

**ANTIOXIDANTS AND CDDP-INDUCED  
NEPHROTOXICITY**

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**ABSTRACT**

*Cis-dichlorodiammineplatinum(II) (cisplatin (CDDP) ) is one of the most effective antitumor agents currently available for cancer therapy. However, its clinical use has been limited by its severe side effects, especially nephrotoxicity. To evaluate the effect of radical scavengers on CDDP nephrotoxicity in rats, CDDP and Vitamin C were given intraperitoneally. Remarkable protective effects of Vitamin C against nephrotoxicity of CDDP were observed when Vitamin C and GSH were administered to rats before or with CDDP injection. Vitamin C which has radical scavenging effect directly reduced nephrotoxicity of CDDP in vivo. Thus, it seems that free radical is the cause of CDDP nephrotoxicity. Also, combination treatment did not reduce anticancer activity of CDDP. The present results indicate that Vitamin C, and/or GSH when they were given before or with CDDP, may provide protection against CDDP nephrotoxicity without reducing anticancer activity. The aim of the present study was to investigate whether or not the renal antioxidant system plays also an important role in renal damage induced CDDP (2 mg/kg intraperitoneally at a single dose in rats). In order to elucidate it, serum creatinine and urea levels, renal glutathione and thiobarbituric acid-reactive substances (TBARS) content, as well as renal catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSHpx) activities were measured in the kidney homogenates and additionally of gene expression of GSHpx and glutathione reductase (GRD) were determined.*

## INTRODUCTION

Cancer is one of the most dreaded diseases and currently taking the heaviest toll of human lives, with distant hope of finding an effective cure unless detected and treated in early stages. Chemotherapy and radiotherapy are the most common modalities of cancer treatment. CDDP is currently one of the most important chemotherapeutic drugs used in treatment of a wide range of solid tumors - head, neck, ovarian and lung cancers. However, the clinical usefulness of this drug is limited due to nephrotoxicity induction, a side effect that may be produced in various animal models. CDDP gets accumulated in the tubular epithelial cells of proximal kidney tubule, causing nephrotoxicity, characterized by morphological destruction of intracellular organelles, cellular necrosis, loss of microvilli, alterations in the number and size of the lysosomes and mitochondrial vacuolization, followed by functional alterations including inhibition of protein synthesis, GSII depletion, lipid peroxidation and mitochondrial damage (Joy, 2008).

CDDP is one of the most effective chemotherapeutics against a wide range of cancers including testicular, ovarian, bladder, and neck. It is also used in the management of endometrial cancer, non-small lung cancer, malignant melanoma, penile cancer and adrenocortical carcinoma (Santos *et al.*, 2007 and Martins *et al.*, 2008). However, its therapeutic use is limited by its severe toxic side effects, namely nephrotoxicity (Servais *et al.*, 2008). Although nephrotoxicity is the major limiting factor in its chemotherapeutic use (Servais *et al.*, 2008), liver toxicity may occur when CDDP is administered at high doses (Martins *et al.*, 2008). Moreover, recent studies demonstrated that CDDP induces mitochondrial oxidative damage and apoptosis in rat liver, thus, suggesting mitochondria as a target to the hepatotoxicity of this drug (Santos *et al.*, 2007 and Martins *et al.*, 2008). The exact mechanism by which CP produces renal damage is unknown. After a single dose of CP, there is

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referential sequestration of the drug in the kidneys, liver, intestine, and testes, with concentrations in the kidneys reaching levels higher than 37 times those of plasma (**Sheikh -Hamad, 2008**).

CDDP-DNA crosslink's causes cytotoxic lesions in tumors and other dividing cells. DNA damaging agents usually have less toxicity in non proliferating cells, yet the quiescent proximal tubule cells are selectively damaged by CDDP. The mechanism for this renal cell injury has been the focus of intense investigation for many years, and recent studies suggest that inflammation, oxidative stress injury, and apoptosis probably explain part of this injury. Understanding the mechanisms) for this side effect should allow clinicians to prevent and/or treat this problem better and provides a model for investigating drug-induced nephrotoxicity in general (**Schrier *et al.*, 2004**). Morphologic changes after CDDP-induced kidney injury are most prominent in S3 segments of proximal tubules, where loss of brush border membrane, cell swelling, nuclear condensation, and focal areas of tubular cell apoptosis and necrosis are seen (**Sheikh - Hamad *et al.*, 2004**).

Oxidative stress injury is actively involved in the pathogenesis of CDDP - induced acute kidney injury. Reactive oxygen species (ROS) directly act on cell components, including lipids, proteins, and DNA, and destroy their structure. ROS are produced via the xanthine-xanthine oxidase system, mitochondria, and NADPH oxidase in cells. In the presence of CDDP, ROS are produced through all these pathways and are implicated in the pathogenesis of acute CDDP-induced renal injury (**Kawai *et al.*, 2006**). CDDP induces glucose-6- phosphate dehydrogenase and hexokinase activity, which increase free radical production and decrease antioxidant production (**Yilmaz *et al.*, 2004**). It increases intracellular calcium level which activates NADPH oxidase and to stimulates ROS production by damaged mitochondria (**Kawai *et al.*,**

2006). Super-oxide anion ( $O_2^{\bullet}$ ) (Davis *et al.*, 2001), hydrogen peroxide ( $H_2O_2$ ) (Kadikoylu *et al.*, 2004), and hydroxyl radical ( $\bullet OH$ ) (Shino, *et al.*, 2003), are increased in CDDP-treated kidneys. These free radicals damage the lipid components of the cell membrane by peroxidation and denature proteins, which lead to enzymatic inactivation. Free radicals can also cause mitochondria dysfunction. Antioxidant enzymes are inhibited by CDDP, and renal activities of SOD, glutathione peroxidase, and CAT are significantly decreased (Badary *et al.*, 2005). Antioxidants melatonin, (Sewer, *et al.*, 2000), vitamin C (Kadikoylu *et al.*, 2004) and vitamin E have been shown to prevent CDDP-induced acute nephrotoxicity (Weijl, *et al.*, 2004).

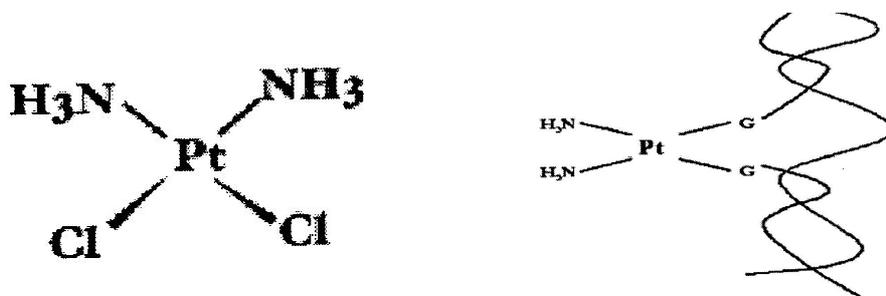
Agents such as SOD, dimethyl thiourea and GSH have been shown to reduce the degree of renal failure and tubular cell damage when administered simultaneously with CDDP in rats (Sadzuka *et al.*, 1992). Much attention has been given to the possible role of dietary antioxidants in protecting the kidney against CDDP induced nephrotoxicity. There is a large body of evidence on the chemo-protecting activities of vit.C, E., curcumin, selenium, bixin and other dietary components that scavenge free radicals induced by exposure to CDDP (Antunes *et al.*, 2001).

It is well established that CDDP forms platinum-DNA adducts that initiate tumor cell death (Brabec and Kasparkova, 2002; Wang and Lippard, 2005) where N7 of neighboring and intrastrand purines is the preferred binding site (Fichtinger-Schepman *et al.*, 1987). Such CDDP-DNA adducts initiate DNA fragmentation and apoptosis (Henkels and Turclri, 1997). The cytotoxic effect of CDDP when bound to targets other than DNA may also be important. For instance,

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Chemical structure of CDDP and its proposed interaction with DNA

The most common adverse effect limiting the efficacy of this drug is nephrotoxicity which develops primarily in the S3 segment of the proximal tubule. Although the exact mechanism of CDDP-induced nephrotoxicity is not well understood, but according to the previous results, some mechanisms are involved such as: depletion of sulfhydryl (SH) groups, impaired anti-oxidant defense system and mitochondrial dysfunction by inhibition of complexes I to IV of the respiratory chain in proximal tubules (**Baek *et al.*, 2003**).

The enzymes, SOD and GSHpx, in the cells are important components of several naturally occurring antioxidant defense mechanisms to prevent oxidative injury (**Weijl *et al.*, 1997**). There is also a hypothesis that decreased levels of natural antioxidants vitamins and diminished scavenging enzyme capacity may be responsible for the excess of ROS observed in CDDP-induced toxicity (**Antunes *et al.*, 2001**). GSH plays an important role in the cellular defense system. Against oxidative injury, it is a cofactor for GSHpx, which catalyzes the reduction of hydrogen peroxide to water and oxygen, hence limiting the formation of hydroxyl radical, the highly toxic reactive oxygen species (**Hsu *et al.*, 2002**).

Vitamin C is an antioxidant that has been demonstrated to be an effective free radical scavenger (**Anderson, 1996**) and it has been previously shown that VC successfully reduced the clastogenic effect of many antitumor agents and radiation in in vivo assays (**Konopacka et al., 1998**) also has in vivo anticlastogenic effects against chromosomal damage induced by CDDP in rodents (**Antunes et al., 1999**). Supplementation of the antioxidant vitamin C and selenium has been reported to inhibit lipid peroxide in various conditions such as CDDP-induced nephrotoxicity (**Carry and Frenkel, 2000**).

CDDP has been thought to bind to the renal base transport system. Cisplatin induces hypomagnesemia through its renal toxicity possibly by a direct injury to mechanisms of magnesium reabsorption in the ascending limb of the loop of Henle as well as the distal tubule. Cisplatin preferentially accumulates in cells of the S3 segment of the renal proximal tubules and is toxified to form a reactive metabolite intracellularly by hydration. The primary symptoms of cisplatin nephrotoxicity are inhibition of protein synthesis and intracellular GSH and protein-SH depletion, resulting in lipid peroxidation and mitochondrial damage (**Badccry et al., 2005**). The peroxidation of membrane lipids may account for its nephrotoxicity (**Satoh et al., 2005**). Available evidence suggests that cisplatin exerts its nephrotoxic effects by the generation of free radicals (**Kawai et al., 2006**). GSH and protein-SH form the major cellular anti-oxidant defense systems, which control lipid peroxidation. From these pathomechanisms of cisplatin nephrotoxicity, it is clear that the nephrotoxicity of cisplatin involves reactive radicals. Thus the reasonable cellular-protective agents against cisplatin toxicity may have at least some antioxidant properties to prevent GSH depletion and/or scavenge the intracellular reactive oxygen species.

## **MATERIAL & METHODS**

A total of 60 Adult male Sprague-Dalcy rat (200 250g) were used in the experiment. The animals were purchased from the Central Animals House of Faculty of Medicine Zigzag University housed in an environmentally (t = 25°C) and air humidity (60%) controlled room with a 12-hour light-dark cycle, and kept on a standard laboratory diet and drinking water ad libitum.

Animals were maintained in stainless steel cages in the biochemistry Department under hygienic condition. The rats were acclimatized for 2 days and randomly divided to six groups. Group I was injected with saline intraperitoneally, this group served as control. Groups II were treated with GSH for 15 days and then received CDDP plus GSH for 5 days. Groups III was treated with vitamin C for 15 days then injected with CDDP plus GSH for 5 days. Groups IV was treated with CDDP plus GSH for 5 days. This group treated with CDDP plus GSH for 5 days. Group V was treated with (DDP plus vitamin C for 5 days. Group VI was treated with CDDP only for 5 days.

CDDP was injected i.p. by a dose of 02 mg/kg body weight, Vit.C was administered orally by gavages by a dose of 250 mg/kg body weight GSH was Injected i.m. by a dose of 600 mg/kg body weight according to (**Giri *et al.*, 1998; Iusania *et al.*, 2002; and Cascinu, *et al.*, 1995**) respectively.

Seven Days post the last injection , food was withheld from all animals for 12 hours before blood collection , blood samples were collected by sacrificing the animals and received into heparinized tubes plasma was harvested by centrifuged of the blood at 3000 rpm for biochemical analysis of urea and creatinine using assay kits, kidneys were removed. Small part was taken from kidney as possible as before rats death, weighted and immediately were kept in liquid

nitrogen until be used for determination of gene expression of GSH<sub>Px</sub> and GRD. One gram of kidney was removed from each animals and they are washed out from contaminated blood with cold water was homogenized in 10 ml of distilled water using electrical homogenizer, centrifuged at 3000 rpm for 15 minutes, the resulting supernatant were collected and used for estimation of GSH, antioxidant enzymes (SOD, CAT and GSH<sub>Px</sub> and MAD levels (Sidhu *et al.*, 2004). Kidney from animals was preserved at -20°C until performing the investigations.

## **RESULTS**

The results are expressed as mean  $\pm$  SEM. Data were analyzed by one-way analysis of variance. Sequential difference among mean were calculated at the level of  $P < 0.05$ , using Tukey contrast analysis as needed.

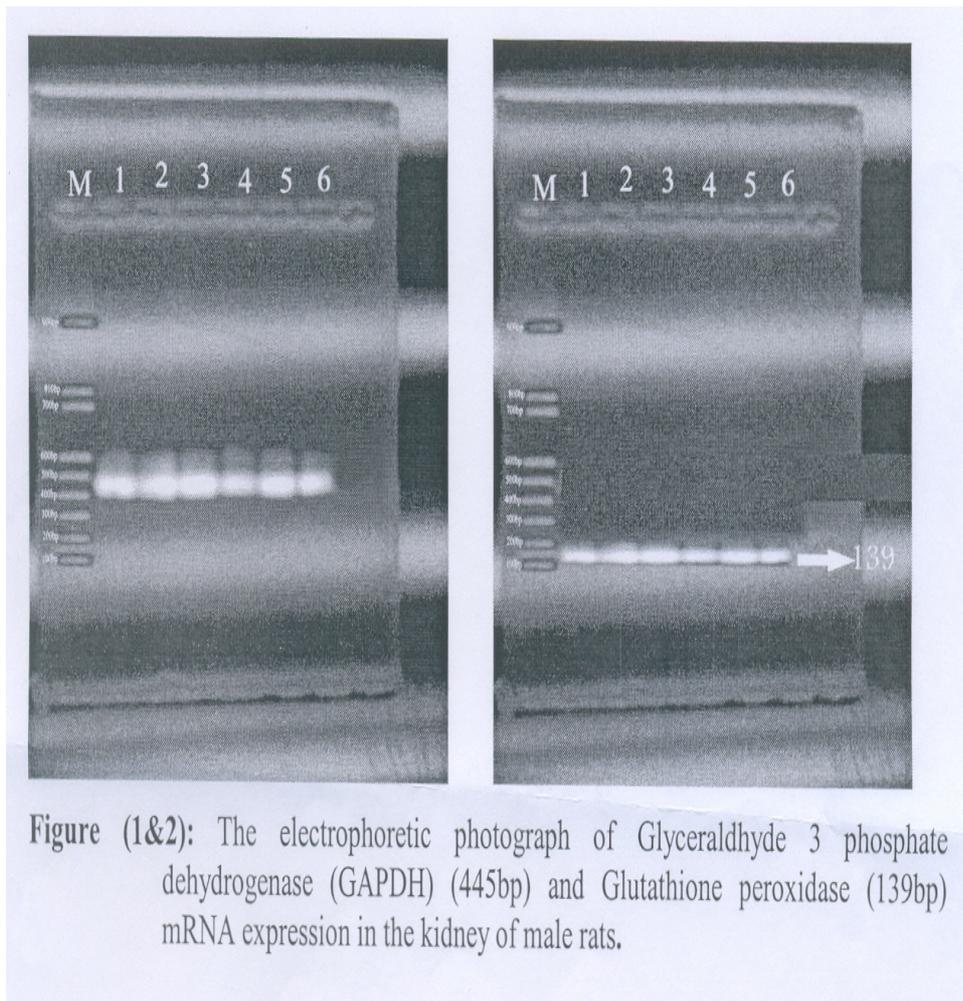
The internal control gene showed almost stable pattern of expression in the kidney tissues of male rats in treated and control groups.

The level of gene expression of GSHPX and GRD were decrease in kidney homogenate of all groups treated with CDDP, this decrease is of great degree in the group treated with CDDP only but was of lesser degree in the groups treated with GSH or Vit. C plus CDDP that may represent their cure effect in the decrease of the effect of CDDP.

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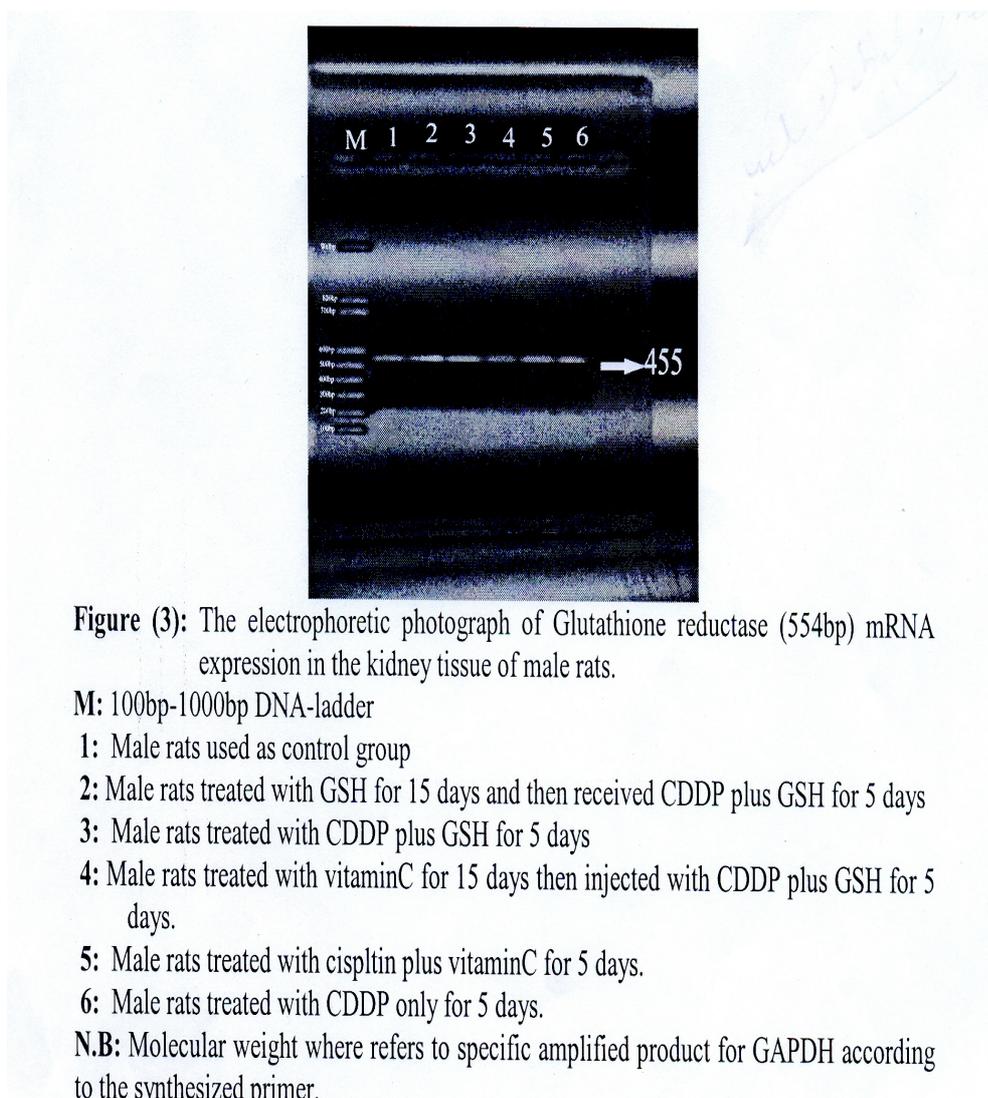


**Table (1):** Antioxidant status of rat kidney after treatment with CDDP alone or with vit.C and GSH. Each value represents mean  $\pm$  SD of ten rats.

Group	urea	creatinine	mda	(GSH <sub>px</sub> )	gsh	CAT	sod
Control	34.42 $\pm$ 2.1 <sup>d</sup>	0.856 $\pm$ 0.088 <sup>e</sup>	2.53 $\pm$ 0.144 <sup>c</sup>	0.308 $\pm$ 0.055 <sup>a</sup>	51.11 $\pm$ 1.7 <sup>ab</sup>	248.8 $\pm$ 13.2 <sup>a</sup>	72.96 $\pm$ 2.8 <sup>a</sup>
Glutathione treated for 15 days then treated with CDDP and glutathione for 5 days	119.75 $\pm$ 7.6 <sup>b</sup>	2.38 $\pm$ 0.26 <sup>bc</sup>	2.73 $\pm$ 0.112bc	0.206 $\pm$ 0.036 <sup>ab</sup>	47.1 $\pm$ 3.4 <sup>bc</sup>	250.42 $\pm$ 21.69 <sup>a</sup>	56.31 $\pm$ 5.2 <sup>b</sup>
Vit. C treated for 15 days then treated with CDDP and glutathione for 5 days	137.59 $\pm$ 0.99 <sup>a</sup>	2.7 $\pm$ 0.45 <sup>b</sup>	2.92 $\pm$ 0.18 <sup>bc</sup>	0.212 $\pm$ 0.01 <sup>ab</sup>	56.68 $\pm$ 2.4 <sup>a</sup>	235.98 $\pm$ 13.35 <sup>a</sup>	33.32 $\pm$ 7.4 <sup>c</sup>
CDDP and glutathine treated group	69.92 $\pm$ 5.96 <sup>c</sup>	1.41 $\pm$ 0.05 <sup>de</sup>	3.34 $\pm$ 0.25 <sup>b</sup>	0.212 $\pm$ 0.02 <sup>ab</sup>	50.18 $\pm$ 2.7 <sup>abc</sup>	246.12 $\pm$ 14.19 <sup>a</sup>	35.06 $\pm$ 6.7 <sup>c</sup>
CDDP and vitamin C treated group	108.7 $\pm$ 7.9 <sup>b</sup>	1.71 $\pm$ 0.27 <sup>cd</sup>	3.18 $\pm$ 0.26 <sup>b</sup>	0.236 $\pm$ 0.07 <sup>ab</sup>	55.22 $\pm$ 1.6 <sup>a</sup>	225.04 $\pm$ 8.67 <sup>a</sup>	41.86 $\pm$ 5.8bc
CDDP treated group	149.25 $\pm$ 3.92 <sup>a</sup>	4.58 $\pm$ 0.16 <sup>a</sup>	4.26 $\pm$ 0.16 <sup>a</sup>	0.14 $\pm$ 0.014 <sup>b</sup>	43.4 $\pm$ 1.69 <sup>c</sup>	208.6 $\pm$ 3.8 <sup>a</sup>	15.96 $\pm$ 2.67 <sup>d</sup>
F-Test	**	**	**	N.S	**	N.S	**
L.S.D	15.4	0.773	0.626	-----	6.747	-----	13.92

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**Figure (3):** The electrophoretic photograph of Glutathione reductase (554bp) mRNA expression in the kidney tissue of male rats.

**M:** 100bp-1000bp DNA-ladder

**1:** Male rats used as control group

**2:** Male rats treated with GSH for 15 days and then received CDDP plus GSH for 5 days

**3:** Male rats treated with CDDP plus GSH for 5 days

**4:** Male rats treated with vitaminC for 15 days then injected with CDDP plus GSH for 5 days.

**5:** Male rats treated with cisplatin plus vitaminC for 5 days.

**6:** Male rats treated with CDDP only for 5 days.

**N.B:** Molecular weight where refers to specific amplified product for GAPDH according to the synthesized primer.

Tabel: (2) Analysis of product of mRNA in kidney homogenate of male rats

Groups	Lane	GRD	GSH px	GAPDH
Control	1	1.568 $\mu$ g	$\mu$ 2.892g	4.45 $\mu$ g
GSH treated for 15 days, then treated with CDDP and GSH for 5 days	2	1.298 $\mu$ g	2.694 $\mu$ g	4.51 $\mu$ g
Vit: treated for 15 days then treated with CDDP and glutathione for 5 days	3	1.165 $\mu$ g	2.764 $\mu$ g	4.48 $\mu$ g
CDDP GSH and treated group	4	1.201 $\mu$ g	2.790 $\mu$ g	4.39 $\mu$ g
CDDP and vit. C treated group	5	1.246 $\mu$ g	2.843 $\mu$ g	4.46 $\mu$ g
CDDP treated group	6	1.082 $\mu$ g	2.398 $\mu$ g	4.42 $\mu$ g

## **DISCUSSION**

Using an experimental model of CDDP-induced nephrotoxicity in rats, we evaluated the renal activity of the key antioxidant enzymes (SOD, GSH-Px), the glutathione level and TEARS content of tissue as an index of lipid peroxidation. The cytotoxic effects of CDDP are postulated to occur via several mechanisms, including the inhibition of protein synthesis, mitochondria injury and DNA damage (**Park *et al.*, 2002**). Several studies have now documented the importance of ROS in CDDP-induced nephrotoxicity (**Ueda *et al.*, 2000**). Oxidative damage of mitochondria might be the initial event causing renal injury by CDDP (**Chang *et al.*, 2002**). In the present study we assessed in vivo the mitochondria involvement in CDDP-induced nephrotoxicity.

Reports obtained from our study indicate that CDDP increases lipid peroxidation in the treated tissue of rat. The drug is also involved in altering the thiol status of the tissue with concomitant alterations in the enzymatic antioxidants. GSH, SOD and GSH<sub>Px</sub> levels were significantly decreased after CDDP therapy. This effect may be a secondary event following the CDDP-induced increase in free radical generation and/or decrease in lipid peroxidation protecting enzymes. CDDP can cause the generation of oxygen free radicals, such as hydrogen peroxide, superoxide anions and hydroxyl radicals. The hydroxyl radical is capable of abstracting a hydrogen atom from polyunsaturated fatty acids in membrane lipids to initiate lipid peroxidation. These radicals can evoke extensive tissue damage, reacting with macromolecules, such as membrane lipids, proteins and nucleic acids (**Emerit *et al.*, 2001**). Moreover, depletion of glutathione may contribute to CDDP -induced lipid peroxidation (**Antunes *et al.*, 2000**). Thus, an alteration in enzymatic antioxidant

status with increase in lipid peroxidation and nitric oxide indicates that the enzymes play an important role in combating free radical induced oxidative stress on the tissue.

Our results demonstrated that treatment with CDDP (2mg/kg, intraperitoneally) induced biochemical and histological signs of renal injury with significant reduction in the activity of SOD and GSH<sub>px</sub>. However, several studies demonstrated that CDDP induced acute nephrotoxicity is mediated by depletion of renal GSH and by impaired activity of SOD and GSH<sub>px</sub> as well as an increase in renal lipid peroxidation (**Kadikoylu *et al.*, 2004**). In relation to renal GSH levels, whereas some reports show GSH depletion in acute CDDP nephrotoxicity, others show increased or normal GSH levels under the same experimental conditions.

In the present study, it has been shown that administration of CDDP to rat caused an elevation in plasma creatinine and urea levels. Also, CDDP-induced nephrotoxicity was accompanied by an increase in MDA, reduction of total thiol and GSI-I-Px concentrations in kidney tissue. These results are in accordance with several studies have reported that the alterations induced by CDDP in the kidney functions were characterized by signs of injury, such as increase in products of lipid peroxidation and changes in total thiol concentration in kidney tissue as well as glucose, protein, creatinine and urea levels, in urine and plasma samples (**Atessahin *et al.*, 2005**).

The impairment of the mitochondria antioxidant defense system (decrease in GSH and NADPH levels, GSH/GSSG ratio, increase in GSSG level) in the CDDP group. This depletion of the antioxidant system could be explained as secondary to the oxidative stress caused by CDDP, or it could explain the oxidative stress itself, in the case of a direct effect of CDDP on enzymatic and non-enzymatic antioxidant system. In fact, it has been reported that CDDP causes a

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decrease in the activities of antioxidant enzymes, including SOD, CAT and GSH-Px (**Antunes *et al.*, 2000**) and GRD (**Kadikoylu *et al.*, 2004**) in kidney tissues.

The depletion of GSH and the accumulation of GSSG could, therefore, be explained by the decreased activity of GDR resulting from a direct attack of CDDP. Additionally, there is evidence from both in vivo and cell-culture studies that CDDP is metabolized to a nephrotoxin through a GSH-conjugate intermediate. It has been shown that inhibiting the conjugation of CDDP to GSI-I reduces CDDP nephrotoxicity (**Hanigan and Devarajan, 2003**). The formation of the Platinum-GSH conjugate could also account for mitochondrial GSH depletion and eventually for the alteration of the redox state in kidney mitochondria.

GSH plays a role in the regulation of K<sup>\*</sup>-channel kinetics (**Ruppersberg *et al.*, 1991**). This regulation is probably based upon the fast and reversible formation of disulfide bridges between different functional parts of the K<sup>\*</sup> channel. The depletion of intracellular GSH may have profound effects on the function of cells that are dependent on the normal kinetics of this ion channel. It is also possible that the kinetics of other ion channels is also regulated by intracellular GSH levels. This could be another mechanism by which GSH influences nerve cell function and health. Finally, neuroprotection may be a consequence of the action of GSH on kidney function. Some data support this hypothesis. First, the excretion of CDDP remains constant during treatment, as demonstrated by **Bohm, et al., (1991)**. This indicates minimal kidney damage. Otherwise, GSH pretreatment does not influence the pharmacokinetic parameters of CDDP and does not change the bound fraction of CDDP in plasma (**Leone *et al.*, 1992**).

The decreased concentration of GSH increases the sensitivity of organ to oxidative and chemical injury. The role of GSH, non-

protein thiols in the cells, in the formation of conjugates with electrophilic drug metabolites most often formed by cytochrome P-450 linked monooxygenase is well established (**Rana *et al.*, 2002**). Reduced renal GSH can markedly increase the toxicity of CDDP. The depletion of GSH also seems to be a prime factor that permits lipid peroxidation in the CDDP treated group. Concomitant treatment of vitamins rendered protection due to the increase in GSH concentration and could protect the renal cells from oxidants attack.

The decreased SOD activity could cause the initiation and propagation of lipid peroxidation in the CDDP treated group. This decreased activity may be either due to loss of copper and zinc, which are essential for the activity of enzyme or due to reactive oxygen species-induced inactivation of enzyme proteins (**Ajith *et al.*, 2007**). The activity of CAT and GPx also decreased in the CDDP alone treated group, which in turn increased the H<sub>2</sub>O<sub>2</sub> concentration and enhanced the lipid peroxidation. Hence the concentration of MDA, as a result of lipid peroxidation, increased in the CDDP treated group. Treatment with vitamins prevented the lipid peroxidation by enhancing the renal CAT and GSH-Px activities.

In the present investigation, treatment with Vit C inhibited the increase in lipid peroxidation induced by CDDP in renal tissue which was measured in terms of MDA, a stable metabolite of the free radical mediated lipid peroxidation cascade. MDA level was increased significantly in CDDP-treated group. VitC has reversed the enhancement of MDA level to a considerable extent, thereby confirming its antioxidant role in CDDP acute renal failure (**Hosseinzadeh *et al.*, 2005**). The ability of vit.C to significantly decrease renal lipid peroxidation caused by CDDP supports the idea that supplementation with dietary antioxidants could maintain the integrity of lipids in kidney tissue under oxidative stress induced during chemotherapy.

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As Vit.C has been shown to protect against various kinds of injuries and neoplasms involving oxidative stress, it is possible that treatment of animals with Vit.C may attenuate CDDP -induced lipid peroxidation, glutathione depletion, and a decrease in the glomerular filtration rate. (**Antunesu *et al.*, 2000**). Moreover, Vit.C should be able to diminish DNA damage by reducing radical species directly, decreasing formation of reactive species such as lipid hydroperoxides or preventing radical attack on proteins that repair DNA. Vit.C as an antioxidant can prevent nitrosamine formation, so subsequent formation of some reactive nitrogen species is prevented. Once nitrosamines give rise to reactive nitrogen species, prevention of mutagenic activity by Vit.C is less effective in prevention of DNA damage (**Odin, 1997**).

A link between Vit.C and DNA repair has been suggested. Intracellular Vit.C calls protect human lymphocytes against oxidative DNA damage in a dose-dependent manner (**Cooke *et al.*, 1998**). Furthermore, DNA levels of 8-oxodG, a biological marker of oxidative DNA damage in vivo, decrease significantly after ascorbate supplementation, and this decrease is strongly correlated with increases in the plasma Vit.C concentration (**Lenton *et al.*, 1999**). These pieces of evidence suggest that antioxidant defences, together with DNA repair, may contribute to give a steady-state level of damage, which represents a minimal risk for the cell. Although interesting, the exact correlation between Vit.C and tile DNA repair machinery has not been extensively investigated.

The present observations support the hypothesis that the mechanism of ncphrotoxicity is related to the depletion of the antioxidant defense system. CDDP treatment has been shown to induce loss of copper and zinc in the kidneys. The decrease in SOD activity in renal tissues following CDDP administration might be due to the loss of copper and zinc (Sharma, 1985). The activity of CAT

and GPx is also found to decrease after CDDP administration resulting in the decreased ability of the kidney to scavenge toxic hydrogen peroxide and lipid peroxides. The results from the present study indicate that the vitamin C and GSH significantly reduced the depletion of GSH levels and antioxidant enzyme activity in the renal cortex of mice treated with CDDP.

Similar to these studies (St. Clair *et al.*, 1991), have shown that over-expression of MnSOD attenuates the toxic effects of paraquat with no affect on endogenous copper/zinc SOD, CAT, or glutathione peroxidase expression. These results are consistent with our observations with CDDP in that over-expression of MnSOD also demonstrated the role of superoxide radicals in paraquat cytotoxicity. Furthermore, studies in mouse proximal tubular cells have demonstrated that antioxidants, including SOD and CAT, protect these cells from ROS in apoptosis that is induced by either growth factor deprivation (Lieberthal *et al.*, 1996) or CDDP (Lieberthal *et al.*, 1998).

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