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Time dependent study to evaluate the efficacy of zinc on hepatic marker enzymes and elemental profile in serum and liver of protein deficient rats

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Abstract

This study was designed to determine the time dependent protective effects of zinc sulfate on the serum and liver marker enzymes along with elemental profile in protein deficient Sprauge Dawley (S.D.) female rats. Zinc sulfate in the dose of 227 mg/l in drinking water was administrated to normal control as well as protein deficient rats for a total duration of 8 weeks. The effects of different treatments were studied on enzymes like alkaline phosphatase (ALP), aspartate aminotransferases (AST) and alanine aminotransferases (ALT) in rat serum at different time intervals of 1, 2, 4 and 8 weeks and in the rat liver at the end of study. The status of different essential elements in liver was also studied. The serum ALP activity got significantly depressed when estimated at the intervals of 4 and 8 weeks. Activity of serum ALT was significantly increased after 4 weeks interval in protein deficient rats and the increasing trend continued upto 8 weeks of protein deficiency. On the other hand, activity of AST showed a significant increase just after 2 weeks and activity continued to be increased up to 8 weeks. Moreover activities of all the hepato marker enzymes showed a significant increase in liver of protein deficient rats. Interestingly, supplementation of Zn to protein deficient rats helped in regulating the altered activities of ALP, AST and ALT both in serum and liver. However, zinc treatment alone to normal rats did not indicate any significant change in the activities of all the enzymes in liver as well serum except at the interval of 2 weeks where a marginal increase in the activity of AST was seen. It has also been observed that concentrations of zinc, copper, iron and selenium were found to be decreased significantly in protein deficient animals. However, the levels of these elements came back to within normal limits when zinc was administrated to protein deficient rats.

Introduction

It has been well documented that protein energy malnutrition (PEM) or protein calorie malnutrition is capable of inducing many physiologic, functional and biochemical changes in the body (Kumari *et al.* 1993; Tandon *et al.* 1999). Low protein diet involves a reduction in the rate of protein synthesis in most body tissues (Wykes *et al.* 1996). The increase in serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in animals fed on a protein deficient diet, suggests hepatocellular dysfunctions (Tandon *et al.* 1998; Obatolu *et al.* 2003). The protein calorie malnutrition affects synthesis of the mRNA suggesting that a defect occurs at a pre-transcriptional level that results in reduced concentrations of mRNA (Adames *et al.* 1996).

Persons afflicted with protein malnutrition are mostly deficient in a variety of micronutrients. Protein deficiency led to reduction in the hepatic zinc, copper and manganese contents but increase in the hepatic iron content (Takeda *et al.* 1996; Squali *et al.* 2001). Alterations in the levels of trace elements result in number of diseases like hypoalbuminemia, and anemia in malnourished children

(Singla *et al.* 1996). Dietary zinc has been considered necessary for the proper development and growth of mammals including humans (Burch & Sallivan 1975). Combined protein deficiency along with zinc might stimulate salivary gland functions that in turn affect the liver zinc contents (Johnson *et al.* 1995). Protein deficiency leads to depletion of liver zinc contents (Gonzalez *et al.* 1998). Zinc in serum has been found to be in low concentrations in conditions of PEM and its depletion hampers the growth in children and results in hypogonadism and loss of taste (Sharda & Bhanderi 1977).

Zinc supplementation during nutritional rehabilitation of PEM hastens the recovery from protein deficiency and helps in gaining the body weights (Srivastava *et al.* 1993). Supplementation of zinc restores the serum thymulin activity and improves nutritional status of the elderly people i.e., food intake, serum albumin and transthyretin concentrations (Driscoll & Bettger 1992; Boukaiba 1993). Also, zinc increases the total globulin, total protein and albumin levels in the blood (Tekeli 2002). The amount of zinc bound to liver metallothionein (MT) is known to increase by fasting (Richards & Cousins 1976). There are also reports that increased MT synthesis under varying stressful conditions may be accompanied by a reduction of plasma zinc (Bremmer *et al.* 1975; Deagen *et al.* 1978).

In view of lack of information on the levels of various essential elements in liver and status of hepatic marker enzymes as a function of time, the present time dependent study was designed to evaluate the protective role of zinc in conditions of protein deficiency in rat liver and serum.

Materials and methods

Grouping of animals

Female rats in the weight range of 110–120 g of Sprague Dawley (S.D.) strain were procured from the Central Animal House, Panjab University, Chandigarh. The animals were housed in polypropylene cages in the animal house of the Department of Biophysics, under hygienic conditions and were acclimatized for at least 1 week before putting them on different treatments. Thereafter, the animals were randomly and equally divided into the four groups each having 10 animals.

The Registration number for the use of experimental animals obtained from the Ministry of Social Justice and Empowerment, Government. of India is 45/1999/CPCSEA dated 25.01.2000.

G-1, Normal control
Animals in this group served as normal controls and were fed with diet containing normal protein contents (18%). Composition of the diet used was as described by Kaur *et al.* 1992 and given in the following table.

G-2, Protein deficient, (PD)
Protein deficiency was induced in the animals of this group by maintaining them on the laboratory prepared protein deficient diet with 8% protein contents. Composition of the diet used was as described by Kaur *et al.* 1992 and given in the following table.

G-3, Zinc treated, (Zn)
Animals in this group were given zinc in the form of $ZnSO_4$ at a dose level of 227 mg/l in drinking water and the animals had free access to the drinking water containing zinc and the normal diet.

G-4, Zn + PD treated
Animals in this group were given protein deficient diets as given to G-2 animals and in addition were subjected to Zn treatment as mentioned for G-3 animals.

Composition of diets (weight %)

The composition of the diet is given in the following table (Kaur *et al.* 1992).

	Normal (18%) protein diet	Low (8%) protein diet
Casein (g)	18	8
Starch (g)	25	31.5
Sucrose (g)	25	31.5
Cellulose (g)	14	11
Corn oil (ml)	10	10
Vitamin mixture (g)	2	2
Salt mixture (g)	6	6

Blood samples were taken by puncturing the retro-orbital plexus of the animals by using sterilized glass capillaries at the intervals of 1, 2, 4 and 8 weeks after giving light ether anesthesia. The collected blood samples were kept at room temperature for 30 min and then were centrifuged at 2,000 rpm for 10–15 minutes to separate the serum. Serum was used for the estimation of the protein content, albumin/globulin ratio, liver marker enzymes viz., ALP, aspartate aminotransferase (AST) and ALT.

The treatments of rats continued for a period of 8 weeks. At the end of the treatment, the animals were weighted and were sacrificed by exsanguination under light anesthesia. Livers were removed immediately and were perfused and rinsed in normal saline (NaCl, 9 g/l/w/v). One lobe was preserved by freezing for the determination of various trace elements and the other was processed immediately for various biochemical studies.

Biochemical estimations

Protein
Protein assay was done by the method of Lowry *et al.* (1951).

Albumin
Albumin was estimated by a routine method using Biuret reagent (Layne 1957).

Preparation of Biuret reagent Nine grams of sodium potassium tartarate were dissolved in 500 ml of 0.2 N sodium hydroxide (NaOH). To this, 3 g of copper sulfate ($CuSO_4$) and 5 g of potassium iodide (KI) were added and the final volume was adjusted to 1000 ml with 0.2 N NaOH.

Estimation of albumin in serum 5.5 ml of 27% sodium sulfite solution was added to 0.5 ml of serum and 2 ml of ether was also added to it. The solution was mixed gently by inverting the tube and then vortexed. The contents were centrifuged at 3000 rpm for 10 min. The globulin precipitates, that were formed as a ring between aqueous and ether phases in the tube, were discarded. Three milliliters of supernatant were taken and 5 ml of biuret reagent was added to it. The contents were incubated at 37 °C for 10 min and optical density was measured at 540 nm.

Calculation of globulin in Serum Serum proteins comprise of albumin and globulins. Alpha, beta and gamma globulins in totality constitute globulins. Therefore, concentration of globulins can be expressed as below:

$$\text{Concentration of globulins} = \text{concentration of total proteins} - \text{concentration of albumin}$$

$$\text{A/G ratio} = \text{concentration of albumin/concentration of globulins}$$

Estimation of liver marker enzymes in liver

The enzyme activity of ALP was measured by the method of Wooton (1964) and the enzyme activities of aspartate aminotransferases (AST) and Alanine ALT were estimated according to the procedure of Reitman & Frankel (1957).

Elemental analysis of liver samples
Estimations of various elements in the liver samples of different treatment groups were carried using energy depressive X-ray fluorescence (EDXRF) technique. EDXRF technique is the most suitable analytical method to analyze trace elements because of its properties such as non-destructive, sensitivity up to μg/g and multi-elemental analysis.

Sample preparation for EDXRF The liver tissues of all the animals were oven dried at 70 °C to a constant weight and then ground with the help of Agate Pestle and Mortar. Three hundred milligram dried powder of the tissue so obtained was weighed and mixed with equivalent amount of Hoechst Wachs (wax) to make self-supporting pellets using a specially designed pure steel dye and a hydraulic press from Paul weber, Germany. A force of approximately 45 kN (kilo newtons) was applied at the dye top in order to make pellets of uniform thickness.

EDXRF Set-up In the present work, the pellets of tissues were analyzed using an EDXRF SPECTRO X-Lab, 2000 from Germany to determine the levels of various elements. The X-lab, 2000 spectrometer involved a 0.4 kW Pd anode X-ray tube as source of excitation. The power of the X-ray tube was adjusted on line for each individual measurement by the spectrophotometer software to secure optimum acquisition parameters for the current analysis.

Presently, different X-ray energies and excitation modes are being used but the most important mode used was of 40 kV and excitation used was polarized X-ray. A Si (Li) detector coupled with computer (Pentium, 600 MHz, software package SPECTRO X-LAB^{PRO} 2.2) was used to collect the flourescent X-ray spectra from the samples. The X-ray tube, secondary exciter, target and the Si (Li) detector were placed in a triaxial geometry mode. This geometry was used to minimize the background due to scattered photons. The X-Lab 2000 spectrometer was pre-calibrated with the standards from National Institute of Standards and Technology, NIST, USA and International Atomic Energy Agency, IAEA, Austria, by the manufacturer to estimate the concentration of an element in an unknown sample within an accuracy of 5%. The quality control tests are regularly performed in the laboratory by running the NIST standards.

Results

The results of all the experiments conducted during the current study are depicted in various tables. All the results of various treatment groups have been compared with their normal controls. Results of zinc + protein deficient (G-4) treated group have been compared with the results of the protein deficient group (G-2) also.

Statistsical analysis

The statistical significance of the values has been determined by using one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls test. The determinations are represented as mean ± SD.

Body weights

The variations in the body weights of the animals subjected to different treatments are shown in Table 1. It was observed that the body weights of normal control and zinc treated rats, increased progressively throughout the study. Protein deficiency resulted in a significant ($P < 0.001$) decrease in the body weights after 8 weeks when compared to normal control rats. Zinc treatment to the protein deficient rats tended to improve the body weight growth in comparison to protein deficient (G-2) rats but was still statistically different from normal control rats.

Serum total protein, albumin, globulin and albumin/globulin ratio

The serum total protein contents, albumin levels, globulin levels and A/G ratio are presented in Table 2. Protein deficient rats showed significant reduction ($P < 0.01$) in serum total protein and albumin. Administration of Zn to the animals of protein deficient groups tended to restore the altered serum albumin levels.

Hepatic protein contents

The Table 1 shows the hepatic protein contents in various treatment groups expressed as mg g^{-1} tissue. Protein deficient animals showed a highly significant ($P < 0.001$) reduction in the hepatic protein contents as compared to normal control group. However, zinc administration to the protein deficient rats helped in raising the hepatic

Table 1. Effect of zinc treatment on the body weights and hepatic protein in protein deficient rats

Groups	Weight (g)	Hepatic protein (mg g^{-1} tissue)
G-1 Normal control	199 ± 7	156 ± 5
G-2 Protein deficient	146 ± 29^{a3}	112 ± 5^{a3}
G-3 Zinc treated	180 ± 22	162 ± 7
G-4 Protein deficient + zinc	161 ± 30^{a1}	151 ± 9^{b3}

Values are mean ± SD.
By Newman–Keuls test:
$^{a1}P < 0.05$, $^{a2}P < 0.01$ and $^{a3}P < 0.001$ in comparison to G-1.
$^{b1}P < 0.05$, $^{b2}P < 0.01$ and $^{b3}P < 0.001$ comparison of G-4 with G-2.

Table 2. Effect of zinc on the serum protein, serum albumin, serum globulin and albumin: globulin ratio (A/G ratio) in protein deficient rats

Groups	Serum protein (g/l)	Serum albumin (g/l)	Serum globulin (g/l)	Albumin/globulin (A/G) ratio
G-1 Normal control	74 ± 3	54 ± 5	20 ± 6	2.6 ± 0.7
G-2 Protein deficient	60 ± 5[a3]	39 ± 4[a3]	22 ± 4	2.0 ± 0.5
G-3 Zinc treated	77 ± 3	53 ± 6	24 ± 7	2.5 ± 1.2
G-4 Protein deficient + zinc	64 ± 6[a3]	41 ± 3[a3]	20 ± 5	2.2 ± 0.7

Values are mean ± SD.
By Newman–Keuls test:
[a1]$P < 0.05$, [a2]$P < 0.01$ and [a3]$P < 0.001$ in comparison to G-1.
[b1]$P < 0.05$, [b2]$P < 0.01$ and [b3]$P < 0.001$ comparison of G-4 with G-2.

protein contents as compared to their respective controls.

Alkaline phosphatase (ALP)
The serum activities of ALP are shown in Table 3. The serum alkaline phosphatase activity got significantly depressed ($P < 0.001$) when estimated at 4 and 8 weeks intervals following protein deficiency as compared to normal control rats. Whereas, administration of Zn as an additional treatment to protein deficient rats helped in raising ($P < 0.01$) the decreased activity due to protein deficiency.

The hepatic activities of alkaline phosphatase are shown in Table 6. Hepatic alkaline phosphatase activity showed a significant elevation ($P < 0.001$) at 8 weeks due to protein deficiency. Interestingly, zinc administration to protein deficient rats could brought the raised levels to within normal range.

Aspartate aminotransferase (AST)
Activities of serum AST as a function of time are shown in Table 4. No significant change in serum AST was seen up to 1 week in rats subjected to different treatments. However, activity of AST showed a significant increase at an interval of 2 weeks in protein deficient rats and the increasing trend continued up to 8 weeks. However, simultaneous zinc treatment to protein deficient animals could not restore completely the altered levels of AST.

Activities of hepatic AST are shown in Table 6, which indicate a statistically significant increase ($P < 0.001$) in protein deficient animals. However, zinc was able to restore the elevated levels in such conditions to within normal range.

Alanine aminotransferase (ALT)
Activity of serum ALT in the protein deficient rats was found to be raised significantly ($P < 0.001$) after 4 and 8 weeks of treatment, as compared to normal control rats as shown in Table 5. Interestingly, zinc administration to protein deficient animals resulted in restoring the raised levels of serum ALT near to normal levels.

Table 6 depicts the hepatic observations of ALT where significant ($P < 0.001$) elevation of ALT

Table 3. Effect of zinc on the serum alkaline phosphatase (ALP) activity as a function of time in protein deficient rats

μmol phenol produced min^{-1} l^{-1} serum Group	1st week	2nd week	4th week	8th week
G-1 Normal control	80.57 ± 0.79	76.28 ± 0.35	77.1 ± 2.2	78.5 ± 1.2
G-2 Protein deficient	78.00 ± 2.28	75.91 ± 0.55	74.3 ± 1.4[a2]	74.1 ± 1.0[a3]
G-3 Zinc treated	78.29 ± 0.88	76.00 ± 0.51	76.5 ± 2.6	77.9 ± 1.3
G-4 Protein deficient + zinc	77.54 ± 1.13[a1]	75.62 ± 0.55	77.0 ± 1.5[b2]	77.3 ± 0.9[b3]

Values are mean ± SD.
By Newman–Keuls test:
[a1]$P < 0.05$, [a2]$P < 0.01$ and [a3]$P < 0.001$ in comparison to G-1.
[b1]$P < 0.05$, [b2]$P < 0.01$ and [b3]$P < 0.001$ comparison of G-4 with G-2.

Table 4. Effect of zinc on the serum aspartate aminotransferase (AST) activity as a function of time in protein deficient rats

μ moles of pyruvate formed min^{-1} l^{-1} serum Group	1st week	2nd week	4th week	8th week
G-1 Normal control	46.8 ± 2.3	44.3 ± 1.4	49.3 ± 1.3	53.7 ± 0.9
G-2 Protein deficient	45.5 ± 1.2	45.9 ± 1.5^{a1}	54.0 ± 1.5^{a3}	59.4 ± 1.9^{a3}
G-3 Zinc treated	45.3 ± 1.4	45.8 ± 0.7^{a1}	50.8 ± 0.6	53.7 ± 1.9
G-4 Protein deficient + zinc	46.1 ± 1.0	46.8 ± 1.1^{a2}	51.2 ± $1.9^{a1, b2}$	57.2 ± 3.0^{a1}

Values are mean ± SD.
By Newman–Keuls test:
$^{a1}P < 0.05$, $^{a2}P < 0.01$ and $^{a3}P < 0.001$ in comparison to G-1.
$^{b1}P < 0.05$, $^{b2}P < 0.01$ and $^{b3}P < 0.001$ comparison of G-4 and G-2.

Table 5. Effect of zinc on the serum alanine aminotransferase (ALT) activity as a function of time in protein deficient rats

μ moles of pyruvate formed min^{-1} l^{-1} serum Group	1st week	2nd week	4th week	8th week
G-1 Normal control	37.8 ± 4.0	40.7 ± 3.9	39.1 ± 2.0	39.4 ± 1.4
G-2 Protein deficient	39.7 ± 3.2	41.8 ± 2.9	46.8 ± 2.9^{a3}	45.1 ± 3.2^{a2}
G-3 Zinc treated	35.5 ± 3.5	40.2 ± 2.4	40.8 ± 4.5	38.7 ± 2.5
G-4 Protein deficient + zinc	37.3 ± 1.9	40.2 ± 2.9	43.8 ± 3.0^{a1}	43.5 ± 3.1^{a1}

Values are mean ± SD.
By Newman–Keuls test:
$^{a1}P < 0.05$, $^{a2}P < 0.01$ and $^{a3}P < 0.001$ in comparison to G-1.
$^{b1}P < 0.05$, $^{b2}P < 0.01$ and $^{b3}P < 0.001$ comparison of G-4 with G-2.

Table 6. Effect of zinc on the hepatic liver marker enzymes in protein deficient rats

Groups	Alkaline phosphatase activity (nmoles phenol produced min^{-1} mg^{-1} protein)	Aspartate aminotransferase (μ moles of pyruvate formed min^{-1} g^{-1} tissue)	Alanine aminotransferase (μ moles of pyruvate formed min^{-1} g^{-1} tissue)
G-1 Normal control	1.03 ± 0.06	2.59 ± 0.17	3.02 ± 0.05
G-2 Protein deficient	1.67 ± 0.09^{a3}	3.50 ± 0.09^{a3}	4.75 ± 1.07^{a3}
G-3 Zinc treated	1.00 ± 0.05	2.56 ± 0.07	3.05 ± 0.06
G-4 Protein deficient + zinc	1.12 ± 0.05^{b3}	2.51 ± 0.05^{b3}	3.18 ± 0.21^{b3}

Values are mean ± SD.
By Newman–Keuls test:
$^{a1}P < 0.05$, $^{a2}P < 0.01$ and $^{a3}P < 0.001$ in comparison to G-1.
$^{b1}P < 0.05$, $^{b2}P < 0.01$ and $^{b3}P < 0.001$ comparison of G-4 with G-2.

levels has been observed in protein deficient animals. However, zinc treatment to normal rats brought the altered levels of ALT to within normal range.

Hepatic concentration of various elements

Table 7 shows the concentrations of different elements in liver tissue of various treated animals. The zinc levels were found to be decreased signif-

Table 7. Effect of zinc on the levels of different hepatic elements in protein deficient rats

Group/elements	Zinc (μg/g)	Copper (μg/g)	Iron (μg/g)	Selenium (μg/g)
G-1 Normal control	58 ± 6	13 ± 2	194 ± 29	3.81 ± 0.34
G-2 Protein deficient	40 ± 5[a3]	7 ± 1[a3]	149 ± 39	1.05 ± 0.11[a3]
G-3 Zinc treated	58 ± 8	11 ± 2	208 ± 51	2.42 ± 0.39
G-4 Protein deficient + zinc	51 ± 5[b2]	12 ± 3[b3]	179 ± 61	2.12 ± 0.09[a3, b3]

Values are mean ± SD.
By Newman–Keuls test:
[a1]$P < 0.05$, [a2]$P < 0.01$ and [a3]$P < 0.001$ in comparison to G-1.
[b1]$P < 0.05$, [b2]$P < 0.01$ and [b3]$P < 0.001$ comparison of G-4 with G-2.

icantly in protein deficient animals. However, zinc concentrations were brought back to within normal levels in these groups when zinc was administrated along with other treatments. Tissue copper concentrations were reduced significantly ($P < 0.001$) in protein deficient animals. Administration of zinc to protein deficient animals restored the lowered values of copper near to normal levels. Tissue iron concentrations were decreased in protein deficient animals. However, zinc brought back the levels of iron to near normal concentrations in these animals. The levels of selenium were found to be decreased significantly in protein deficient animals when compared to normal animals. However, administration of zinc to these groups raised the selenium levels significantly as compared to respected controls but these are still lower than the concentration in normal control animals.

Discussion

We observed that the body weights of normal control and zinc treated rats increased progressively throughout the study. Protein deficiency resulted in a significant ($P < 0.001$) decline in the body weights after 8 weeks when compared to normal control rats. In an earlier study from our laboratory, it was also seen that protein deficiency leads to significant growth retardation in animals (Tandon *et al.* 1999). Loss in body weight is characteristic of protein malnutrition. It has been reported that retardation in body weight growth over a period is not due to low intake of diet but due to deficiency in protein intake (Eisenstein & Harper 1991). Zinc treatment to protein deficient rats tended to improve the body weight growth. The protective effects of zinc could be attributed to its ability to reduce collagen accumulation in liver and also its physiological role in regulating the structure and function of cell (Dhawan & Goel 1994a, b).

Protein deficient rats showed a significant reduction ($P < 0.01$) in serum albumin contents as well as total protein contents both in liver and serum. The reduction in serum albumin contents can be due to depletion in amino acid precursors of albumin synthesis, which in turn results in reduction of protein contents (Davenport *et al.* 1994). Administration of Zn to the animals of protein deficient group tended to restore the serum albumin levels, but still the levels were significantly different from normal controls. This property of Zn could be attributed to its role in the induction of MT (Zn-binding protein) thereby regulating the amino acid precursors for albumin synthesis (Dhawan *et al.* 1992; Yang *et al.* 1995; Tekeli 2002).

The serum alkaline phosphatase activity was significantly depressed following protein deficiency as compared to normal control rats. This could be attributed to the slow rate of protein synthesis and amino acid mobilization or it could be due to the increased hydrolysis of serum ALP that might be needed to combat the protein deficiency state (Kumari *et al.* 1993). Hepatic alkaline phosphatase activity was increased significantly in condition of protein deficiency in the present study. Probably, the activated macrophages including the Kupffer cells are the cellular source for the increased levels of ALP in conditions of liver damage caused by protein deficiency (Hultberg & Isakasson 1983). Davenport *et al.* (1994) also postulated that many hepatic and extrahepatic conditions could result due to protein-restricted diets that in a way caused increased production of alkaline phosphatase isoenzymes from bone and

hepatobiliary source. Role of Zn in regulating the altered alkaline phosphatase level could be attributed to its property as an important co-factor for the expression of enzyme activity and thereby helping in smooth transportation of phosphates across cell membranes (Conard & Barton 1978). The antioxidative potential of the zinc and improved histoarchitecture of the damaged hepatocytes on zinc supplementation as observed by us earlier might also have lead to the regulation of ALP activity (Dhawan *et al.* 1992; Dhawan & Goel 1994; Ajay Goel & K. Dhawan 2001).

The observed increased activities of serum and hepatic AST and ALT in the animals given LP diet are in conformity with the study of Pond *et al.* (1992). They reported that the increase in ALT activity and total bilirubin concentration in pigs fed a protein deficient diet suggested altered liver function, although microscopic anatomy revealed no evidence of excessive fat accumulation or of pathologic changes. Similar observations were noticed by Kumari *et al.* (1993) who estimated ALT and AST activities in the serum samples of 30 cases of pediatric PEM. Their observations also noticed a significant increase in marker enzymes of liver in these cases. These authors explained that during hepatobiliary disorder, amino acids are released form exaggerated tissue breakdown. In order to metabolize these amino acids, the process of transmutation gets enhanced leading to increased activity of the related enzymes AST and ALT. On the contrary, Davenport *et al.* (1994) did not observe any alteration in the activities of serum AST and ALT in protein deficient rats. The rise in the activity of both the transaminases with a concomitant increase in serum bilirubin is suggestive of the acute liver injury (Tokha-el-Sherif 1970).

AST is also present in high concentrations in the heart, skeletal muscles and kidney apart from the liver, therefore elevated levels of serum AST even just after 2 weeks of protein deficiency might reflect some kind of non-specific or extrahepatic dysfunction also. However, ALT is a liver specific enzyme and is present in the liver in abundant quantities, therefore it is more specific marker of hepatocellular function. The normalization of AST and ALT activities following Zn supplementation to protein deficient rats are in agreement with the available reports (Dhawan *et al.* 1992; Dhawan & Goel 1994a, b). These reports emphasized the hepatoprotective efficacy of zinc under CCI_4 induced liver injury, as zinc treatment helped in the maintaining the homeostasis through regulation of protein synthesis.

It is now well understood that liver has an important function in the regulation of trace element metabolism (Parsad & Oberleas 1976, Dhawan & Goel 1996). Further, trace elements serve as cofactors for many enzymes in numerous metabolic pathways; therefore, changes in the distribution of these essential elements in the body can have both nutritional and toxicological consequences with regard to the metabolism of other metals (Dhawan & Goel 1994a, b). Metals that have similar chemical and physical properties would often interact biologically and antagonize or embellish each other's function (Singh *et al.* 1994). Nutritional stress in the form of protein deficiency is quite prevalent in the developing countries and it has been shown that nutritional status of an individual may have a profound influence on the absorption of metals (Wallwork *et al.* 1983). In view of all these facts, we have made an effort to investigate the effects of various treatments on the trace elemental composition of different essential elements and we further aimed at to delineate any possible correlation amongst them.

In the present study, observations of depressed Zn levels in the liver of protein deficient rats are in conformity with previous studies (Martel *et al.* 1992; Tandon *et al.* 1999). Lowered Zn concentrations could be explained on the basis that either it is excessively being utilized in providing antioxidant defense mechanism or there is some defect in the absorption/metabolism of zinc in toxic conditions created by protein deficiency. Studies carried out in animals and humans had shown that Zn is essential for utilization of aminoacids (Sandstead *et al.* 1976). Conversely, protein malnutrition plays a major role on liver zinc depletion (Martel *et al.* 1992). Thus, protein and zinc nutritions are interdependent.

Protein restricted diet led to marked reduction in copper contents in the present study which is in agreement with earlier reports (Martel *et al.* 1992; Tandon *et al.* 1999). Copper depletion is associated with depressed hepatic Cu–Zn superoxide dismutase (SOD) activity as protein deficiency results in reduction in SOD activity (Huang & Fwu 1993). Zinc supplementation to protein deficient animals resulted in normalized zinc and

copper concentrations in liver, which may be due to increase in production of Zn-MT.

In the present study, the hepatic Fe contents were found to be significantly depressed in protein deficient rats as compared to normal control group. Reduction in Fe contents following protein deficiency could be explained on the basis that protein plays an important role in the absorption of iron from the gastrointestinal tract (Klavins *et al*. 1962; Tandon *et al*. 1999). However, zinc treatment to protein deficient animals, has lead to considerable regulation of Fe levels but they still were different from normal animals. This could be due to the property of zinc to prevent the iron induced lipid oxidation (Zago & Oteiza 2001).

Selenium, which is an essential trace metal and inactivates sulfhydryl groups in certain enzymes and is also a component of glutathione peroxidase (Rukgauer *et al*. 2001). This ubiquitous enzyme located in both cytosol and mitochondrial matrix uses glutathione to reduce organic hydroperoxides, thereby, prevents oxidative damage to various cell organelles. In the present study, selenium was decreased significantly in protein deficient animals. However, zinc administration to these groups raised the selenium levels significantly as compared to respective controls but these are still lower than the concentration in normal control animals. This observation is in consonance with earlier studies where it was seen that essential micronutrients selenium and zinc interact with one another to regulate the cellular homeostatic zinc system (Eisenstein & Harper 1991; Jacob *et al*. 1998).

Conclusion

It may be concluded from the present study that the activities of liver specific enzymes in serum get altered when rats are subjected to protein deficiency for a minimum period of 4 weeks and zinc has the potential in regulating the activities of hepatic marker enzymes as well as essential hepatic elements.

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