Pak. J. Biochem. Mol. Biol. 2012; 45(2): 68-72

Effect of zinc supplementation on antioxidant enzymes in liver cirrhosis

*Syeda Nuzhat Fatimah and Tabassum Mahboob**
Clinical Biophysics Research Unit, Department of Biochemistry, University of Karachi, Karachi, Pakistan

Abstract: The present study was designed to evaluate the effects of zinc sulphate 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) for first phase and saline for second phase, Group III, received thioacetamide (200mg/kg b.w, i.p for 12 weeks, twice a week) for first phase and zinc sulphate(6mg/kg b.w/day,i.p,for six weeks) for second phase. Group IV, received zinc sulphate(6mg/kg b.w/day,i.p,for six weeks) in the 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. Zinc sulphate 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 zinc sulphate successively attenuates the thioacetamide induced liver cirrhosis.

Keywords: Zinc sulphate, thioacetamide, liver enzymes, SOD, GSH, catalase, MDA.
Received: March 21, 2012 **Accepted:** May 10, 2012
***Author for Correspondence:** tab60@hotmail.com

INTRODUCTION

Zinc is an essential trace mineral with important anti-inflammatory[1], antiapoptotic[2], and antioxidant effects[3]. Various biological effects of zinc depend on its catalytic, structural and regulatory role in a large number of enzymes and zinc finger motifs[4]. Nearly 300 different enzymes contain zinc as an integral component[5]. Zinc finger motif stabilizes the structure of a number of proteins which are involved in various cellular processes such as replication and repair, transcription and translation, metabolism and signaling, cell proliferation and apoptosis[6]. Zinc plays central role in aging[7] as it is required for cell cycle progression[8] DNA repair[9] and for the prevention of neoplastic cell growth[10]. Zinc exerts a considerable effect in the stress response, affects the compensatory capacity, and acts as a neuromodulator[11]. The role of zinc in antioxidant defense mechanism includes the protection due to redox active transition metals such as copper and iron, and the protection of sulfhydryl groups of protein from oxidative damage. The chronic antioxidant effects of zinc results in the induction of metellothionein synthesis and the CU/ZN superoxide dismutase stabilization. Metallothionein act as scavangers of toxic metals and a number of antioxidant molecules and make a connection between cellular zinc and its redox state[12]. Other antioxidant effects exerted by zinc include its protective role against vitamin E depletion[13] and apoptosis, induction of cell-proliferation and inhibition of NADPH oxidases[14].

Effects of zinc deficiency include growth failure, reduced gonadal development in male, cell-mediated immune disorders, mental lethargy, skin changes, delayed wound healing,poor appetite and neurosensary disorders[15]. Zinc deficiency causes the tissue oxidative damage and modulation of selected signaling cascades in the liver[16]. Zinc deficiency causes impairment of cellular immunity[17], susceptibility to infections, induction of oxidative stress and its related conditions such as susceptibility to hepatitis, reduced acute phase response protection against hepatitis, and lipid oxidation, by changing the cellular redox state which results in activation of oxidant-sensitive transcription factors that affects the cell function and leads to induction of disease.

The role of zinc is very important in antioxidant defense mechanism as well as in regeneration of damaged cells. In views of above mentioned previous studies it is hypothesized that cirrhosis of the liver could be prevented by the supplementation of zinc. The present study was designed to examine the protective role of zinc in thioacetamide induced liver cirrhosis in experimental rat's model.

MATERIALS AND METHODS

Total 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).

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 18 weeks, divided into two phases. Thioacetamide and zinc sulphate were administered in either phase.

Thioacetamide and zinc sulphate 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+ Zinc Sulphate treated
Group IV: Zinc Sulphate treated

In Phase I, TAA-treated and TAA+Zinc Sulphate groups received, thioacetamide dissolved in 0.9% NaCl and was injected intraperitoneally at a dosage of 200mg/kg b.w, twice a week for 12 weeks. Zinc Sulphate group received saline in first phase. In phase II, TAA-treated group received saline for six weeks, TAA+Zinc Sulphate group received Zinc Sulphate (intraperitoneally at a dosage of 6mg/kg b.w/ day starting from 13th week for six weeks) after TAA in first phase to study the hepatocorrective role of zinc and zinc sulphate group received Zinc sulphate (intraperitoneally at a dosage of 6mg/kg b.w/ day for 6 weeks). 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 -70ºC until analysis.

Estimation of plasma ALT and bilirubin

Plasma ALT[18], total and direct bilirubin[19] were analyzed using commercially prepared reagent kits from Randox.

Preparation of Post mitochondrial supernatant (PMS)

Liver homogenate was prepared by taking 1gm 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 4ºC to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500g for 20 minutes at 4ºC to get postmitochondrial supernatant which was used to assay SOD, Catalase, MDA, and glutathione reductase activity.

Estimation of lipidperoxidation

The malonyldialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thio- barbituric acid reacting substances (TBARS)[20]. Briefly, the reaction mixture consisted of 0.2ml of 8.1% sodium dodecyle sulphate, 1.5ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2ml of 10% (w/v) of PMS. The mixture was brought up to 4.0ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0 ml distilled water and 5.0ml of the mixture of n-butanol and pyridine (15:1v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532nm 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 previously described method[21]. Briefly, the assay mixture consisted of 1.96ml phosphate buffer (0.01M, pH 7.0), 1.0ml hydrogen peroxide (0.2M) and 0.04ml PMS (10%) in a final volume of 3.0ml. 2ml dichromate acetic acid reagent was added in 1ml of reaction mixture, boiled for 10 minutes, cooled. Changes in absorbance were recorded at 570nm.

Estimation of SOD

Levels of SOD in the cell free supernatant were measured[22]. Briefly 1.3ml of solution A (0.1M EDTA containing 50mM Na_2CO_3, pH 10.0), 0.5 ml of solution B (90µmNBTnitro blue tetra zolium dye) and 0.1ml of solution C (0.6%Triton X-100 in solution A), 0.1ml 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 560nm. 0.1ml of the supernatant was added to the last minute at 560nm. 0.1ml 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[23]. In a clean glass test tube, 0.3mL of 10% BSA, 1.5mL of 50mM potassium phosphate buffer (pH 7.6), 0.35mL of 0.8mM ß-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 25ºC 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 µmolxcm and expressed in U/gram tissue.

Statistical analysis

Results are presented as mean±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

Effects of thioacetamide and zinc sulphate treatment on liver weight and liver to body weight ratio in control and treated rats

Increased liver weight and relative liver weight was observed in TAA group after 12 week administration of TAA as compare to control (7.11±0.2 P<0.01) (0.033±0.001 P<0.01) (Table 1) where as reduction in the liver weight and relative liver weight was observed in TAA+Zinc Sulphate group as compare to control (6.80.15, P<0.01) (0.036±0.001 P<0.01) respectively. An increase in liver weight was observed in zinc sulphate treated rats (7.1±0.34, P<0.01) as compare to control where as relative liver weight was almost normal (0.036±0.001, P<0.01) as compare to control.

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

Groups	Liver Weights	Relative Liver Weights
Control	6.33±0.31*	0.033±0.001*
TAA-treated	7.11±0.2*	0.04±0.001*
TAA+Zinc Sulphate treated	6.8±0.15*	0.036±0.001*
Zinc sulphate treated	7.1±0.34*	0.031±0003*

Values are mean±SD. Significant difference among control, Thioacetamide and Thioacetamide+sodium selenite treated groups by t-test, *P<0.01.

Effects of thioacetamide and zinc sulphate treatment on plasma 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±0.2, P<0.01) whereas, in TAA+Zinc Sulphate treated group, zinc sulphate supplementation brought those increased levels almost to the normal concentrations as compare to control (0.75±0.01, P<0.01). There was a slight increase in plasma total bilirubin level in zinc sulphate treated group as compare to control (0.87±0.2, P<0.01). Increased levels of direct bilirubin was shown by TAA-treated group as compare to control (3.31±0.2, P<0.01) where as zinc sulphate supplementation brought those higher levels almost to normal levels as compare to control (1.15±0.02, P<0.01) . Alone zinc sulphate had no significant effects on plasma direct bilirubin concentration. Plasma Alanine aminotransferase level was markedly increased in TAA-treated group as compare to control (1021.3±68.19, P<0.01). Alanine amino transferase level was significantly decreased in TAA+Zinc Sulphate group as compare to control (250.8.2±13.6, P<0.01). Alone zinc sulphate had no significant effect on plasma ALT activity.

Table 2: Effects of thioacetamide and zinc sulphate treatment on plasma bilirubin and ALT activity in control and treated rats.

Parameters	Control	TAA-treated	TAA+Zinc Sulphate	Zinc Sulphate
Total bilirubin (U/L)	0.58 ±0.04	3.19 ±0.2*	0.75 ±0.01*	0.87 ±0.2*
Direct bilirubin (U/L)	1.50 ±0.2	3.31 ±0.2*	1.15 ±0.02*	1.36 ±0.02
Alanin-amino transferase (U/L)	200.7 ± 11.7	1021.3 ±68.9*	250.8 ±13*	198 ±10.1*

Values are mean±SD. Significant difference among control, Thioacetamide and zinc sulphate treated groups by t-test, *P<0.01.

Effects of thioacetamide and zinc sulphate treatment on hepatic concentration of glutathione reductase

Hepatic concentration of glutathione reductase was significantly reduced in TAA-treated group as compare to control (0.031±0.001, P<0.01). TAA+zinc sulphate showed almost normal level of glutathione reductase as compare to control (0.79±0.01, P<0.01). Alone zinc sulphate had no effect on glutathione concentration as compare to control.

Effects of thioacetamide and zinc sulphate treatment on hepatic concentration of MDA

Level of MDA was markedly increased in TAA-treated group as compare to control (128.8±1.8, P<0.01). Zinc sulphate administration in TAA+zinc sulphate group decreased the concentration of MDA as compare to control (46.1±1.2, P<0.01) while zinc sulphate treated group showed a slight increase in MDA level as compare to control (58.2±1.5,P<0.01) (Table 3).

Table 3: Effects of thioacetamide and zinc sulphate treatment on hepatic concentration of glutathione reductase, superoxide dismutase, malondialdehyde and catalase.

Parameters	Group I	Group II	Group III	Group IV
Glutathione reductase (U/gm)	0.82 ±0.02	0.031 ±0.001*	0.79 ±0.01*	0.80 ±0.03
Superoxide dismutase (U/gm)	961.4 ±2.1	430±1.5*	968.2 ±1.9*	631.5 ±3.1*
MDA (nmol/gm)	54.4 ±1.4	128.8±1.8*	46.1 ±1.2*	58.2 ±1.5*
Catalase (nmol/gm)	6.3 ±0.02	40.1±0.01*	9.2 ±0.01*	6.5 ±0.3*

Values are mean±SD. Significant difference among control, thioacetamide, thioacetamide and zinc sulphate and sodium selenite treated groups by t-test, *P<0.01

Effects of thioacetamide and zinc sulphate 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 (430±1.5, P<0.01). TAA+zinc sulphate group, after zinc sulphate supplementation, showed normal levels of SOD activity (968.2±1.9, P<0.01) as compare to control. SOD activity reduced in zinc sulphate-treated group (631±3.1, P<0.01) as compare to control.

Effects of thioacetamide and zinc sulphate treatment on hepatic concentration of catalase

Concentration of catalase was significantly increased in TAA-treated group (40.1±0.01, P<0.01) as compare to control. Administration of zinc sulphate in TAA+zinc sulphate group significantly reduced catalase level (9.2±0.01, P<0.01) as compare to control. Alone zinc sulphate treatment had no effect on catalase level (6.5±0.3, P<0.01) (Table 3).

DISCUSSION

The present study describes the long term administration of thioacetamide resulted in the development of severe liver injury in rats. Administration of zinc in cirrhotic rats causes a significant decrease in the level of plasma bilirubin and ALT activity (Table 2). Our results are in agreement with the studies of Hussein Dashti who found that zinc supplementation normalizes the plasma bilirubin and ALT activity in cirrhotic rats[24].

The repeated thioacetamide treatment resulted in increased lipidperoxidation and decreased antioxidant enzyme levels. Dashti reported that thioacetamide administration is easy and reliable for the induction of liver cirrhosis in experimental animal models and Muller reported that resulting disease resembles the human cirrhosis[25]. Thioacetamide exihibits its toxicity through its reactive metabolites, thioacetamide sulfoxide and thioacetamide-s-s-oxide[26].

Previous studies showed that thioacetamide induced liver cirrhosis can be prevented by the use of radical scavangers and antioxidants[27]. The property of Zinc to stop oxidative reactions has been known for years. Zinc exerts its antioxidant action through acute and chronic effects. Acute effects include stabilization of protein sulfhydryls or reduction in the formation of hydroxyl from hydrogen peroxide through the antagonism of redox active transition metals[28]. Chronic effects of Zinc results in induction of metallothioniens in many body organs such as liver, kidney and intestine. Metallothioniens make a connection between cellular zinc and redox state of the cell[29] and show antioxidant properties under different circumstances such as oxidative stress caused by reactive oxygen and nitrogen species[30], have ability to neutralize hydroxyl radicals[31], function as strong copper chelators[32], toxicity caused by the use of some anticancer drugs or others, toxicity induced by ethanol, exposure by radiations, and oxidatively mediated mutagenesis[33]. A long term zinc deprivation results in increased susceptibility to injury or disease due to a number of oxidative stresses[34]. Liver cirrhosis is found to have a poor zinc status. M.Ozaslan reported ability of zinc to regenerate damaged cells and its important antioxidant property[34]. In our study, data of increased liver weight, liver to body weight ratio, with respect to the treatments of thioacetamide and zinc sulphate administration (Table 1), the restorage of the level of total and direct bilirubin, ALT activity (Table 2). Furthermore, the reversal of levels of glutathione reductase and MDA and the catalase and superoxide dismutase activity (Table 3) indicate that zinc may play important role in treatment of liver cirrhosis.

REFERENCES

1. An WL, Pei JJ and Cowburn RF. Zinc-induced anti-apoptotic effects in SH-SY5Y neuroblastoma cells via the extracellular signal-regulated kinase ½. *Mol. Brain Res*., 2005; 135. 40-47.
2. Powell SR. The antioxidant property of zinc. *J. Nutr.,* 2000; 130: 1447S-1454S.
3. Christianson DW. Structural biology of zinc. *Adv. Protein Chem*., 1991; 42: 281-355.
4. Keith A. Mc Call, Chih-Chin and Carol A. Function and mechanism of zinc metalloenzymes. *J. Nutr.,* 2000; 130: 1437S-1446S
5. Mocchegiani E. Zinc and ageing: third zincage conference. Immunity and ageing 2007; pp 4:5.
6. SmithPJ and Errington R. Impact of overexpression of metallothionein-1 on cell cycle progression and zinc toxicity. *Am. J. Physiol.,* 2008; 38: 114-119.
7. Song Y and Emily HO. Zinc deficiency affects DNA damage, and DNA repair in rats. *The American Institute of Nutrition.* 2009.
8. Xiao TZ and Longley BJ. MAGEI Transcription factors regulate KAP1 and KRAB Domain zinc finger transcription factor mediated gene repression. *Plos ONE,* 2011; 6: e23747.
9. Mocchegiani E, Bertoni-Freddari C, Marcellini F and Malavolta M. Brain, aging and neurodegeneration: role of zinc ion availability. Prog. Neurobiol., 2005; 75:367-390.
10. Maret W and Krezel A. Cellular zinc and redox buffering capacity of metallothionein/ thionein in health and disease. *Mol. Med.,* 2007; 13: 371-375.
11. Goode HF, Purkins L, Heatley RV and Kelleher J. The effect of dietary vitamin E deficiency on plasma zinc and copper concentration. *Clin. Nutr.,* 1991; 10: 233-235.
12. Chvapil M, Janet C, Ludwig I, Sipes G and Ronald L. Inhibition of NADPH oxidation and related drug oxidation in liver microsomes by zinc. *Biochem. Pharmacol.,* 1976; 1787-1791.
13. Parsad AS. Zinc in growth and spectrum of human zinc deficiency. *J. Am. Coll. Nutr.,* 1988; 7: 377-384.
14. Oteiza P, Clegg MS, Paolazago M and Keen CL. Zinc deficiency induces oxidative stress and AP-1 activation in 3T3 cells. *Radical Biol. Med.,* 2000; 28: 1091-1099

15. Bogden JD. Influnce of zinc on immunity in the elderly. *J. Nutr. Health Ageing*, 2004; 8: 48-54.
16. Ohkawa H, Ohishi N and Yagi K. Assay for lipidperoxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.*, 1979; 95: 351-358.
17. Sinha KA. Colorimetric assay of catalase. *Anal Biochem.*, 1972; 47: 389-394.
18. Kono Y. Generation of superoxide radical during auto-oxidation of hydroxylamine and an assay for superoxide dismutase. *Arch. Biochem. Biophys.*, 1978; 186: 189-195.
19. Carlberg I and Mannervik B. Glutathione reductase. *Methods Enzymol.*, 1985; 113: 484-490.
20. Dashti H, Jeppsson B, Hagerstrand I, Hultberg B, Srinivas U and Abdulla M. Thioacetamide and carbontetrachloride-induced liver cirrhosis. *Eursurg. Res.*., 2001; 21: 83-91.
21. Muller A, Machnik F, Zimmermann T and Schubert H. Thioacetamide-induced cirrhosis-like liver lesions in rats. Usefulness and reliability of this animal model. *Exp. Pathol.*, 1988; 34: 229-236.
22. Stankova P, Ottokucera A, Lotkova H, Rousar T, Endlicher R and Cervinkova Z. The toxic effect of thioacetamide on rat liver in vitro. *Toxicol. In vitro*, 2010; 24: 2097-2103.
23. Bruck R, Shirin H, Aeed H, Matas Z, Hochman A, Pines M and Avni Y. Prevention of hepatic necrosis in rats by hydroxyl radical scavengers. J. Hepatol., 2001; 35: 457-464.
24. Powell SR. The antioxidant property of zinc. J. Nutr., 2000; 130: 1447S-1454S.
25. Maret W. The function of zinc metallothionein, a link between cellular zinc and redox state. *J. Nutr.*, 2000; 130: 1455S-1458S.
26. Ebadi M, Leuschen MP, Rafaey H, Hamada FM and Rojas P. The antioxidant properties of zinc and metallothionein. *Neurochem. Int*., 1996; 29:159-166.
27. Viarengo A, Burlando B, Ceratto N and Panfoli I. Antioxidant role of metallothioneins: a comparative overview. *Cell Mol. Biol*., 2000; 46: 407-417.
28. Kawai K, Liu S, Tyurin VA, Tyurina YY, Grigory G, Jiang JF, Claudette M, Fabisiak JP, Pitt BR and Kagan VE. Antioxidant and antiapoptotic function of metallothioneins in HL-60 cells challenged with copper Nitrilotriacetate. *Chem Res. Toxicol*., 2000; 13: 1275-1286.
29. Basu A and Lazo J. A hypothesis regarding the protective role of metallothioneins against the toxicity of DNA interactive anticancer drugs. *Toxicol. Lett*., 1999; 50: 123-136.
30. Zhou Z, Sun X, Lambert JC, Saari JT and Kang YJ. Metallothionein-independent zinc protection from alcoholic liver injury. *Am. J. Pathol.*, 2002; 160: 2267-2274.
31. Rossman TG and Goncharova EI. Spontaneous mutagenesis in mammalian cells is caused mainly by oxidative events and can be blocked by antioxidants and metallothionein. *J. Nutr*., 1998; 402: 103-110.
32. Rink L and Gabriel P. Zinc and the immune system. *Proc. Nutr. Soc.*, 2000; 59: 541-552.
33. Ozaslan M, Kilic IH and Ayetekin T. Investigation of antioxidant effect of zinc biochemically and histopathologically in rats. *Biochem. Biotechnol*., 2005; 19: 1-5.
34. Pathak A, Mahmood A, Pathak R and Dhawan D. Effect of zinc on hepatic lipid peroxidation and antioxidant enzymes in ethanol fed rats. J. Appl. Toxicol., 2002; 22: 207-210.