CHAPTER 8
NEPHRO PROTECTIVE EFFECT AND PREVENTION OF
DRUG INDUCED OXIDATIVE DAMAGE BY THE
EXTRACTS OF PLEUROTUS FLORIDA AND
PLEUROTUS SAJOR-CAJU
8.1 INTRODUCTION

The kidneys are exposed to many potential toxins because of its anatomy and physiology. Prerenal factors affecting cardiac output, drugs altering intrarenal haemodynamics and those directly toxic to the renal parenchyma may cause life-threatening renal impairment. Cisplatin (Cisplatinum (II) diammine dichloride) is a highly effective and extensively used anticancer drug against a variety of cancers. Higher doses of cisplatin are more efficacious for the treatment. However high dose chemotherapy of this drug manifests acute nephrotoxicity, ototoxicity, and other toxicities (Bodenner et al., 1986, Hamers, 1993). This deleterious side effect is one of the limiting factors for its use in cancer chemotherapy. A number of chemotherapeutic agents have been reported to render protection against cisplatin induced nephrotoxicity (Tognella, 1990). However, none of them is known to be clinically effective as a complete protective agent. Several lines of evidence suggest that free radicals are involved in the nephrotoxicity induced by cisplatin and the damage is the consequence of decreased renal antioxidant enzyme activity with the enhanced lipid peroxidation (Ajith et al., 2002). Administration of antioxidants has been shown to ameliorate cisplatin induced nephrotoxicity in animals (Babu et al., 1995).

Mushrooms are widely distributed in the world and some of them have been used in traditional Chinese medicines. In the last 15-20 years medicinal mushrooms have been a subject of intensive investigation in several laboratories for their therapeutic use. Since, oyster mushrooms have been
reported to possess a large number of medicinal properties, investigations were
carried out to find out the nephroprotective effect of *P. florida* and *P. sajor-
caju*. Methanolic extracts of these mushrooms used for the studies and the
findings are reported in this chapter

**8.2 MATERIALS AND METHODS**

**8.2.1 ANIMALS**

Male Swiss albino mice of 6-8 weeks of age weighing 25 ± 2 g were
used.

**8.2.2 DETERMINATION OF NEPHROPROTECTIVE ACTIVITY OF
*P. FLORIDA***

Nephroprotective activity of the extract was determined by cisplatin
induced nephrotoxic model described by Somani et al., (2000) with some
modifications. Animals were divided into 4 groups of 6 animals in each group
and treated as follows. Group I (normal) administered with normal saline
intraperitoneally (i.p). Group II was given 16 mg/Kg cisplatin i.p (16 ml/Kg) as
a single dose kept as normal. Group III and IV were given 500 mg/Kg and
1000 mg/Kg body weight methanolic extract of *P. florida* respectively in
normal saline (i.p) 30 min before the injection of cisplatin (16 mg/kg body
weight, i.p). Mice in all groups were sacrificed 72 h after the cisplatin
injection. The blood was collected directly from the heart; serum was separated
for creatinine and urea analysis. The kidneys were dissected and stored at -70°C
until analyses were completed. The kidneys were homogenized in 50 mM
phosphate buffer (pH 7.0) to give a 10% homogenate (w/v) (section 2.2.2).
Serum creatinine and urea were estimated by the method of Brod and Sirota (1980) and Marsh et al., (1980) respectively. Tissue homogenate was used for assay of SOD, CAT, GPx, GSH, MDA and protein as described in the sections 7.2.3.6, 7.2.3.7, 7.2.3.8, 7.2.3.5, 4.2.3.3.1 and 4.2.3.3.2 respectively.

8.2.2.1 DETERMINATION OF SERUM UREA

Serum urea was determined according to the method of Marsh et al., (1980).

PRINCIPLE

Urea on heating with diacetylmonoxime under acidic condition condenses with diacetyl to form a pink colored diazine complex. The reaction was catalyzed by thiosemicarbazide and Fe$^{3+}$ ions. The absorbance of the complex was measured at 525 nm.

PROCEDURE

Reagents used were from Span diagnostic kit. 5 ml of diluted urea reagent (1:5 with distilled water) were mixed with 0.02 ml serum and 0.5 ml of diacetylmonoxime. Mixed well and kept in boiling water bath for 10 min. Cooled and absorbance was measured at 525 nm against reagent blank. A standard solution of urea (30 mg/dl) was treated in the same way.

Calculation

\[
\text{Serum urea (mg/dl)} = \frac{O.D_T \times 30}{O.D_s}
\]

\(O.D_T\) – Optical density of test

\(O.D_s\) – Optical density of standard
8.2.2.2 DETERMINATION OF SERUM CREATININE

Serum creatinine was determined according to method of Brod and Serota as described in Text book of Clinical Biochemistry, Varley (1980)

PRINCIPLE

Creatinine forms a yellow-orange colored complex in alkaline medium with picric acid. The intensity of the color is measured at 500 nm. The concentration of the dye formed over a specific reaction time is the measure of the creatinine concentration.

PROCEDURE

Reagents used were from Merk diagnostic kit. 0.2 ml of serum was mixed with 0.5 ml of buffer (313 mM NaOH and 12.5 mM phosphate, pH 8), 0.5 ml of 8.73 mM picric acid. The absorbance is measured immediately after 1 min (O.Dt1) and exactly after 5 min (O.Dt2) at 500 nm. A standard creatinine solution (1 mg/dl) was treated in the same way.

Calculation

Creatinine concentration (mg/dl) = \( \frac{O.Dt_2 - O.Dt_1}{O.Ds_2 - O.Ds_1} \)

8.2.3 DETERMINATION OF NEPHROPROTECTIVE ACTIVITY OF P.SAJOR-CAJU

To determine the nephroprotective effect of methanolic extract of P.sajor-caju all experiments described above were repeated using methanolic extract of this mushroom. Serum urea and creatinine were assayed as above. Activities of SOD, CAT, GPx, GSH, MDA and protein content in the tissue
Homogenate were determined as described in sections sections 7.2.3.6, 7.2.3.7, 7.2.3.8, 7.2.3.5, 4.2.3.3.1 and 4.2.3.3.2 respectively.

8.2.4 EFFECT OF PRETREATMENT OF *P. FLORIDA* AND *P. SAJOR-CAJU* EXTRACTS ON ANTITUMOUR ACTIVITY OF CISPLATIN

Efficacy of cisplatin on antitumour activity in concomitant administration of methanolic extract of *P. florida* was determined. Animals were injected with 1 x 10^6 viable cells of Daltons Lymphoma Ascites (DLA) in phosphate buffered saline in the right groin and divided into 3 groups of 6 animals each. After 24 hr of tumour implantation, animals were treated as follows; Group 1 treated with saline intraperitonealy was kept as control, group 2 treated with *P. florida* (1000 mg/Kg, i.p) 30 minutes before cisplatin injection (3 mg/Kg body weight i.p) and group 3 was given cisplatin injection (3 mg/Kg body weight i.p). The treatments continued for 10 consecutive days. At the end of 5th week, the animals were sacrificed and tumours extirpated and weighed. The percent inhibition was calculated using the formula (1-B/A) X 100 where, A is average tumour weight of the control group and B is that of treated.

The Effect of pretreatment of methanolic extract of *P. sajor-caju* on efficacy of antitumour activity of cisplatin was also studied. The experiment described above was repeated employing methanolic extract of *P. sajor-caju*. 
8.3 RESULTS

8.3.1 NEPHROPROTECTIVE ACTIVITY OF *P. FLORIDA* AND *P. SAJOR-CAJU*

Serum creatinine levels registered an eight-fold increase in cisplatin treated mice compared to normal group. Administration of methanolic extract of *P. florida* and *P. sajor-caju* (500 and 1000 mg/Kg body weight) significantly reduced the increased serum creatinine levels to almost normal (Table 8.1 and 8.2). Serum urea level was also increased over six-fold in cisplatin treated group than the normal. The methanolic extract of *P. florida* and *P. sajor-caju* (500 and 1000 mg/Kg body weight) significantly reduced the increase in serum urea level in a dose-dependent manner (Table 8.1 and 8.2).

Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities in the kidney significantly decreased in cisplatin treated group of mice compared to the normal. Administration of methanolic extract of *P. florida* and *P. sajor-caju* (500 and 1000 mg/Kg body weight) prior to cisplatin treatment significantly enhanced these enzyme activities (Table 8.3 and 8.4).

The concentration of renal GSH was significantly decreased in cisplatin treated group of animals compared to normal. Administration of methanolic extract of *P. florida* and *P. sajor-caju* (500 and 1000 mg/Kg body weight) prior to cisplatin treatment increased the level of GSH significantly (Figure 8.1 and 8.2).
The concentration of renal malondialdehyde significantly increased in cisplatin treated group of animals compared to control. Administration of methanolic extract of *P. floridajloa* and *P. sajor-caju* (500 and 1000 mg/Kg body weight) prior to cisplatin treatment registered a significant decrease in malondialdehyde levels (Figure 8.3 and 8.4).

### 8.3.2 PRETREATMENT OF *P. FLORIDA* AND *P. SAJOR-CAJU* EXTRACTS ON ANTITUMOUR ACTIVITY OF CISPLATIN

Administration of methanolic extract of *P. floridajloa* prior (30 min) to the injection of cisplatin did not interfere with the tumour reducing activity of cisplatin. Cisplatin plus methanolic extract and cisplatin alone reduced the tumour development by 98.3% and 95.2% respectively compared to the control group. The tumour weight of the animals in the control group was $11.11 \pm 1.03$ g whereas the same for the animals treated with cisplatin plus *P. floridajloa* extract and cisplatin alone was $0.178 \pm 0.03g$ and $0.513 \pm 0.110g$, respectively (Table 8.5).

Similarly, methanolic extract of *P. sajor-caju* prior (30 min) to the injection of cisplatin did not interfere with the tumour reducing activity of cisplatin. Cisplatin plus methanolic extract of *P. sajor-caju* and cisplatin alone reduced the tumour development by 98.2% and 95.5% respectively compared to the control group. The tumour weight of the animals in the control group was $12.432 \pm 1.030$ g whereas the same for the animals treated with cisplatin plus extract and cisplatin alone was $0.212 \pm 0.040$ g and $0.560 \pm 0.080$ g, respectively (Table 8.6).
Table 8.1: Effect of methanolic extract of *P. florida* on the increase in serum urea and creatinine levels induced by cisplatin administration

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments (mg/kg)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Saline</td>
<td>61.55 ± 3.89&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.42 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (cisplatin)</td>
<td>16</td>
<td>370.20 ± 7.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.58 ± 0.34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methanol extract + Cisplatin</td>
<td>500</td>
<td>99.50 ± 6.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.77 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>70.38 ± 5.48&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.50 ± 0.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6 animals. Any two values having a common letter are not significantly different at 5% level, lsd= 0.5.876 (Urea), lsd= 0.0.202 (Creatinine)

Table 8.2: Effect of methanolic extract of *P. sajor-caju* on the increase in serum urea and creatinine levels induced by cisplatin administration

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatments (mg/kg)</th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Saline</td>
<td>59.15 ± 2.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.46 ± 0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (cisplatin)</td>
<td>15</td>
<td>363.22 ± 4.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.87 ± 0.61&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methanol extract + Cisplatin</td>
<td>500</td>
<td>95.37 ± 7.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.63 ± 0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>64.35 ± 4.86&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.43 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6 animals. Any two values having a common letter are not significantly different at 5% level, lsd= 0.5.876 (Urea), lsd= 0.0.375 (Creatinine)
Table 8.3: Effect of methanolic extract of *P. florida* on renal SOD, CAT and GPx activities induced by cisplatin

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Saline</td>
<td>11.79 ± 0.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td>48.11 ± 4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.22 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (cisplatin)</td>
<td>16</td>
<td>6.75 ± 0.88&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.22 ± 0.59&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.28 ± 0.70&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methanol extract + Cisplatin (16mg/mg)</td>
<td>500</td>
<td>8.81 ± 1.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>30.18 ± 4.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.81 ± 2.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>13.7 ± 0.92&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.57 ± 2.43&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.5 ± 3.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6 animals. Any two values having a common letter are not significantly different at 5% level, lsd= 1.132 (SOD), lsd= 3.202 (CAT), lsd= 3.2 (GPx)

Table 8.4: Effect of methanolic extract of *P. sajor-caju* on renal SOD, CAT and GPx activities induced by cisplatin

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment (mg/kg)</th>
<th>SOD (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Saline</td>
<td>12.85 ± 1.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.58 ± 4.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.32 ± 4.89&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control (cisplatin)</td>
<td>16</td>
<td>7.78 ± 0.99&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.07 ± 1.15&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.80 ± 1.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methanol extract + Cisplatin</td>
<td>500</td>
<td>9.29 ± 0.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.45 ± 5.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.87 ± 2.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>13.83 ± 1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45.26 ± 6.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.58 ± 3.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6 animals

Any two values having a common letter are not significantly different at 5% level, lsd= 1.501 (SOD), lsd= 5.837 (CAT), lsd= 3.10 (GPx)
Table 8.5: Effect of cisplatin, cisplatin + methanolic extract of *P. florida* on solid tumour

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg/Kg)</th>
<th>Tumor Volume (cm$^3$)</th>
<th>Tumor weight (g)</th>
<th>%Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1.345 ± 0.08$^a$</td>
<td>11.110 ± 1.030$^a$</td>
<td>---</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>4</td>
<td>0.061 ± 0.21$^b$</td>
<td>0.513 ± 0.110$^b$</td>
<td>95.2</td>
</tr>
<tr>
<td>Cisplatin + <em>P. florida</em> extract</td>
<td>1000</td>
<td>0.027 ± 0.10$^b$</td>
<td>0.178 ± 0.030$^b$</td>
<td>98.3</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6 animals. Any two values having a common letter are not significantly different at 5% level, lsd= 0.063 (Tumor volume), lsd= 0.7353 (Tumor weight).

Table 8.6: Effect of cisplatin, cisplatin + methanolic extract of *P.sajor-caju* on solid tumour

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg/Kg)</th>
<th>Tumor Volume (cm$^3$)</th>
<th>Tumor weight (g)</th>
<th>%Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>1.187 ± 0.120$^a$</td>
<td>12.432 ± 1.030$^a$</td>
<td>---</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>4</td>
<td>0.070 ± 0.220$^b$</td>
<td>0.560 ± 0.080$^b$</td>
<td>95.5</td>
</tr>
<tr>
<td>Cisplatin + <em>P.sajor-caju</em> extract</td>
<td>1000</td>
<td>0.028 ± 0.100$^b$</td>
<td>0.212 ± 0.040$^b$</td>
<td>98.2</td>
</tr>
</tbody>
</table>

Values are mean ± S.D, n=6 animals. Any two values having a common letter are not significantly different at 5% level, lsd= 0.0996 (Tumor volume), lsd= 0.364 (Tumor weight)
Fig 8.1: Effect of methanolic extract of *P. florida* on GSH level induced by cisplatin administration
A: Normal, B: Control, C: Methanolic extract of *P. florida* (500mg/Kg body weight)
D: Methanolic extract of *P. florida* (1000mg/Kg body weight)
Values are mean ± S.D, n=6 animals. Any two values having a common letter are not significantly different at 5% level, Isd= 1.125
Fig 8.2. Effect of methanolic extract of *P.sajor-caju* on renal GSH level caused by cisplatin administration

A: Normal, B: Control, C: Methanolic extract of *P.sajor-caju* (500mg/Kg body weight)

D: Methanolic extract of *P. sajor-caju* (1000mg/Kg body weight)

Values are mean ± S.D, n=6 animals

Any two values having a common letter are not significantly different at 5% level, lsd= 0.1.198
Fig 8.3: Effect of methanolic extract of *P. florida* on lipid peroxidation (MDA level) caused by cisplatin administration

A: Normal, B: Control, C: Methanolic extract of *P. florida* (500mg/Kg body weight)

D: Methanolic extract of *P. florida* (1000mg/Kg body weight)

Values are mean ± S.D, n=6 animals

Any two values having a common letter are not significantly different at 5% level, lsd= 0.076
Fig 8.4: Effect of methanolic extract of *P.sajor-caju* on increase of lipid peroxidation (MDA level) induced by cisplatin administration

A: Normal, B: Control, C: Methanolic extract of *P.florida* (500mg/Kg body weight)
D: Methanolic extract of *P.florida* (1000mg/Kg body weight)

Values are mean $\pm$ S.D, n=6 animals

Any two values having a common letter are not significantly different at 5% level, $lsd = 0.114$
8.4 DISCUSSION

Results of the investigations show that methanolic extracts of *P. florida* and *P. sajor-caju* render significant protection against cisplatin induced nephrotoxicity in mice. Several lines of evidence indicate that free radicals and reactive oxygen species are involved in cisplatin induced oxidative stress because of depletion of reduced GSH concentration and antioxidant enzyme activity in the kidneys (Hannemann and Baumann, 1988). The decrease in superoxide dismutase activity after cisplatin administration might be due to the loss of copper and zinc, which are essential for enzyme activity (Sinet and Carber, 1981). Cisplatin has been demonstrated to induce the loss of copper and zinc in the kidneys. The decreased superoxide dismutase activity is insufficient to scavenge the superoxide anion produced during the metabolic process. The superoxide anion can thus cause initiation and progression of lipid peroxidation.

The activity of catalase and glutathione peroxidase also decreased after cisplatin administration. This resulted in the decreased ability of the kidney to scavenge toxic H$_2$O$_2$ and lipid peroxides. The restoration of renal superoxide dismutase, catalase and glutathione peroxidase activities by pretreatment of *P. florida* and *P. sajor-caju* extracts suggests that the oyster mushroom extracts are capable to protect these enzymes even three days after cisplatin administration.

GSH depletion can markedly increase the toxicity of cisplatin. Therefore lipid peroxidation due to cisplatin administration is a consequence of
GSH depletion and impaired antioxidant enzyme activities. The increased GSH levels render protection, which is evident from the treatment of the extract prior to cisplatin administration. The experimental findings thus indicate the nephroprotective effect of oyster mushroom. Since oyster mushrooms are excellently edible and nontoxic, the findings suggest the potential use of their extracts in cancer chemotherapy.