Abstract
This study was designed to determine the toxic effects of nickel sulfate on the biochemical and elemental profile of liver in protein deficient rats. Nickel sulfate in the dose of 800 mg/l in drinking water was administered to Sprague Dawley (S.D) normal control as well as protein deficient rats for a total duration of eight weeks. The effects of nickel treatment and protein deficiency when given separately and in combination were studied on rat liver marker enzymes like Alkaline phosphatase (ALP), Glutamate oxaloacetate transaminase (GOT), Glutamate pyruvate transaminase (GPT) and also on the status of essential elements in rat liver. Protein deficient, Ni treated as well as combined protein deficient and nickel treated rats showed significant reductions in the body weight and hepatic protein contents as compared to normal control rats. Hepatic alkaline phosphatase activity and alanine aminotransferase showed a significant elevation in rats subjected to protein deficiency, nickel treatment and combined protein deficiency and nickel treatment. As regards to hepatic levels of aspartate aminotransferase a significant elevation was observed in protein deficient and nickel treated protein deficient animals. Nickel administration to normal and protein deficient rats has resulted in a significant increase in concentrations of nickel, phosphorus and sulfur in liver tissue. The concentration of zinc and copper in liver tissue decreased significantly in protein deficient, nickel treated and nickel treated protein deficient animals. Tissue iron concentrations were found to be decreased in protein deficient animals, but the concentrations of iron got elevated significantly in nickel treated and nickel treated protein deficient animals. It has been observed that nickel was not able to augment the hepatotoxicity in protein deficient rats.

P. Sidhu, M. L. Garg, M. Morgenstern, J. Vogt, T. Butz, D. K. Dhawan*  
Institute of Physiology and Experimental Pathophysiology, Friedrich-Alexander University, Erlangen-91054, Germany. 
Department of Biophysics, Panjab University, Chandigarh-160014, India. 
Umweltforschungszentrum Leipzig-Halle, Leipzig, Germany. 
Fakultät für Physik und Geowissenschaften, Universität Leipzig, Leipzig, Germany. 
Department of Biophysics Panjab University Chandigarh, India.

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Correspondence: 
P. Sidhu  
E-mail: pardeepsidhu@yahoo.co.uk

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Introduction

Nickel has been shown to interact with number of trace elements. It catalyzes the excretion of copper, zinc and manganese. Nickel mobilizes and affects the absorption and uptake of 65Zn.

Protein malnutrition has very low levels of the thymulin hormone, which is a sensitive indicator of zinc status in the body. It is known that dietary protein variation affects the synthesis of the mRNA suggesting that a PEM defect occurs at a pre-transcriptional level that results in the hepatotoxicity in protein deficient rats.

In the developing countries like India, protein deficiency is a prominent cause of energy malnutrition (PEM), which is a matter of concern to elucidate their role in manifesting many biochemical disorders of different organs. The present investigations were designed to study the hepatotoxic effects of nickel in rats subjected to protein deficiency.

Materials and methods

Persons afflicted with protein malnutrition are more prone to nickel toxicity. The protein calorie malnutrition (PEM), is a prominent cause of many biochemical disturbances like hypoalbuminemia, and anemia in malnourished children.

The increases in serum alanine aminotransferase and alkaline phosphatase in animals when compared to normal animals. The elevation of selenium in nickel treated animals when compared to normal animals.

Results

Liver, being a major metabolic organ, plays an important role in the regulation of biochemical and trace element metabolism. The present investigations were intended to study the hepatotoxic effects of nickel in rats subjected to protein deficiency.

Nickel affects the iron metabolism and intensifies the erythropoiesis with induced hemolytic anemia. It is known that dietary protein variation affects the synthesis of the mRNA suggesting that a PEM defect occurs at a pre-transcriptional level that results in the hepatotoxicity in protein deficient rats.

Ineffectiveness of nickel in augmenting function of body metabolism, which is linked to nickel toxicity. Malnourished people living in the vicinity of nickel industrial and smelting units are more prone to nickel toxicity. Trace elements including iron, zinc, copper, manganese, magnesium in experimental animals were mobilized and affect the liver marker enzymes (ALT, AST, ALP). Alterations in the levels of zinc, manganese, copper, calcium and magnesium in tissue needs further research. Since, nutrients like hypoalbuminemia, and anemia in malnourished children suffer from severe protein energy malnutrition (PEM) has been shown to decrease the hepatic levels of zinc, manganese, copper, calcium and magnesium in experimental animals.

Protein deficiency was induced in the animals of G-2, Protein deficient, (PD) and Nickel treated groups of animals. Thereafter, the animals were randomly and equally divided into the four groups each having ten animals. One group was kept untreated as normal controls, one group was treated with nickel and one group was nickel treated and nickel treated protein deficient animals were fed with diet containing normal protein contents and the other was fed with diet containing normal protein contents.

Animals in this group served as normal controls and were fed with diet containing normal protein contents. nickel (Ni) industrial and smelting units are more prone to nickel toxicity. The protein calorie malnutrition (PEM), is a prominent cause of energy malnutrition (PEM), which is a matter of concern to elucidate their role in manifesting many biochemical disorders of different organs. The present investigations were intended to study the hepatotoxic effects of nickel in rats subjected to protein deficiency.

Key words: Nickel. Protein deficiency. Liver marker enzymes. Trace elements.
pared protein deficient diet with 8% protein contents. Composition of the diet used was as described by Kaur et al., 1992 (16) and given in table below.

G-3, Nickel treated, (Ni)
Animals in this group were given nickel in the form of NiSO_4\cdot6H_2O at a dose level of 800 mg/L in drinking water and the animals had free access to the drinking water containing nickel and the normal diet.

G-4, Ni+PD treated
Animals in this group were given protein deficient diet as given to G-2 animals and in addition were subjected to Ni 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).

<table>
<thead>
<tr>
<th>Diet</th>
<th>Normal (18%)</th>
<th>Low (8%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (g)</td>
<td>18</td>
<td>8</td>
</tr>
<tr>
<td>Starch (g)</td>
<td>25</td>
<td>31.5</td>
</tr>
<tr>
<td>Sucrose (g)</td>
<td>25</td>
<td>31.5</td>
</tr>
<tr>
<td>Cellulose (g)</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Corn oil (ml)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Vitamin mixture (g)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Salt mixture (g)</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

The treatments of rats continued for a period of eight 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 (17).

Estimation of liver marker enzymes in liver
The enzyme activity of Alkaline Phosphatase (ALP) was measured by the method of Wooton (18) and the enzyme activities of aspartate aminotransferases (AST) and Alanine Aminotransferase (ALT) were estimated according to the procedure of Reitman and Frankel (19).

Elemental analysis of liver samples
Estimations of various elements in the liver samples of different treatment groups were carried out using Energy Depressive X-ray Fluorescence (EDXRF) technique. This is one of the most suitable analytical methods for analysis of trace elements because of its properties such as non-destructive, sensitivity up to ppm 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. 300 mg of the tissue powder was weighed and mixed with equivalent amount of Hoechst Wachs (wax) to make self-supporting pellets. The pellets were made by applying a force of approximately 45 KN (Kilo newtons) at the dye top in order to make pellets of uniform thickness.

– EDXRF Setup
In the present work, the pellets of tissues were analyzed using an EDXRF X-Lab, 2000 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. Currently, 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-LABPRO 2.2) was used to collect the fluorescent 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.

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 nickel + protein deficient (G-4) treated group have been compared with the results of the protein deficient group (G-2) also.

Statistical Analysis
The statistical significance of the values has been determined by using one way analysis of variance (20).
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 I. It was observed that the protein deficiency resulted in a significant (p < 0.001) decrease in the body weights after eight weeks, when compared to normal control rats. Nickel treatment to normal control rats resulted in some decrease (p < 0.05) in the body weights but nickel treatment to protein deficient rats resulted in appreciable reduction (p < 0.001) in the body weights as compared to normal control rats.

Hepatic protein Contents
The hepatic protein contents in various treatment groups expressed as mg g⁻¹ tissue are shown in table 1. Protein deficient, Ni treated as well as combined protein deficient and nickel treated rats showed significant (P < 0.001) reductions in the hepatic protein contents as compared to normal control rats.

Alkaline phosphatase
Hepatic alkaline phosphatase activity showed a significant elevation (p < 0.01) in rats subjected to protein deficiency, nickel treatment and combined protein deficiency and nickel treatment as shown in table II.

Aspartate Aminotransferase
As regards to hepatic levels of AST, a significant (p < 0.001) elevation was observed in protein deficient and nickel treated protein deficient animals (table II).

Alanine Aminotransferase
Table II depicts the hepatic observations of ALT where significant (p < 0.001) elevation in ALT levels has been observed in protein deficient, nickel and nickel treated protein deficient animals.

Table I

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight (Grams)</th>
<th>Hepatic Protein (mg g⁻¹ tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-1 Normal Control</td>
<td>199 ± 7</td>
<td>156.31 ± 5.38</td>
</tr>
<tr>
<td>G-2 Protein Deficient</td>
<td>146 ± 29 a3</td>
<td>112.37 ± 5.02 a3</td>
</tr>
<tr>
<td>G-3 Nickel Treated</td>
<td>167 ± 20 a1</td>
<td>140.96 ± 2.03 a3</td>
</tr>
<tr>
<td>G-4 Protein deficient + Nickel</td>
<td>141 ± 30 a3</td>
<td>120.00 ± 9.48 a3, b1</td>
</tr>
</tbody>
</table>

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 II

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hepatic Alkaline Phosphatase activity (nmoles phenol produced min⁻¹)</th>
<th>Hepatic Aspartate aminotransferase (µ moles of pyruvate formed min⁻¹)</th>
<th>Hepatic Alanine aminotransferase (µ moles of pyruvate formed min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-1 Normal Control</td>
<td>1.03 ± 0.06</td>
<td>2.59 ± 0.17</td>
<td>3.02 ± 0.05</td>
</tr>
<tr>
<td>G-2 Protein Deficient</td>
<td>1.67 ± 0.09 a3</td>
<td>3.50 ± 0.09 a3</td>
<td>4.75 ± 1.07 a3</td>
</tr>
<tr>
<td>G-3 Nickel Treated</td>
<td>1.36 ± 0.07 a3</td>
<td>2.50 ± 0.04</td>
<td>5.79 ± 0.77 a3</td>
</tr>
<tr>
<td>G-4 Protein deficient + Nickel</td>
<td>1.55 ± 0.11 a3, b2</td>
<td>3.20 ± 0.15 a3, b3</td>
<td>5.77 ± 0.48 a3, b3</td>
</tr>
</tbody>
</table>

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.

Hepatic concentration of various elements
The concentrations of various elements have been depicted in table III. Nickel administration to normal and protein deficient rats has resulted in a significant (p < 0.001) increase in concentrations of nickel in liver tissue.

The concentration of zinc and copper in liver tissue got decreased significantly (p < 0.001) in protein deficient, nickel treated and nickel treated protein deficient animals.

Tissue iron concentrations were found to be decreased in protein deficient animals, but the concentrations of iron got elevated significantly (p < 0.001) in nickel treated and nickel treated protein deficient animals.

It has been observed that selenium got decreased significantly (p < 0.001) in protein deficient, nickel treated and nickel treated protein deficient animals when compared to normal animals. The elevation of selenium in nickel treated protein deficient animals was also significantly (p < 0.05) higher when compared to protein deficient animals.
Significant (p < 0.001) decrease has been observed in potassium concentration in nickel treated and nickel treated protein deficient animals. On the other hand phosphorus and sulfur concentrations were found to be increased significantly in nickel treated and nickel treated protein deficient animals.

Discussion

We observed a significant (p < 0.001) decline in the body weights of rats subjected to protein deficiency for a period of eight weeks, when compared to normal control rats. Loss in body weight is characteristic of protein malnutrition. In an earlier report from our laboratory it has been observed that protein deficiency leads to significant growth retardation in animals2. Many other workers have also reported the decrease in body weight due to protein deficiency20,21. It has been seen in these reports that retardation in body weight growth over a period is not due to low intake of diet but deficiency in protein intake.

Nickel treatment to normal control and protein deficient rats resulted in marked reduction in the body weights as compared to normal control rats. The reduction in body weights following nickel treatment has also been reported earlier22,23. The decrease in body weight may not solely be attributed to protein deficiency alone as the Ni treatment alone also has caused significant decline in body weight. The decrease in body weight due to nickel treatment has been connected by researchers to be not due to low intake of diet consumption of the rats following toxic treatment with nickel, vis a vis normal rats, and thus it is anticipated that this effect could possibly be due to the overall increased degeneration of lipids and proteins as a result of nickel toxicity23,24.

Rats in protein deficient, Ni treated and combined PD+Ni treated groups, showed a highly significant (P < 0.001) reduction in the hepatic protein contents as compared to rats of normal control group. Davenport y cols., 199425 demonstrated that in protein deficient states, the reduction in protein contents are due to depletion in amino acid precursors. Nickel in earlier reports, has also been able to cause significant depression in protein levels26,27. Nickel diminishes the DNA and RNA polymerase activity and decreases DNA replication fidelity28 which in turn can reduce the protein synthesis.

Hepatic alkaline phosphatase activity followed a significant elevation due to protein deficiency and nickel treatment. This elevation could be anticipated to the reason that ALP is bound to the intracellular membranes, and does not leak out with the increased permeability of the cell membranes. Moreover, Hultberg and Disaksson, 198329 proposed that activated macrophages including the Kupffer cells are the cellular source for the increased levels of ALP in conditions of liver damage. Davenport y cols., 199425 also postulated that many hepatic and extrahepatic conditions could also result due to protein-restricted diets that in a way caused increased production of alkaline phosphatase isoenzymes from bone and hepatobiliary source.

The aminotransferases are intracellular enzymes, which are active in operating the reversible exchange of aminoacids between alpha–amino and alpha-keto acids. As all the naturally occurring amino acids can undergo amino transfer reactions thus this class of intracellular enzymes act as catalysts in the transfer of amino groups over a variety of acceptor amino acids. The transaminases are classified into two families, the first family of transaminases, which include the alanine and aspartate transaminases, are involved in the synthesis of the amino group donor, whereas the second family of transaminases, which includes the glutamate transaminase, are involved in the utilization of the amino group donor. The transaminases are also involved in the metabolism of branched chain amino acids and in the urea cycle. The transaminases are also involved in the metabolism of branched chain amino acids and in the urea cycle. The transaminases are also involved in the metabolism of branched chain amino acids and in the urea cycle. The transaminases are also involved in the metabolism of branched chain amino acids and in the urea cycle. The transaminases are also involved in the metabolism of branched chain amino acids and in the urea cycle.
Ineffectiveness of nickel in augmenting hepatic levels of AST and ALT got significantly (p < 0.001) elevated in protein deficient and nickel treated protein deficient animals. The observed increased activities of hepatic AST and ALT in the animals given nickel treatment, which has also been reported by Klavins et al., 1999. They concluded that protein plays an important role, not only in oxygen delivery, but also in the degradation of xenobiotics.

The present study has been carried out in an attempt to understand the role of nickel in hepatic functions during protein deficiency. A significant inhibition is observed in copper concentration following nickel treatment. Protein restricted diet led to marked reduction in copper contents following protein deficiency has previously been reported by Pond et al., 1992. They reported that the copper contents in the present study which is in agreement with earlier reports. Copper depletion is associated with depressed hepatic Cu-Zn superoxide dismutase (SOD) activity and it is well known that Cu-Zn SOD is one of the most important antioxidant enzymes.

The administration of nickel to normal and protein deficient rats caused an increase in hepatic iron contents in the animals, which is in conformity with the earlier reports. It is now well authorized that the liver has an important link between protein and carbohydrate metabolism and that proteins play a vital role in the regulation of trace element metabolism. It has been so severe that more and more of this metal has been produced in the world and as a result, the environment has become contaminate with it. This has been so severe that more and more of this metal has been produced in the world and as a result, the environment has become contaminate with it.
inactivates sulfhydryl groups in certain enzymes and in the form of enhanced ALP, AST and ALT levels.

and transmission of electrochemical impulses. It has potassium. Its deficiency affects the activity of muscles inhibition of ATPase leading to decreased levels of po-

tain normal osmotic pressure and water balance hence balance with the extracellular ionized sodium to main-

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another dose of nickel in the present study.

which seems to have been marginalized due to high

exceed the iron binding capacity of transferrin a

affected in stress conditions. When serum iron levels

neration pathways, which could have been adversely

ion is transported to the liver, as it serves as a cofactor

S-adenosylmethionine (SAM) and glutathione

Selenium, which is an essential trace metal and

Kolkata and ICMR, New Delhi.

Acknowledgements

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the hepatotoxicity in protein deficient rats

Ineffectiveness of nickel in augmenting


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