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# Zinc protects rat liver histo-architecture from detrimental effects of nickel

Pardeep Sidhu[1], M. L. Garg[2] & D. K. Dhawan[2,*]
[1]*Department of Physiology and Pharmacology, The University of Western Ontario, London, Ontario, Canada N6A 5C1;* [2]*Department of Biophysics, Panjab University, Chandigarh, 160014, India;* *Author for correspondence (Tel: +91-172-2534121; Fax: +91-172-2534118; E-mail: pardeepsidhu@yahoo.co.uk)*

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**Abstract**

This study was designed to examine the protective potential of zinc on the histoarchitecture distortion induced by nickel in rats. Male Sprauge Dawley (S.D) rats received either nickel alone in the form $NiSO_4 \cdot 6H_2O$ at a dose of 800 mg/l in drinking water, zinc alone in the form of $ZnSO_4 \cdot 7H_2O$ at a dose of 227 mg/l in drinking water, or nickel plus zinc or drinking water alone for a total duration of eight weeks. The effects of different treatments were studied on rat liver histoarchitecture by using both light and transmission electron microscopes. Normal control and zinc treated animals revealed normal histology of liver, however, nickel treated animals resulted in drastic alterations of normal hepatic histoarchitecture, after 8 weeks of treatment. Administration of zinc to nickel treated rats resulted in marked improvement in the structure of hepatocytes, thus emphasizing the protective potential of zinc in restoring the altered hepatic histoarchitecture close to the histoarchitecture of normal animals.

## Introduction

Food is the dominant source of nickel exposure in the non-smoking, non-occupationally exposed population. Nickel levels in food are generally in the range 0.01–0.1 mg/kg, but with large variations (Dabeka & McKenzie 1995). Although the environmental hazards of nickel are not great, yet there are many occupational dangers due to this element (Henry *et al.* 1974). Occupational exposure to nickel occurs primarily via inhalation and ingestion of metal-containing dusts. However, in addition to this main exposure route, workers are also indirectly exposed to nickel by ingestion of nickel contaminated drinking-water and food, and through skin contact. Tobacco smoking is also an important source of nickel exposure. Nickel compounds of commercial importance are used in preparation of nickel alloys, ground coated enamels, in cooling of ceramics and glass, electroplating, nickel-iron storage batteries, electronic components and to prepare nickel catalysts (Denkhaus & Salnikow *et al.* 2002). Nickel breaks down the immunity by affecting the T-cell system and suppresses the activity of natural killer cells in rats and mice (Goutet *et al.* 2000; Condevaux *et al.* 2001). Nickel induced free radical production has been shown to promote cell damage by promoting lipid peroxidation (Chen *et al.* 2003) which in turn affects the structural architecture of the tissue. Nickel compounds are known to cause cancer in humans and induce tumors in experimental animals (Salnikow & Kasprzak 2005).

Zinc is required for the normal growth, development and function of different organs of mammals. It is an essential element of more than 200 metalloenzymes, including some of the antioxidant enzymes like superoxide dismustase and affects their conformity, stability, and activity. Zinc is also important for the proper functioning of the immune system, and for glandular, reproductive and cell health. Abundant evidence demonstrates the antioxidant role of zinc (Rostan *et al.*

2002). Chvapil *et al*. (1972) have shown that $CCI_4$ induced lipid peroxidation in the microsomal fraction of liver homogenates was inhibited by adding zinc to the incubation medium. Dhawan & Goel (1994) supported these findings by subjecting $CCI_4$ treatment to rats along with dietary Zn supplementation. Indications of biological antagonism between nickel and zinc have been reported earlier (Waalkes *et al*. 1985; Kazimierz *et al*. 1986). Zinc protects the cell membrane from the injury caused by nickel (Bettger & Dell 1981) and has the potential to stablise the liver functions by regulating the levels of essential elements as well as the key liver marker enzymes under the toxic conditions of nickel (Sidhu *et al*. 2004). Michael *et al*. (1984) concluded that zinc prevents some, but not all the toxic effects of nickel.

The present study was designed to explore further the protective potential of zinc with regard to maintaining the structural integrity of liver in conditions of nickel toxicity.

## Materials and methods

### *Experimental design*

#### *Grouping of animals*

Male rats in the weight range of 110–120 g of Sprague Dawley (S.D.) strain were procured from the Central Animal House, Punjab University, Chandigarh. The animals were housed in polypropylene cages under hygienic conditions and were acclimatized for at least one week before putting them on different treatments. During the course of the study, the animals had free access to water and diet.

Animals were segregated into four different groups of ten animals each. One served as normal control (G-1) and the other three were given treatments of nickel alone (G-2), zinc alone (G-3) and nickel plus zinc (G-4). Ni was administrated in the form of $NiSo_4{\cdot}6H_20$ in drinking water at a dose level of 800 mg-Ni/l (Sidhu *et al*. 2003) of water while Zn was given in the form of $ZnSO_4{\cdot}7H_2O$ in drinking water at a dose level of 227 mg Zn/l of water (Dhawan & Goel 1994). The treatments of rats continued for a period of 8 weeks. The animals were sacrificed by exsanguination under light anesthesia. Livers were removed immediately and one part of the lobe was preserved by freezing for the determination of nickel and zinc. The other part was processed immediately for the estimations of lipid peroxidation. Further, the other lobe was used for light and electron microscopic studies.

### *Biochemical estimations*

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

*Lipid peroxidation (LPO)*. Lipid peroxidation was estimated by the method of Ohokawa *et al*. (1979).

### *Elemental analysis of liver samples*

Estimations of nickel and zinc concentrations in the liver samples of different treatment groups were carried out by using Energy Depressive X-ray Fluorescence (EDXRF) technique. In the present work, the pellets of tissues were analyzed using an EDXRF SPECTRO X-Lab, 2000 spectrometer, from Germany to determine the levels of various elements. The spectrometer was already calibrated using different standards to give concentration within accuracy of few percent in the unknown samples.

#### *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 mg dried powder of the tissue so obtained was weighed and mixed with equivalent amount of Hoechst Wachs (wax) to make self supporting pellets. The pellets were made by 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.

### *Histopathological studies*

*Light microscopic studies*. Fresh tissue pieces of liver were immediately immersion fixed in 10% phosphate buffered formalin. Following an overnight fixation, the specimens were dehydrated in ascending grades of alcohol, cleared in benzene and embedded in paraffin wax (58–60 °C). Blocks

were made and 5–7 $\mu$m thick sections were double stained with hematoxylin and eosin and observed under light microscope.

*Electron microscopic studies*. Liver samples were processed immediately after sacrificing the animals for histopathological examination, at the electron microscopic levels. A small lobe of liver was incised out and immersion fixed in the fixative having formaldehyde and glutaraldehyde made in 0.2 M sodium cacodylate buffer (pH 7.2) for 10–12 h at 4 °C. The specimens were then thoroughly washed 3 times in cacodylate buffer and then post fixed for 60 min in 1% osmium tetraoxide ($OsO_4$) made in the same buffer. The tissues were then thoroughly washed in buffer to remove the extraneous traces of $OsO_4$ and then dehydrated in ascending series of acetone, allowing 20 min in each change, in each concentration of acetone. Specimens were then infiltrated and subsequently embedded in Durcupan resin kit supplied by Polaron Scientific Inc., U.S.A. Specimen blocks (3–5 blocks) from each animal were made out by polymerization of the pure embedding resin at 60 °C for 48–72 h.

Ultrathin sections of various specimen blocks were cut using a Reichert Jung Ultramicrotome. Initially, semi-thin sections of thickness about 1 $\mu$m were cut using very sharp glass knives (prepared on LKB knife marker) to locate the area of interest in different treatment groups. These semi-thin sections were stained with 0.5% toludine blue made in 1% Borax solution. Ultrathin sections of interference colors from golden to silver were cut and loaded on fine copper grids of 100–300 mesh size. Sections were double stained with uranyl acetate and lead citrate. These ultra thin sections were finally viewed under Transmission Electron Microscope at Department of Anatomy, All India Institute of Medical Sciences (AIIMS), New Delhi.

*Statistsical analysis*. All the results of various treatment groups have been compared with their normal controls. Results of the nickel + zinc (G-4) treated group have also been compared with the results of the nickel treated group (G-2). 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 ± S.D.

## Results

### *Body weights*

The variations in the body weights of the animals subjected to different treatments are shown in Table 1. The body weights of normal control and zinc treated rats, showed steady increase and at the end of the study, weights of the Zinc treated animals were not different from that of the normal controls. Nickel treatment to normal control rats and as well as to zinc treated rats resulted in significant reduction ($P < 0.05$) in the body weights when compared to normal control rats.

### *Hepatic protein contents*

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

### *Hepatic lipid peroxidation*

The results of hepatic, lipid peroxidation, in different treatment groups are given in the Table 2. A significant ($P < 0.001$) increase in lipid peroxidation was observed in nickel treated group in the present study. Interestingly, Zn treatment to Ni treated animals resulted in lowering the process of LPO ($P < 0.001$) to a significant extent as compared to their respective controls.

*Table 1* Effect of zinc treatment on the body weights and hepatic protein in rats subjected to nickel treatment.

| Groups | | Weight (Grams) | Hepatic protein (mg $g^{-1}$ tissue) |
|---|---|---|---|
| G-1 | Normal control | $199 \pm 7$ | $156 \pm 5$ |
| G-2 | Nickel treated | $167 \pm 20^{a1}$ | $141 \pm 2^{a3}$ |
| G-3 | Zinc treated | $180 \pm 22$ | $162 \pm 7$ |
| G-4 | Nickel + Zinc | $166 \pm 14^{a1}$ | $152 \pm 5^{c2}$ |

Values are Mean ± S.D. By Newman–Keuls test. $^{a1}P < 0.05$, $^{a2}P < 0.01$ and $^{a3}P < 0.001$ in comparison to G-1. $^{c1}P < 0.05$, $^{c2}P < 0.01$ and $^{c3}P < 0.001$ comparison between G-2 and G-4.

*Table 2.* Effect of zinc on the hepatic lipid peroxidation (LPO) in rats subjected to nickel treatment.

| Groups | | MDA levels (n mol $mg^{-1}$ protein) |
|---|---|---|
| G-1 | Normal control | $0.99 \pm 0.00$ |
| G-2 | Nickel treated | $1.42 \pm 0.17^{a3}$ |
| G-3 | Zinc treated | $1.00 \pm 0.00$ |
| G-4 | Nickel + Zinc | $1.11 \pm 0.19^{c3}$ |

Values are Mean ± S.D. By Newman–Keuls test. $^{a1}P < 0.05$, $^{a2}P < 0.01$ and $^{a3}P < 0.001$ in comparison to G-1. $^{c1}P < 0.05$, $^{c2}P < 0.01$ and $^{c3}P < 0.001$ comparison between G-2 and G-4.

*Table 3.* Effect of zinc on the levels of nickel and zinc levels in rats subjected to nickel treatment.

| Group/elements | | Nickel ($\mu$g/g) | Zinc ($\mu$g/g) |
|---|---|---|---|
| G-1 | Normal control | $4.1 \pm 1.4$ | $58 \pm 6$ |
| G-2 | Nickel treated | $12.1 \pm 2.7^{a3}$ | $35 \pm 4^{a3}$ |
| G-3 | Zinc treated | $1.9 \pm 0.7$ | $58 \pm 8$ |
| G-4 | Nickel + Zinc | $4.9 \pm 1.8^{c3}$ | $53 \pm 4^{c3}$ |

Values are Mean ± S.D. By Newman–Keuls test. $^{a1}P < 0.05$, $^{a2}P < 0.01$ and $^{a3}P < 0.001$ in comparison to G-1. $^{c1}P < 0.05$, $^{c2}P < 0.01$ and $^{c3}P < 0.001$ comparison between G-2 and G-4.

### *Hepatic concentrations of nickel and zinc*

The concentrations of nickel and zinc have been depicted in Table 3. The nickel administration to normal rats have resulted in a significant ($P < 0.001$) increase in concentrations of nickel in liver tissue. However, zinc restored the elevated levels of nickel significantly ($P < 0.001$) to within normal range in these animals when compared to their respective control groups.

The levels of Zinc got decreased significantly ($P < 0.001$) in nickel treated animals. However, following zinc treatment to nickel treated animals, the concentrations of zinc got elevated significantly ($P < 0.001$), to within normal levels when compared to respective nickel treated control group.

### *Hepatic histoarchitecture*

#### *Light microscopic observations*

Histological studies of liver from various treatment groups are represented in Figures 1–4. Normal control animals revealed clear cut hepatic lobules, separated by interlobular septa, traversed by portal veins (Figure 1). Within the lobule, hexagonal arrangement of hepatic plates was observed radiating outward towards periphery from a central vein. Between the laminae, hepatic sinusoids were quite clear. The hepatocytes were almost polyhedral. The nuclei were round and their size was almost equal with the exception of a few binucleated cells. Similar observations were

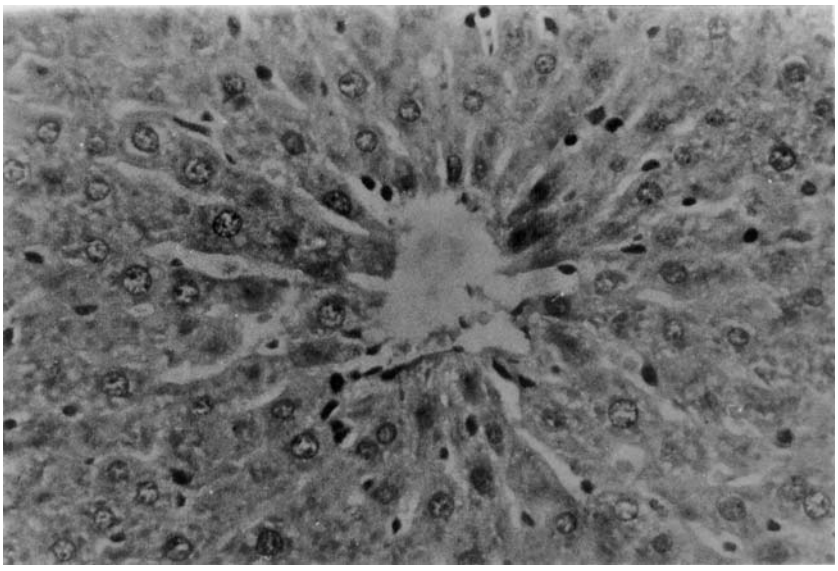

*Figure 1.* Liver section from a normal control rat. Hematoxylin + Eosin (×400).

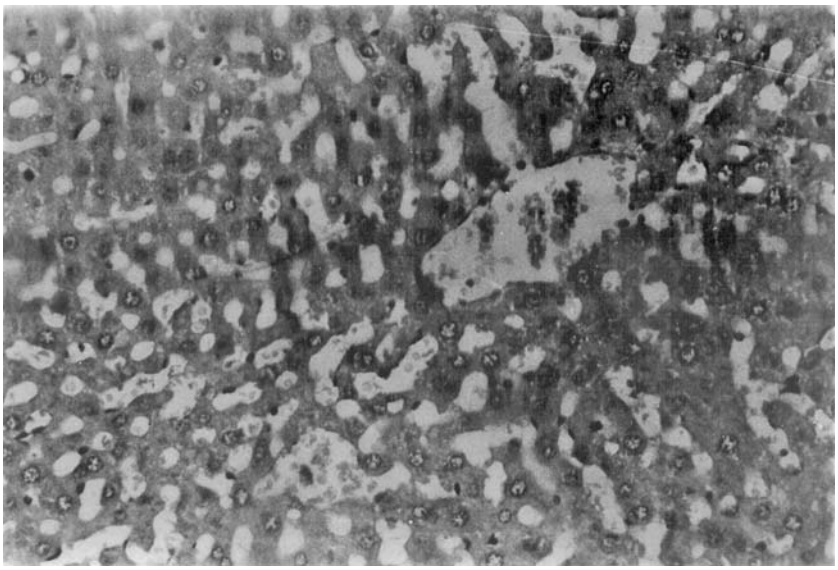

*Figure 2.* Liver section from a nickel treated rat. Hematoxylin + Eosin (×400).

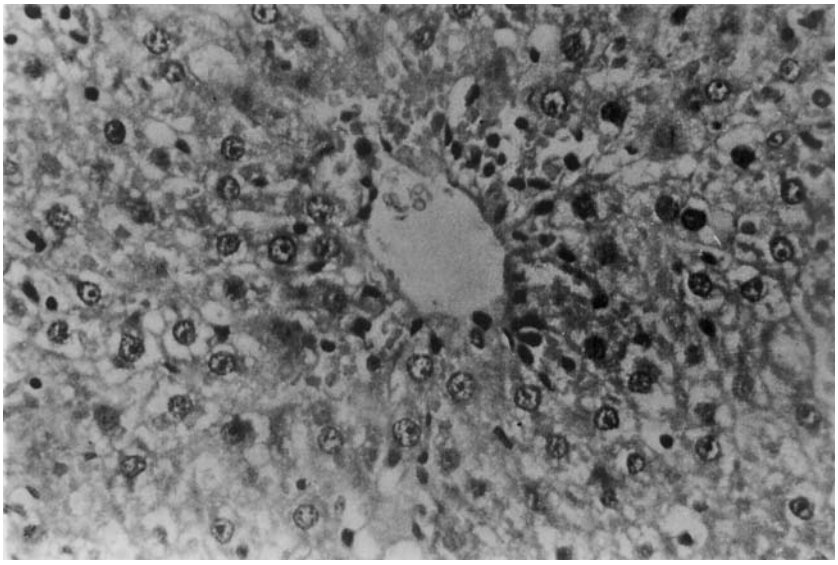

*Figure 3.* Liver section from a zinc treated rat. Hematoxylin + Eosin (×400).

made for the normal control animals given zinc treatment (Figure 3).

Nickel treated animals resulted in drastic alterations of hepatic histoarchitecture after 8 weeks of treatment. The hepatic cords were disrupted at most of the places (Figure 2). A few of the hepatocytes were vacuolated and were not having the usual polyhedral shapes. Vacuolization was more severe near the portal tracts with a resultant widening of the sinusoidal spaces. In general, some degree of hepatic hypertrophy was evident, with the cytoplasmic structures becoming more and more light opaque. More number of binucleated cells were also seen.

Administration of zinc to nickel treated rats resulted in marked improvement in the structure of hepatocytes (Figure 4), but still a great number of binucleated cells were seen. Though, the lamellar pattern of hepatocytes was restored to almost normal, still the sinusoidal spacing was prominent

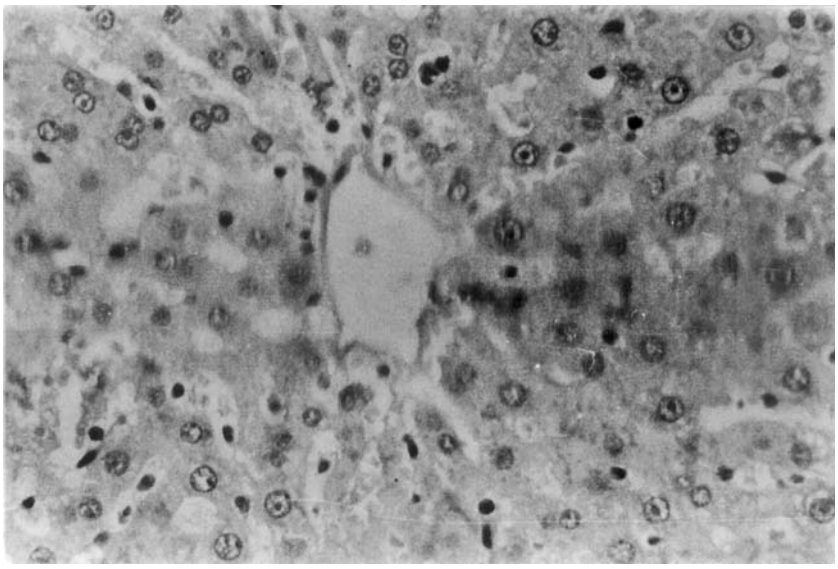

*Figure 4*. Liver section from a zinc + nickel treated rat. Hematoxylin + Eosin (×400).

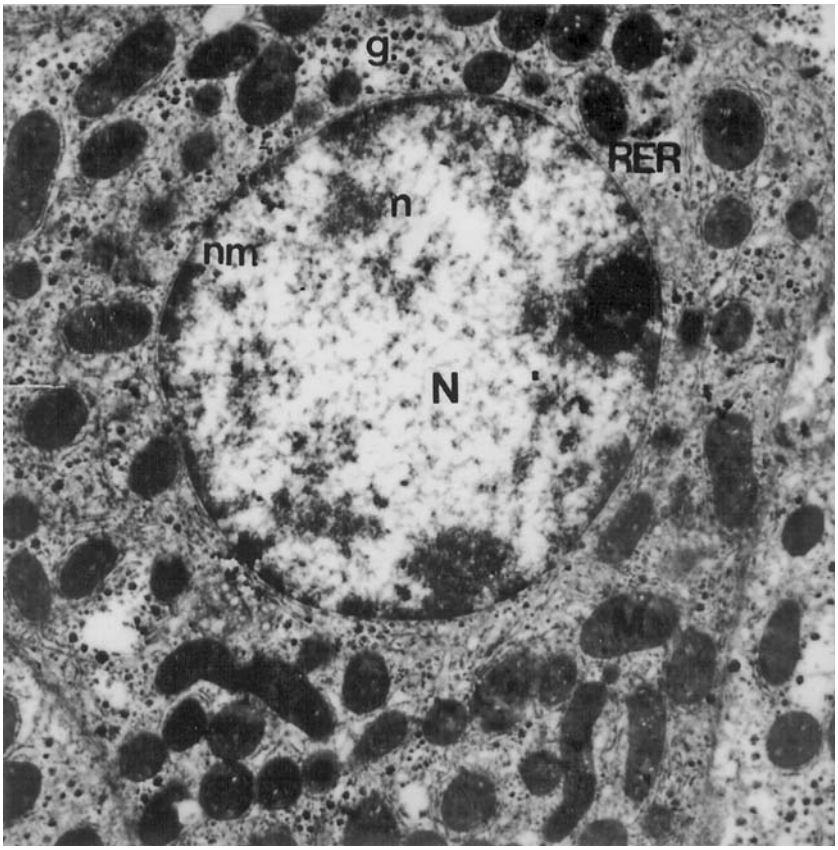


*Figure 5*. Electron micrograph of liver cell from a normal control rat revealing a well defined nucleus (N) with well defined nuclear membrane (nm) and clear nucleolus (n). Mitochondria (M) are uniformly round and scattered amongst rosettes of glycogen (g). Parallel plates of rough endoplasmic reticulum (RER) are also visible (×3000).

in these animals, in comparison to the normal control animals. Furthermore, very fine vacuolizations noticed were of very small proportions and were present around the hepatoportal tracts.

*Ultrastructural observations of liver*

Transmission electron microscopy was undertaken to assess the effects of various treatments in livers of the animals at the end of the study. Normal control animals, which remained without any treatment showed clear and fine hepatic cellular structures (Figures 5 and 6). All the cell organelles were present in a uniform and usual pattern. Nuclear structures were well intact, with fine nucleolar chromatin and well intact nuclear membranes. Mitochondria were uniform in size with structures varying from round to oval in appearance. Mitochondrial cristae were quite intact. These mitochondrial structures were surrounded by parallel pleats of rough endoplasmic reticulum (RER) dispersed throughout the cytoplasm. Fine ribosomes were studded onto the lamellae of RER, which was indicative of normal protein synthesis in these normal control animals. Abundant star shaped rosettes of glycogen were scattered amongst the endoplasmic reticulum and mitochondiral structures.

Nickel treatment to normal rats led to marked disruptions in the hepatic ultra structure (Figure 7). Nucleolus got retracted but perichromatin granules did not disappear. The presence of lots of lipid droplets and many phagocytic vesicles were depicted. Some reduction in RER and ribosomes was also noticed.

Normal animals, which were given zinc treatment, did not show any major alternations in the hepatic histoarchitecture in comparison to normal rats, except a few swollen mitochondria were noticed (Figures 8 and 9). Zinc treatment to nickel treated animals resulted in a significant improvement

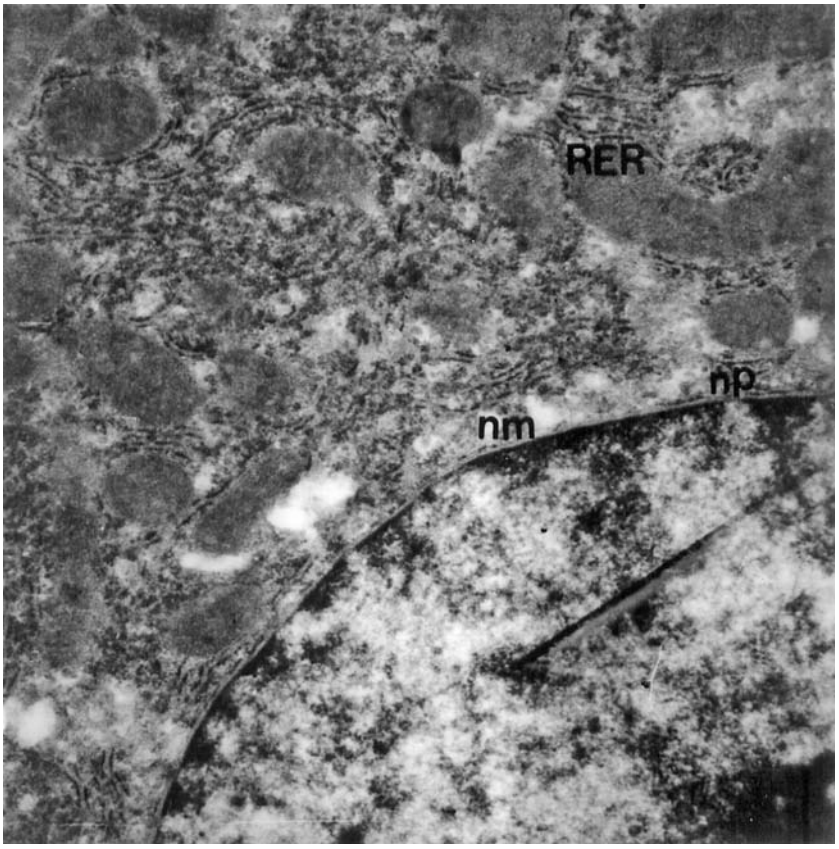


*Figure 6*. Electron micrograph of the hepatocyte from a normal control rat showing a well intact nuclear membrane (nm) and nuclear pore (np). Mitochondria (M) are uniformly round and scattered amongst rosettes of glycogen (g). Parallel plates of rough endoplasmic reticulum (RER) are also prominent (×11,500).

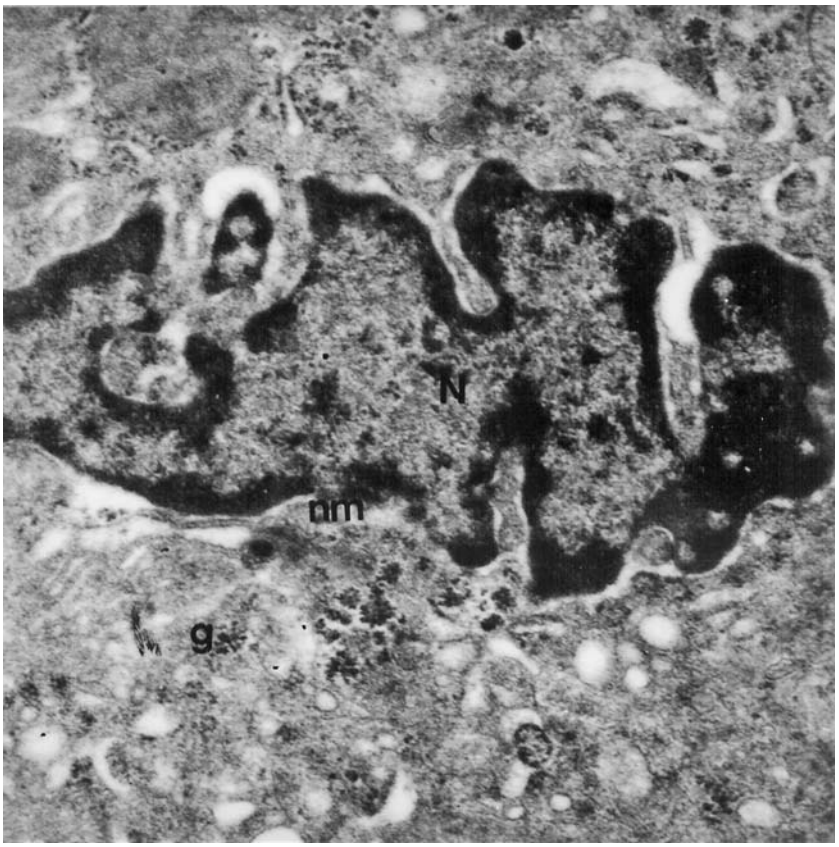


*Figure 7.* Electron micrograph of liver cell from a nickel treated rat revealing a distorted nucleus (N) with broken nuclear membrane (nm). Few glycogen (g) moieties are also seen (×8400).

in the overall histoarchitecture, but still it was not completely normal (Figure 10). Some degree of mitochondrial swelling with some variation in the size was noticed. However, nuclear structures along with glycogen content were normal in these animals, thus emphasizing the protective potential of zinc in restoring the altered hepatic histoarchitecture close to the histoarchitecture of normal animals.

## Discussion

Nickel treatment to normal control 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 earlier (Cempel & Janicka 2002). The decrease in body weight due to nickel treatment has been connected by researchers possibly due to the overall increased degeneration of lipids and proteins as a result of nickel toxicity (Dieter *et al.* 1988; Cempel & Janicka 2002). Zinc treatment to the Ni treated rats tended to improve the body weight growth, though it was not significant. Similar protective effects of zinc in improving the body weight gain of the animals have also been emphasized in other studies, where radiations or carbontetrachloride was used to cause liver injury (Dhawan & Goel 1994; Yao *et al.* 2001; Chen *et al.* 2001). The protective effects of zinc could be attributed to its ability to reduce collagen accumulation in liver and also it exerts critical physiological role in regulating the structure and function of cell.

Nickel treated group showed a highly significant ($P < 0.001$) reduction in the hepatic protein contents as compared to normal control group. Nickel diminishes the DNA and RNA polymerase activity (Sirover & Loeb 1976) which in turn can reduce the protein synthesis. To substantiate this contention (Das & Dasgupta 2000), has observed the decrease in nucleic acids and protein concentration following nickel toxicity. However, zinc

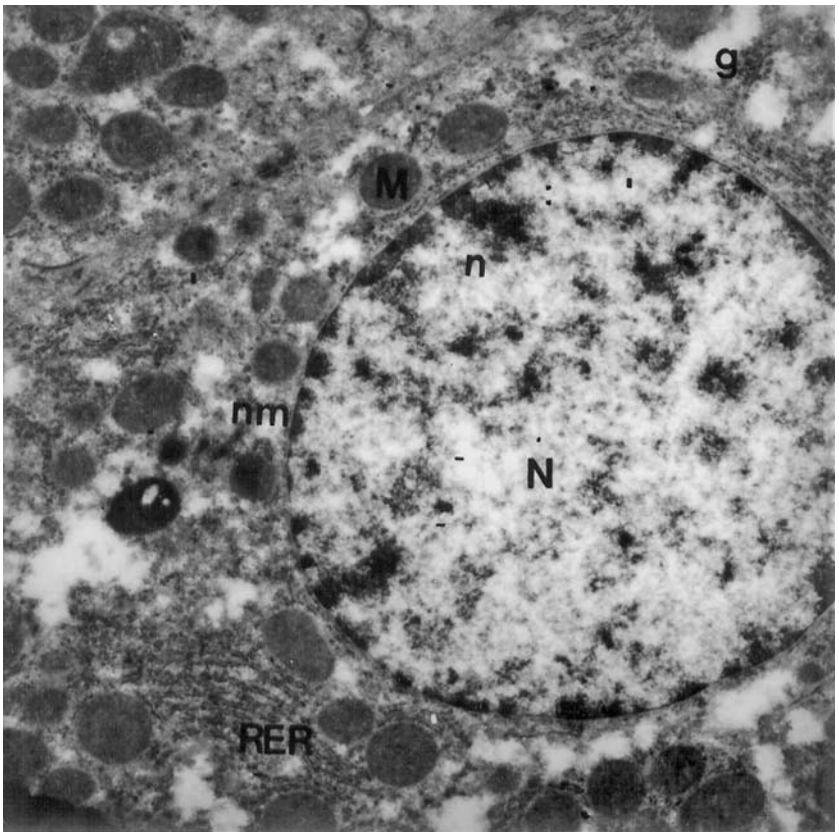


*Figure 8*. A hepatocyte from a zinc treated rat showing intact nucleus (N) with compact nucleolus (n) and the nuclear membrane (nm). Mitochondria (M) are round to oval, abundant glycogen (g) and pleats of rough endoplasmic reticulum (RER) are also visible (×3000).

administration to this group helped in raising the hepatic protein contents ($P < 0.01$). Other authors have also shown conclusively that zinc stimulates protein synthesis (Ehara & Yamaguchi 1997) and that Zn deficiency weakens protein synthesis (Giugliano & Millward 1987) making zinc an essential factor for protein synthesis.

Lipid peroxidation is the process of oxidative degradation of polyunsaturated fatty acids (PUFA) and because of its biomedical implications, the process has been the subject of strenuous research. Nickel treatment to normal control rats indicated increase in the hepatic LPO. Increase in lipid peroxidation following nickel treatment has also been reported earlier (Shirali *et al*. 1994; Chen *et al*. 1998). Current studies on cellular injury by environmental toxicants implicate peroxidation of PUFA, leading to the degradation of phospholipids in a variety of phathological states, which has been anticipated as an index of cellular deterioration (Tappel 1973; Abou-Donia 1981). The normalization of LPO due to Zn administration could be attributed to its antiperoxidative property. Srivastava *et al*. (1993) suggested that Zn–MT serve as an efficient antagonist in inhibiting nickel-mediated lipid peroxidation compared to Cd–MT or Ag–MT. We have shown earlier that Zn causes inhibition of both endogenous as well as induced lipid peroxidation to stabilize biomembranes (Dhawan *et al*. 1992; Dhawan & Goel 1996).

Nickel concentration has been found to be increased in liver tissue following the administration of nickel to normal rats. Our results are in agreement with earlier reports (Obone *et al*. 1999; Cempel & Janicka 2002). However, administration of zinc along with nickel treatment brought the altered nickel levels to near normal limits, thereby showing the antagonistic effects of zinc towards nickel toxicity. Indications of biological antagonism between nickel and zinc have also been

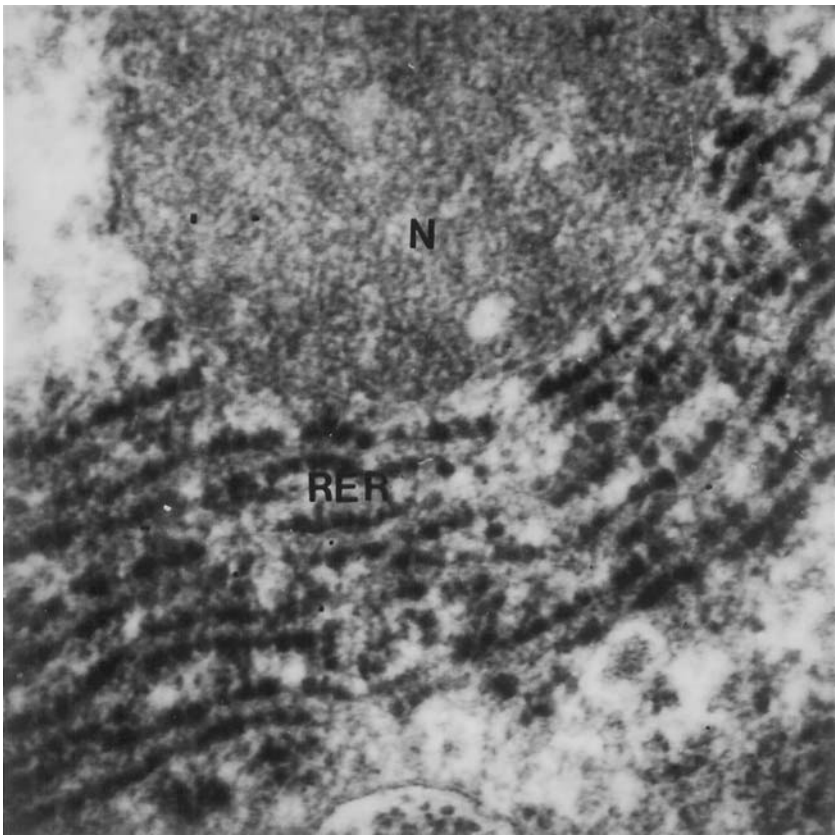


*Figure 9*. Electron micrograph of liver cell from zinc treated rat showing very prominent parallel plates of rough endoplasmic reticulum (RER) along with well-defined nucleus (N) (×30,000).

reported earlier (Fisher *et al*. 1986; Kazimierz *et al*. 1986). The decrease in the concentration of nickel following zinc administration may be because the uptake of nickel in the intestinal wall gets suppressed by concomitant perfusion with high levels of zinc (Foulkes & McMullen 1986).

In present study, we have observed decrease in hepatic zinc concentration following nickel toxicity, which may be because nickel mobilizes and promotes the excretion of copper, zinc and manganese from organs (Cempel & Janicka 2002). Abnormalities in Zinc metabolism leading to its deficiency are generally attributed to various factors like, malabsorption, decreased intestinal Zinc binding factors or increased excretion of Zinc (McClain & Su Le 1983).

In the present study, the hepatic histoarchitecture of the nickel treated animals resulted in vacuolization of the hepatocytes and sinusoids got dilated appreciably in comparison to the normal rats. The number of binucleated cells were also more in these animals, in proportion to the normal control animals. In the earlier studies also, similar observations were recorded following nickel toxicity (Mathur *et al*. 1977). The present observations indicated marked changes in the overall histoarchitecture of liver in response to nickel treatment, which could be explained on the basis that nickel manifested its toxic effects primarily by the generation of reactive oxygen species. The resulting effect was production of significant amount of lipid peroxidation which caused damage to the various membranous components of the cell. The necrotic conditions observed in these animals are in good agreement with our observed biochemical studies, wherein we have noticed increased levels of lipid peroxidation which are indicative of hepatic injury.

Furthermore, electron microscopic observations of the present study with regard to nickel toxicity revealed predominant effects on the mitochondria, endoplasmic reticulum, and glycogen

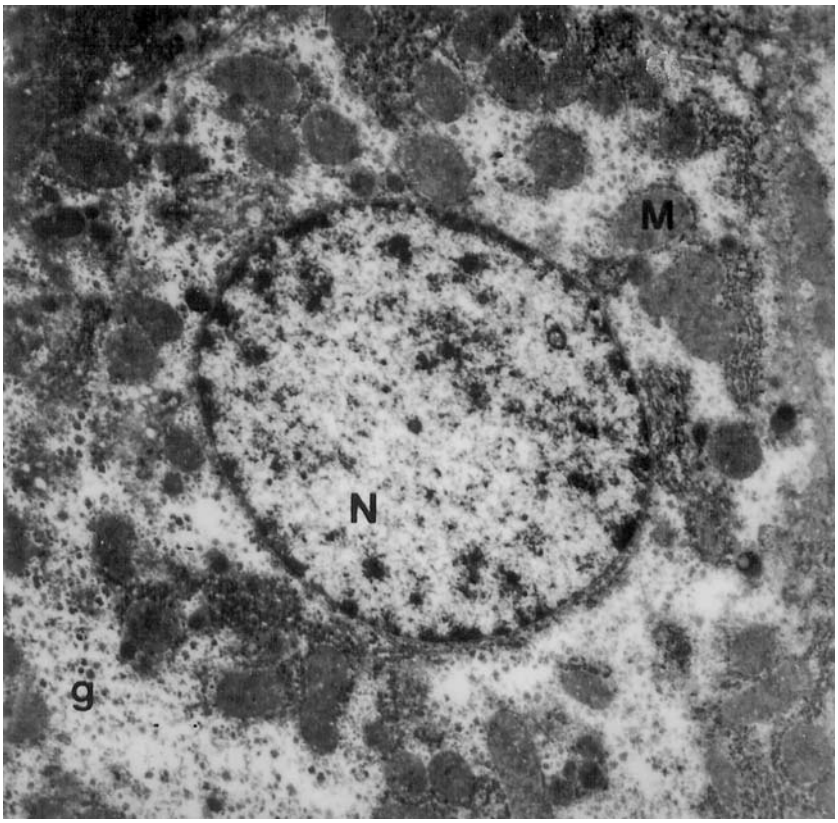


*Figure 10*. Electron micrograph of the hepatocyte from nickel + zinc rat depicting intact nucleus (N), nucleolus (n) and nuclear membrane (nm). Moderately swollen mitochondria (M) and glycogen (g) granules are visible (×3000).

content. Ours is probably the only study so far to examine the effects of nickel on the ultrastructure of liver and we have observed reasonably convincing correlation between the extent of hepatic damage and various biochemical indices envisaged in this study. In our study, nickel treatment to rats resulted in significant effect on nuclear structures of hepatocytes whereby their membranes were broken and shapes got deformed. These effects are suggestive of the decreased rate of DNA synthesis as there are reports which indicate the effect of nickel on DNA synthesis by diminishing the DNA and RNA polymerase activity and decreasing DNA replication fidelity (Sirover & Loeb 1976). Moreover, marked swelling and increase in the number of mitochondria was another prominent feature of the present study in nickel intoxicated animals which might be indicative of the increased energy requirements of the cells in an effort to overcome the noxious effects of the nickel.

Administration of zinc to nickel treated animals resulted in normalizing the hepatic histoarchitecture quite appreciably. Such hepatoprotective effects of zinc have also been observed by us earlier while evaluating its potential in $CCl_4$ toxicity (Dhawan & Goel 1994). Similar results are evident in this study, and could well be attributed to the antioxidant effects of zinc, which maintains integrity of the membrane either by some direct mechanism or indirectly by scavenging the free radicals responsible for increased lipid peroxidation. The normalization of histoarchitecture of liver following zinc administration may also be attributed to the recovery accorded by zinc in regulating the levels of different enzymes as indicated in our earlier study (Dhawan & Goel 1996). Thus, it may be concluded from the present study that zinc plays an important role in regulating the levels of LPO as well as nickel and is quite effective in maintaining the structural integrity of liver in conditions of nickel toxicity.

## Conclusions

In the present study it has been found that zinc is quite effective in maintaining the structural integrity of hepatocytes in conditions of nickel toxicity as evidenced by light and electron investigations.

## Acknowledgements

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