CHAPTER IV

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During the tenure of the present investigation, the toxic effects of nickel and/or chromium salts in the form of nickel chloride (NiCl₂) and potassium dichromate (K₂Cr₂O₇) were studied with respect to structure and physiology of some vital organs of adult female albino mice (Mus musculus) of Swiss strain. The role of antioxidants like Vitamin-A (Vit A) and Vitamin-E (Vit E) on NiCl₂ and K₂Cr₂O₇ combination induced toxicity were also evaluated. The experimental animals were given nickel chloride (NiCl₂) at dosages of 8 mg/kg (low dose) and 16 mg/kg body weight (high dose) orally for 30 days. The doses of potassium dichromate were 5 mg/kg (low dose) and 10 mg/kg body weight (high dose) orally for 30 days. Vitamin A and Vitamin E at doses of 0.2 mg/kg and 2 mg/kg body weight respectively were fed orally to NiCl₂ + K₂Cr₂O₇ (HD) intoxicated mice separately.

At the end of each treatment period, the animals were killed by cervical dislocation and the tissues like liver and kidney were used for biochemical, histopathological and electron microscopic (EM) studies.

Parameters studied were gravimetric and biochemical tests of vital organs like liver, kidney and blood. Gravimetric studies included were absolute body and organ weight of liver and kidney after each respective treatments.

The biochemical parameters studied in the liver were protein, alkaline and acid phosphatases (ALKPase and ACPase), succinate dehydrogenase (SDH), phosphorylase, glycogen, cholesterol, lipid peroxidation, glutathione, total ascorbic acid (TAA),
dehydroascorbic acid (DHA), reduced ascorbic acid (RAA), superoxide dismutase (SOD) and catalase in all groups. Biochemical tests in kidney were protein, alkaline and acid phosphatases (ALKPase and AC Pase) and creatinine. In blood, haemoglobin (Hb), RBC and WBC counts were done in all groups, and in serum, marker enzymes of liver toxicity like serum glutamate pyruvte transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) were investigated along with serum protein and cholesterol levels.

Chromium and nickel individually are considered potential health hazards. Health effects of nickel and chromium share some similarities. Nickel along with chromium has been identified as the most frequently occurring contact allergen. Both metals also exert effects on organs like the kidney and liver at high exposure level in animal experiments (NAS, 1974, NAS 1975). Although, much work has been done on the toxic effects of chromium and nickel alone, there is still a need to study their combined toxicity and remedial sources to their poisoning. Hence this study has been undertaken and results thus obtained are discussed here.

GRAVIMETRIC STUDIES

Whole body and organ weights are good indicators for toxicity of many xenobiotics.

In the present study, no significant changes in body weights were observed after various NiCl₂ treated groups. Similarly previous reports from our laboratory have found no significant change in body weights of male mice treated with NiCl₂ (Rao and Rajvanshi, unpublished data). But Seidenberg et al (1986) found decreased body weight by nickel exposure. Decreased body weight has also been observed in rats given single
intramuscular injection of nickel chloride (Smialowicz et al., 1987). The discrepancy between above studies could be due to differences in age, animal species, dose, duration and mode of administration of nickel salts.

After NiCl₂ (LD) treatment, no significant alteration in the weights of liver and kidney were observed but NiCl₂ (HD) treatment brought about a significant decline in these weights. In support to the above results, decreased liver weight was observed in rats and mice exposed to nickel chloride (American Biogenics Corporation, 1988). Also changes in kidney weight were observed in rats exposed to ≥ 1.5 mg nickel/kg/day as nickel salts for ≤ 9 months (American Biogenics Corporation, 1988).

Feeding of chromium brought about a reduction in the body weights in the present study. Intoxication with chromium also showed a decrease in liver and kidney weights of mice. Moreover, Trivedi et al. (1989) and Junaid et al. (1996b) also reported a fall in body weights of mice exposed to potassium dichromate. Similarly Chowdhary and Mitra (1995) reported that rats treated with sodium dichromate by gavage for 90 days at 40 mg Cr (VI)/kg/day had a decrease in body weight. Glaser et al. (1990) also noticed a decrease in body weights in rats exposed to sodium dichromate in support of our above observations.

Treatment of NiCl₂ + K₂Cr₂O₇ at both dosage level also brought a significant reduction in body, liver and kidney weights of mice in our study. The decline in the body and organ weights as obtained in the present study might be attributed to reduced food and water intake due to chromium and/or nickel intoxication.

The changes in the body and organ weights in this study underlines the necessity for seeking other, more sensitive indices of metabolic and structural disturbances caused...
by chronic exposure of chromium and/or nickel which are discussed further in this chapter

**BIOCHEMICAL STUDIES**

Liver is the largest organ of the body which has important functions viz., secretion, excretion, phagocytosis, detoxification, storage and synthesis of glycogen as well as some vitamins, catabolism of some hormonal and non-hormonal substances. It is the first organ that comes into contact with enterally absorbed nutrients and xenobiotics via the portal blood. Hence the liver with its metabolic detoxifying function is extremely vulnerable to harmful substances.

Since the entire range of toxic metabolites in the body is excreted mainly from the kidney, any alteration in the structure of kidney would affect its function. Kidney is the main target organ for chromium accumulation after oral or dermal absorption which can result in acute tubular necrosis in human (Franchini et al., 1978, Langard and Norseth, 1979) and in animal models (Mathur et al., 1977, Tandon et al., 1979, Tandon, 1982). Similarly, once nickel enters the body, it mainly goes to the kidneys (ATSDR, 1996). Thus high chromium and nickel concentration may cause impaired kidney function. In order to investigate the effects of chromium and/or nickel on kidney functions, the concentration of proteins, creatinine and the activities of acid and alkaline phosphatases were determined as mentioned earlier.
In the present investigation, the treatments of nickel chloride and potassium dichromate alone and in combination to mice resulted in a decline in protein levels in liver and kidney in a dose-dependent manner.

Previous study in our laboratory has already reported a reduction in protein levels in liver of mice after 45 days treatment with nickel and chromium salts (Rao and Rajvanshi, unpublished data) In support of our data, fresh water fish, *Channa punctatus*, under the stress of nickel (NiSO$_4$·6H$_2$O) at various concentrations, brought about a gradual decline in protein levels of liver (Desai et al., 2002).

Results obtained by Horak et al. (1980) and Gumbleton and Nichollas (1988) who observed an increased urinary excretion of protein after exposure of rats to nickel and chromium revealed a significant decline in protein levels in kidney in support of our data. Similarly Gitlitz et al. (1975) also reported nephrotoxicity manifested by proteinurea and enzymurea in NiCl$_2$ treated rats. Dieter et al. (1988) observed that oral exposure to nickel caused protein loss accompanied by renal tubular damage in animals. Previous studies of workers exposed to Cr (VI) in chromate production industry have found increased urinary levels of low molecular weight proteins indicative of renal damage (Franchini and Mutti, 1988). Rats given Cr (VI) also manifested an increase in protein and a decrease in urine volume (ATSDR, 1998). Proteinurea was also seen in rabbits after administration of Cr (VI) (Nomiyama et al., 1982). Thus the above studies are in agreement to our present findings of reduction in protein levels of liver and kidney by metal poisoning.

The decline with K$_2$Cr$_2$O$_7$ might be related to impairment of protein synthesis by chromium ions. It is also known that heavy metals bind with -SH groups of protein.
molecules rendering them inactive and/or inhibiting protein synthesis (De Brum, 1976; Rao, 1997) Chromium is also known to affect the rate of cellular protein synthesis. Recent studies by Bagchi et al. (2002) noted that Cr (VI) induced an oxidative stress through enhanced production of ROS leading to deterioration of proteins in tissues. It is also known that Ni (II) binding to some chromatin proteins in somatic cells may result in oxidative and structural damage to the proteins (Kasprzak et al., 2003). Nickel ions have a higher affinity for proteins and amino acids and have shown to produce oxidation of proteins in cells as measured by carbonyl formation (Costa et al., 1994). From *in vitro* and *in vivo* studies, it is known that nickel interacts with proteins resulting in toxic effects (WHO, 1991).

Similar mechanism might also be responsible for the reduced protein concentrations in liver and kidney by the combined treatment of nickel and chromium. Thus, it is evident that the overall decline in protein levels could be the outcome of its disturbed metabolism due to chromium and/or nickel treatment which could subsequently lead to a decline in activities of several enzymes in the affected tissues, their secretion as well as their structural integrity.

**ALKALINE PHOSPHATASE (ALKPase)**

Phosphatases are significantly associated with many functions at the cellular level. A wide range of industrial pollutants are known to cause adverse effects on alkaline and acid phosphatase activities.

Alkaline phosphatases are a group of enzymes which hydrolyse phosphatase at alkaline pH. Alkaline phosphatase has a ubiquitous distribution in all tissues of the body.
especially in the cell membrane where it is associated with the transport of metabolite across the membrane. It is highly sensitive to different heavy metals and its inhibition leads to disturbances to the cellular functions (Thaker et al., 1997).

In the present study, a marked decrease in alkaline phosphatase activity was observed in a dose dependent manner by nickel and/or chromium salts in the liver and kidney of mice. Previous work in our laboratory have also found a decline in the enzyme activity in liver by nickel and chromium salts administrated alone (Rao et al., 2003a,b, Rajvanshi, 2002). Dey et al. (2001) also reported a significant decline in alkaline phosphatase activity in rat liver and kidney after chromium treatment. Similar results were also obtained by Thakker et al. (1997) in the vital organs of mudskipper, _Periopthalmus dipes_ treated with Cr (VI). Rats treated with Cr (VI) also exhibited changes in alkaline phosphatase activity in liver and kidney (Kumar et al., 1985, ATSDR, 1998). Gumbleton and Nichollas (1988) also noticed kidney damage with elevation in the rate of urinary excretion of alkaline phosphatase in male rats treated with potassium dichromate.

Nickel chloride treatment at high dose level also exhibited a decline in the enzyme activity. Previous studies by Chatterjee et al. (1979) also reported changes in its activity in liver and kidney by ingestion of nickel sulphate in rats. Thus all these results are in agreement with our present findings.

The resulted inhibition in the enzyme activity might be due to an alteration in the membrane permeability brought about by binding of the heavy metal ions to the enzyme. It is also possible that the synthesis of this enzyme is impaired due to the direct effect of
heavy metal ions on the serine residue at the active site of the key protein synthesizing enzymes (Mehendru and Agarwal, 1983, Kumar and Ansari, 1986, Thaker et al., 1997)

ACID PHOSPHATASE (ACPase)

Acid phosphatase (ACPase), a lysosomal enzyme is involved in a number of activities such as phagocytosis, autolysis, dissolution of tissue components, fat absorption in intestine, cellular differentiation and keratinization (Novikoff, 1961).

The present investigation revealed a gradual decline in ACPase activity in the liver and kidney of mice treated with potassium dichromate and/or nickel chloride. In corroboration with the above data, drastic inhibition in the activity of acid phosphatase was observed in liver and kidney of mudskipper by the treatment of Cr (VI) (Thaker et al., 1997). Similarly changes and relocalization of acid phosphatase was observed in liver and kidney of rats exposed to Cr (VI) (Kumar et al., 1985, ATSDR, 1998). Similar results were obtained in the liver of chromium or nickel treated mice in our laboratory (Rao and Rajvanshi, unpublished data). Moreover, gradually heavy metal cations are known to exert marked effects upon phosphatase in various tissues (De Bruin, 1976) as noticed in our investigation.

Inhibition of this lysosomal enzyme, as observed from the results of the present investigations, may be due to the alteration in the membrane permeability of the lysosome since the heavy metal ions act as labilizing agents. As a result, release of all hydrolytic enzymes occurred (Kumar et al., 1985). These released enzymes quickly diffused in the cytoplasm and start cell autolysis ultimately causing cell death (Inskip and Petrowski, 1985, Thaker et al., 1997).
CREATININE

Creatinine concentration is an important measure of renal function. The data of current investigation revealed a decline in levels of creatinine in the kidney of mice by nickel and/or chromium treatments. Previous study by Ahmed et al. (1999) reported enhanced serum creatinine level by nickel administration to female rats indicating renal injury. Verschoor et al. (1988) also reported a significant increase in serum creatinine levels by Cr (VI) compounds in stainless steel welders. The decline in the tissue creatinine levels obtained in the present study might be due to enhanced serum creatinine levels. Thus the present study suggests an impairment in glomerular functions by the above mentioned treatments.

GLYCOGEN AND PHOSPHORYLASE

In the present study a significant increase in glycogen contrary to a decreased phosphorylase activity is observed in a dose dependent manner by feeding of chromium and nickel salts alone and in combination to mice for 30 days. In any instances of poisoning, the changes in glycogen storage is found to be correlated with dysfunctional and/or dystrophic changes in the liver (De Bruin, 1976). Changes in liver enzymes indicating altered carbohydrate metabolism were reported in rats after single dermal application of potassium dichromate (Merkur eva et al., 1982). In our laboratory, studies with chromium treatment indicated liver toxicosis by affecting glycogen metabolism in support to our findings (Rao and Rajvanshi, unpublished data). Further, Shagufta (1999) also reported that NiCl₂ feeding to mice brought about an increase in liver glycogen.
Followed by reduced phosphorylase activity. Gradual decrease in liver glucose breakdown was also observed in freshwater fish, *Channa punctatus* under the stress of nickel (NiSO₄·6H₂O) at various concentrations for 30 days (Desai et al., 2002). Intravenous application of nickel doses above 1 mg/kg to animals also inhibited glucose metabolism (Schafer et al., 1999). All the above data support our findings.

Glycogen is degraded to a limited extent by phosphorylase. Hence if the activity of phosphorylase decreases, glycogen is not converted to glucose which may lead to cell lysis. This can be correlated with the histology and ultrastructural changes in the liver which is discussed later in this chapter. Thus the above study suggests that carbohydrate metabolism is significantly affected in liver by these treatments.

**SUCCINATE DEHYDROGENASE (SDH)**

In the present study, the SDH activity had a significant decline in the liver of chromium and/or nickel treated mice. Succinate dehydrogenase is an oxidative enzyme involved in Krebs cycle. The decrease in its activity could affect the conversion of succinate to fumarate and can cause a block in the Krebs cycle altering the energy metabolism of the tissue. Moreover, SDH being a mitochondrial enzyme, a decline in its activity indicates a possible alteration in mitochondrial structure and function. Most metals are known to cause alteration in SDH activity and protein production (Wataha et al., 1995).

An inhibition of the mitochondrial enzymes in mammalian tissue and cells by nickel is demonstrated by binding with –SH groups of proteins (Shimada et al., 2000). Further in support to our data, Rubanvi and Balogh (1982) demonstrated nickel caused
mitochondrial structural damage affecting enzyme activity Chatterjee et al (1979) also reported changes in SDH activity in the liver by ingestion of nickel sulphate. The liver mitochondria are reported to accumulate Cr (VI) and hence inhibits the activity of SDH in animal mitochondria (Fernandes et al, 2002) Bag et al (1999) also reported a significant decline in liver SDH activity while studying the toxic affects of sludge–supplemented diets contaminated with nickel and chromium along with other heavy metals. Recent study in our laboratory also reported a significant fall in SDH activity in liver by nickel or chromium salts in male mice (Rao and Rajvanshi, unpublished data, Rao et al, 2003a,b) All the above studies corroborate with the present results. An immediate decrease in oxygen consumption might occur in the presence of nickel and/or chromium which may also cause alterations in the energy metabolism of mitochondria and the whole liver cells.

**CHOLESTEROL**

In the present study, no significant alteration in cholesterol levels was obtained by NiCl₂ or K₂Cr₂O₇ (low dose) treatment to mice but the high dose administration as well as combined treatment brought about a significant decline in its level. The decline in cholesterol of liver could probably be attributed to alteration in its synthesis, conversion and transport. In support of our data, chromium supplementation to normal adult men, as well as diabetics has been reported to increase high density lipoprotein cholesterol and decrease triglycerides and total cholesterol (Anderson, 1986). Similarly a study at Auburn university showed that chrome mate (chromium nicotinate) reduced LDL cholesterol in human by an average of 14%. In fact, chrome mate has been
awarded a patent for lowering cholesterol. Contrary to our result, toxicological studies carried out by Desai et al. (2002) on fresh water fish under stress of nickel sulphate revealed higher liver cholesterol level, while a biochemical study on nickel sulphate toxicity in rats showed almost no alteration in the hepatic lipid composition (Chatterjee et al., 1979). This discrepancy between above studies could be due to difference in animal species, dose, duration and mode of administration of chromium and nickel salts. Thus the present investigation by chromium and/or nickel treatment indicates an imbalance between the synthesis, transport and utilization of cholesterol and hence alteration in its levels.

**ANTIOXIDATIVE DEFENCE SYSTEM**

Certain meals are known to act as catalyst for the production of free radicals/ROS in biological systems (Panda, 2003). Free radicals/ROS are highly reactive species that have unpaired electrons e.g., the hydroxyl and superoxide radicals. The macromolecules like protein, nucleic acids, lipids and carbohydrates are at risk of oxidative damage due to their destabilization by increase in ROS. The most important consequences are lipid peroxidation increase and change in permeability of the cell membrane (Subramaniam et al., 1994).

Lipid peroxidation is a free radical mediated process that has been implicated in a variety of disease states. Lipid peroxidation involves the formation and propagation of lipid radicals, the uptake of oxygen and a rearrangement of the double and unsaturated lipids, resulting in a variety of degraded products that eventually cause destruction of membrane lipids. Increased peroxidation can result in changes in cellular metabolism of
hepatic and extra hepatic tissues (Das et al., 2001) Lipid peroxidation is often discussed as a cause of metal induced toxicity (Ames et al., 1982).

In the present study, lipid peroxidation was assayed as a marker of oxidative damage to the lipids in the liver of mice treated with nickel and/or chromium salts. The current data revealed a significant increase in the lipid peroxidation levels in liver of treated mice. This increased lipid peroxidation suggests an increase of phospholipase activities during peroxide decomposition of different suborganelles and plasma membrane lipids. The peroxide generated must stimulate phospholipase A$_2$ (PLA$_2$) activity on the cell membrane. Activation of PLA$_2$ causes the production of a variety of eicosanoids, which are responsible for different metabolic disorders and cell injury (Halliwell and Gutteridge, 1989).

It is reported earlier that parenteral administration of nickel chloride (NiCl$_2$) to rats enhanced lipid peroxidation in liver (Sunderman et al., 1985; Donskoy et al., 1986). Similarly Stinson et al. (1992) also reported nickel chloride induced hepatic lipid peroxidation under in vitro and in vivo conditions. Nickel sulfate also induced lipid peroxidation in liver of male rats (Das et al., 2001). Misra et al. (1990) further noted that high level of lipid peroxidation in liver and kidney might result in increased concentration of H$_2$O$_2$ in these tissue by nickel and this increase concurred with transiently decreased activity of H$_2$O$_2$ scavenging enzymes like catalase that are known to be inhibited by nickel (Rodriguez and Kasprzak, 1989). These two effects combined could augment the potential of oxidative cell damage. Chromium is also found to effect LPO at both concentrations thus leading to cell injury. Mao et al. (1995) in their study demonstrated increased levels of LPO in the liver having the higher activity, thus
demonstrating the potential of chromium to generate free radicals. Chromium nitrate or potassium dichromate also induced LPO (Ueno et al., 1988). Chen and Lin (1998) too reported that the effect of oxidative stress is to damage membrane lipids in a process known as lipid peroxidation. Bagchi et al. (2002) reported an increase in lipid peroxidation in mice liver by Cr (VI) treatment. Moreover, recent studies in our laboratory indicated increased levels of LPO in liver of male mice treated with chromium or nickel (Rajvanshi, 2002). All the above results are in agreement to our present investigation.

The data of current investigation revealed that chromium and nickel induced lipid peroxidation followed by a decrease in the activities of superoxide dismutase and catalase, -SH groups and glutathione levels discussed further in this chapter.

There are several physiological mechanisms (antioxidative defense mechanism) that dispose off free radicals/ROS by directly scavenging them or by interrupting the already occurring lipid peroxidation chain reaction and thus limit their tissue damaging effects. The antioxidants which are involved in curbing these harmful effects include water soluble compounds such as ascorbate, -SH groups and glutathione and enzymes such as superoxide dismutase and catalase.

In the present study, total -SH groups had a significant decline by the treatment of chromium and nickel alone and in combination to mice. One of the mechanisms by which heavy metals produce toxic effects is through their interaction with cellular sulphhydryl groups in proteins or elsewhere. Sulphhydryl groups thus not only serve as a source of electrons for reduction but they also mediate the methylation process. When the availability of free thiol groups is low, enhanced expression of toxicity in form of
oxidative damage might occur. The total thiol groups reduction in our study thus indicates toxicity status of tissue induced by heavy metal poisoning.

Glutathione comprises up to 90% of the non-protein thiol content of mammalian cells and performs a pivotal role in maintaining their metabolic and transport functions. It acts as a nucleophilic 'scavenger' of many compounds and their metabolites via enzymatic and chemical mechanism, converting electrophilic centers to ether bonds. Its depletion to about 20% to 30% of total glutathione levels can impair cell defenses against toxic actions, which may lead to cell injury and death (Reed, 1990). Glutathione conjugation helps in detoxification by binding electrophiles that could otherwise bind to proteins or nucleic acid, resulting in cellular damage and genetic mutation (Seidegard and Ekstron, 1997, Lauterberg et al., 1980).

In the present investigation, after nickel and/or chromium treatment, the liver glutathione levels registered a decrease. This could be because GSH is involved in the mechanism of detoxification of various xenobiotics (Meister and Anderson, 1983), inhibition of lipid peroxidation by scavenging free radicals (Li et al., 1999, Satsangi and Dua, 2000) as well as reducing dehydroascorbic acid to the reduced form.

Chromium is found to affect GSH levels in various tissues. This could be due to the participation of glutathione (GSH) in the formation of chromium complexes which are excreted, thus exerting protection over toxicosis of the liver. If glutathione is depleted or markedly reduced in liver, the hepatotoxicity would be expected to be enhanced (Lewin, 1976). Corroborating with our report, a significant decline in glutathione (GSH) levels in the hepatic tissue was observed by the administration of chromium picolinate in all the treated groups (Bagchi et al., 2002). Nickel feeding also resulted in reduced GSH.
levels with an increase in lipid peroxidation. Misra et al. (1990) also observed that nickel treatment depletes pre-existing GSH levels which can be explained by the concept that nickel binds to GSH thus reducing its levels. Further in support to our data, Das et al. (2001) also showed that nickel induced a decrease in glutathione levels which may be due to its increased use in protecting –SH containing proteins from lipid peroxides. Similar mechanism might be responsible for the reduced glutathione levels in combined treatment of chromium and nickel in the present study.

The significantly suppressed GSH levels would further aggravate the toxic effects of chromium and nickel. Since GSH is also known to scavenge free radicals, its depletion might lead to their impaired clearance and cause the probable accumulation of heavy metals and lipid peroxides in different tissues of treated mice.

Ascorbic acid (AA) is known to be a powerful reducing reagent which helps in activating several enzymes and acts as an antioxidant for detoxifying several toxic substances (Kutsky, 1973). Depletion of ascorbic acid indicates its involvement in overcoming stress (Chinoy, 1978). A decrease in levels of total and reduced ascorbic acid (TAA and RAA) concomitant with an increase in the dehydroascorbic acid (DHA) was obtained in liver of mice administered with chromium or nickel alone and in combination in the present study. This suggests that the stored ascorbic acid is rapidly oxidized in liver under chromium and/or nickel induced stress and is converted to its dehydro form which consequently increases gradually. In support to our data, gradual decrease in the levels of liver ascorbic acid was observed in fresh water fish Channa punctatus under the stress of nickel (Desai et al., 2002). Similarly Das et al. (2001) also reported altered ascorbate metabolism and hence severe liver damage by nickel treatment. The protective action of

It is thus evident that both chromium and/or nickel caused a disturbance in the utilization and probably synthesis of liver ascorbic acid leading to a change in its metabolism which might be influenced by decrease in total –SH groups including glutathione (GSH) in the present study, as GSH is involved in reducing dehydroascorbic acid to reduced form.

The enzyme superoxide dismutase (SOD) catalyzes dismutation of superoxide radical leading to formation of hydrogen peroxide which in turn is detoxified by the enzyme, catalase (Rzeuski et al., 1998).

In the present study, chromium and/or nickel caused a decrease in the activities of free radical scavenging enzymes viz superoxide dismutase (SOD) and catalase but increased the lipid peroxidation in liver of mice. In corroboration with the above findings, Misra et al. (1990) demonstrated decreased activity of catalase in the liver of nickel treated rats. According to Lefebvre and Pezerat (1994) ROS production is to be inhibited by catalase. Das et al. (2001) also reported decreased activity of SOD and catalase with an increase in lipid peroxidation in liver of nickel treated rats. These results are in agreement with those of Bagchi et al. (2002) who observed a decrease in SOD and catalase activities in hepatic tissue with increased lipid peroxidation.

The results obtained in the present study support the view that SOD and catalase are enzymes that scavenge free radicals during lipid peroxidation induced in liver by chromium and/or nickel salts. It is also concluded that the cytotoxicity of molecular
oxygen is checked by the delicate balance between the rate of generation of the partially reduced oxygen species and the rate of their removal by different defense mechanisms. A shift in this delicate balance can lead to cellular damage (Step, 1993).

**HAEMATOLOGY**

The present data showed a reduction in haematological parameters viz total erythrocyte counts, total leucocyte counts and haemoglobin content by chromium and/or nickel intoxication to mice.

The gradual reduction in total erythrocyte count leads to physiological dysfunction of haemopoietic system which may be due to a decrease in rate of erythrocyte synthesis (erythropoiesis) and destruction of erythrocyte directly (Siddiqui et al., 1987). Further, the decrease in RBC counts was also correlated with a significant fall in the blood haemoglobin levels causing anaemia.

WBC counts also had a significant decline in mice fed these toxicants. These effects are probably related to the accumulation of chromium in haematopoietic tissue (ATSDR, 1998). Decreased haemoglobin was also observed by Mancuso (1951) in workers occupationally exposed to chromium. Effects like anaemia, leukocytosis and decreased haemoglobin have been observed in human (ATSDR, 1998) in support of our data.

Nickel treatment also followed the same trend as that of chromium. Previous studies documented a reversible decrease in hemoglobin content and leucocyte counts in rats after oral exposure to nickel (Whanger, 1973; Węscher et al., 1980, American
Thus the above data reveals the toxic effect of nickel and/or chromium on blood parameters in mice.

Serum parameters are generally considered as diagnostic indicators of liver damage. Some marker enzymes for liver toxicity, viz. serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) along with serum protein and serum cholesterol levels were investigated after the administration of chromium and/or nickel salts. The results of the present investigation exhibited a dose dependent increase in the activities of SGOT and SGPT and serum cholesterol levels while a gradual decrease in serum protein levels.

Increase in levels of serum enzymes are usually associated with damage or death of cell. The elevation of transaminases in serum indicate pathological changes such as necrosis of hepatocytes which in turn result in membrane lesions. Previous studies by Fristed et al. (1965) and Kaufman et al. (1970) reported increased SGOT and SGPT activities accompanied by liver necrosis in support of our data. Chatterjee et al. (1979) also observed increased SGOT activity in rat administered toxic doses of nickel.

The determination of serum cholesterol is considered to be significant in various liver diseases under certain pathological conditions. In the present study, increase in serum cholesterol levels could be attributed to its altered metabolism and transport due to the stress imposed by chromium and nickel. Previous study by Glaser et al. (1990) also reported increased serum cholesterol by heavy metal exposure which supports our data. Serum cholesterol accumulation might be responsible for its reduction in tissue as noted in this study.
Serum protein analysis can diagnose liver disorders. Decrease in total protein values is associated with cirrhosis of the liver and other liver disorders and neoplastic disease. In the present study, the decline in total serum protein levels could be the outcome of its disturbed protein synthesis due to chromium and/or nickel salt poisoning.

Thus, the results of the present investigation confirm hepatocellular damage and change in liver function as evident by the alteration in the above hematological parameters, histopathological and ultrastructure studies described later as a result of metal poisoning.

**HISTOPATHOLOGICAL AND ULTRASTRUCTURAL STUDIES**

All the above biochemical changes that affected liver and kidney are further supported by the histopathological studies. In the evaluation of liver response to a toxicant, the morphological status is one of the main parameters to be considered. Pathological disturbances in the hepatic cell are expressions of molecular derangements. Hepatotoxins like chromium and nickel disturb the metabolic network of the liver leading to morphologically evident lesions. The liver of chromium and/or nickel treated rats showed a variety of histopathological lesions.

In the present study, liver of nickel fed mice exhibited degenerative changes varying from cell necrosis to cytoplasmic vacuolization which corroborates with the observation of others (Mathur et al., 1977, WHO, 1991). Earlier investigations by Donskoy et al. (1986) have demonstrated microvesicular steatosis, fatty metamorphosis, mild hydropic degeneration and foci of inflammation in livers from rats treated with NiCl₂. Our findings have been further substantiated by histopathological changes in liver.
of nickel treated lake white fish which included areas of focal necrosis and altered bile ducts (Ptashynski et al., 2002)

Similarly liver of potassium dichromate treated mice also exhibited extensive degenerative changes like vacuolization, necrosis, disorganization of hepatocytes, pyknotic nuclei and fatty deposition. In corroboration with the above results, hepatocyte cytoplasmic vacuolization was reported in male and female rats treated with Cr (VI). Marked liver necrosis was also reported by Kaufman et al. (1970) in an incidence of Cr (VI) induced toxicity. Similar studies by Baines (1965), Gale (1978) and Laborda et al. (1986) also observed histopathological changes in liver of mammals by Cr (VI). Cr (VI) has also been reported to cause liver effects in workers exposed to chrome plating industry which includes derangement of cells in liver, necrosis, lymphocytic and histiocytic infiltration and increase in Kupffer cells (ATSDR, 1998).

The kidney of chromium and/or nickel treated mice also showed histopathological lesions characterized by increased periglomerular space, increased peritubular space, shrunken and necrotic glomerular tuft, deposition of hyaline casts, loss/necrosis of epithelial cells and pyknosis. In corroboration with the present investigation, nephrotoxicity manifested by glomerular and tubular histopathology is reported in NiCl₂ treated rats (Gittelz et al., 1975). Previous studies by Foulkes and Blanck (1984) reported that nickel is a nephrotoxin in animals with the glomerular epithelium being the target of nickel toxicity. Renal tubular damage was also observed in animals after oral exposure to nickel (Dieter et al., 1988). Similarly, renal failure and necrosis of renal tubules have been reported in case of fatal or near fatal ingestion of Cr (VI) compounds by human (Clochesy, 1984). Several other renal studies in rabbits, hamsters and rats also
marked tubular necrosis, derangement of glomeruli and infiltration of lymphocytes after Cr (VI) treatment (Mathur et al., 1977; Gale, 1978, Laborda et al., 1986) in light of our data.

The biochemical and histological changes are further supported by ultrastructural studies. Ultrastructural studies in liver of mice treated with nickel and/or chromium revealed altered mitochondrial morphology with denudation of cristae, extensive vacuolization and increased glycogen granule deposition in the cytoplasm. The morphology of mitochondria was altered in accordance with the metabolic state of the cell and with pathological processes. Disintegration of few mitochondria indicates structural changes of inner mitochondrial membrane which in turn leads to a loss of ATP synthesizing ability and a failure of ATP dependent functions viz., functioning of sodium pump at plasma membrane (Chongwan and Dathei, 1988). The fragmentation of mitochondria and degranulation also suggest suppression of protein synthesis. The decrease in SDH activity by chromium and/or nickel could also be attributed to the altered ultrastructure of mitochondria. Further, increased glycogen granule deposition by Cr and/or Ni treatment could be correlated with the altered carbohydrate metabolism in liver as evidenced by increase in the glycogen levels and decrease in the phosphorylase activity.

The ultrastructural studies of kidney in Ni and/or Cr treated mice revealed disorganized podocytes with denuded and stumpy foot processes which appeared to be reduced in number. Thickening of glomerular capillary basement membrane was also observed. Changes in the renal tubules mainly included are disorganization of the tubular basement membrane, changes in the morphology of nucleus with irregular nuclear...
membrane and remarkable changes in the mitochondrial morphology. Vacuoles were observed in the renal tubule by the combined treatment of Ni and Cr. Mitochondrial changes included ruptured membrane and disintegration of cristae which caused vacuolization in mitochondria. All these changes were more pronounced by the combined treatment of Ni and Cr leading to impairment of kidney functions which is evidenced by alteration in creatinine levels. The fragmentation of mitochondria might cause suppression of protein synthesis which in turn affects the activity of other enzymes in kidney. Thus the biochemical, histopathological as well as the ultrastructural studies confirm hepatocellular as well as renal damage by chromium and/or nickel treatments which seemed to be more pronounced with higher doses and combined treatment.

In the present study, individual supplementation of vitamin A and vitamin E along with NiCl₂ + K₂Cr₂O₇ treatment exhibited mitigating effects in liver and kidney. The enzymes studied viz SDH, phosphorylase, phosphatases, catalase, SOD etc did not alter significantly and the results obtained were comparable to the control values. The histology and ultrastructural studies also revealed no significant variation by vitamin supplementation and were almost comparable to the control. The therapeutic agents individually produced no effect.

Previous studies by Iyama et al (1996) reported that beta-carotene can suppress lipid peroxidation in mouse liver and kidney. According to Moreno et al (2002), vitamin A administration tended to reduce the total number of persistent hepatocyte nodules. Another study by de Freitas et al (2003) documented that administration of vitamin A significantly reduced hepatic fibrosis induced by biliary obstruction. Thus the above studies confirm that vitamin A is a therapeutic antioxidative agent capable of minimizing...
the toxicity of various natural and man made mutagens due to its free radical scavenging nature (Rajvanshi, 2002)

Vitamin E has come under much scrutiny for its possible therapeutic roles in numerous disease states especially involving oxidation related events (Phelps, 1987). It is a proven antioxidant with a property of scavenging free radicals and hence maintaining the integrity of lipid structure \textit{in vivo}. Leibovitz et al. (1990) reported that vitamin E exhibited greatest protection against lipid peroxidation in liver and kidney. Experimental studies by Sokol (1996) and Appenroth et al. (2001) have reported that antioxidants of the vitamin E family have protective effects against metal toxicity. Sugiyama et al. (1991) noted that vitamin E can protect cells from the clastogenic and mutagenic action of chromate compounds possibly through its ability to scavenge \textit{Cr} (V) and/or free radicals. Studies by Susa et al. (1996) reported that pre-treatment with vitamin E normalized the levels of non enzymatic antioxidants such as glutathione and vitamins suppressed by dichromate. Thus the above studies corroborate with the present investigation and confirms the role of vitamin E as a scavenger of free radicals, thus preserving the structural and functional integrity of sub-cellular organelles thereby protecting tissue/organ functions.

Thus, it is clear that chromium and/or nickel induced toxic effects in liver and kidney of treated mice due to their probable accumulation in them. These effects are more pronounced by combined treatment as they might act synergistically in biosystems. By simultaneous feeding of vitamins, these effects were not observed due to their antioxidant properties. Hence vitamins may be of clinical significance in population exposed to heavy metal poisoning.