Protective effect of *Pongamia pinnata* flowers against cisplatin and gentamicin induced nephrotoxicity in rats

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Ethanol extract of flowers of *Pongamia pinnata* was studied for its protective effect against cisplatin and gentamicin induced renal injury in rats. When the extract (300 & 600 mg kg−1) was administered orally for 10 days following cisplatin (5 mg kg−1 i.p.) on day 5, toxicity of cisplatin, as measured by loss of body weight, elevated blood urea and serum creatinine declined significantly. Similarly in gentamicin (40 mg kg−1 s.c.) induced renal injury, the extract (600 mg kg−1) normalized the raised blood urea and serum creatinine levels. Reversal of cisplatin and gentamicin renal cell damage as induced by tubular necrosis i.e. marked congestion of the glomeruli with glomerular atrophy, degeneration of tubular epithelial cells with casts in the tubular lumina and infiltration of inflammatory cells in the interstitium was confirmed on histopathological examination. In the preventive regimen, co-administration of the extract with gentamicin significantly prevented the renal injury both functionally and histologically. Ethanol extract of flowers had a marked nitric oxide free radical scavenging effect, suggesting an antioxidative property. Two flavonoids, known for their antioxidant activity viz. kaempferol and 3, 5, 6, 7, 8-pentamethoxy flavone were isolated from the extract. The results suggested that the flowers of *Pongamia pinnata* had a protective effect against cisplatin and gentamicin induced renal injury through antioxidative property.

Various studies have reported that the nephrotoxicity associated as a limiting side-effect of the antineoplastic, cisplatin and the amino glycoside antibiotic, gentamicin is due to the involvement of oxidative stress via free radical formation2. Synthetic antioxidants and free radical scavengers like glycine3 and selenomethionine5 have been found to show partial protection against damage caused by cisplatin. Similarly cyclodextrin sulphates7, polyaspartic acid9 etc also have been found to partially reduce gentamicin induced renal damage. Search for nephroprotective agents has made man turn to alternative sources viz. indigenous system of medicine. It is a well-documented fact that most medicinal plants are enriched with bioflavonoids, which have antioxidative property. *Pongamia pinnata* (L.) Pierre (Fabaceae) is one such plant containing a number of bioactive compounds. Commonly known as Indian Beech Tree, it is a medium sized (up to 18 m high) glabrous tree, found in India, Burma, Malaya, N. Australia, Polynesia, China and Philippine Islands. Flowers of this plant are rich in bioflavonoids and extensively used for various skin diseases, diabetes and in renal disorders by the tribes of Chittoor District7 (Andhra Pradesh, India). A survey of literature showed the absence of any experimental data whatsoever to justify the nephroprotective role of the flowers of this plant. Thus, the present study was undertaken to evaluate the efficacy of the flowers of *Pongamia pinnata* against nephrotoxicity induced by commonly known nephrotoxic agents cisplatin and gentamicin.

Materials and Methods

Plant material — Flowers of *Pongamia pinnata* were collected from Kolar District, Karnataka, India, in the month of April-May, 2001 and authenticated by botanist Dr. Gopalkrishna Bhat, Professor of Botany, Poorna Prajna College, Udupi, India. A herbarium specimen bearing voucher No. PP. 508 has been deposited in the Department of Pharmacognosy, College of Pharmaceutical Sciences, Manipal, India.

Drugs and chemicals — Cisplatin and gentamicin were obtained from Biochem Pharmaceutical Industries, Mumbai; Urea estimation kit and Creatinine estimation kit were procured from Agappe Diagnostics, Maharashtra and Dr. Reddy’s Laboratories, Hyderabad respectively.

Preparation of alcoholic extract — The shade dried coarsely powdered flowers (1 kg) were subjected to Soxhlet extraction using ethanol(95%) for 6 hr. The solvent was removed in *vacuo* and the extract was used for chemical and pharmacological studies.
Phytochemical screening — Preliminary phytochemical screening revealed the presence of steroids, carbohydrates, phenolic compounds, flavonoids, fixed oils and fats.

Chemical studies — Alcoholic extract was fractionated into petroleum ether, ether, ethyl acetate fraction. Ether fraction (13 g) was dissolved in a small quantity of methanol and mixed with silica gel-G (200 g) column prepared in chloroform. The column was eluted with chloroform followed by varying percentages of methanol in chloroform. The eluates with 1% methanol in chloroform deposited a light brown compound which had ultra violet spectral data matching with 3, 5, 6, 7, 8-pentamethoxy flavone. The eluates (4 and 5% methanol) in chloroform precipitated a yellow coloured compound which showed UV spectral characteristics matching that of kaempferol.

Pharmacological study

Animals — The study was performed on male Wistar albino rats (60-90 days old) weighing 150-200 g. They were maintained on standard conditions (temperature and humidity controlled), diet (Hindustan Lever Ltd) and water ad libitum. The study was conducted after obtaining local animal ethical committee clearance.

Acute toxicity studies — Animals were fed with increasing doses of (30, 100, 300, 600, 1000 and 3000 mg/kg) of alcoholic extract of flowers of *P. pinata* suspended in 2% w/v gum acacia. The animals were observed continuously for 2 hr for the gross behavioral changes and then intermittently once every 2 hr and finally at the end of 24 and 72 hr to note any other toxic signs including death.

Cisplatin-induced renal injury — Seven groups (*n* = 8) were used to study the effect of *P. pinata* on cisplatin induced renal toxicity in rats. Group 1 administered with equivalent volumes of vehicle for 10 days, served as normal control. Group 2 was treated orally with *P. pinata* extract (600 mg kg⁻¹) for 10 days. The following day renal function was assessed. Groups 3, 4, 5 and 6 were administered with cisplatin (5 mg kg⁻¹ body weight; single dose, ip)¹⁰. The blood was withdrawn on day 5 in Group 3 and day 15 in Group 4 to check the persistence of renal injury. Groups 5 and 6, serving as curative regimen, were treated with *P. pinata* extract (300 and 600 mg kg⁻¹ po respectively) from day 6 onwards for 10 days. The following days renal function was assessed. Group 7, which served as preventive regimen, was treated with 600 mg kg⁻¹ of extract for 1 hr prior to the administration of cisplatin. Treatment was continued for next 4 days.

Gentamicin-induced renal injury — Five groups of rats were used. Group 1 served as normal control. Remaining groups were administered with gentamicin (40 mg kg⁻¹ sc) for 13 days¹¹. Blood was withdrawn on day 14 in Group 2 and on day 24 in Group 3 to check the persistence of renal injury. Group 4 animals were treated orally with 600 mg kg⁻¹ of extract from day 14 onwards for 10 days, after which blood was assessed for renal clearance. In Group 5 gentamicin and the flower extract was administered simultaneously for 13 days following which renal function tests were performed.

Assessment of renal function — (a) Percentage change in the body weight was measured for each rat before treatment and at the end of the treatment; (b) blood urea level was estimated by urease enzymatic method using UV spectrometer Shimadzu UV-240; and (c) serum creatinine level was measured by alkaline picrate method using UV spectrometer Shimadzu UV-240.

Histopathological studies — Two animals from each group were sacrificed on the day of blood withdrawal and kidneys were isolated. The kidney sections were stained with hematoxylin and eosin and observed under light microscope.

Statistical analysis — The data were analysed using one way analysis of variance followed by post hoc Student-Newman-Keuls Test using SPSS computer software. Statistical significance was set at *P* < 0.05.

In vitro antioxidant study

Nitric oxide scavenging activity — Nitroprusside (5 mM) was mixed with different concentrations of test extract and incubated at 25°C for 5 hr. After 5 hr Greiss reagent was added and absorbance of chromophore formed was read at 546 nm. Control experiment was also carried out in similar manner. The experiment was repeated in triplicate. Percentage scavenging effect was calculated and the results are presented in Fig. 3.

Results and Discussion

Pharmacological studies

Toxicity — Oral administration of the alcoholic extract of *Pongamia pinata* produced no toxic effects up to 3 g/kg in rats even after 24 and 72 hr.

Cisplatin-induced renal damage — Results on effects of oral administration of *P. pinata* on cisplatin induced elevation of blood urea, serum creatinine and
on reduction of body weight in rats are given in Table 1. The control animals had an increase in body weight gain of 13%. The body weight of animals, which received cisplatin (ip), was reduced by 30% at the end of two weeks. However, when *P. pinnata* extract of 300 and 600 mg kg⁻¹ was given in curative regimen the extent of reduction in body weight showed significant decline at both the doses. Co-administration of cisplatin and flower extract, failed to prevent the reduction in body weight. The levels of blood urea and serum creatinine increased significantly in cisplatin treated animals and this elevation persisted for 2 weeks. The elevations of serum markers were significantly reduced following 300 and 600 mg kg⁻¹ of flower extract. However, the extent of elevation in the serum markers was not affected when the flower extract was administered simultaneously.

The sections of the kidneys isolated from rats treated with cisplatin exhibited marked congestion of the glomeruli with glomerular atrophy, desquamation of tubular epithelial cells with casts in the tubular lumen, infiltration of inflammatory cells in the interstitium indicating cisplatin induced acute renal necrosis (Fig. 1). Following the treatment with extract (600 mg kg⁻¹) in the curative regimen congestion of the glomeruli were reduced, degeneration of tubular cells were not observed and a very few tubular casts were observed in the interstitium indicating marked protection against the injury caused by cisplatin. However, in preventive regimen of 600 mg kg⁻¹ features of tubular necrosis persisted.

**Gentamicin- induced renal damage**—The results are depicted in Table 2. The daily subcutaneous administration of gentamicin (40 mg kg⁻¹) for 13 days caused renal impairment. This was evidenced by significant decrease in body weight after 13 days of injection. However, the body weight rose gradually in the next 10 days. This may be due to sodium water retention. The extract at 600 mg kg⁻¹ normalized the change in body weight both in curative and preventive regimen.

Renal dysfunction was observed further as significant increase in blood urea and serum creatinine levels after 13 days of gentamicin administration, which persisted even after 10 days of cessation of therapy. The treatment of the rats with flower extract (600 mg kg⁻¹) after gentamicin induced damage caused a marked decrease in the blood urea and serum creatinine levels. The co-administration of the extract also showed the same effect indicating its protective

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Fig. 1 — Photomicrographs of (a) normal control, (b) cisplatin intoxicated and (c) *Pongamia pinnata* extract treated rat kidney. [Inflammatory cells, casts in the tubular lumen (arrow) and tubular degeneration (arrow head) in b which was normalized in c. G-Glomerulus, T-Renal tubules, H X E stained, Scale bar = 40μ.]
activity. The histopathologic examination reported features of acute renal necrosis like tubular desquamation along with number of casts in the tubular lumen, after 10 days of cessation of therapy (Fig. 2), which was not seen on day 13. In fact, the relationship among tubular cell dysfunction, morphological damage, impairment of glomerular filtration and renal blood flow in acute renal failure are poorly understood\(^\text{13}\). Hence, it could be reasoned that functional damage or renal dysfunction occurred before structural damage. The flower extract normalized the histopathological features of acute tubular necrosis by gentamicin in the curative regimen.

The present results of our study confirmed that cisplatin at 5 mg kg\(^{-1}\) ip and gentamicin 40 mg kg\(^{-1}\) sc produces significant nephrotoxicity as characterized by increase in blood urea, serum creatinine and renal tubular necrosis. Induction of nephrotoxicity by cisplatin is assumed to be a rapid process involving reaction with proteins in renal tubules. As renal damage occurs within an hour after administration of cisplatin, it is important that the protective agent be present in sufficient concentration in the renal tubules before injury occurs. This might explain why even oral administration of the flower extract in multiple doses failed to protect the rats of group 7 after cisplatin administration.

A relationship between oxidative stress and nephrotoxicity has been well demonstrated in many experimental animal models\(^\text{2-3}\). Evidence points out that cisplatin and gentamicin induce nephrotoxicity partly via oxidative stress. One of the mechanisms proposed, by which cisplatin induces free radical damage, is by increasing the activity of calcium-independent nitric oxide synthase. Flavonoids are potent antioxidants and are known to modulate the activities of various enzyme systems due to their interaction with various biomolecules\(^\text{2}\). The flowers of

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**Table 1** — Effect of alcoholic extract of *P. pinuta* in cisplatin induced renal damage  
[Values are mean ± SE of 8 replications]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment regimen</th>
<th>Change in body weight (%)</th>
<th>Blood urea (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>13.20 ± 2.24</td>
<td>32.60 ± 3.61</td>
<td>0.96 ± 0.05</td>
</tr>
<tr>
<td>2</td>
<td>Alc. extract</td>
<td>13.20 ± 2.15</td>
<td>31.42 ± 1.7</td>
<td>0.90 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>Cisplatin 5(^{th}) day</td>
<td>-13.39 ± 1.43*</td>
<td>90.96 ± 7.14*</td>
<td>1.81 ± 0.08*</td>
</tr>
<tr>
<td>4</td>
<td>Cisplatin 15(^{th}) day</td>
<td>-30.68 ± 3.40*</td>
<td>71.53 ± 4.51*</td>
<td>1.61 ± 0.04*</td>
</tr>
<tr>
<td>5</td>
<td>Cisplatin + alc. extract</td>
<td>-5.03 ± 2.86*</td>
<td>50.27 ± 1.46*</td>
<td>1.40 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>300 mg/kg (curative regimen)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Cisplatin + alc. extract</td>
<td>-3.30 ± 1.73c*</td>
<td>28.21 ± 2.43*</td>
<td>1.29 ± 0.50f*</td>
</tr>
<tr>
<td></td>
<td>600 mg/kg (curative regimen)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Cisplatin + alc. extract</td>
<td>-1.54 ± 1.28</td>
<td>109.0 ± 8.11</td>
<td>1.65 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>600 mg/kg (preventive regimen)</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

\(^{*}P<0.05\) vs group 1; \(^{c}P<0.05\) vs group 4; \(^{e}P<0.05\) vs group 2  
One way ANOVA followed by post hoc Student-Newman-Keuls Test

**Table 2** — Effect of alcoholic extract of *P. pinuta* in gentamicin induced renal damage  
[Values are mean ± SE of 8 replications]

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment regimen</th>
<th>Change in body weight (%)</th>
<th>Blood urea (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=8)</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>13.19 ± 2.24</td>
<td>32.60 ± 3.61</td>
<td>0.96 ± 0.057</td>
</tr>
<tr>
<td>2</td>
<td>Alc. extract</td>
<td>13.20 ± 2.15</td>
<td>31.42 ± 1.7</td>
<td>0.90 ± 0.06</td>
</tr>
<tr>
<td>3</td>
<td>Gentamicin 14(^{th}) day</td>
<td>-9.79 ± 1.7*</td>
<td>55.34 ± 7.14*</td>
<td>1.72 ± 0.064*</td>
</tr>
<tr>
<td>4</td>
<td>Gentamicin 24(^{th}) day</td>
<td>13.61 ± 3.76*</td>
<td>68.92 ± 4.51*</td>
<td>1.29 ± 0.029*</td>
</tr>
<tr>
<td>5</td>
<td>Gentamicin + alc. extract</td>
<td>-6.73 ± 0.83*</td>
<td>39.34 ± 1.09*</td>
<td>1.07 ± 0.04*</td>
</tr>
<tr>
<td></td>
<td>(600 mg/kg) (curative regimen)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Gentamicin + alc. extract</td>
<td>1.54 ± 1.62*</td>
<td>46.72 ± 2.12*</td>
<td>1.04 ± 0.03*</td>
</tr>
<tr>
<td></td>
<td>(600 mg/kg) (preventive regimen)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{*}P<0.05\) vs group 1; \(^{c}P<0.05\) vs group 4; \(^{e}P<0.05\) vs group 3  
One way ANOVA followed by post hoc Student-Newman-Keuls Test
Fig. 2—Photomicrographs of (a) gentamicin intoxicated and (b) Pongania pinnata extract treated rat kidney. [Casts in the tubular lumen (arrow) and tubular degeneration (arrow head) in a which was normalized in b. G-Glomerulus, T-Renal tubules, H×E, stained, Scale bar=40μl.]

Fig. 3—Nitric oxide scavenging activity of alcoholic extract of Pongania pinnata flowers

P. pinnata are known to contain number of bioflavonoids like kaempferol, quercetin, karanjin, kanjone, pongaglabrone, gammatin, pongaglabol, kanugin etc.14. In vitro studies of the alcoholic extract of P. pinnata flowers revealed marked nitric oxide scavenging activity (Fig. 3) suggesting a potent antioxidative property. Kaempferol and quercetin are well known free radical scavengers. Hence the possible mechanism by which the flower extract exerts nephroprotection could be attributed to its free radical scavenging property. The exact mechanism of nephroprotection has to be still investigated and isolation of active constituents is required.

References