PROTECTIVE EFFECT OF STEM BARK ETHANOL AND AQUEOUS EXTRACTS OF FICUS RACEMOSA AGAINST CISPLATIN INDUCED NEPHROTOXICITY IN MICE

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ABSTRACT
Cisplatin (cis-diamminedichloroplatinum(II), CDDP) is an antineoplastic drug used in the treatment of many solid-organ cancers, including those of the head, neck, lung, testis, ovary, and breast. While toxicities include ototoxicity, gastrotoxicity, myelosuppression, and allergic reactions, the main dose-limiting side effect of cisplatin is nephrotoxicity. Reactive oxygen species and oxidative damage are the most important factors in cisplatin-induced acute renal failure. In the present study the protective effects of stem bark ethanol and aqueous extracts of Ficus racemosa in cisplatin induced mice were studied. The results of this study indicated that Ficus racemosa bark ethanol and aqueous extracts significantly protected the cisplatin induced nephrotoxicity in mice. The serum urea, creatinine, blood urea nitrogen and lipid peroxidation levels in cisplatin alone treated groups were significantly elevated (P < 0.01) and catalase and renal glutathione (GSH) levels were declined when compared to control group. The serum urea, creatinine and blood urea nitrogen levels were reduced and, catalase and renal GSH levels elevated significantly (p<0.01) in the mice treated with stem bark ethanol and aqueous extracts of Ficus racemosa (200 and 400 mg/ kg, p.o). The results suggested that the stem bark extract of Ficus racemosa showed protective effect against cisplatin induced nephrotoxicity in mice, which may probably mediated by its antioxidant property.

Keywords: Ficus Racemosa, Cisplatin, Oxidative Damage, Lipid Peroxidation.

INTRODUCTION
There are several nephrotoxic drugs in the market for treating cancer and infectious diseases caused by gram negative bacteria namely cisplatin and gentamicin etc. Cisplatin is a potent anticancer drug used in the treatment various solid tumors. But the major dose limiting side effect associated with cisplatin is nephrotoxicity¹. Cisplatin (cis-diamminedichloroplatinum(II), CDDP) is an antineoplastic drug used in the treatment of many solid-organ cancers, including those of the head, neck, lung, testis, ovary, and breast. While toxicities include ototoxicity, gastrotoxicity, myelosuppression, and allergic reactions, the main dose-limiting side effect of cisplatin is nephrotoxicity. Cisplatin is cleared by the kidney by both glomerular filtration and tubular secretion. Cisplatin concentrations within the kidney exceed those in blood suggesting an active accumulation of drug by renal parenchymal cells. Previous studies
using kidney slices, cultured renal epithelial cells and isolated perfused proximal tubule segments have provided evidence for basolateral-to-apical transport of cisplatin. However there is lack of experimental to justify its nephroprotective effect. Hence a systematic study the neproprotective effect against experimentally induced renal damage has been carried out. The roots, bark-skin, fruits, latex and leaves of Ficus racemosa have great medicinal value. The traditional uses of Ficus racemosa are: wound healing, stomatitis, sore throat, toothache, diarrhea, dysentery, ulcerative colitis, anorexiant, mouth wash, haemoptysis. The antidiabetic, hypolipidemic, anticholinesterase, antibacterial, anthelmintic, antioxidant, radioprotective, hepatoprotective, and antiuicretic activities of Ficus racemosa were reported. The stem bark of Ficus racemosa contains tannin, wax, saponin gluanol acetate, β-sitosterol, leucocyanidin, 3 - O - β - D - glucopyranoside, eucopeelargonidin - 3 - O - β - D - glucopyranoside, leucopelargonidin - 3 - O - a - L - rhamnopyranoside, lupeol, ceryl behenate, lupeol acetate, α-amyrin acetate, lucoanthocyanidin, and lucoanthocyanin from trunk bark, lupeol, β-sitosterol and stigmasteral 4.

MATERIALS AND METHODS
Collection and extraction
The stem barks of Ficus racemosa were collected and identified and authenticated by a qualified botanist. The shade dried and powdered stem bark of Ficus racemosa (1 kg) were extracted with 95% ethanol in a Soxhlet apparatus (55°C; 25–30 cycles), followed by water extraction on a hot water bath (70°C; 3–4 h). The ethanol and aqueous extracts were concentrated to a small volume and then evaporated to dryness. These extracts (EFR and AFR) were kept in air tight containers.

Animals
Swiss albino mice (25-30g) were used for the present study. Animals were housed in a ventilated room under a 12/12 hr light/dark cycle at 24±2°C and had free access to water and food. The animal care and experimental protocols were made in accordance with CPCSEA/IAEC.

Method
The mice were selected and randomized into 6 groups, each group consisting of 6 mice. Nephrotoxicity in mice was induced with the administration of cisplatin (12mg/kg). The stem bark ethanol and aqueous extracts of Ficus racemosa (EFR and AFR) were administered (200, 400mg/kg) to mice orally 1 h before the administration of cisplatin and at 24h and 48h after cisplatin injection. The parameters were studies 72 h after cisplatin administration. Normal control group was not administered with either extract or cisplatin. The mice were selected and randomized into 6 groups, each group consisting of 6 mice. After 72h of last treatment the mice were anaesthetized by chloroform and sacrificed. Blood was then collected by cardiac puncture and kidneys were dissected out immediately.

Post mitochondrial supernatant (PMS) preparation
The kidneys were perfused immediately with ice-cold normal saline and homogenized in chilled potassium chloride (1.17%w/v) using a homogenizer. The homogenate was centrifuged at 800 g for 5 min at 4°C in a refrigerated centrifuge to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 g for 20 min at 4°C to get the post mitochondrial supernatant (PMS) which was used to assay LPO activity, glutathione, superoxide dismutase(SOD) and catalase.

Estimation of serum urea
During the metabolism of protein in the body, the liver creates ammonia, which is broken down into a by-product called urea. Kidneys filter excess urea into the urine and in sweat, but some goes into the blood stream as serum urea. Serum urea concentration is important to determine if the kidneys are functioning correctly. A high level of serum urea means the kidneys are not filtering properly. Urea reacts directly with diacetyl monoxime under strongly acidic conditions to give a yellow condensation product. This reaction is intensified by the presence of ferric ions and thiosericarbide and the resulting red colored complex formed is a measure of the urea concentration. The serum urea present in the mice blood samples was estimated using semi autoanalyzer.

Creatinine estimation
Creatinine reacts with picric acid in alkaline medium to produce reddish yellow colored compound. The alkaline medium is provided by sodium hydroxide. In Jaffe’s reaction, the
sodium salt of picric acid, sodium picrate is formed in the first phase. The sodium picrate reacts with creatinine to form reddish yellow crystals of creatinine picrate and upon acidification with HCl these crystals are dissolved. The intensity of the color is directly proportional to the amount of creatinine present in the sample. Creatinine present in the mice serum was estimated using semi automatic analyzer.

**Lipid peroxidation (LPO)**

Lipid peroxides are unstable and decompose to form a complex series of compounds including reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) and 4-hydroxyalkenals (HAE) upon decomposition, and the measurement of MDA and HAE has been used as an indicator of lipid peroxidation. The method in this assay is designed to assay either MDA alone (in hydrochloric acid) or MDA in combination with HAE (in methane sulfonic acid.) The assay is based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole (R1), with MDA and 4-hydroxyalkenals at 45°C. One molecule of either MDA or 4-hydroxyalkenal reacts with 2 molecules of reagent R1 to yield a stable chromophore with maximal absorbance at 586 nm. The MDA content was assayed by TBARS method. The reaction mixture was prepared by mixing 0.2ml of 8.1%w/v sodium lauryl sulphate, 1.5 ml of 20% w/v acetic acid. The pH was adjusted by adding NaOH, to this 1.5ml of 0.8% w/v aqueous solution of thiobarbituric acid and 0.2ml of 10w/v of PMS were added. The resultant was made up to 4ml with distilled water. The mixture was heated at 95°C for 60min. It was cooled with tap water and 1ml distilled water and 5ml mixture of n-butanol and pyridine (15:1) were added and centrifuged. The organic layer was separated and its absorbance measured at 532nm. The content of LPO was reported as nmole MDA per mg protein. Tissue protein was estimated using the Biuret method of the protein assay.

**Glutathione estimation**

Reduced glutathione was determined using the modified method of Ellman (1951). An aliquot of 1.0 ml of supernatant of liver homogenate was treated with 0.5 ml of Ellman’s reagent (19.8 mg of 5, 5'-dithiobisnitro benzoic acid (DTNB) in 100 ml of 0.1 % sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was measured at 412 nm. The percentage inhibition of GSH was calculated using the following equation:

\[
\text{% reduced glutathione inhibition} = \left( \frac{A_o - A^1}{A_o} \right) \times 100
\]

Where; \(A_o\) is the absorbance of the control and \(A^1\) is the absorbance of the sample extract.

**Catalase estimation**

1ml \(H_2O_2\) is added to 1.95ml of phosphate buffer (0.05M, pH 7.0) and 0.05 ml of PMS (10%). Changes in absorbance were measured in spectrophotometer at 240nm. The catalase activity was measured by using its extinction coefficient. One unit of activity is equal to one mM of \(H_2O_2\) degraded/ min and is expressed as units/ mg of protein.

**DISCUSSION**

The results of the present study indicated that the stem bark ethanol and aqueous extract of Ficus racemosa protected the cisplatin-induced in vivo nephrotoxicity in mice. However, the mechanisms underlying the cisplatin-induced acute renal failure have not been fully understood, several investigators have shown that the ROS or free radicals are closely related to the acute renal failure induced by cisplatin. Free radicals formation is one of the mechanisms of nephrotoxicity induced by cisplatin and antioxidants have protective effect against renal toxicity. Constituents of Ficus racemosa bark such as flavonoids, tannins and glutathione have antioxidant activity. So this plant may inhibit lipid peroxidation by scavenging free radicals and increasing intracellular concentration of glutathione. Several studies have reported that the alterations induced by cisplatin 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 catalase, creatinine, LPO and urea levels, in urine and plasma samples. Cisplatin is a potent anticancer drug preferred in the treatment of solid tumors. As CP is a lipophilic in nature it crosses the plasma membrane and migrates to the nucleus and causes the alteration in DNA structure. It is thought to be beneficial to administer free radical scavengers like anti-oxidants along
with nephrotoxic drugs so as to reduce their toxicity without affecting the therapeutic efficacy\^{10,11}.

**CONCLUSION**

In conclusion, findings of the present study show that *Ficus racemosa* is a protective agent against gentamicin-induced nephrotoxicity in rat. However, the exact protective mechanism(s) of *Ficus racemosa* is unknown and its mechanism is need to be investigated.

**Table 1:** Table Showing Serum Urea, Creatinine, LPO in Control, Cisplatin, EFR and AFR Treated Mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Serum urea (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
<th>LPO (nM/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>40.24±0.022</td>
<td>0.85±0.098</td>
<td>33.88±0.122</td>
</tr>
<tr>
<td>II</td>
<td>CP</td>
<td>12</td>
<td>106±0.280</td>
<td>1.78±0.988</td>
<td>165.33±0.864</td>
</tr>
<tr>
<td>III</td>
<td>CP +EFR</td>
<td>12+200</td>
<td>80.49±0.362**</td>
<td>1.23±0.743**</td>
<td>131.24±0.540**</td>
</tr>
<tr>
<td>IV</td>
<td>CP +EFR</td>
<td>12+400</td>
<td>74.83±0.178**</td>
<td>1.05±0.243***</td>
<td>128.53±0.922***</td>
</tr>
<tr>
<td>V</td>
<td>CP +AFR</td>
<td>12+200</td>
<td>76.22±0.875**</td>
<td>1.45±0.758**</td>
<td>141.24±0.674*</td>
</tr>
<tr>
<td>VI</td>
<td>CP +AFR</td>
<td>12+400</td>
<td>71.89±0.976***</td>
<td>1.27±0.392**</td>
<td>132.22±0.792**</td>
</tr>
</tbody>
</table>

Mean percentage ±SEM; *n* = 6 animals in each group. The treated groups are compared by Student t test with the group II 
\**P < 0.001, **P < 0.01, *P < 0.05

**Table 2:** Table showing reduced glutathione (GSH), catalase in control, cisplatin, EFR and AFR treated mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>GSH (µg/mg protein)</th>
<th>Catalase (units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>-</td>
<td>35.33±1.028</td>
<td>33.33±1.121</td>
</tr>
<tr>
<td>II</td>
<td>CP</td>
<td>12</td>
<td>26.34±0.928</td>
<td>19.33±0.798</td>
</tr>
<tr>
<td>III</td>
<td>CP +EFR</td>
<td>200</td>
<td>30.12±2.229**</td>
<td>26.43±1.022*</td>
</tr>
<tr>
<td>IV</td>
<td>CP +EFR</td>
<td>400</td>
<td>32.89±0.006***</td>
<td>28.14±0.976**</td>
</tr>
<tr>
<td>V</td>
<td>CP +AFR</td>
<td>200</td>
<td>29.36±1.032**</td>
<td>28.32±0.231**</td>
</tr>
<tr>
<td>VI</td>
<td>CP +AFR</td>
<td>400</td>
<td>32.21±0.872***</td>
<td>33.22±1.212***</td>
</tr>
</tbody>
</table>

Mean percentage ±SEM; *n* = 6 animals in each group. The treated groups are compared by Student t test with the group II 
\**P < 0.001, **P < 0.01, *P < 0.05

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