**ABSTRACT**

Cisplatin is the potent antineoplastic drug with significant nephrotoxic effects. The present study is to evaluate the nephrotoxic effects on varying doses of cisplatin such as 5mg/kg, 6mg/kg and 7mg/kg doses on male albino rats of Wistar strain. Various parameters determining nephrotoxicity such as blood urea nitrogen (BUN), urea, kidney weight and total body weight were assessed along with the in vivo antioxidant parameters like lipid peroxidation, reduced glutathione and catalase. Based on the histopathological evaluation this study has significantly shown the severity in the damage of the glomerular, tubular and epithelial cells on varying doses of cisplatin dose dependently.

**INTRODUCTION**

Nephrotoxicity is one of the most common kidney problems which are mostly caused due to exposure to environmental or industrial chemicals and drugs. The chemicals like sodium oxalate, ethylene glycol, carbon tetrachloride, heavy metals such as mercury, lead, arsenic and cadmium and chemotherapeutic agents such as non steroidal anti-inflammatory drugs and aminoglycoside antibiotics induces nephrotoxicity. Kidney has high blood supply and the presence of cellular transport systems causes accumulation of these compounds within the nephron epithelial cells which lead to acute renal failure, chronic interstitial nephritis and nephritic syndrome. Kidney disease is one of the common causes for hospitalization (Arunachalam et al., 2013; Ramya pydi et al., 2011; Sreedevi et al., 2010).

Cisplatin is a platinum compound and one of the most potent antineoplastic agents used for the treatment of solid tumours such as neck, lung, testicular, ovarian and cervical cancers. The cytotoxic mechanism involves the interaction with DNA by forming the covalent adducts between certain DNA bases and the platinum compound (Sudhavani et al., 2010; Srinivasan et al., 2011). Besides its clinical applications, cisplatin is associated with several dose dependent toxicities which include nephrotoxicity, hepatotoxicity, neurotoxicity (Mustafa et al., 2013) and cardiotoxicity, but the prevalence of nephrotoxicity is most prominent. The general toxicity mechanism includes oxidative stress by the generation of reactive oxygen species such as hydrogen peroxide, superoxide anion and hydroxyl radicals which are generated under normal cellular conditions and are immediately detoxified by the endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and non enzymatic compounds as reduced glutathione (GSH). But the excessive accumulation caused by cisplatin causes an imbalance in antioxidants due to loss of copper and zinc in kidneys in case of depleted SOD and leads to peroxidation of lipids and depletion of glutathione (Ammar et al., 2013; Ahmed et al., 2012; Srinivasan et al., 2011). The synthetic and free radical scavengers such as glycine and selenomethionine show partial protection against cisplatin damage (Annie et al., 2003).

Many approaches have been made since many years to alleviate these side effects. One strategy is to synthesize the novel cisplatin analogues that have lesser side effects in normal tissues which led to the synthesis of Carboplatin with less severe side effects. Another approach is made to well hydrate the patients with normal saline and mannitol. Sometimes reduction in the dose of cisplatin or discontinuation of the treatment. Despite these efforts, the side effects of cisplatin limit its use and efficacy in cancer therapy (El-sayed et al., 2011; Sahat et al., 2010; Xin Yao et al., 2007).

According to Wasim et al., 2010, the elevated levels of blood urea nitrogen and serum creatinine are the indicators of nephrotoxicity along with the acute tubular injury observed in the histopathological studies when compared with the normal histopathological studies. The main aim of the present study is to evaluate the changes in the levels of various parameters involved in nephrotoxicity on varying doses of cisplatin administration i.e 5mg, 6mg and 7mg/kg body weight of the animal.
MATERIALS AND METHODS

Chemicals & Drugs
Cisplatin injection available as Cytoplatin 50mg/50ml manufactured by Cipla is used. The biochemical kits used are obtained from coral and Span. All the chemicals used were of analytical grade.

Animals & Experimental design
Male albino rats of Wistar strain weighing 200-250g are purchased from Teena labs, Hyderabad. The animals are subjected to acclimatization for one week provided with food and water *ad libitum*. The animals were maintained at a controlled temperature under 12hrs dark/light cycle in the animal house at CMR College of pharmacy, Hyderabad approved by CPCSEA.

The experimental animals used in the study were divided into four groups containing six in each. The first group is the control group which is administered with normal saline. The second, third and fourth groups are treated with 5mg/kg, 6mg/kg and 7mg/kg cisplatin (*i.p* route) respectively. The drug cisplatin is administered on day one for the experimental animals. On fifth day the blood was withdrawn by retro-orbital puncture under light ether anaesthesia and the animals were sacrificed by spinal dislocation (Salma et al., 2011). The body weights of all the animals were noted on first day before the administration of cisplatin and on fifth day before sacrificing the animals (Kundan et al., 2013).

Biochemical estimations
The serum obtained from the blood was used for the evaluation of biochemical parameters like blood urea nitrogen (BUN) and serum creatinine. Kidneys were rapidly excised, trimmed of connective tissue and the wet weights are noted. The trimmed kidneys were subjected to washing with ice-cold normal saline to make free from blood and were used for the preparation of post mitochondrial supernatant for *in vivo* antioxidant studies and histopathological studies.

In vivo antioxidant studies
Kidney homogenate is chilled in phosphate buffer pH 7.4 using a tissue homogenizer. The post mitochondrial supernatant (PMS) collected after centrifuging at 10000 rpm for 20 mins was used for *in vivo* antioxidant parameters like lipid peroxidation (Niehaus et al., 1968), Catalase (Hugo, 1984) and reduced glutathione (Jollow et al., 1974).

Estimation of Lipid Peroxidation (LPO)
The procedure was followed according to Niehaus et al., 1968. The PMS of volume 0.5ml was allowed to react with 0.5ml tris hydrogen chloride buffer and incubated at 37°C for 2hrs followed by addition of 1ml ice cold trichloracetic acid and centrifuged at 1000rpm for 10 mins and 1ml supernatant was added to thiobarbituric acid and boiled for 10 mins. After cooling 1ml distilled water is added and the absorbance was measured at 532 nm against the blank without tissue homogenate.

Estimation of Reduced Glutathione (GSH)
According to Jollow et al., 1974, the PMS of volume 0.75ml is mixed with 0.75ml of 4% sulfoasalicylic acid and centrifuged. 0.5ml of supernatant was added with 4.5ml of 0.01M DTNB and the absorbance was measured at 412 nm against the blank without tissue homogenate.

Estimation of Catalase (CAT)
The tissue homogenate in volume 0.4 ml was diluted 20 times with phosphate buffer pH 7.0 and for 2ml of the diluted homogenate 1ml of hydrogen peroxide was added and the absorbance was measured at 240 nm against the blank without tissue homogenate (Hugo, 1984).

Statistical analysis
The statistical analysis was carried out using one way ANOVA and unpaired student’s ‘t’ test and the values were expressed as mean ± SEM. Values are considered statistically significant at P<0.001

RESULTS

Histopathological Studies
Pieces of kidney from each group were fixed immediately in 10% formalin solution for at least 24hrs then dehydrated in alcohol (50-100%), embedded in paraffin, cut into 4-5µm thick sections and stained with hematoxylin-eosin. The sections obtained are evaluated for the pathological signs of nephrotoxicity which includes necrosis, fatty infiltration, lymphocyte infiltration and fibrosis (Abubaker et al., 2012).

Thin Sections of kidney

Fig 1 A photomicrograph of normal rat kidney showing normal organization of glomerular (G) and tubular epithelial cells.

Fig 2 A photomicrograph of rat kidney treated with Cisplatin 5mg/kg showing glomerular congestion(G) but not significant.
DISCUSSION

Kidney is an essential organ which has a prominent role in maintaining body homeostasis by excreting the metabolic waste products and conserving necessary products depending on the needs of the body. Renal failure is a common clinical syndrome of nephrotoxicity which causes a rapid decline in renal functioning due to decreased glomerular filtration rate (GFR) and the inability of the kidney to excrete the toxic metabolic substances produced in the body resulting in abnormal retention of renal biomarkers i.e. serum creatinine and blood urea nitrogen (Arunachalam et al., 2013).

Nephrotoxicity is a complex process which has been reported to involve DNA damage, caspase activation, mitochondrial dysfunction, formation of reactive oxygen and nitrogen species, polymerase overactivation, and inflammation leading to necrosis and apoptosis. (Dong et al., 2007). Glomerular, tubular and interstitial cells frequently encounter with significant concentrations of medications and their metabolites, which can induce changes in kidney function and structure. The renal tubular cells such as proximal tubular cells of inner cortex and outer medulla which are the major sites for cisplatin induced renal damage absorb high amounts approximately five times of drug compared with extracellular concentration after its administration which promotes the generation of ROS (Hao et al., 2009).

Cisplatin can induce nephrotoxicity after its single Intraperitoneal administration, which is a rapid process involves reaction with the proteins in the renal tubules which occurs mostly in the first hour of administration (Sreedevi et al., 2010). Approximately, 25-35% of the patients treated with single dose (50-100mg/m²) of cisplatin show renal dysfunctioning associated with high mortality (Gholamreza et al., 2010; Sahar et al., 2010).

Cisplatin is stable in the blood stream but it is hydrolyzed in the chloride-poor cellular environment and the hydrolyzed metabolite binds DNA, RNA, proteins, and phospholipids, causing cytotoxicity (Miller et al., 2010). Recent studies suggest that inflammatory mediators such as macrophages and leukocytes are released due to cisplatin induced damage. Tumour necrosis factor (TNF)-α mediated apoptic pathway is reported to be one of the major parts in cisplatin induced damage (Sudhavani et al., 2010; Srinivasan et al., 2011).

The oxidative stress is associated with lipid peroxidation on cellular lipids, which is determined by measurement of thiobarbituricacid reacting substance. The concentration of LPO products reflects the degree of oxidative stress and the malondialdehyde (MDA) concentration is a measure for lipid peroxidation. The levels of the products of LPO was significantly increased in varying doses of cisplatin (Niehaus et al., 1968). GSH is a major non-protein thiol which is the endogenous antioxidants that counter balances free radical mediated damage. It is involved in the protection of normal cell structure and function by maintaining homeostasis. In this

Table 1 Effect of Cisplatin treatment in varying doses on body weight, BUN and serum creatinine

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment group</th>
<th>Changes in kidney weight (g)</th>
<th>Changes in body weight (%)</th>
<th>BUN (mg%)</th>
<th>Serum Creatinine (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal saline</td>
<td>0.43 ± 0.05</td>
<td>10.9 ± 3.55</td>
<td>18.5 ± 2.72*</td>
<td>0.7 ± 0.27*</td>
</tr>
<tr>
<td>2</td>
<td>Cisplatin 5mg/kg</td>
<td>0.69 ± 0.06*</td>
<td>-11.7 ± 2.52*</td>
<td>31.8 ± 1.72*</td>
<td>2.5 ± 0.10*</td>
</tr>
<tr>
<td>3</td>
<td>Cisplatin 6mg/kg</td>
<td>0.70 ± 0.07*</td>
<td>-11.9 ± 2.10*</td>
<td>31.5 ± 2.45*</td>
<td>2.1 ± 0.16*</td>
</tr>
<tr>
<td>4</td>
<td>Cisplatin 7mg/kg</td>
<td>0.75 ± 0.07*</td>
<td>-13.1 ± 2.78*</td>
<td>33.5 ± 2.67*</td>
<td>2.9 ± 0.14*</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SEM (n=6 animals per group). *P<0.001 Vs Group 1

Table 2 Effect of Cisplatin treatment in varying doses of In vivo antioxidant studies

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment group</th>
<th>LPO nmol/mg of tissue</th>
<th>GSH nmol/mg of tissue</th>
<th>CAT nmol/mg of tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal saline</td>
<td>0.26 ± 0.03</td>
<td>0.70 ± 0.02</td>
<td>0.83 ± 0.008</td>
</tr>
<tr>
<td>2</td>
<td>Cisplatin 5mg/kg</td>
<td>0.82 ± 0.02*</td>
<td>0.60 ± 0.03*</td>
<td>0.66 ± 0.01*</td>
</tr>
<tr>
<td>3</td>
<td>Cisplatin 6mg/kg</td>
<td>0.83 ± 0.01*</td>
<td>0.52 ± 0.01*</td>
<td>0.60 ± 0.01*</td>
</tr>
<tr>
<td>4</td>
<td>Cisplatin 7mg/kg</td>
<td>0.94 ± 0.01*</td>
<td>0.34 ± 0.01*</td>
<td>0.47 ± 0.01*</td>
</tr>
</tbody>
</table>

Values were expressed as mean ± SEM, *P<0.001 Vs Group 1
study the increasing dose of cisplatin increases the amount of free radicals produced which significantly decreases the levels of this endogenous antioxidant as it is involved in scavenging the reactive oxygen species (Jollow et al., 1974). While CAT is a hemoprotein which is localized to microperoxisomes reduces hydrogen peroxide by dismutation reaction and prevents generation of hydroxyl radicals thereby protecting the cellular constituents from oxidative damage in peroxisome. The enzyme catalyses the decomposition of hydrogen peroxide to water and oxygen and thus protecting the cell from oxidative damage. The increase in the levels of the hydroxyl radicals counteract with CAT and cause its significant decrease according with the dose (Hugo, 1984).

In the present study, it is determined that the blood parameters related to nephrotoxicity include blood urea nitrogen and serum creatinine have shown the elevated levels in the cisplatin treated groups when compared with control group. According to the results illustrated the weight of the animal and the percentage change in body weight has significantly decreased on cisplatin treatment dose dependently on comparison with control rats. These findings have shown the nephrotoxic effect of cisplatin which has produced marked reduction of rat’s body weight with a significant increase in relative kidney weight.

Generally, the animals increase in the body weight along the duration of time as in case of the group one. The loss of weight is closely related to the gastrointestinal toxicities and concomitant loss of animal appetite with subsequent reduction of food ingestion or due to excessive loss of water, salts and proteins possibly due to the tubular damage with subsequent dehydration and weight loss. The rise in weight of kidney might be due to the inflammatory mechanisms. The glomerular damage caused in cisplatin treated group might be due to ROS generation and that possibly elevate the serum creatinine and blood urea levels. The oxidative stress inhibits the antioxidant enzymes and generates the ROS that destroys the lipid, protein and DNA components of the cell with subsequent enzymatic inactivation and mitochondrial dysfunction which would be the major pathway of nephrotoxicity (Dilek et al., 2013; Nasr, 2013).

Cisplatin is one of the commonly used drugs for inducing nephrotoxicity. It can be potentially used to evaluate the nephroprotective effect of various natural and synthetic compounds. The microscopical evaluation of the sections revealed the marked tubular degeneration, necrosis and desquamation of the tubular epithelial cells with cystic formation, interstitial cellular infiltration, wide capsular space and congested glomerular capillary tufts which was dose dependent. Cisplatin 7mg/kg dose could be the optimum dose for inducing nephrotoxicity in rats with less mortality. The higher doses can significantly show high mortality (Marcos et al., 2009) and several experimental deviations.

CONCLUSION
From the study it can be concluded that increasing doses of cisplatin significantly decreases the levels of various invivo antioxidants such as CAT & GSH and increase the levels of nephrotoxicity evaluating parameters like BUN and serum creatinine. Cisplatin can be extensively used for inducing nephrotoxicity and studying various nephroprotective compounds.

References