

## Experimental Medicine

# Hepatoprotective Influence of Selenium in Experimental Liver Cirrhosis

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Kuwait Medical Journal 2001, 33 (4): 333-336

**ABSTRACT**

**Objective:** To study the hepatoprotective and hepatocorrective role of 20  $\mu\text{M}$  of selenium in thioacetamide-induced (TA 4 mM) cirrhosis in male Wistar rats, by both assaying superoxide dismutase (SOD) in erythrocytes and liver and by liver histology.

**Methods:** Six groups of ten rats each were studied in two phases for 32 weeks. Group I was the control. Groups II, III, IV received TA in phase 1. In phase 2, group II received no treatment, group III received selenium as hepatocorrective, and group IV continued to receive TA.

Group V received selenium as hepatoprotective in phase 1 and TA in phase 2. Group VI received selenium throughout the study. SOD activity and liver histology were studied at the end of phase 2.

**Results:** Prophylactic selenium in group V is associated with no mortality, increased liver weight and liver-to-body weight ratio, maximal SOD activity and least destruction of liver architecture.

**Conclusion:** Selenium mitigates liver injury.

**KEYWORDS:** cirrhosis, hepatoprotective, selenium, superoxide dismutase, thioacetamide

**INTRODUCTION**

The levels of selenium, a naturally occurring micronutrient, were significantly reduced in thioacetamide (TA) treated rats. Supplementation with selenium reversed the changes induced by TA. The mode of action of selenium is unknown but may involve anti-oxidant defense mechanisms<sup>[1]</sup>. TA in concentration of 4mM in drinking water for 10-12 weeks efficiently produces cirrhosis in Wistar rats<sup>[2]</sup>. Free radical formation is postulated with various compounds including TA<sup>[3]</sup>. Aerobic forms of life have evolved an array of enzymatic antioxidant defenses; superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase. These enzymatic antioxidants operate in tandem to decompose free radicals. SOD (EC 1.15.1.1) is the known enzyme whose substrate is only free radicals<sup>[4]</sup> unlike GSHPx which acts on peroxides from various sources<sup>[5]</sup>. SOD activity measured in the erythrocytes and lymphocytes of patients with alcoholic cirrhosis was found to be decreased<sup>[6]</sup>. In patients of alcoholic cirrhosis, chronic active hepatitis and chronic persistent hepatitis, serum levels of selenium have been decreased to 40-80% of the control<sup>[7]</sup>. The literature is replete with the integral association of selenium and GSHPx. However, there is paucity in the work done to determine the relationship between selenium administration and SOD levels, the first enzyme among a series to decompose oxidants.

**MATERIALS AND METHODS**

A total of 60 locally bred male Wistar rats (100-250g) were housed two per cage under standard laboratory conditions and fed standard laboratory feed and water *ad libitum*. They were divided into six groups each of 10 rats. The duration of the study was 32 weeks divided into two phases of 16 weeks each. Thioacetamide (Sigma Chemicals) and sodium selenite  $\text{Na}_2\text{SeO}_3$  (Medispan India Ltd.) in concentrations of 4 mM and 20  $\mu\text{M}$ <sup>[8]</sup>, respectively, were administered in drinking water in either phase as described below. A pilot study using TA and sodium selenite in the doses of 4 mM and 20  $\mu\text{M}$  was done in the same institution. Group I, the control, received neither TA nor sodium selenite in either phase. In phase 1, groups II, III and IV received TA, a known hepatotoxin. In phase 2, group II received no treatment and spontaneous recovery was observed. Group III was given sodium selenite in the second phase after TA in the first phase to study the hepatocorrective role of selenium. Group IV continued to receive TA in the second phase. The sequence of treatment was reversed in group V. In this group, the rats received sodium selenite in phase 1 and TA in phase 2 and the hepatoprotective role of selenium was studied. Group VI was given sodium selenite in both phases to study the cumulative effect of this chemical.

At the end of 32 weeks, rats from all the groups were anaesthetized with ether. Blood was drawn

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**Table 1**  
Lethality, liver weight, liver to body weight ratio, SOD activity

Group (n = 10)	Lethality		Liver weight (g)	Liver-body weight ratio	SOD activity units/ml erythrocytes	SOD activity units/g liver tissue
	(%)	Phase				
I	00	-	12.59 ± 4.42	0.03 ± 0.01	126.11 ± 34.99	1247.70 ± 34.99
II	30	Phase 1 1	12.37 ± 2.73	0.04 ± 0.01	103.95 ± 17.46	1264.33 ± 356.77
		Phase 2 2				
III	50	Phase 1 2	13.72 ± 1.94	0.05 ± 0.01	109.40 ± 12.64	1307.00 ± 196.62
		Phase 2 3				
IV	60	Phase 1 0	21.50 ± 3.24*	0.11 ± 0.01*	77.94 ± 14.56***	830.80 ± 6.06***
		Phase 2 6				
V	00	-	20.82 ± 2.55*	0.07 ± 0.11*	169.27 ± 35.96**	2765.00 ± 1018.12**
VI	20	Phase 1 0	13.50 ± 2.64	0.04 ± 0.01	29.50 ± 66.85***	459.56 ± 115.41***
		Phase 2 2				

\* P < 0.001 as compared to control

\*\* P < 0.001 as compared to the remaining groups

\*\*\* P < 0.001 as compared to control values are expressed as mean ± SD

n = number of observations

**Table 2**  
Comparative histopathological assessment of liver

Rat No.	Group I	Group II	Group III	Group IV	Group V	Group VI
1	Normal liver	Moderate cirrhosis	Moderate cirrhosis	Severe cirrhosis	Normal liver	Normal liver
2	Normal liver	Severe cirrhosis	Moderate cirrhosis	Hepatocellular carcinoma (HCC)	Early cirrhosis	Normal liver
3	Normal liver	Severe cirrhosis	Moderate cirrhosis	Severe cirrhosis	Normal liver	Normal liver
4	Normal liver	Moderate cirrhosis	Moderate cirrhosis	Severe cirrhosis	Normal liver	Normal liver
5	Normal liver	Moderate cirrhosis	Early cirrhosis	Severe cirrhosis	Early cirrhosis	Normal liver
6	Normal liver	Moderate cirrhosis	Early cirrhosis	Severe cirrhosis	Early cirrhosis	Normal liver
7	Normal liver	Moderate cirrhosis	Early cirrhosis	Severe cirrhosis	Early cirrhosis	Normal liver
8	Normal liver	Moderate cirrhosis	Early cirrhosis	Severe cirrhosis	Early cirrhosis	Normal liver
9	Normal liver	Moderate cirrhosis	Early cirrhosis	Severe cirrhosis	Early cirrhosis	Normal liver
10	Normal liver	Moderate cirrhosis	Moderate cirrhosis	Severe cirrhosis	Moderate cirrhosis	Normal liver
	Normal 10	Normal 0	Normal 0	Normal 0	Normal 3	Normal 10
	Early 0	Early 0	Early 5	Early 0	Early 6	
	Moderate 0	Moderate 8	Moderate 5	Moderate 0	Moderate 1	
	Severe 0	Severe 2	Severe 0	Severe 9		
				HCC 1		

by cardiac puncture and collected in heparinised syringes for measurement of SOD activity in erythrocytes. The liver was dissected, weighed and two pieces were taken. One piece was fixed in formalin, processed and paraffin blocks were made. Five micron sections were then cut and stained with haematoxylin and eosin and studied under light microscope. The other piece, approximate 1g, was kept frozen for measurement of SOD activity. At the end of the experiment, all the animals were sacrificed.

Preparation of erythrocyte suspension and haemolysate was according to the method of McCord and Fridovich<sup>[9]</sup>. Preparation of liver homogenate, 1g of liver tissue in 10 ml of 5 mM potassium phosphate buffer (pH 7.8), was taken to prepare liver homogenate using a homogeniser.

The unbroken cell and cell debris were removed by centrifugation using a refrigerated centrifuge. The supernatant contained SOD activity. SOD was assayed according to the method of Beauchamp and Fridovich<sup>[10]</sup>. Statistical analyses of data were done using Student's 't' test, results were expressed as mean ± SD. AP value of <0.001 was considered significant.

## RESULTS

Table 1 gives the mortality, mean liver weight, mean liver to body weight ratio and mean SOD activity in erythrocytes and liver tissue of all animals that survived in all the groups. There were no deaths in group I (control) or group V that received selenium treatment as hepatoprotective. Groups IV and V had a higher mean liver weight

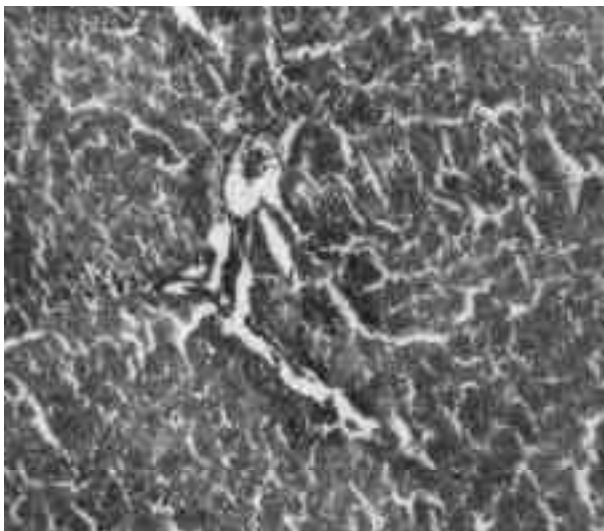


Fig. 1: Normal liver tissue showing central vein with cords of normal liver cells radiating from it. H & E 45 x 10.

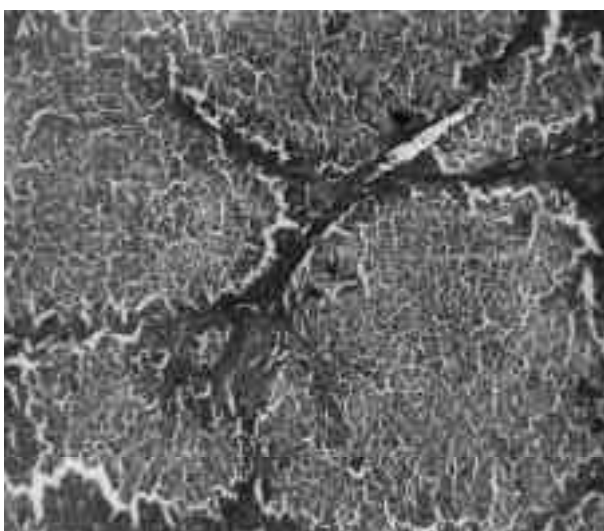


Fig. 2: Cirrhotic liver - Nodules of regenerating liver cells without central vein, separated by fibrous septae. H & E 45 x 10.

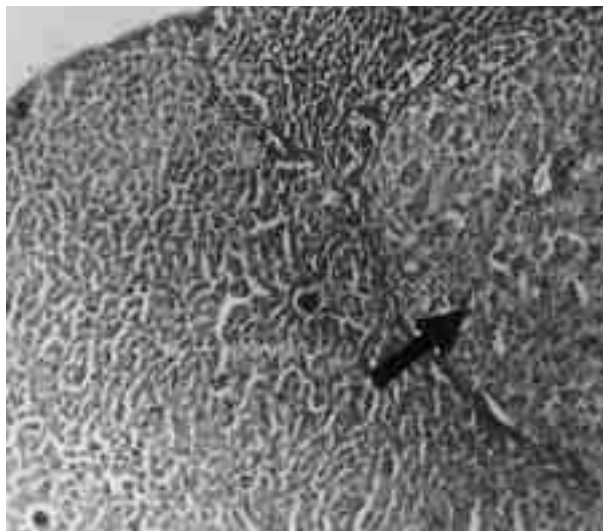


Fig. 3: Cirrhotic liver with dysplastic nodule (arrow) made up of large cells with slightly hyperchromatic and pleomorphic nuclei. H & E 10 x 10. Staining technique. Liver tissue bits were fixed in formalin, processed and paraffin blocks were made, 5 micron sections were cut and stained with haematoxylin and eosin and studied under light microscope.

and higher mean liver to body weight ratio that was significant compared to the control. The highest level of SOD activity was measured in group V. The increase in activity was 134% and 220% in erythrocytes and liver tissue, respectively. This was significant compared to the remaining groups. The lowest level of SOD activity was measured in group VI that received selenium treatment throughout the study and group IV that received TA in phase 1 and 2. The decrease in activity was 24% and 62% in erythrocytes and 37% and 66% in liver tissue of groups VI and IV, respectively. This was significant compared to the control. Table 2 gives the histopathology of the liver of all the animals included in the study (Figs. 1, 2, 3). Microscopic evidence of liver with cirrhosis was based on the following: fibrous septa that dissect and surround nodules of liver cells, the septa contained variable number of lymphocytes

and some bile duct proliferation. The changes of cirrhosis were graded as early, moderate and severe based on: a) amount of fibrosis b) disorganization of normal liver architecture c) stages of nodule formation and disorientation of vascular architecture. Of the four groups that received the hepatotoxin TA, group V had the least distortion of normal liver architecture compared to groups II, III and IV. In group IV, the histopathology of one rat liver that was enlarged showed microscopic evidence of well differentiated hepatocellular carcinoma. Liver histology of animals in group VI was normal. The intake of sodium selenite in groups III, V and VI was .06, .07 and .09 mg per rat per day, respectively.

## DISCUSSION

Prophylactic administration of the micronutrient selenium in a dose of 20  $\mu$ M for 16 weeks, served as a useful hepatoprotective against damage induced by the hepatotoxin, TA, absence of mortality during the period of the study, increased liver to body weight ratio, maximal SOD activity and histopathological finding of the least destruction of liver architecture (group V). Dashti et al<sup>[1,8]</sup> have also reported some improvement in the microscopic appearances of the liver, body weight, liver weight, liver-to-body weight ratio when selenium was administered to thioacetamide-induced liver cirrhosis. What mechanism might explain the role played by selenium in group V, where in spite of a level of liver injury, the animals were provided protection from mortality? Our data of increased liver weight, increased liver-to-body weight ratio and maximal SOD activity underscores the suggestion of hepatocyte proliferation leading to

stimulated hepatocellular regeneration and tissue repair, which are the critical determinants of survival from liver injury. Studies with colchicine used for antimitosis to block cell division and thymidine incorporation into nuclear DNA synthesis preceding cell proliferation<sup>[11]</sup> and estimation of selenium levels that were not done due to technical limitations may have confirmed the above explanation. An equally important observation, though with less circumstantial evidence, is that the micronutrient may play a modest role as a hepatocorrective. The liver architecture of animals in group III was better preserved than in animals in group II which were observed for spontaneous recovery. This histopathological finding is in spite of the higher percentage of mortality recorded in group III as compared to group II.

Selenium is a micronutrient that has a narrow margin between beneficial and harmful levels. This observation is substantiated in our study by the following two findings in group VI where prolonged administration of selenium in the same dose for 32 weeks for the cumulative effect was associated with the lowest level of SOD activity and mortality. These findings may be indicative of the pro-oxidant nature of selenium and its ability to generate free radicals and super oxides on long-term administration. Low levels of SOD activity in group IV animals exposed to hepatotoxin over a prolonged period, which has also been mentioned in the study of Behbehani et al<sup>[12]</sup>, is indicative of oxidative stress that may have been responsible for maximal destruction in liver architecture and mortality. The small number of animals studied in each group may account for the lack of uniformity in the pattern of mortality of groups II, III and IV. Studies on a larger number of animals may result in more convincing data.

The observations in this study help us to conclude that selenium in carefully titrated doses mitigates the damage in experimentally induced liver cirrhosis. This finding may offer scope for the use of this micronutrient as a dietary supplement. Intake of foods such as seafood, meat and whole grains, which are generally good sources of selenium (over .2 ppm), may decrease the risk of

hepatic damage in vulnerable sections of the populations. As a corollary we add, a diet poor in selenium sources such as fruits and vegetables can accelerate hepatic damage in those predisposed to liver damage.

#### ACKNOWLEDGEMENTS

The authors wish to thank the Karnataka State Council for Science and Technology for financial support.

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