Discussion
4. DISCUSSION

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Cisplatin is an important chemotherapeutic agent used for the treatment of a large variety of solid tumors. The principle limiting factor of this drug is its renal toxicity. The acute toxicity of cisplatin is well known (Jones et al., 1980). The pathological changes in nephrotoxicity consecutive to platinum administration consist essentially in tubular disease (Choie et al., 1981).

Glutathione ester effectively transported into cells and converted intracellularly into glutathione (Anderson, 1997). Glutathione, a major non-protein cellular thiol, participates in numerous cellular functions including protection from free radical damage and detoxification of chemotherapeutic agents (Bernstein et al., 1995). In this study, the modulating efficacy of glutathione ester on cisplatin induced nephrotoxicity is highlighted by discussing with various biochemical parameters.

4.1 Body weight and blood glucose

The body weight of cisplatin treated rats were significantly reduced. Cisplatin causes an appetite suppression which limits food intake, since the treatment with cisplatin induces the loss of body weight (Tokunaga et al., 1996). It remains, however, open as to whether the reduced food intake is causally linked to damage of the intestinal mucosa. Luminal sulphate and D-glucose transport was inhibited due to cisplatin administration correlating with the degree of weight loss (Ammer et al., 1993).
Glucosuria induced by cisplatin was not due to a direct impairment of glucose transporter in brush border membranes but due to an inhibition of Na⁺-pump activity and a decrease in area for active glucose reabsorption in the proximal tubule (Kim et al., 1995). Halabe et al. (1991) reported that a significant decrease in sodium dependent glucose transport was observed in cisplatin treated rats. It appears to involve a depletion of intracellular glutathione or attachment of sulphydryl groups (-SH) of proteins necessary for enzyme function (Clarkson, 1972). -SH groups play an important role in a variety of active transport processes (Meister, 1989).

4.2 Nephrotoxicological parameters

Elevation in urea concentration is considered to be an indicator of nephrotoxicity in cisplatin treated rats (Reeves and Saari, 1990; Li et al., 1994). Urea "recycling", indicating urea entry between the late surface proximal tubule and early distal tubule was absent in cisplatin treated rats and instead urea reabsorption was seen (Safirstein et al., 1981). Since the pars recta of the proximal tubule is selectively damaged by cisplatin, Safirstein et al. (1981) suggested that failure of urea secretion in this nephron segment could account for this finding. Failure of urea recycling in the loop of Henle would undoubtedly contribute to reduced interstitial solute concentration.

Uric acid level was significantly increased in cisplatin treated rats. The pars recta, a site of intracellular accumulation of platinum, is a major site of uric acid secretion (Holmes et al., 1972). On the other hand, high uric acid
levels may indicate an underlying renal abnormality, ie., the cisplatin could further affect an already "damaged" kidney (Nanji et al., 1986).

Nosaka et al. (1992) reported that the creatinine level was significantly increased in the serum of cisplatin treated rats. Since, creatinine is filtered in the glomerulus, the increased level of creatinine in serum may be due to a primary decrease in glomerulus filtration and tubular fluid back leak in addition to tubular obstruction (Choie et al., 1981; Chopra et al., 1982).

Creatinine clearance was found to be decreased in cisplatin administration. This parameter was included in the study as a marker of glomerular filtration rate (GFR) and glomerular haemodynamics. Acute renal failure indicated by a reduced creatinine clearance occurred development of tubular necrosis in cisplatin induced nephrotoxicity (Gaedeke et al., 1996; Bokemeyer et al., 1996).

Glutathione significantly depressed cisplatin induced blood urea nitrogen and was also maintained on the 14th day after cisplatin administration (Sadzuka et al., 1992a). Bernstein et al. (1995) reported that modulation of cisplatin nephrotoxicity is by glutathione. Since, kidney metabolises large amount of glutathione, one might have expected preferential protection of the kidney through the utilisation of exogenous glutathione.

The glutathione mediated decrease of creatinine level was also observed in methylthiobenzoic acid treatment (Husain et al., 1996).
Similarly, we found reduced level of urea, uric acid, creatinine and increased creatinine clearance in cisplatin and glutathione ester administered rats.

Administration of glutathione ester along with cisplatin could accumulate glutathione in the kidney, which is a major organ metabolising serum glutathione which preferentially could have reduced cisplatin induced renal toxicity.

Albumin and globulin levels were was significantly decreased in cisplatin induced nephrotoxic rats. Cisplatin is rapidly and virtually irreversibly bound to plasma albumin and other proteins (Repta and Long, 1980). This suggests that cisplatin binding to albumin may sequester the cisplatin, there by reducing kidney uptake. Alternatively, the possible effect of albumin on platinum distribution in kidney and its effect on cisplatin nephrotoxicity could be mediated through its effect on peritubular capillary resorption and intra renal haemodynamics (Brenner and Troy, 1971).

Glomerular proteinuria can result from increased permeability of the glomerulus which is due to either an enlargement of pores or a loss of the negative fixed charges of the glomerular capillary walls. Restriction by negatively charged glomerular capillary components seems to be important for the reduction in filtration of polyanions such as albumin (Bridges et al., 1982; Brenner et al., 1976). Another explanation for increased excretion of high - molecular weight protein is incomplete reabsorption by the proximal tubules.
The lowering of renal function is reflected by a decrease in tissue protein concentration in the kidney. Such a decrease in protein concentration compared to tissue weight is caused by the inhibitory effect of cisplatin on DNA biosynthesis (Sadzuka et al., 1991). The another possible mechanism of tubular toxicity may result from the intrinsic property of cisplatin to depress DNA, RNA and protein synthesis (Tay et al., 1988; Yasumasu et al., 1992).

Silibinin a radical scavenger, although it also has membrane stabilising and regenerative properties (Ferenci et al., 1989). It is known to up-regulate the function of a DNA-dependent RNA polymerase I in liver cells (Sonnenbichler and Zetl, 1987) and may thereby counteract the decrease in synthetic activity of the kidney. Silibinin advantages compared with intracellular radical scavenger, such as sodium thiosulphate.

Similarly, the administration of glutathione ester modulates the albumin, globulin and total protein levels in cisplatin induced nephrotoxicity.

4.3 Lysosomal and transaminase enzymes

Transport enzymes are of biological importance within the kidney and elsewhere throughout the organism. Inactivation of these enzymes would tend to alter both the function and the viability of kidney tubules. In our study, the levels of alkaline phosphatase and acid phosphatase were found to be significantly decreased in kidney. Acid phosphatase present in lysosomes throughout the kidney and increased substantially after cDDP treatment which is similar to that of alkaline phosphatase. In the case of acid phosphatase
lysosomal buildup occurs until the cell lyse, releasing their enzymes in the urine in a functional form. The decrease in the membrane enzyme levels in the kidney may corresponds to an increase in urinary alkaline phosphatase, suggesting a possible discharge into the urine (Batzer and Aggarwal, 1986).

Hepatopathy, one of the cisplatin induced adverse effects, has not been well understood. There have been reports on cisplatin hepatopathy (Sadzuka et al., 1994a; Treskes et al., 1992), but clinically useful modalities are very few. Renal failure in rats treated with cisplatin could be the result of the lower enzymatic activities in the kidney as evidenced by the decreased activities of aspartate aminotransferase and alanine aminotransferase along with the acid and alkaline phosphatases (Bogin et al., 1994).

The effect of cisplatin was much greater on the kidney than the liver. This could be evidenced by slight increases in the activities of the enzymes studied (Table 5) in liver.

The serum enzymes alkaline phosphatase, acid phosphatase, aspartate aminotransferase and alanine aminotransferase are commonly elevated following cellular damage as a result of enzymes leakage from cells to the blood. Cisplatin treated animals have been reported to have increased activities of serum marker enzymes (Sadzuka et al., 1994a; Treskes et al., 1992; Ueda et al., 1998).

It has been postulated that the nephrotoxic mode of action of the drug cisplatin is similar to that of other heavy metals and is related to the decrease
in the intracellular concentrations of glutathione and protein-bound sulphydryl groups, which are required for normal cellular functions (Levi et al., 1980; Litterst and Weiss, 1987; Borch and Markman, 1989).

Glutathione is a potent endogenous antioxidant that protects major organs from oxidant injury. However, present nutrition regimens may inadequately support tissue stores of this tripeptide during critical illness (Robinson et al., 1992).

Administration of glutathione ester thought to be a cellular protectant by which it maintains a large pool of glutathione normalising the lysosomal and transaminase enzymes level in kidney, liver and serum of cisplatin treated rats.

4.4 Non-enzymic antioxidants

The decrease in glutathione level and depression of macromolecule synthesis in kidney may play a role in cisplatin induced nephrotoxicity (Goldstein and Mayor, 1983). Glutathione and endogenous free thiol, has been reported to decrease cisplatin nephrotoxicity (Somani et al., 1995). This depletion of glutathione seems to be a prime factor that permits lipid peroxidation (Younes and Siegers, 1981). However, free radical production has been suggested in cisplatin nephrotoxicity, causing oxidative damage (Hannemann and Baumann, 1988a; Sugihara et al., 1987).

Cisplatin treatment significantly decreased the glutathione activity in both kidney and liver (Dwivedi et al., 1996). Cellular glutathione plays an
important role in the detoxification of metals. Depletion of glutathione may increase susceptibility of animals to the toxicity or lethality of mercury (Baggett and Berndt, 1986; Singhal et al., 1987).

In our study, ascorbic acid level was found to be significantly decreased in cisplatin induced renal damage. In vitro, vitamin C was shown to be able to ameliorate cisplatin induced injury (Gemba and Fukuishi, 1991).

One of the vital roles of ascorbic acid (Vitamin C) is to act as an antioxidant to protect cellular components from free radical damage as does vitamin E. Additionally, vitamin C has been proven to protect membranes and other hydrophobic compartments from such damage by regenerating the antioxidant form of vitamin E (Packer et al., 1979; Beyer, 1994).

The concentration of vitamin E in renal tissue was significantly decreased after the administration of cisplatin. Sugihara and Gemba, (1986) suggested that the toxic effects of cisplatin may be related to free radical induced damage. The concentration of vitamin E in the renal tissue of adult rats was shown to decrease significantly after the administration of cisplatin (Appenroth et al., 1997).

The interaction of vitamin E and cisplatin induced nephrotoxicity described few. α-tocopherol was found to be able to prevent the increase in lipid peroxides (Sadzuka et al., 1992a).

Vitamin E functions as a trap for lipid peroxyl and other radicals by effectively inhibiting the peroxidation of cellular membranes (Witting, 1980).
The decreased levels were observed in total thiol, protein thiol and non-protein thiol in cisplatin treated rats. The nephrotoxic effects of cisplatin might be due to -SH binding with the heavy metal. Platinum(II) shows high affinity for sulphur-containing species such as protein bound -SH groups (Howe-Grant and Lippard, 1980; Melius and Friedman, 1977). Similarly, mercury also the most extensively studied (Magos, 1971; Clarkson, 1972) and is known to react with sulphydryl groups (Cafruny et al., 1955).

The nephrotoxicity of the heavy metals appears to be related to a depletion of intracellular glutathione or attachment to -SH groups of proteins necessary for enzyme function (Clarkson, 1972). Levi et al. (1980) findings, suggested that the possibility of the nephrotoxic effects of cisplatin may be related to depletion of -SH groups.

In the rat renal proximal tubule, cDDP induces a decrease in protein-bound thiols which like glucosuria, occurs before the elevation of blood urea nitrogen levels (Mistry et al., 1991).

In our study, decreased activity of ceruloplasmin in cisplatin induced nephrotoxicity was observed. Ceruloplasmin - bound copper does not accelerate radical reactions, i.e., ceruloplasmin inhibits copper-dependent lipid peroxidation. However, a more important antioxidant feature of ceruloplasmin is shown to depend on its ferroxidase activity. Ceruloplasmin catalyses the oxidation of Fe$^{2+}$ to Fe$^{3+}$ with simultaneous reduction of O$_2$ to H$_2$O. Ceruloplasmin is shown to have some superoxide dismutase activity, i.e., O$_2^-$ scavenging activity (Goldstein et al., 1979).
Glutathione serves as major reductant that maintains certain important proteins and enzymes in the reduced, active state through a thiol-disulphide interchange reaction (Mannervik et al., 1989) and regenerates other small molecular weight antioxidant such as α-tocopherol (Chan et al., 1991) and ascorbate (Martensson and Meister, 1992). Depletion of cellular glutathione may reduce the cellular capacity to scavenge free radicals and reactive oxygen species, thereby raising the general oxidative potential in the cells.

Administration of glutathione ester which is converted into glutathione and is very easily transported into cells, replenishing the antioxidant pool. Thus, glutathione ester modulates the observed disturbances in non-enzymic antioxidant levels in cisplatin treated rats.

4.5 Antioxidant enzymes

Superoxide dismutase plays an important role in the dismutation of superoxide anions by catalysing their conversion to hydrogen peroxide and singlet oxygen.

Cisplatin inhibits activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in rat kidneys (Sadzuka et al., 1992b; Sakthisekaran et al., 1992) and SOD treatment in vivo attenuates cisplatin nephrotoxicity in rats (McGinness et al., 1978), suggesting that cisplatin cytotoxicity results from the generation of reactive oxygen species (ROS). In fact, it has been reported that the direct interaction
of cisplatin and DNA generates superoxide in cell-free system (Masuda et al., 1994). Therefore, it is expected that SOD and catalase would exert protective effects against cell injury and lipid peroxidation.

Superoxide dismutase has been reported to contain arginine and histidine in its active site (Malinowski and Fridovich, 1979). Metal ions like copper and zinc plays an important role in the activity of superoxide dismutase and interaction of hydroxyl radicals was reported to induce loss of activity (Fee and Brigs, 1975). Sharma, (1985) has also demonstrated that renal excretion of magnesium, zinc and copper were markedly increased during cisplatin treatment. In cisplatin treated rats, there was a significant decrease in superoxide dismutase activity.

The activity of the antioxidant enzyme catalase was found to be significantly decreased in cisplatin treated rats. Catalase has been shown to be responsible for the detoxification of significant amounts of hydrogen peroxide (Deduve and Baudhuin, 1966; Chance et al., 1979) and the decrease in catalase activity could be due to free radicals, which are expected to be generated by the administration of cisplatin in experimental rats. Catalase has also been shown to be inactivated by hydrogen peroxide and superoxide anions (Sinet and Carber, 1981). Sadzuka et al. (1992b) found that the activity of catalase was significantly decreased after cisplatin administration.

The specific decrease in the activity of catalase and glutathione peroxidase which are hydroperoxide scavenging enzymes in the kidney after cisplatin administration, enhances the production of hydroperoxides.
The selenium dependent glutathione peroxidase activity was significantly decreased in cisplatin treated rats. Selenium is an essential trace element in human beings and it plays a key role in the activity of glutathione per oxidase. A decrease in glutathione peroxidase activity is thought to be linked with a decrease in oxygen metabolic generation (Chow, 1979). The activity of glutathione peroxidase was decreased significantly in cisplatin treated kidney mitochondria. These results suggested that mitochondrial dysfunction was closely related to a decrease in the activity of glutathione peroxidase (Sugiyama et al., 1989).

Glutathione peroxidase plays a role in the destruction of lipid hydroperoxides and hydrogen peroxide (H$_2$O$_2$) generated by enzymic and non-enzymic reactions in the cell. Therefore, a decrease in the glutathione peroxidase activity could cause accumulation of H$_2$O$_2$ and lipid hydroperoxides, thus leading to deleterious effects. These results correlate well with those of Vernie et al. (1988) and Milano et al. (1988) who showed that cisplatin inhibited erythrocyte glutathione peroxidase.

Husain et al. (1996) showed decreased activities of renal SOD, CAT and GPx in cisplatin administration. The inhibition of antioxidant enzyme activity (AOE) may be because of: (1) direct binding of cisplatin to essential sulphhydryl groups at the active sites of these enzymes, (2) depletion of copper and selenium which are essential for SOD and GPx activities (DeWoskin and Reiviere, 1992), (3) increased reactive oxygen species and organic peroxides which inactivate antioxidant enzymes (Pigeolet et al., 1990), and (4) depletion of GSH and NADPH which are essential for GPx activity.
Glutathione is a non-protein sulphur compound with numerous biological functions that include inactivation of peroxides and free radicals, production of sulphhydryl groups of proteins and detoxification of foreign substances.

Administration of glutathione ester prevents lipid peroxidation by protecting the free radical scavenging enzymes such as SOD, CAT and GPx in cisplatin induced renal damage.

The decrease in cisplatin-induced lipid peroxidation in the kidney and the liver of rats treated with \( \alpha \)-tocopherol and glutathione might have been related to favourable changes in the activity of lipid peroxidation protecting enzymes (Sadzuka et al., 1992b).

Cisplatin inhibits the renal antioxidant system and 4-methylthiobenzoic acid mediated protection is related to sparing of glutathione levels and activities of SOD, CAT and GPx. The increased renal glutathione level in 4-methylthiobenzoic acid protected rats suggests a role of intracellular glutathione in preventing cisplatin induced nephrotoxicity (Husain et al., 1996). Similarly, glutathione ester does, also suggests the importance of intracellular glutathione in protection against cisplatin induced nephrotoxicity (Anderson et al., 1990).

4.6 Lipid peroxidation

McGinness et al. (1978) and Sugihara et al. (1987) suggested that cisplatin-induced nephrotoxicity is related to increases in lipid peroxide levels
Sadzuka et al. (1991) reported that these levels increased after cisplatin treatment, especially in the kidney. The increase in in vivo lipid peroxide level was mediated by a mechanism other than the direct peroxidation of membrane lipids induced by cisplatin.

A significant increase in lipid peroxide levels in the kidney has been suggested by endogenous or exogenous generation of free radicals in cells (Gemba and Sugihara, 1986) showed that cisplatin-induced lipid peroxidation in the kidney is a direct action of cisplatin or may be related to free radical mediated kidney damage.

A significant increase in cisplatin induced lipid peroxidation may be due to abnormal levels of reactive oxygen radicals which react with membrane lipids, causing an increase in lipid peroxidation (Babu et al., 1995). Due to the decreased levels of antioxidant enzymes, the lipid peroxidation reaction is not neutralised and hence, there is enhanced susceptibility to lipid peroxidation.

The lipid peroxidation reaction was found to be increased in rats treated with cisplatin. Elevated iron levels have been observed in kidneys of rats following cDDP treatment (Shenberg et al., 1989) and iron overload may enhance the rate of lipid peroxidation in animal organs (Younes et al., 1989). This suggests that cisplatin treated rats may be less resistant and more susceptible to lipid peroxidation in the presence of promoters like ascorbate and ferrous sulphate (Anuradha and Selvam, 1993). These results provide evidence that cisplatin may generate free radicals, which can interact with
membrane lipids and consequently cause the production of lipid hydroperoxides.

The malondialdehyde concentration decreased in kidney tissues with subsequent administration of a thiol compound, diehtyldithiocarbamate. This shows the protective effect of thiol compounds against cisplatin induced renal damage (Somani et al., 1995).

Sadzuka et al. (1992) suggested that glutathione decreases cisplatin induced lipid peroxidation by replenishing the antioxidant enzymes, or by increasing the concentration of antioxidants and without affecting the tissue concentration of cisplatin required for its antitumor activity.

Glutathione plays an important role in maintaining membrane structure and participates in a variety of active transport process. It is known to protect the cellular system against the toxic effects of lipid peroxidation (Nicotera and Orrenius, 1986).

The above results confirmed well with the protective role of glutathione ester, one of the thiol compound against cisplatin induced lipid peroxidation.

4.7 Glutathione metabolising enzymes

γ-Glutamyl transpeptidase (γ-GT) is a particulate glycoprotein which is localised within kidney in the brush border membrane of the proximal tubule (Glossman and Neville, 1972).
In our study, cisplatin administration significantly reduced the level of $\gamma$-GT in kidney tissue and was reverted by glutathione ester.

Leyland-Jones et al. (1983) showed urinary loss of $\gamma$-GT that occurred after cDDP treatment. Gordon et al. (1986a) also reported the increased $\gamma$-GT excretion which was associated with the loss of microvilli. The marked enhancement of $\gamma$-GT excretion most likely reflects the accumulation of cisplatin in the corticomedullary region of the kidney, with the resultant loss of the brush border membrane.

Similar type of $\gamma$-GT loss was also observed in the rat kidney treated with cisplatin (Bogin et al., 1994) and cadmium (Sumathi et al., 1996).

The increased activity of $\gamma$-GT observed during glutathione ester administration subsequently replenish the glutathione and also glutathione ester might be expected to maintain the $\gamma$-GT in kidney tissue by regenerating the brush border membrane and microvilli.

Glutathione-S-transferases are thought to play a physiological role in initiating the detoxification of potential alkylating agents (Trakshel and Maines, 1988).

Feinfeld et al. (1986) showed that rats have been given a single toxic dose of cisplatin, excreted detectable amount of glutathione-S-transferase in their urine and suggested that this activity is a marker for proximal renal tubular injury from cisplatin. They confirmed their findings and show that
there is an accompanying loss of enzyme from the renal cortex (Bompart et al., 1990).

Glutathione-S-transferase has basic neutral and acidic isoenzymes and these have multiple functions such as the glutathione conjugation of various drugs and the reduction of organic hydroperoxides. Cisplatin induced nephrotoxicity in rats was due to a decrease in GST-α activity in the kidney after cisplatin administration (Sadzuka et al., 1994a; Sadzuka et al., 1994b).

The nephrotoxicity induced by cisplatin is due to covalent binding of platinum to protein thiol groups (Levi et al., 1980). A decrease in glutathione level and glutathione-S-transferase activity and depression of macromolecule synthesis in the kidney may play a role in cisplatin nephrotoxicity. Administration of diallyl disulphide and diallyl sulphide are organosulphur compounds with cisplatin could enhance the renal glutathione-S-transferase activity and lower cisplatin induced nephrotoxicity (Dwivedi et al., 1996).

Hence, it can be suggested that glutathione ester also normalised the enzyme activity by providing sufficient thiol group in the form of glutathione.

Glutathione reductase catalyses the reduction of GSSG by NADPH. Exposure of the cell to an "oxidant stress" such as hyperoxia may overwhelm the system, thereby causing GSSG to accumulate. Because, GSSG diffuses more easily than GSH from the cell, its formation and subsequent efflux leads to depletion of the intracellular pool of GSH. GSSG can also react with cellular
proteins via mixed disulfide reactions resulting in impaired protein function (Fedorcsak et al., 1977) and depletion of cellular GSH.

The role of glutathione reductase is to increase the availability of reduced glutathione for reaction with free radicals and peroxides, an essential feature in the metabolism of xenobiotics. Cisplatin has been reported to react with -SH groups of several compounds such as glutathione and metallothionein (Odenheimer and Wolf, 1982). Kidney glutathione reductase activity were significantly decreased when compared to controls (Bompart et al., 1990).

Inhibition of complex I (NADH ubiquinone reductase) in combination with reduced level of GSH-reductase activity resulted in reactive oxygen species formation. Cisplatin excerts both effects; therefore, cisplatin induced reactive oxygen formation can be mediated via simultaneous inhibition of complex I and glutathione reductase (Kruidering et al., 1997).

Glutathione reductase generates GSH from GSSG, a concomitant increase in GSSG and decrease in GSH is compatible with a depletion of glutathione reductase activity (Bompart et al., 1991).

The administration of glutathione ester normalised the glutathione reductase activity by replenishing glutathione content to directly quench the free radicals, peroxides and various metabolic xenobiotics.

The decreased activity of γ-glutamylcysteine synthetase was found in cisplatin induced nephrotoxicity. The activity of γ-glutamylcysteine synthetase
is feedback-regulated by GSH and is limited by the supply by cysteine (Meister, 1984).

Maines, (1986); Mayer and Maines, (1990); Meister, (1985) reported that in cisplatinum treated rats, a time-dependent and pronounced inhibition of activities of \( \gamma \)-glutamylcysteine synthetase; the rate-limiting enzyme in the production of glutathione.

Glutathione sulphur transferases catalyse the reaction between the -SH group of GSH and potential alkylating agents, thereby neutralising their electrophilic sites and rendering them more water soluble and suitable for excretion.

Administration of glutathione ester provides not only an effective glutathione but also normalises the \( \gamma \)-GCS in rats during cisplatin induced renal damage.

The decreased activity of glucose-6-phosphate dehydrogenase (G6PD) enzyme was observed in cisplatin administration.

Inhibition of glucose-6-phosphate dehydrogenase causes a decreased supply of reducing equivalents (NADPH) for the conversion of oxidised glutathione to its reduced form in the presence of glutathione reductase. Under conditions of oxidative assaults, the NADP/NADPH ratio will switch in favour of NADP, indicating decreased glucose-6-phosphate dehydrogenase activity (Sumathi et al., 1996) and this statement proves true in metal administered
rats. The paucity of NADPH production will in turn decrease the catalase activity (Nicholls and Schonbaum, 1963).

Glucose-6-phosphate dehydrogenase from several sources is believed to have an essential sulphydryl group for its function and the enzyme from yeast was inhibited by $K_2PtCl_4$ (Aull et al., 1979).

### 4.8 Key enzymes of glycolytic and gluconeogenic pathways

A significant decrease in glycolytic enzymes such as hexokinase, aldolase, phosphoglucoisomerase and lactate dehydrogenase was observed in cisplatin treated rats. The observed decrease might be due to an inhibition in the transport of glucose by cisplatin.

Courjault-Gautier et al. (1995) reported that the inhibition of $Na^+$-dependent glucose uptake may result from a direct interaction between cDDP and the co-transport protein, because this inhibition has been observed in brush-border membrane (BBM) vesicles exposed to cDDP *in vitro*.

Moreover, the $Na^+$/glucose co-transport protein in renal cortex BBM vesicles possesses -SH groups that are essential for its activity (Lo and Silverman, 1994) and may also involve other nucleophilic groups (Potdevin et al., 1998).

Appearance of glucosuria in cisplatin treatment also responsible for the impairment of low-affinity $Na^+/glucose$ cotransport system (Elsas and Longo, 1995).
Glucose inside the cell has only one fate: it is phosphorylated by ATP to form glucose-6-phosphate. Hexokinase, an enzyme, that transfers a phosphoryl group from ATP to an acceptor is highly decreased in cisplatin treatment.

A well documented ATP depletion in cisplatin administration is by an uncoupling of mitochondrial oxidative phosphorylation (Brady et al., 1993) and also by the inhibition of glycolysis (Aggarwal, 1993).

The decreased activity of phosphoglucoisomerase was found in cisplatin induced renal damage.

A sulphydryl group participates in the proposed reaction mechanism of aldolase, one of the enzymes found to be inhibited by platinum compounds (Aull et al., 1979).

In cisplatin treated rats, decreased activity of lactate dehydrogenase (LDH) was observed. Cisplatin is known to inhibit the activity of many enzymes (Daley-Yates and McBrien, 1982; Friedman and Teggins, 1974), especially sulphydryl-group containing enzymes.

The rat renal cytosolic marker enzyme LDH was also inhibited by cisplatin, transplatin or cisplatin hydrolysis products. A concentration of 0.25/ml cisplatin in the incubation medium showed a strong reduction of the LDH activity. A decrease in the intracellular activity of LDH and a decrease in the slice or cell to medium ratio of the activity of LDH could stimulate an increased leakage of the enzyme (Hannemann and Baumann, 1988b).
The decreased activity of glycolytic enzymes such as hexokinase, phosphoglucoisomerase, aldolase and lactate dehydrogenase were also observed in gentamicin induced nephrotoxicity (Sandhya et al., 1995).

Glutathione is involved in maintaining the intracellular thiol/disulphide ratio that is vital for the function of a variety of enzymes (Kosower and Kosower, 1983).

Administration of glutathione ester which leads to very efficient cellular delivery of glutathione, protects the glycolytic enzymes in cisplatin treated rats.

Direct inhibition of gluconeogenic enzymes by cisplatin or its metabolites might play a role in inducing the inhibition of gluconeogenesis. Since, cisplatin or its metabolites are known to inhibit the activity of enzymes (Aull et al., 1979), it shows an inhibitory effect on the activity of the gluconeogenic enzyme, glucose-6-phosphatase.

Depression of gluconeogenesis and glucose-6-phosphatase activity by cisplatin might be a direct effect of inhibition of glucose-6-phosphatase via lipid peroxidation that has been observed in cisplatin treated rats (Ernster et al., 1982; Hannemann and Baumann, 1990).

GSH depletion caused by cDDP is associated with cDDP induced lipid peroxidation and inhibition of gluconeogenesis, thus cDDP induced depletion of glutathione might be a critical event in oxidative stress and subsequent cytotoxicity (Zhang et al., 1992).
In in vivo, only cDDP hydrolysis products exhibited a significant inhibitory effect on renal glucose-6-phosphatase activity. In in vitro, rat renal and hepatic microsomal glucose-6-phosphatase activity was decreased by cDDP which was both time-and concentration-dependency (Hannemann et al., 1991).

In our study, the decreased activity of fructose-1,6-diphosphatase was observed in cisplatin treated rats. The gluconeogenic key enzyme fructose-1,6-diphosphatase, which is localised exclusively in the proximal nephron segment as a marker compound to monitor injury of the proximal nephron segment during nephrotoxic therapy. The combined administration of cisplatin, etoposide and ifosfamide resulted in a pronounced proximal tubular injury as shown by the release of fructose-1,6-diphosphatase into the urine (Pfaller et al., 1944).

The strong and significant inhibition of rat renal gluconeogenesis by mersalyl, a diuretic takes place with different kinds of substrate, entering at different levels of gluconeogenic pathway, in both fed and fasted rats (Croen et al., 1986).

The effect of mercurials on thiol groups inhibiting different cell proteins is well known. Thus, it has been proposed that mersalyl acts as a nephro-toxin, when it accumulates in the renal tubules (Kessler, 1960). Cisplatin causes an appetite suppression which limits food intake in animals and also includes a loss of body weight (Tokunaga et al., 1996). When the tubules were obtained from 24h starved rats the degree of renal gluconeogenesis inhibition was lower, probably due to enzymes involved in the
gluconeogenic pathway being induced as a consequence of starvation (Garcia-Salguero and Lupianez, 1988; Lupianez et al., 1976).

Amores et al. (1994) also reported the inhibition of gluconeogenesis in isolated rat kidney cortex tubules by mersalyl. This inhibition was due to a significant decrease in the activity of the key gluconeogenic enzymes.

Administration of glutathione ester effectively modulated the gluconeogenic enzymes which demonstrates the prevention of lipid peroxidation and also the possible regeneration of renal proximal tubules.

4.9 Tricarboxylic acid (TCA) cycle marker enzymes

The decreased activities of TCA cycle enzymes such as isocitrate dehydrogenase, succinate dehydrogenase, malate dehydrogenase and α-ketoglutarate dehydrogenase were observed in cisplatin induced nephrotoxicity.

Cisplatin has a site-specific nephrotoxic effect and pathological studies have shown that the major site of renal injury by cisplatin in rats is the S₃ segment of the proximal tubule located in the outer stripe of the outer medulla (Dobyan et al., 1980; Chopra et al., 1982). Cells of the proximal convoluted tubule are generally rich in mitochondria and the cells of the metabolism of glutathione (Berndt and Davis, 1988).

Mitochondria seem to play an important role in cisplatin induced nephrotoxicity (Gordon and Gattone, 1986b; Brady et al., 1993). Accordingly,
Kruidering et al. (1994) demonstrated that exposure of freshly isolated porcine proximal tubular cells suspension to cisplatin resulted in loss of mitochondrial membrane potential (ΔΨ). The decrease in ΔΨ preceded cell death, implying that damage to the mitochondria is an early event in the cascade of events leading to cell death.

Binet and Volfin, (1977) have observed platinum complex inhibition of phosphate transport (no absorbance modifications were observed in potassium phosphate medium), a decreased accumulation of Ca^{2+} and an increase in the passive permeability of Ca^{2+}. The stimulation of state 4 respiration, which is similar to an apparent uncoupling of oxidative phosphorylation might also be related to modifications of membrane permeability.

Cisplatin induced mitochondrial dysfunction is caused by inhibition of complexes I to IV of the respiratory chain, which results in decreased intracellular levels of ATP. This selectivity for mitochondria is probably caused by accumulation of cisplatin in the negatively charged inner space of the mitochondria because of the positive charge of aquated complexes of cisplatin. Indeed, cisplatin has been reported to accumulate in mitochondria of kidney cells in vitro (Gemba and Fukuishi, 1991; Kameyama and Gemba, 1991a and b) as well as in mitochondria of kidney and liver cells in vivo (Singh, 1989; Gemba et al., 1987; Rosen et al., 1992).

The activities of the electron transport chain enzyme such as NADH cytochrome c reductase, succinate cytochrome c reductase and
cytochrome c oxidase was found to be decreased by cisplatin administration (Sugiyama et al., 1989).

The markedly inhibited activity of MDH (58%) observed at the 80mg/kg dose of gentamicin would probably reduce the production of oxaloacetate from malate which is required for the continuation of TCA cycle as well as gluconeogenesis (Abdel - Gayoum et al., 1994).

The depressed activities of aspartate aminotransferase and malate dehydrogenase, the enzymes involved in the malate-aspartate shuttle, which would diminish the flux of reducing equivalents and gluconeogenic intermediates across the mitochondrial barrier. These results are also in agreement with the report of Ross et al. (1981), who demonstrated the inhibition of gluconeogenesis in isolated rat kidney by the addition of inhibitors of transaminases and malate dehydrogenase.

Several enzymes have been inhibited by platinum compounds. MDH and LDH are inhibited by Pt complexes (Friedman and Teggins, 1974) and all have catalytically important -SH groups (Banaszak and Bradshaw 1975). Moreover, cisplatin hydrolysis products were more inhibitory on beef heart MDH (Kohl et al., 1979).

ICDH and SDH enzymes activities were decreased in kidney during cisplatin treatment. Lowered enzyme activities in kidney as well as the less efficient oxidative phosphorylation and ATP production in mitochondria may be the related consequences of cisplatin treatment. Similarly, lower level of
ICDH activity was also observed in the kidney of cisplatin treated rats (Bogin et al., 1994).

α-Ketoglutarate dehydrogenase enzyme activity was also lowered in cisplatin treatment. Remus and Firman, (1990) reported that the decrease in TCA cycle action may be related to the decline in the activity of α-ketoglutarate dehydrogenase and pyruvate dehydrogenase complex.

Similarly, decreased activities of isocitrate dehydrogenase, succinate dehydrogenase and malate dehydrogenase were reported in mitochondria of gentamicin induced nephrotoxicity (Sandhya et al., 1995).

Glutathione ester gets transported efficiently into cells and split intracellularly to yield glutathione (Anderson et al., 1985). The high glutathione levels found in heart mitochondria (Martensson and Meister, 1989) and lung mitochondrial integrity (Martensson et al., 1989a) after administration of glutathione ester suggest that this compound may be a useful agent for protection against toxicity.

Administration of glutathione ester increased the activities of the mitochondrial enzymes such as ICDH, SDH, MDH and α-KGDH and render protection against cisplatin induced nephrotoxicity which suggests that glutathione ester is efficient in maintaining the mitochondrial membrane integrity.
4.10 Renal lysosomal enzymes

In our study, the lysosomal marker enzymes such as N-acetyl-β-D-glucosaminidase (NAG), β-glucuronidase (β-Glu) and cathepsin D (Cat D) registered a decrease in their activities in cisplatin induced proximal tubular damage and the lysosomal enzymes are released into the urine from the damaged kidney.

The kidney has been found to be the principle storage depot for platinum. There is an increase in the number of large lysosomes after platinum drug use (Miller and Aggarwal, 1992; Dobyän et al., 1981; Choie et al., 1980) and like Hg, Pt has been shown to accumulate in the lysosomes and peroxisomes in both the kidney and liver (Levi et al., 1980). The lysosomes have been demonstrated to be very fragile and are probably responsible for cell lysis (Aggarwal et al., 1980a) with the liberation of enzymes into the urine in a functional state (Batzer and Aggarwal, 1986). cDDP tends to induce a significant decrease in the transport enzyme activity in cell membranes with corresponding increase in urinary enzyme levels (Batzer and Aggarwal, 1986).

A detailed ultrastructural analysis of histopathological changes in the kidney from cisplatin treated rats (Aggarwal et al., 1980) has revealed mitochondrial swelling, electron density elevation and thickening of the basement membrane, changes in apical pinocytosis vessels and an increase in size and number of lysosomes. Lysosomes eventually rupture and this may be important in the pathogenesis of proximal tubular cell necrosis.
NAG, a lysosomal enzyme whose activity is located in both the proximal and distal tubules is a sensitive indicator of tubular damage (Donta and Lembke, 1985).

Chemically induced lipid peroxidation was shown to result in an accelerated liberation of lysosomal enzymes. NADPH-dependent microsomal lipid peroxidation in the presence of ADP/Fe$^{3+}$ was shown to produce OH; which promotes the lysis of lysosomes (Fong et al., 1973). Younes et al. (1983) have reported that agents which promotes lipid peroxidation will lead to a release of lysosomal β-glucuronidase \textit{in vitro}.

Nucleophilic thiol reagents have a potential for reacting with and inactivating toxic cisplatin metabolites and their action being based upon the affinity of sulphur containing ligands for platinum(II) complexes. Similarly, sodium thiosulphate and diethyldithiocarbamate are the metabolites of disulfiram that has been used as a chelating agent in the treatment of metal poisoning (Howell et al., 1983; Bodenner et al., 1986).

Administration of glutathione ester modulates cisplatin induced nephrotoxicity suggesting, an increase in the stability of the lysosomal membrane of proximal tubular cells, the important sites for cisplatin nephrotoxicity, thus decreasing the capacity of lysosomes to release hydrolytic enzymes.
Membrane bound Adenosine triphosphatases

In renal tubular epithelia, Na⁺,K⁺-ATPase is exclusively expressed at the basolateral membrane and provides the energy for net transcellular Na⁺ reabsorption. In view of its important 'housekeeping' and specialised functions, it is expected that any dysfunction or dysregulation of this enzyme would have severe pathophysiological consequences. A defect in Na⁺,K⁺-ATPase activity has been suggested to be the underlying cause for a number of disorders with nephrogenic origin including hypertension (Laski and Kurtzman, 1996).

In the present study, a significant decrease in Na⁺,K⁺-ATPase activity was observed in cisplatin treated rats. Previous studies have demonstrated that cisplatin interferes with mitochondrial function (Gordon and Gattone, 1986b; Brady et al., 1993), an effect which indirectly reduces Na⁺,K⁺-ATPase activity by inhibiting ATP synthesis, as well as by directly inhibiting Na⁺,K⁺-ATPase activity (Nechay and Neldon, 1984; Uozumi and Litterst, 1985; Tay et al., 1988). Inhibition of ATPase activity by cisplatin is considered to be one of the possible mechanisms of cisplatin induced nephrotoxicity (Guarino et al., 1979).

The structure within the nephron that specifically demarcates the red medulla from outer portions of the kidney is the thick ascending limb of Henle's loop. Na⁺,K⁺-ATPase is higher in the thick ascending limb than it is in the proximal tubules (Kats, 1986). Na⁺,K⁺-ATPase inhibition by ouabain decreases Na⁺ reabsorption in the proximal tubule and the thick ascending limb (Rocha and Kokko, 1973). A decreased filtration of sodium consequent to
a 50% reduction of glomerular filtration rate in cisplatin rats could decrease 
the Na\(^{+},K^{+}\)-ATPase in proximal tubules and may consequently account for the 
observed reduction in Na\(^{+},K^{+}\)-ATPase activity in the renal cortex (Seguro et al., 
1989).

Daley-Yates and McBrien (1982) investigated renal ATPase activity. They found that 
cisplatin inhibits Mg\(^{2+}\) and Na\(^{+},K^{+}\)-ATPase with equal 
effectiveness. In isolated perfused rat kidney preparations, sodium 
reabsorption was inhibited by cisplatin, which may be due to the inhibition of 
Na\(^{+},K^{+}\)-ATPase; Mg\(^{2+}\)-ATPase which are involved in the oxidative 
phosphorylation in mitochondria. The swelling and vacuolation of mitochondria 
may be the result of osmotic forces initiated by ionic imbalance in mitochondria 
with impaired ATPase activity.

Intracellular calcium functions as a second messenger in the 
regulation and control of cellular processes. It plays a central role in mediating 
muscle contraction, neuro secretion and other Ca\(^{2+}\)-mediated cell functions. 
Membrane systems have distinctive pathways for the uptake and release of 
Ca\(^{2+}\) and cells are provided to regulate these diverse functions by temporal and 
spatial changes in intracellular Ca\(^{2+}\) level (Carafoli, 1987; Alkon and 
Rasmussen, 1988). Perturbations in Ca\(^{2+}\) homeostasis are therefore important 
targets for the action of various toxicants (Pounds, 1988; Orrenius et al., 1989).

Inhibition of ATP synthesis by various combinations such as 
uncoupling of mitochondrial oxidative phosphorylation inhibition of glycolysis 
resulting in rapid ATP depletion and depletion of Na\(^{+},K^{+}\)-ATPase in the cell
membrane through limitation of ATP synthesis results in an increased cytosolic Na\(^+\) and decreased Na\(^+\)-Ca\(^{2+}\) exchange, forcing increased cytosolic Ca\(^{2+}\) and activation of Ca\(^{2+}\)-calmodulin complexes. ATP deficiency would also deactivate Ca\(^{2+}\)-ATPase at the plasma membrane resulting in an increased cytosolic Ca\(^{2+}\) (Trump and Berezesky, 1984).

The present study shows that cisplatin induced toxicity markedly increased the Ca\(^{2+}\)-ATPase activity. The increase in renal endoplasmic reticulum calcium pump activity may be responsible for an increase in the cytosolic Ca\(^{2+}\) concentration and/or the increased activity could disrupt the normal Ca\(^{2+}\) homeostasis of the cell and cause toxicity to the kidney (Jones et al., 1985).

Administration of nephrotoxic doses of antitumor platinum compounds is associated with an increase in renal endoplasmic reticulum calcium pump activity. It is postulated that the mechanism of platinate nephrotoxicity could involve a disruption of calcium homeostasis. Disruption of the regulation of cytosolic Ca\(^{2+}\) may be important in cellular toxicity and the activity of the endoplasmic reticulum calcium pump may be a useful biomarker for the toxicity of various agents (DeWitt et al., 1988).

Since, ATPases are thiol dependent enzymes, Na\(^+\),K\(^+\)-ATPase, Mg\(^{2+}\)-ATPase and Ca\(^{2+}\)-ATPase require -SH groups to maintain their structure and function (Garner et al., 1983; Shalev et al., 1981).
The protective action of glutathione may have a special role in maintaining renal function and membrane structure. GSH contains a sulphhydryl group on the cysteinyl amino acid and is the major component of the intracellular non-protein sulphhydryl pool. It acts as a detoxifying agent through its free radical stability and its thiolate anion nucleophilicity (Jakoby, 1978; Arrick and Nathan, 1984).

Hence, it can be suggested that the administration of glutathione ester, increased the activity of Na\(^+\),K\(^+\)-ATPase, Mg\(^{2+}\)-ATPase and decreased the activity of Ca\(^{2+}\)-ATPase in cisplatin induced renal damage.

4.12 Retention of platinum

Nephrotoxicity is the dose-limiting effect as expected due to the rapid excretion of cisplatin through urine and the high concentration of platinum found in the kidney depends upon its ability to accumulate cisplatin (Litterst et al., 1977; Safirstein et al., 1987).

Mason et al. (1986) report confirms that in cisplatin treated rats, several low molecular weight platinum containing fractions are formed in the blood and are lost rapidly by protein binding, urinary excretion and uptake by kidneys. Since, most of the platinum containing species in the urine are either actively secreted or reabsorbed in the kidney tubules (Daley-yates and McBrien, 1983). The localisation and severity of tubular necrosis following cisplatin treatment may be related to the prolonged retention of platinum in the kidney (Choie et al., 1980).
Total concentrations of platinum are generally lower in liver than in kidney. The large storage capacity of the liver or the efficient detoxification of substances, eg., metallothioneins (MT), that are produced in large amounts in the liver. It might also indicate that high peak levels (as in the kidney) cause more damage to normal tissues than does long term retention of cDDP. It could also reflect that cells with high initial levels of cDDP-DNA adducts died off. The prolonged retention of platinum indicates that an increasing portion of the "waste" platinum was accumulated in forms other than being bound to DNA in vital tumor cells, tentatively, in necrotic areas (Johnsson et al., 1995).

Sodium thiosulphate is known to be a strong nucleophile and reacts irreversibly with cDDP to form pt(S₂O₃)₄ in plasma (Ishizawa et al., 1981; Pfeifle et al., 1985). It has been suggested that increased urine output might accelerate platinum excretion and might dilute the platinum concentration in urine (Iwamoto et al., 1985). Accelerated platinum excretion in urine has been reported both in humans and in animals (Hirosawa et al., 1987; Nagai et al., 1995). The use of chelating agents to remove platinum from the liver and kidney subsequent to the use of cisplatinum was reported by Jones and Basinger, (1989) and Graziano et al. (1981).

Cisplatin reacts with sulphur containing nucleophiles such as glutathione (Long and Repta, 1981), GSH has numerous biological functions: protection of -SH groups of proteins, inactivation of peroxides, free radicals and detoxification of foreign substances (Ross, 1980). Therefore investigations were carried out to elucidate the role of intracellular glutathione in cisplatin nephrotoxicity that could involve direct reaction of cisplatin and glutathione
with the protection of critical cellular sites or indirect maintenance of cell viability (Appenroth and Winnefeld, 1993; Nakano and Gemba, 1989).

4.13 Minerals

In our study, cisplatin treated rats showed an increased excretion of selenium due to the proximal tubular damage. Glutathione peroxidase is a selenium containing enzyme found in cytosol and mitochondria. Somani et al. (1995) reported lipid peroxidation due to the administration of cisplatin with an impaired glutathione peroxidase activity in kidney. Moreover, the increased excretion of selenium like other electrolytes is the major consequence of cisplatin induced nephrotoxicity.

Few studies have addressed a cDDP-copper interaction and the results are mixed. Kinsler and Bell, (1985) observed no drop in male Sprague-Dawley rat kidney or liver copper upto 48h post cDDP. Sharma, (1985) observed a 23% loss of Wistar rat kidney copper and postulated that this initial copper loss was from metal inducible copper proteins such as superoxide dismutase or metallothionein. Mason et al. (1984) observed a 49% reduction in the kidney cytosolic copper in male Wistar rats 24h post cDDP, but total kidney copper concentrations remained the same. The intracellular distribution of the remaining soluble copper was also unchanged.

Mason and Edwards, (1985) suggest that the persistent low kidney copper was due to cDDP inhibition of tubular reabsorption (not inhibition of MT synthesis). The magnitude of the initial loss of kidney copper observed in
the study, especially in the copper-pretreated rats, suggests that the copper was displaced.

Diethyldithiocarbamate, a sulphur containing compound like glutathione ester effectively lowers kidney platinum concentrations, lessens the loss of kidney copper and ameliorates cisplatin nephrotoxicity (DeWoskin and Riviere, 1992).

Hypomagnesemia caused by renal magnesium (Mg) wasting as a well known side effect of cisplatin chemotherapy (Bell et al., 1985; Buckley et al., 1984; Mavichak et al., 1988). Although Mg is mainly an intracellular cation and a deal of enzyme activities are impaired by Mg depletion in cells (Durlach, 1988).

Besides the well known effects on tubular function with renal Mg wasting, cisplatin is likely to interfere with Mg metabolism at cellular and subcellular levels (Durlach, 1988). Mg is an integral part of the structure of many membranes and plays a key role in regulating membrane transport system (Altura and Altura, 1984).

The aetiology of cisplatin nephrotoxicity is still not completely solved. It has been demonstrated that the final common pathway for cDDP nephrotoxicity is damage to the proximal tubular epithelial cell, resulting in magnesium wasting nephropathy (Mavichak et al., 1985; Sartori et al., 1993).

Taking into account, load dependency of sodium reabsorption in the loop of Henle, an increased fluid load usually produces an increased
reabsorption rate in distal nephron segments and thus the reabsorption of sodium, potassium, magnesium and calcium (Daugaard et al., 1988a). Hypomagnesemia and hypocalcemia are in fact considered to be among the earliest signs of cDDP toxicity. In some cases, severe hyponatremia and hypokalemia may occur. Magnesium deficiency and metabolic alkalosis associated with cDDP induced vomiting may contribute to the hypokalemia (Schilsky and Anderson, 1979).

The K+ balance in the human body: The daily K+ intake of 100mEq is mostly excreted into urine via the kidneys. It is also important to maintain the extracellular K+ concentration for the Na+-transport epithelia (kidney, intestine). As the Na+-coupling co transporters (such as Na+-glucose transporter, etc.,) are electrically charged their translocation across the cell membrane should be regulated by the cell-negative potential. Therefore K+ homeostasis is an essential issue for the organism (Kawahara and Anzai, 1997).

Berliner, (1961) suggested that some distal tubules may be responsible for the urinary K+ excretion which is insensitive to changes in glomerular K+ filtration by renal clearance experiments. Pump-leak coupling is one of the most important functions which maintain the cellular homeostasis of ionic compositions and cell volume in Na+-transporting epithelia. It is well known that a drop in Na+ reabsorption results in a decrease in the K+ permeability of the basolateral membrane (Matsumura et al., 1984; Lapointe et al., 1990).
Cisplatin had induced a distal tubular impairment, because in functionally normal nephrons, a change in proximal fluid delivery is accompanied by an almost parallel change in absolute sodium and water reabsorption rates in the ascending limb of the loop of Henle without changing fractional reabsorption rates (Schnerman, 1968).

The principal focus of this study was an examination of proximal tubule cell electrolyte composition in the very earliest period following administration of cisplatin. When taken in conjunction with accepted models for sodium reabsorption by this epithelium (Rector, 1983), data of this kind are capable of defining the cell membrane surface at which the drug converts its primary action in inhibiting sodium transport. Thus a primary inhibitory effect on passive sodium entry across the apical membrane would be expected to lead to a fall in cell sodium as an active basolateral Na⁺ extrusion via the Na⁺, K⁺-ATPase continued, while a direct effect on the Na⁺,K⁺-ATPase itself would lead to a rise in cell sodium (Gyory et al., 1985).

The period immediately following an intravenous dose of cisplatin, proximal tubule cell sodium content is reduced at a time when sodium reabsorption is impaired. This observation implies that cisplatin acts to inhibit the entry of sodium into proximal cells, possibly by interfering with the function of the Na-H exchanger in the apical membrane (Field et al., 1989).

Both animal and human studies indicate that the incidence of cisplatin associated nephrotoxicity is dose-dependent and may be manifested by renal magnesium wasting, acute renal failure, impaired water metabolism
and massive loss of electrolytes such as sodium, chloride, calcium, magnesium and phosphate (Blachley and Hill, 1981; Bjornson and Stephenson, 1983).

The exogenous administration of glutathione ester along with cisplatin was found to be effective in reducing the accumulation of platinum and restored the minerals from disturbances observed in cisplatin treated rats.

4.14 Urinary marker enzymes

Measurement of urinary enzymes has been recognised as non-invasive, sensitive procedure for monitoring cellular integrity within the kidney (Price, 1982; Stroo and Hook, 1977). Enzymes appearing in the urine originate owing to leakage from damaged cells or remain in the urine owing to inadequate reabsorption by the proximal tubules.

The use of urinary markers for nephrotoxicity, traced the acute and chronic effects of cisplatin therapy on renal tubular cells.

Bulger and Dobyan (1984) reported that a single dose of cisplatin administered to rats was capable of inducing a relative increase in the number of proximal tubular like cells having a brush border and numerous lysosomes.

Alkaline phosphatase is present in the lysosomes throughout the kidney which increases substantially after cDDP treatment similar to that of acid phosphatase. In the case of acid phosphatase, lysosomal buildup occurs until the cells lyse, releasing their enzymes in the urine in a functional form. The increase in urinary alkaline phosphatase corresponds to a decrease in the
membrane enzyme levels in the kidney, suggesting a possible discharge into
the urine (Batzer and Aggarwal, 1986).

Excretion of N-acetyl-β-D-glucosaminidase (NAG), a high-molecular
mass lysosomal enzyme found in the proximal and distal renal tubules (Le Hir
et al., 1979). Urinary levels of NAG could be increased as a result of enhanced
secretion of lysosomal enzymes or extrusion of lysosomes from necrotic cells.
Fujita, (1985) demonstrated that cisplatin was found to elevate urinary activity
of the enzyme NAG, which is rich in lysosomes of renal cells in patients. NAG
excretion is a sensitive and useful indicator of cisplatin nephrotoxicity
(Harauchi and Yoshizaki, 1990; Brillet et al., 1993; Takeda et al., 1994).

Kuhn et al. (1980) followed nephrotoxicity of cisplatinum by the
measurement of urinary β-glucuronidase, an urinary enzyme as sensitive
parameter of tubular damage, because it leaks into the urine only after the
tubular cells get impaired.

γ-Glutamyl transpeptidase (γ-GT) is an enzyme with maximal activity
along the brush border membranes of the proximal tubular epithelium (Beck,
1983). Cisplatin accumulates in an area rich in γ-GT. γ-GT is only one of a
number of renal enzymes that have been systematically studied in urine as a
potential marker of nephrotoxic and ischemic renal injury in experimental
animals and in humans (Vanderlinde, 1981; Stroo and Hoak, 1977).

The observed pattern in urinary γ-GT excretion can perhaps be best
explained from histologic evidence that confirms previous morphologic studies
on cisplatin induced renal injury (Dobyan et al., 1980). The marked early enhancement of $\gamma$-GT excretion most likely reflects the accumulation of cisplatin in the corticomedullary region of kidney with the resultant loss of the brush border and development of blebs consequent to toxicity of the drug. The measurement of urinary excretion of the brush border enzyme $\gamma$-GT may serve as an useful non-invasive predictor of cisplatin nephrotoxicity (Gordon et al., 1986a; Dierickx, 1981).

Cathepsin D is a major renal lysosomal endoproteinase which is distributed in various anatomical and functional areas of the normal rat kidney. In cisplatin treated rats, urinary excretion of cathepsin D level was found to be increased. Urinary protein is known to stimulate pinocytosis and upregulate the quantity of lysosomal enzymes in kidney tissue (Olbricht et al., 1986; Maack et al., 1971). Furthermore, lysosomal enzymes are known to be concentrated in proximal tubule cells of the nephron and have recently been demonstrated in the glomerular basement membrane matrix as well (Singh, 1993). In addition, marked proteinuria is thought to cause lysosomes to engorge and rupture, releasing their contents, enzymatically damaging the renal tubular epithelium (Maack et al., 1979).

Jackson et al. (1996) hypothesize that the preeclamptic patient has proximal tubule epithelial injury, which leads to the release of lysosomal enzymes and a significant increase in the fractional excretion of lysosomal hydrolysis.
The nephrotoxic effect of cisplatin results in an increased urinary excretion of lactate dehydrogenase (LDH) also. The leakage of the cytosolic enzyme LDH has been used to monitor the nephrotoxicity of cisplatin (Zhang et al., 1992; Bret et al., 1993). Ishikawa et al. (1993) and Sadzuka et al. (1994b) have reported that urinary lactate dehydrogenase activity was significantly increased after cisplatin administration.

The renal protection offered by the strong nucleophiles such as thiosulphate, mensa and dimensa occurs rather by neutralisation of the aquated species in the lumen of the renal tubules than by neutralisation of intact cisplatin, and that neutralisation of these species in plasma contributes significantly to their protective efficacy (Leeuwenkamp et al., 1991).

Glutathione is an excellent nucleophilic compound used in direct detoxification which may have a special role in maintaining renal function and structure (Torres et al., 1986; Russo et al., 1986a).

Administration of glutathione ester along with cisplatin protects the renal tubules and thereby prevents the increased excretion of urinary enzymes in cisplatin treated rats.

4.15 Nephrotoxicity indicators of urinary constituents

In our study, cisplatin treated animals were shown an increased excretion of urine. Many heavy metal compounds tend to accumulate in the kidney, predisposing to heavy metal nephrotoxicity. Histologically, the acute tubular necrosis observed in cisplatin treated rats is similar to that produced
in intoxication by mercury (Choie et al., 1981). Rats given a single toxic dose of cisplatin, increased urine volume characteristic of its nephrotoxicity (Feinfeld et al., 1986).

The enhanced renal protein excretion of adult rats after cisplatin has been described previously (Salter et al., 1977; Aggarwal et al., 1980). It could be caused firstly by the disturbed reabsorption mechanism for protein in the proximal tubules and secondly by the excretion of proteins derived from destroyed cells. Appenroth et al. (1997) and Bräunlich et al. (1996) have also been reported an increased urinary protein excretion in cisplatin induced nephrotoxicity.

Cisplatin administration causes impairment of phosphate transport in mitochondria (Aggarwal et al., 1980). Cisplatin also inhibits organic cation transport in brush border and basolateral membranes from rat kidney cortex (Williams and Hottendrop, 1985). An increased phosphaturia was also observed in cisplatin induced nephrotoxicity (Halabe et al., 1991).

Cisplatin induces glucosuria and oliguric acute renal failure in rabbits. Glucosuria induced by cisplatin was not due to a direct impairment of glucose transporter in brush border membranes, but due to an inhibition of \( \text{Na}^+ \)-pump activity and a decrease in area for active glucose reabsorption in the proximal tubules (Kim et al., 1995). It has also been demonstrated that cisplatin treatment induces glucosuria in rats (Goldstein et al., 1981; Kramer, 1989; Ishikawa et al., 1993).
Increased level of uric acid in urine is associated well with cisplatin induced nephrotoxicity (Nanji et al., 1986). Cisplatin just like other diuretic agents such as mannitol and furosemide (Holmes et al., 1972) is also associated with increased urinary excretion of uric acid.

Glutathione is a sulphur containing nucleophile that protects against cisplatin induced renal toxicity without reducing the antitumor activity of the cytotoxic agent (Bohm et al., 1991). Glutathione ester administration, diminished the toxic effects of cisplatin and also normalised the urinary excretion of protein, glucose, phosphate and uric acid along with urine volume in cisplatin treated rats.