome P-450 content may be considered as an indication of improved protein synthesis in hepatic tissue (20).

It is clearly suggest that there is a decline in protein content, which could affect the metabolic activity of the liver. A protein being involved in the architecture and also in the physiology of the cell seems to occupy a key role in the cell metabolism (21). The decline in protein levels indicates an acceleration of protein catabolism during CML intoxication. The fall in protein

content during stress may be due to increased proteolytic activity and decreased anabolic activity of protein. The decreased protein content might also be due to tissue destruction by necrosis or disturbance of cellular fraction and consequent impairment in protein synthetic machinery (22).

Hepatomegaly is also one of the earliest signs of chronic alcohol abuse (23). This results from the accumulation of triglycerides due to increased synthesis from the carbohydrate load and reduced protein synthesis. Continued high alcohol intake may cause cirrhosis of the liver with resultant decreased serum albumin concentration, portal hypertension, coagulation defects, cardiomyopathy and peripheral neuropathy (23)

Renzoni (24) is supporting the possible detoxification effect of Selenium on various toxicity. Our findings show the clear-cut effective protection against the impact of CML toxicity on protein. Similarly reduced GSH too detoxifies the various toxic effect on the total protein studied in all the tissues (25, 26). Since the reduced GSH is a sulphhydryl rich compound, it could be possible to suggest that the rehabilitation of protein content in the experimental animal groups must be due to GSH.

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