

Role of selenium in protection of liver cirrhosis

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Abstract: Selenium is an essential trace element and has been shown to protect the rats against dietary liver necrosis. This study was designed to evaluate the effects of selenium supplementation on different biochemical parameters in thioacetamide induced cirrhotic rats. For this purpose 24 male Albino wistar rats were divided into four groups (n=6). Group I, remained healthy control rats, Group II, received thioacetamide (at a dose of 200mg/kg b.w, i.p, for 12 weeks, twice a week) in first phase and saline in second phase, Group III, received thioacetamide (200mg/kg b.w, i.p for 12 weeks, twice a week) in first phase and sodium selenite ((1mg/kg b.w, i.p. for 12 weeks, three times a week) in second phase and Group IV, received sodium selenite (1mg/kg b.w, i.p. for 12 weeks, three times a week) in first phase and saline in second phase. Biochemical analysis was evaluated by total and direct bilirubin, liver specific enzymes, and antioxidant enzymes. Marked increase in total and direct bilirubin and ALT activity was the indicative markers of liver cirrhosis while reduced antioxidant activity (SOD and GSH) and increased MDA and Catalase levels were observed in cirrhotic group. Sodium selenite supplementation markedly reduced total bilirubin and ALT activity and restored the antioxidant enzymes (SOD and GSH) and MDA and catalase activity. These results indicate that sodium selenite successively attenuates the thioacetamide induced liver cirrhosis.

Keywords: Liver cirrhosis, Sodium selenite, Thioacetamide, Liver enzymes, SOD, GSH, Catalase, MDA.

INTRODUCTION

Liver cirrhosis is characterized by the replacement of healthy liver tissue by fibrotic scar tissue and by regeneration of hepatocytes which progressively leads to loss of liver function (Maki Sato *et al.*, 2000). Different animals models have been used to study the mechanisms of liver cirrhosis which include treatment of rats with carbon tetrachloride (Mion *et al.*, 1996; Dashti *et al.*, 1989), bile duct ligation (Zimmermann H *et al.*, 1997), alcoholism (Tsukamoto *et al.*, 1995) and through the administration of thioacetamide (Muller *et al.*, 1988; Munos Torres *et al.*, 1991). Preze Tamayo reported that cirrhosis induced by thioacetamide contained more prominent fibrotic tissue and regenerative nodules than cirrhosis induced by carbon tetrachloride (Perez Tamayo, 1983). The pathology of long term induction of thioacetamide in experimental rats resembles to that of cirrhosis of human beings (David *et al.*, 2002) contained oxidative destruction (Teck Yew Low *et al.*, 2004) and resulted in reduction of glutathione reductase level (S Dorgu-abbasoglu *et al.*, 2001), an increase in MDA level (Balkon *et al.*, 2001) decrease in the activity of superoxide dismutase (Fatih Aydin *et al.*, 2010; Feher *et al.*, 1998) and an increase in the activity of catalase (Cruz *et al.*, 2005).

Selenium is an essential trace mineral for animals including humans (Huaweizeng, 2009a). It is critical for antioxidant defense (Sladan, 2001), thyroid hormone metabolism (John, 1991), fertility (Hansen and Deguchi, 1996) immune response (Kiremidjian-Schumacher and

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Stotzky, 1987), muscle development (Alain Lescure *et al.*, 2008) and have a protective effect at different stages of carcinogenesis (Zeng and Combs, 2008). Several mechanisms for selenium anti-cancer action have been proposed including antioxidant protection, enhanced carcinogen detoxification, enhanced immune surveillance, modulation of cell proliferation, inhibition of tumor cell invasion and inhibition of angiogenesis (Huaweizeng *et al.*, 2007b).

Thioacetamide treated rats showed low level of selenium (Zhang and Wanq, 2007). Changes appeared by thioacetamide administration reversed by selenium supplementation (Navarro-Alarcon *et al.*, 2002). Hoffman reported that selenium may have antioxidant defence mechanism (Hoffman, 2002). As the selenium containing enzyme glutathione peroxidase (GSHPX) is responsible for protection of biomembranes from oxidative damage. It is present in high concentrations in cells, decrease in concentration of glutathione peroxidase increases the risk of cellular destruction. Animals with low selenium level showed depressed glutathione peroxidase activity (Hofeman *et al.*, 1974). Combination of different antioxidants such as selenium, vitamin E and superoxide dismutase play a central role in protection of cell against oxidative destruction (Charez, 1979; Thompson *et al.*, 1980).

In views of above mentioned previous studies it is hypothesized that cirrhosis of the liver could be prevented by the use of some antioxidants. The present study was designed to examine the protective role of Selenium in the TAA induced liver cirrhosis in experimental rats model.

MATERIALS AND METHODS

24 male Albino Wistar rats weighing 200-250gm were purchased from the animal house of ICCBS (International center for chemical and biological sciences, Karachi, Pakistan) for the study. Animals were acclimatized to the laboratory conditions before the start of experiment and caged in a quiet temperature controlled animal room (23±4°C). Rats had free access to water and standard rat diet.

Ethical guidelines

The experiments were conducted with ethical guidelines of institutional ERB (Ethical Review Board) and internationally accepted principles for laboratory use and care in animal research (Health research extension Act of 1985).

Study design

The rats were randomly divided into four groups, each of six rats. The duration of the study was 24 weeks, divided into two phases. Thioacetamide (Sigma) and sodium selenite (Na₂SeO₃) were administered in either phase as described below. Thioacetamide and sodium selenite were purchased from Merck and the other chemicals used in present study were purchased from BDH laboratory supplies, Fisher Scientific UK limited and Fluka AG.

Group I: The control (remained untreated)

Group II: TAA-treated

Group III: TAA+ selenium treated

Group IV: Selenium treated

In Phase I, TAA-treated and TAA+selenium treated groups received TAA, dissolved in 0.9% NaCl and was injected intraperitoneally at a dosage of 200mg/kg b.w, twice a week for 12 weeks. Selenium treated group received sodium selenite intraperitoneally at a dosage of 1mg/kg b.w, three times a week for 12 weeks. In Phase II, the animals of TAA+selenium treated group were given sodium Selenite (intraperitoneally at a dosage of 1mg/kg b.w, three times a week, starting from 13th week for 12 weeks) after TAA in first phase to study the hepatocorrective role of selenium. TAA-treated and sodium selenite-treated group received saline in second phase. At the end of experimental period, rats from all the groups were decapitated. The blood was collected from the neck wound in the lithium heparin coated tubes and centrifuged to collect plasma. Liver was excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues then kept in freezer at -70°C until analysis.

Assessment of ALT and total and direct bilirubin

Plasma ALT (Retiman and Franhel, 1957) and total and direct bilirubin (Sherlock, 1951) were analyzed using commercially prepared reagent kits from Randox.

Preparation of post mitochondrial supernatant

Liver homogenate was prepared by taking 1g of liver tissue in 10ml of 5mM potassium phosphate buffer (pH 7.8) by using a homogenizer. The homogenates were centrifuged at 800g for five minutes at 4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500g for 20 minutes at 4°C to get post mitochondrial supernatant which was used to assay SOD, Catalase, MDA, and glutathione reductase activity.

Estimation of thiobarbituric acid substances:

The malonyldialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) by the lipid peroxidation method (Okhawa *et al.*, 1979). Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 ml of 10%(w/v) of PMS. The mixture was brought up to 4.0 ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0 ml distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm and compared with those obtained from MDA standards. The concentration values were calculated from absorption measurements as standard absorption.

Estimation of catalase:

Catalase activity was assayed by the method of Sinha (Sinha *et al.*, 1979). Briefly, the assay mixture was consisted of 1.96 ml phosphate buffer (0.01M, pH 7.0), 1.0 ml hydrogen peroxide (0.2 M) and 0.04 ml PMS (10%w/v) in a final volume of 3.0 ml. 2 ml dichromate acetic acid reagent was added in 1 ml of reaction mixture, boiled for 10 minutes, cooled. Changes in absorbance were recorded at 570 nm.

Estimation of Superoxide dismutase:

Superoxide dismutase levels in the cell free supernatant were measured by the method of (Kono *et al.*, 1978). Briefly 1.3ml of solution A (0.1 M EDTA containing 50 mM Na₂CO₃, pH 10.0), 0.5 ml of solution B (90µM NBT nitro blue tetra zolium dye) and 0.1 ml of solution C (0.6% Triton X-100 in solution A), 0.1 ml of solution D (20 mM Hydroxyl amine hydrochloride, pH 6.0) were mixed and the rate of NBT reduction was recorded for one minute at 560 nm. 0.1 ml of the supernatant was added to the test cuvette as well as reference cuvette, which do not contain solution D. Finally, the percentage inhibition in the rate of reduction of NBT was recorded as described above. One enzyme unit was expressed as inverse of the amount of protein (mg) require in one minute.

Estimation of glutathione reductase:

GSH activity was determined by continuous spectrophotometric rate determination (Calberg and Mannervik, 1985). In a clean glass test tube, 0.3 mL of 10% BSA, 1.5mL of 50mM potassium phosphate buffer (pH 7.6), 0.35mL of 0.8mM β -NADPH and 0.1mL of 30mM oxidized glutathione was taken and finally added 0.1mL of homogenate, mixed well by inversion. Absorbance was recorded at 340nm at 25°C for 5 minutes on kinetic spectrophotometer PRIM 500 (Germany) with automatic aspiration and thermostat. The activity was calculated using the molar coefficient for NADPH of $6.22 \mu\text{mol}^{-1} \times \text{cm}^{-1}$ and expressed in unit/gram tissue.

STATISTICAL ANALYSIS

Results are presented as mean $\pm$ SD. Statistical Significance and difference from control and test values evaluated by Student's t-test. Statistical probability of **P<0.05, *P<0.01 were considered to be significant.

RESULTS**Effect of Thioacetamide and sodium selenite treatment on body weight in control and treated animals**

Decreased body weight was observed after chronic administration of TAA in TAA-treated rats in both phases while in TAA+selenium treated group in phase I. Animals of TAA+selenium treated group regained their body weight after sodium selenite treatment in second phase (fig. 1). Increased body weight was observed in control and selenium-treated group.

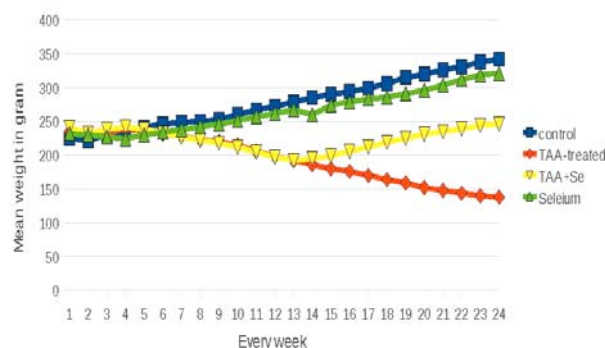


Fig. 1: Effect of Thioacetamide and sodium selenite treatment on body weight in control and treated animals.

Effect of thioacetamide and sodium selenite treatment on liver weight and liver to body weight ratio in control and treated animals.

Increased liver weight and relative liver weight were observed in TAA-treated group as Compare to control (8.38 ± 0.72 P<0.01) (0.33 ± 0.005 P<0.01) table1. where as reduction in the liver weight and relative liver weight was observed in TAA+selenium treated group as compare to control (6.59 ± 0.72 , P<0.01) (0.03 ± 0.005 P<0.01) respectively. Increase in liver weight and relative liver weight was observed in selenium-treated group as compare to control (3.31 ± 0.6 p<0.01) (0.013 ± 0.006).

Table 1: Liver weight, liver to body weight ratio in control and treated rats

Groups	Liver weights	Relative Liver Weights
Control	$2.44 \pm 0.27^*$	$0.010 \pm 0.001^*$
TAA-treated	$8.38 \pm 0.72^*$	$0.33 \pm 0.005^*$
TAA+selenium treated	$6.59 \pm 0.72^*$	$0.03 \pm 0.005^*$
Selenium-treated	$3.31 \pm 0.6^*$	0.013 ± 0.006

Values are mean + SD. Significant difference among control, TAA-treated, TAA+selenium and selenium treated groups by student's t-test **P<0.05, *P<0.01.

Effects of thioacetamide and sodium selenite treatment on total and direct bilirubin and ALT activity in control and treated rats

Table 2 shows a marked increase in total bilirubin level in TAA-treated group as compare to control (3.19 ± 0.22 P<0.01) where as, in TAA+selenium treated group, sodium selenite supplementation brought those increased levels almost to the normal concentrations as compare to control (0.65 ± 0.01 , P<0.01), alone sodium selenite had no significant effect. Increased levels of direct bilirubin was shown by TAA-treated group as compare to control (3.75 ± 0.03 , P<0.01) where as sodium selenite supplementation in TAA+selenium treated group brought those higher levels almost to normal levels as compare to control (1.75 ± 0.03 , P<0.05), in selenium treated group no significant effect on direct bilirubin was found.

Plasma Alanine aminotransferase level was markedly increased in TAA-treated group as compare to control (945 ± 68.1 , P<0.01). Alanine amino transferase level was

Table 2: Effects of thioacetamide and sodium selenite treatment on total and direct bilirubin and ALT activity in control and treated rats

Parameters	Control	TAA-treated	TAA+selenium treated	Selenium-treated
Total bilirubin (Unit/L)	0.61 ± 0.02	$3.19 \pm 0.22^*$	$0.65 \pm 0.01^*$	$0.59 \pm 0.01^{**}$
Direct bilirubin (Unit/L)	1.68 ± 0.05	$3.75 \pm 0.03^*$	$1.75 \pm 0.03^{**}$	$1.62 \pm 0.03^{**}$
Alanin-amino transferase (Unit/L)	203 ± 2.27	$945 \pm 68.1^*$	$210.8 \pm 2.5^*$	$198.5 \pm 2.3^*$

Values are mean + SD. Significant difference among control, TAA-treated, TAA+selenium treated and selenium treated groups by student's t-test **P<0.05, *P<0.01.

decreased significantly in TAA+selenium treated group as compare to control (210.8 ± 2.5 , $P < 0.01$). Alone selenium had no significant effects on ALT activity.

Effect of thioacetamide and sodium selenite treatment on hepatic concentration of glutathione reductase

Hepatic concentration of glutathione reductase was significantly reduced in TAA-treated group as compare to control (0.026 ± 0.01 , $P < 0.01$). TAA+selenium treated group showed normal levels of glutathione reductase as compare to control (0.61 ± 0.01 , $P < 0.01$) table 3. Glutathione reductase was found normal in selenium-treated group as compare to control (0.6 ± 0.01 , $P < 0.01$).

Effects of thioacetamide and sodium selenite treatment on hepatic concentration of MDA

Level of MDA was markedly increased in TAA-treated group as compare to control (124.6 ± 1.8 , $P < 0.01$). Sodium selenite administration in TAA+selenium treated group decreased the concentration of MDA as compare to control (47.2 ± 0.05 , $P < 0.01$) while animals of selenium-treated group showed normal range of MDA level as compare to control (48.8 ± 1.5 , $P < 0.01$) after Sodium Selenite treatment (table 3).

Effect of thioacetamide and sodium selenite treatment on hepatic concentration of superoxide dismutase in control and treated rats

Table 3 showed a significant decrease in SOD activity in TAA-treated group as compare to control (352.4 ± 1.38 , $P < 0.01$). TAA+selenium treated group, after sodium selenite supplementation, showed a significant increase in SOD activity (964.3 ± 0.4 , $P < 0.01$) as compare to control. SOD activity was significantly reduced in selenium-treated group (225.0 ± 0.4 , $P < 0.01$) as compare to control.

Effect of thioacetamide and sodium selenite treatment on hepatic concentration of catalase

Concentration of catalase was significantly increased in TAA-treated group (38.91 ± 0.12 , $P < 0.01$) as compare to control. Administration of sodium selenite in TAA+selenium group significantly reduced catalase level (10.68 ± 0.16 , $P < 0.01$) as compare to control. Level of catalase was slightly increased (11.98 ± 0.16 , $P < 0.01$) in selenium treated group as compare to control (table 3).

Table 3: Effects of thioacetamide and sodium selenite treatment on hepatic concentration of glutathione reductase, superoxide dismutase, malondialdehyde and catalase

Parameters	Control	TAA-treated	TAA+selenium treated	Selenium-treated
Glutathione Reductase (unit/gm of tissue)	0.64 ± 0.02	$0.026 \pm 0.01^*$	$0.61 \pm 0.01^*$	$0.60 \pm 0.01^*$
Superoxide dismutase unit/gm of tissue.	959.6 ± 0.9	$352.4 \pm 1.38^*$	$964.3 \pm 0.4^*$	$225.0 \pm 4.0^*$
Malondialdehyde nmol/gm of tissue.	44.4 ± 1.4	$124.6 \pm 1.8^*$	$47.2 \pm 0.5^*$	$48.8 \pm 1.5^*$
Catalase nmol/gm of tissue.	8.63 ± 0.02	$38.91 \pm 0.12^*$	$10.68 \pm 0.16^*$	$11.98 \pm 0.16^*$

Values are mean + SD. Significant difference among control, TAA-treated, TAA+selenium treated and selenium-treated groups by student's t-test ** $P < 0.05$, * $P < 0.01$.

DISCUSSION

In present study, intraperitoneal administration of thioacetamide produced definite cirrhotic changes in rats, increased concentrations of total and direct bilirubin and alanine aminotransferase were found in TAA-treated group while following selenium administration, there was a significant decrease in the level of total and direct bilirubin and ALT activity. Concentration of Total and direct bilirubin (Torres et al., 1996) and ALT activity (Sturgill and Lambert, 1997) increased in rats by long term thioacetamide induction due to its toxic effects on the bile ductular system (Yamada and Fausto, 1998). Thioacetamide exhibited necrogenic (Perez Tamayp, 1983) and carcinogenic (Gervasi et al., 1989) effects and can be used in animal models for the induction of acute liver failure (Fontana et al., 1996) and cirrhosis of liver (Abul et al., 2000). Thioacetamide was selected for the present study because in rats it produces the liver cirrhosis with similar histological appearances as found in liver cirrhosis of human beings and the intraperitoneal administration of thioacetamide was proved successful in producing cirrhosis within 12 weeks as reported by Teck Yew et al. (2004). Thioacetamide biotransformation resulted in the reduction of dioxygen to superoxide anion and finally in the formation of hydrogen peroxide (H_2O_2) (Karantonis et al., 2010) which leads to liver injury with oxidative destruction (Osada et al., 1986). Induction of cirrhosis in liver resulted in reduction of selenium level from liver (Al-Bader et al., 1998a) as well as a reduction in the synthesis of α -glubulin which serves as a binding protien for selenium indicating selenium loss from both liver and plasma (Abul et al., 2001). Selenium plays an essential role in protection of cell membranes from oxidative destruction as it is an important component of glutathione peroxidase enzyme which destroys lipid damaging peroxides (Rotruck et al., 1973). Bruck reported that loss of selenium causes necrosis in experimental animals (Bruck et al., 1995). According to Mohandas Rai, a dose of $2 \mu m$ of selenium for 16 weeks appeared as a useful hepatocorrective against thioacetamide induced liver cirrhosis. However longterm selenium supplementation resulted in reduced SOD activity as in selenium-treated group which may be because of its pro-oxidant nature and ability of selenium

to form free radicals and super oxides on its prolonged exposure (Mohandas Rai *et al.*, 2001).

Supplementation of selenium to thioacetamide induced cirrhotic rats resulted in improvements in body weight, liver weight, liver to body weight ratio and also in the microscopic appearances of liver (Al-Bader *et al.*, 1998 b). In the present study, data of increased liver weight & liver to body weight ratio, increased body weight, restorage of the levels of ALT activity, total-bilirubin concentration, levels of glutathione reductase, malondialdehyde, SOD activity and catalase activity suggest proliferation of liver cells which leads to stimulate hepatocellular regeneration and tissue repair which are the critical determinants of survival from liver injury. Therefore it can be concluded that selenium, a very important micronutrient can be used successfully to suppress the hepatocellular effects produced by cirrhosis of liver.

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