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Role of glutathione in modifying hepatotoxicity induced by copper in rats

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Abstract

Impact of glutathione against copper poisoning with special reference to liver through enzymological parameters have been studied in rats. Intake of copper significantly inhibited the activities of phosphatases, dehydrogenases, cholinesterase and lipase, however, alkaline phosphatase activity increased insignificantly. Reversal of key enzymes activity after supplementation of glutathione to copper fed rats reflects a repair in membranes.Other pharmacotoxicological aspects of glutathione are also discussed.

Key words: *Glutathione, copper, liver, enzymes, rats.*

Introduction

Glutathione is a small protein composed of three amino acids- cysteine, glutamic acid and glycine- that is involved in detoxification and antioxidant mechanisms. It is one of the most important conjugating compounds in helping the body to eliminate fat soluble toxins such as heavy metals, solvents, and pesticides to transform them into a water soluble form allowing more efficient excretion via the kidney. Reduced level of glutathione increases the risk of health problems (Flagg *et al.* 1994). Its dietary role in enhancing detoxification and protection against several anomalies have been reported (Sechi *et al.*1996, Sen, 1997). But the information on the protection offered by glutathione against heavy metal toxicities particularly copper is meager. Whereas, the toxicity of copper is now well known which constitutes inactivation of enzymes (Kumar and Rana1982), lipid peroxidation (Rana and Kumar 1984), interference with mitochondrial function (Nomiyama et.al. 1985) and DNA breakage (Reeves *et al.* 1994) due to their accumulation in different tissues of animals and man in liver, kidney and brain (Kumar and Chandra 1989). Therefore, the present study reports on the influence of glutathione in modifying hepatotoxicity particularly liver dysenzymia induced by copper in albino rats *Rattus rattus* albino.

Materials and Methods

Thirty healthy, intact, pathogen free, colony bred male albino rats (*Rattus rattus* albino), weighing 100 ± 10 g were selected for this study. Each rat was housed separately in a plastic cage bedded with rice- husk and fed on laboratory diet(Hindustan Lever Ltd., Bombay) and tap water *ad libitum*. The animals were divided at random into three groups each containing 10 rats. Rats of group I served as controls, received the laboratory diet alone and tap water *ad libitum*. Rats of group II and III in addition to receiving pellet diet, were fed by gavage copper as copper sulfate at the dosage of 0.1gm/kg body weight on each day for 30 days. Whereas, rats of group III were supplemented with glutathione at the dosage of 0.25gm/kg body weight, in addition to copper sulfate on each day for the same duration.

After 30 days, all the rats were starved for 24 hr. and then sacrificed by decapitation. Slices of liver were quickly excised from the bodies and immediately frozen at 4°C . 10% (w/v) homogenates of liver

were prepared in 0.25 M ice cold sucrose solution. Temperature near 0° C was maintained throughout the period of homogenization. The homogenates were centrifuged for 20 min. at 1500×g and the clear supernatant fluids were used as the source of enzymes. The activity of alkaline and acid phosphatases (Bodansky, 1933), glucose- 6-phoshatase (Swanson, 1955), fructose-1,6 diphosphatase (Pontremoli and Melloni, 1975), lactate dehydrogenase (Bergmeyer and Bernt, 1975), isocitrate dehydrogenase (Bernt and Bergmeyer, 1965), succinate dehydrogenase (Beatty *et al.* 1966), Cholinesterase (Rappaport et.al.1959), and lipase (Bier, 1955) were determined. The protein contents in the homogenates were determined (Lowry *et al.*1951). The student "t" test (Fisher, 1950) was used to calculate the statistical significance between control and experimental values.

Results and Discussion

Enzymological alteration in the liver of copper poisoned rats and effects of supplementation of glutathione are presented in Table 1, from which it is evident that nutritional conditions did affect the key enzymes.Intake of copper inhibited the activity of phosphatases, dehydrogenases, cholinesterase and lipase significantly. However, an insignificant elevation was observed in the activity of alkaline phosphatase.Whereas, supplementation of glutathione to the diet of copper fed rats were found capable in reversing the lost enzyme activity. Maximal reversal was found in the activity of acid phosphatase.

Heavy metal toxicity in animals can be affected by a variety of dietary components. One of these components is protein/amino acid containing sulfhydryl groups like glutathione, methionine and cysteine (Kumar *et al.*1987, Kumar and Kumar 2003). Protection offered by sulfur containing amino acids against copper toxicity in chicks was observed (Jensen and Maurice 1979). Present study was undertaken to confirm the protective role of glutathione on copper induced hepatic dysenzymia- a major event in metal toxicity. Enzymological study chosen as markers of cellular components and parameters of metabolic pathway revealed that copper inhibited the activities of all the enzymes studied except alkaline phosphatase, probably via I the removal of essential metal ion leaving the apoenzyme and (II) replacement of some of the protein groups giving a mixed enzyme inhibitor metal complex. Thus it appears that the presence of free metal ion caused inhibition. Maximum inhibition was found in the activity of acid phosphatase indicating the damage to lysosoms by copper have been reported earlier also by Ishmael *et al.* (1972) and Kumar and sharma (1987).

The efficiency of any hepatoprotective drug is essentially dependent on its capacity on either reducing the harmful effects or in maintaining the normal physiology, which have been disturbed by a hepatotoxin. In the present study, supplementation of glutathione to copper fed rats provides certain amounts of protection and has the capacity to correct liver dysfunction as evidenced by a significant elevation in the activities of all key enzymes studied. Present results confirmthe protective role of glutathione as reversal of key enzymes activity occur, suggest repair in cell membrane/organelle like lysosomes and plasma membrane and normal metabolic pathway. Reversal effects of glutathione (Dalhoff *et al.* 1992) may be affecting the synthesis or functional level of enzymes directly or indirectly by altering the cytomorphology of hepatic parenchymal cells.This may be due to extensive changes in liver beyond the limits of recovery.

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Table 1: - Effect of glutathione on key enzymes in the liver of copper poisoned rats.

	Experimental				
Control	Copper	Alteration Cu +	Glutathione	Alteration	
Enzyme	Group I	Group II	%	Group III	%
Alkaline phosphatase [a]	0.37± 0.020	0.42 ± 0.040 ^{NS}	13.51(+)	0.48 ± 0.38 ^{NS}	14.28 (+)
Acid phosphatase [a]	0.46 ± 0.012	0.08 ± 0.006 ***	82.60 (-)	0.30 ± 0.020 ***	275.0 (+)
Glucose-6-phosphatase [b]	18.62 ± 0.140	12.20 ± 0.110 **	34.47 (-)	15.60 ± 0.210 *	27.86 (+)
Fructose-1-6-diphosphatase [b]	3.12 ± 0.160	0.96 ± 0.120 ***	69.23 (-)	2.02 ± 0.560 **	110.41 (+)
Lactate dehydrogenase [c]	270.50 ± 12.86	92.40 ± 8.50 ***	65.84 (-)	186.40 ± 10.02 ***	101.73 (+)
Isocitrate dehydrogenase [c]	28.00 ± 2.02	10.20 ± 1.56 ***	63.57 (-)	17.10 ± 2.02 **	67.64 (+)
Succinate dehydrogenase [c]	38.40 ± 1.92	22.20 ± 2.02 **	42.18 (-)	30.16 ± 3.98 **	35.85 (+)
Cholinesterase Units	42.00 ± 3.60	26.00 ± 2.80 **	38.09 (-)	32.00 ± 3.02 *	23.07 (+)
Lipase units	23.00 ± 1.98	10.02 ± 1.08 ***	56.43 (-)	16.80 ± 2.20 **	67.66 (+)

All values are mean ± S.E of 5 observations., (+), % stimulation; (-), % inhibition ; NS, not significant ; a, Activity is expressed in mg of inorganic phosphate liberated / mg of protein /hr at 37ºC ; b, Activity is expressed in µ mole of inorganic phosphate / min / gm fresh tissue; c, values expressesd as international units / gm fresh tissue. Values are significant at *P <0.05 **P< 0.01; *** P< 0.001 (Fisher's 't' test), When values of Group –II compared with Group- I; Group-III compared with Group-II.