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 administrated to Sprauge 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

Original
Ineffectiveness of Nickel in augmenting the hepatotoxicity in protein deficient rats
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Introduction
Nickel has been shown to interact with number of elements. Nickel is known to be associated with many biochemical disturbances including hypoalbuminemia, and anemia in malnourished persons afflicted with protein malnutrition are also significantly higher when compared to protein deficient rats. PEM muscle weakness and functional disorders of different organs are essential for all the fundamental cellular processes including catalysis. Nickel breaks down the immunity by affecting the T-cell system and suppresses the activity of natural killer cells in rats and mice. Nickel mobilizes and promotes the excretion of copper, zinc and manganese. Nickel is a common respirable sized particulate pollutant and nickel compounds of commercial importance to elucidate their role in manifestation of functional disorders of different organs, therefore, it becomes a matter of concern to elucidate their role in manifestation of functional disorders of different organs. Nickel (Ni) industrial and smelting units are more likely to be exaggerated in conditions of heavy metal deficiency has been shown to decrease the hepatic levels of zinc, manganese, copper, calcium and magnesium in experimental animals.

Materials and methods

Animals in this group served as normal controls and were fed with diet containing normal protein contents. The animals were divided into the four groups each having ten animals. Thereafter, the animals were randomly and equally distributed into the four groups. Grouping of animals was done one week before putting them on different treatments.

Protein deficiency was induced in the animals of this group by maintaining them on the laboratory prepared G-2, Protein deficient, (PD) diet as described by Kaur et al., 1992 and given in table below. Composition of the diet used was as described by Bhaskaram and Hemalatha, 1995. The animals, both normal control and protein deficient were housed in polypropylene cages in the Animal House of the Department of Biophysics, Panjab University, Chandigarh. The animals were acclimatized for at least a week in hygienic conditions and were acclimatized for at least a week.

This group was designed to study the hepatotoxic effects of nickel in rats subjected to protein deficiency. Nickel was administered in the diet in the form of nickel chloride. Nickel chloride solution was prepared according to the standard method described by Manz et al., 1971.

The animals of this group were fed with diet containing normal protein contents and nickel chloride solution as described above. The concentration of nickel chloride solution was calculated according to the standard method described by Manz et al., 1971.

Statistical and methods

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

The absorption and uptake of $^{65}$Zn was studied in rats to find out nickel toxicity. The protein calorie malnutrition and this may be associated with adverse energy malnutrition (PEM).

In the developing countries like India PEM mainly due to heavy metal toxicity, the analysis of dietary intake is known to be associated with many biochemical disturbances and heavy metal intoxication affects the iron metabolism and intensifies the erythropoiesis with induced hemolytic anemia.

Results and discussion

The results of the present investigations clearly show that nickel can also be a potent hepatotoxin. The protein deficiency was induced in the animals of this group by maintaining them on the laboratory prepared G-2, Protein deficient, (PD) diet as described by Kaur et al., 1992 and given in table below.

Palabras clave:

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 NiSO₄·6H₂O 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></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/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 using Energy Depressive X-ray Fluorescence (EDXRF) technique. Energy depressive X-ray fluorescence technique is one of the most suitable analytical methods to analyze 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 grounded to a powder with the help of Agate Pestle and Mortar. 300 mg of the powder so obtained was weighed and mixed with equivalent amount of Sample Powder (powder) to make self-supporting pellets. The pellets were made by using a specially designed pure steel die and a hydraulic press from Paul Weber, Germany. A force of approximately 45 KN (Kilo newtons) was applied at the die top in order to make pellets of uniform thickness.

EDXRF Setup
In the present work, the pellets of liver samples 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. 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-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.
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 I. 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.

### Hepatic concentration of various elements

The concentrations of various elements have been depicted in table III. The 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.

### 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, b²</td>
<td>120.00 ± 9.48 a³, b³</td>
</tr>
</tbody>
</table>

Values are Mean ± SD. By Newman-Keuls Test.
a¹p < 0.05, a²p < 0.01 and a³p < 0.001 in comparison to G-1.
b¹p < 0.05, b²p < 0.01 and b³p < 0.001 comparison of G-4 with G-2.

### Table II

<table>
<thead>
<tr>
<th>Groups</th>
<th>Alkaline Phosphatase (nmoles phenol produced min⁻¹)</th>
<th>Aspartate Aminotransferase (µ moles of pyruvate formed min⁻¹)</th>
<th>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.
a¹p < 0.05, a²p < 0.01 and a³p < 0.001 in comparison to G-1.
b¹p < 0.05, b²p < 0.01 and b³p < 0.001 comparison of G-4 with G-2.
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 enzymes can be involved in the metabolic processes of various tissues and organs.
Ineffectiveness of nickel in augmenting animal growth: a study on the role of trace elements.

It is now well established that the liver has an important role in the metabolism of other metals. It is well insinuated that the susceptibility to metal toxicity is greatly increased in children due to their higher metal uptake and excretion rates.

We have studied several cases of pediatric PEM (protein-energy malnutrition) and found that the liver has an important role in the metabolism of proteins, nucleic acids, and carbohydrates. The study of Pond et al., 1992, showed that the liver is important in the absorption of iron from the gastrointestinal tract or via urine. Furthermore, the enzymes cytochromes P450 and X50 involve the metal in the metabolism of compounds like steroids and in the degradation of xenobiotics.

High levels of dietary Ni have been reported to decrease the growth of microorganisms for over hundred years; the inhibition of the growth of microorganisms is due to the metal's metabolism. This can be due to inadequate regulation of these enzymes. It is well known that the body requirement of iron is increased in response to the toxic conditions established by lead intoxication.

During the course of this study, we have also observed a higher dose of nickel could be because of nickel administration of nickel in the diet. Nickel also suggests that the body requirement of iron increases during the course of this study. It is now well established that the liver has an important role in the absorption of iron from the gastrointestinal tract or via urine. Further, the enzymes cytochromes P450 and X50 involve the metal in the metabolism of compounds like steroids and in the degradation of xenobiotics.

The hepatic Fe contents, in the present study, were found significantly depressed in protein deficient rats as compared to normal control group. Reduction in Fe levels in response to the toxic conditions established by lead intoxication.

In the present study, nickel concentration has been found to be increased in liver tissue following the administration of nickel in the diet. Nickel also suggests that the body requirement of iron increases during the course of this study. It is now well established that the liver has an important role in the absorption of iron from the gastrointestinal tract or via urine. Further, the enzymes cytochromes P450 and X50 involve the metal in the metabolism of compounds like steroids and in the degradation of xenobiotics.
is also a component of glutathione peroxidase. This inactivates sulfhydryl groups in certain enzymes and in the form of enhanced ALP, AST and ALT levels. It has been reported that nickel inhibits the Na-K-ATPase.

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2. Tandon A, Nagpaul JP, Bandhu H, Singh N, Dhawan D: Effect of protein deficiency on the host defense system which results in the increased mobilization of phosphorus from bones leading to decreased levels of potassium. Its deficiency affects the activity of muscles and plasma protein deficiency (PD) and nickel treatment which increased requirement of phosphorus either due to the increased mobilization from bones. This could be thought of due to impaired hepatic elimination of phosphorus and the toxic effects of nickel. Hepatic iron overload could be a reason for increased liver iron concentrations post-nickel treatment. Hepatic iron overload may be the consequence of alterations in the levels of host defense system which results in the enhancement of various cell organelles. In the present study, selenium act antagonistically and the detoxifying effect of selenium on nickel toxicity seems to be due to various cell organelles. Selenium deficiency animals have observed a statistically significant decrease in potassium levels in nickel treated and nickel treated protein deficient animals. Rai LC, Rai N, Singh AK, Singh AK, Singh AK and Dubey SK: Effect of four heavy metals on the biology of Nostoc muscorum. Indian J Appl Toxicol 1982; 31:335-47.


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