Protective Effect of Cinnamomum tamala Extract on Gentamicin-Induced Nephrotic Damage in Rabbits

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Abstract

Purpose: To investigate the reno-protective properties of Cinnamomum tamala against gentamicin-induced nephrotoxicity in rabbits.

Methods: Rabbits were randomly divided into four groups (n = 6) including Group-1 (normal saline), Group-2 (gentamicin, 80 mg/kg/day), Group-3 (C. tamala, 200 mg/kg/day) and Group-4 (gentamicin, 80 mg/kg/day and C. tamala, 200 mg/kg/day). Body weight, blood urea nitrogen, serum creatinine, creatinine clearance, serum uric acid, urinary volume and urinary protein excretion were measured followed by histological examination.

Results: Gentamicin-treated animals showed significant renal damage as indicated by rise in blood urea nitrogen (54.18 ± 2.60 mg/dl), serum creatinine (4.02 ± 0.14 mg/dl), serum uric acid (2.34 ± 0.12 mg/dl), urinary proteins (3.86 ± 0.32 mg/dl) and decrease in creatinine clearance (0.76 ± 0.09ml/min), urinary volume (126.00 ± 9.09 ml) and body weight (10.80 ± 1.09 %). However, animals treated with gentamicin and C. tamala significantly protected rabbit kidney from structural and functional changes associated with gentamicin.

Conclusion: Based on the findings, it is apparent that concurrent administration of 200 mg/kg/day of C. tamala leaf extract and gentamicin effectively prevented gentamicin-induced renal damage.

Keywords: Cinnamomum tamala, Renal protection, Gentamicin, Renal damage

INTRODUCTION

Cinnamomum tamala (Lauraceae) is commonly known as tejpat and tejpata in Sanskrit [1]. The plant is widely found in Asia and Australia [2] and its major constituents are a-pinene, myrcene, camphene, p-cymene, limonene, eugenol and methyl eugenol [3]. The plant has different medicinal properties like carminative, diuretic, anti-flatulent effects and is also useful in heart abnormalities [2]. The usefulness of C. tamala in the treatment of anorexia, nausea, mouth dryness, diarrhea and bladder disorder has also been described [4]. The plant possesses hypolipidemic and hypoglycemic properties [5] and has potent antibacterial activity against Escherichia coli, Bacillus subtilis and Saccharomyces cerevisiae [6]. C. tamala has been used in folk medicine as brain tonic and anthelmintic especially for the treatment of anal and rectal diseases [1]. It is reported that C. tamala leaves have gastro-protective effects in experimental animals, may be because of free radical scavenging properties [7]. The plant has also been reported with high phenolic contents and has concentration-dependent antioxidant activities [8]. In view of this, the plant extract may
inhibit oxidative damage and protect against gentamicin-induced renal toxicity. The objective of the present study, therefore, was to investigate the renal-protective activity of *C. tamala* against gentamicin toxicity.

**EXPERIMENTAL**

**Plant material and extraction**

*C. tamala* leaves were collected from Northern areas of Pakistan in the year 2010. Following authentication by Professor Umar Farooq, Department of Botany, Postgraduate College No.1, Abbottabad, Pakistan, a voucher specimen was deposited in the herbarium of the same department.

The fresh leaves were washed, shade-dried and ground with a grinder (ZK-115, Japan). Extraction was done with sufficient quantity of 70% ethanol with intermittent stirring for three weeks. The mixture was filtered and the supernatant evaporated using a rotary evaporator (R-210 Germany) [9].

**Experimental protocol**

Four groups of animal, each having six male rabbits of similar weights, were acclimatized for 15 days before the start of study in the animal house of Frontier Medical and Dental College, Abbottabad, Pakistan. All the rabbits were maintained on the same diet and 12 h light/dark cycle. Animal handling and care was strictly according to the rules and regulations of University of Malakand, along with international laws and policies (National Institutes of Health Guide for the Care and Use of oratory Animals, NIH Publication no. 85-23, 1985) after institutional approval of the study by Research Society of the university. The extract was given orally while gentamicin (Merck, Pakistan) was injected intramuscularly to induce nephrotoxicity according to the treatment schedule in Table 1.

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Group code</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C (control)</td>
<td>0.9% saline (2 ml/kg, im)</td>
</tr>
<tr>
<td>2</td>
<td>G (toxic)</td>
<td>Gentamicin (80 mg/kg, im)</td>
</tr>
<tr>
<td>3</td>
<td>GC-ta</td>
<td>Gentamicin (80 mg/kg, im) + <em>C. tamala</em> (200 mg/kg, po)</td>
</tr>
<tr>
<td>4</td>
<td>C-ta</td>
<td><em>C. tamala</em> (200 mg/kg, po)</td>
</tr>
</tbody>
</table>

Blood samples were collected three times throughout the experimental period for the estimation of serum creatinine, creatinine clearance, blood urea nitrogen and serum uric acid. Urine was also collected from the mesh beneath the cages on days 0, 11 and 21 for the assessment of urinary creatinine, urinary protein and urinary volume. The body weight of each rabbit was measured with digital balance (Excel, China) three times throughout the study period. Determination of blood urea nitrogen (BUN) was performed by Bertholot's indophenol procedure, using a reagent kit (ProDia International, UAE), while determination of serum creatinine and urinary creatinine was performed by the Jaffe reaction [10]. Chemistry analyzer (Power Lab 300, Merck, Germany) was used for the evaluation of serum uric acid using a commercially available kit (ProDia International, UAE) [10].

**Renal histopathology**

On the last day of experiment, half of the rabbits in each group were sacrificed. The kidneys were isolated and cut longitudinally and transversely; and fixed in 10% formal saline solution. The tissues were dehydrated with ethanol in the following alcohol concentration sequence: 50, 70 and 90%. Finally, the absolute alcohol was applied, after using xylene solution for clearing purpose. The tissues were then fixed with help of paraffin wax. Rotary microtome (Micros, Germany) was used for the sectioning of solidified blocks. The slides were stained with hematoxylin and eosin dyes and examined with a light microscope.

**Statistical analysis**

One-way ANOVA was used for comparison of groups following Dunnett test using Graph Pad Prism (version-5) software. The results were expressed as Mean ± SEM. Statistical significance was assumed at *P*-value less than 0.05.

**RESULTS**

**Changes in body weight**

Gentamicin treated animals lost 10.795 ± 1.09% of body weight significantly different from control group animals 0.155 ± 0.91%, *P* < 0.0001. Further, animals treated with *C. tamala* lost 0.95 ± 0.67% of body weight while animals treated with simultaneous administration of *C. tamala* and gentamicin lost Lab 1.26 ± 0.30% of body weight significantly different from gentamicin treated animals (*P* < 0.0001) as given in Table 2.
Table 2: Mean body weight (mean ± SEM, n = 6) of rabbits on days 0, 11 and 21

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Day 0</th>
<th>Day 11</th>
<th>Day 21</th>
<th>Weight loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>1.312 ± 0.16</td>
<td>1.308 ± 0.16</td>
<td>1.310 ± 0.16</td>
<td>0.16 ± 0.91***</td>
</tr>
<tr>
<td>G</td>
<td>1.210 ± 0.10</td>
<td>1.166 ± 0.10</td>
<td>1.080 ± 1.09</td>
<td>10.80 ± 1.09</td>
</tr>
<tr>
<td>GC-ta</td>
<td>1.302 ± 1.26</td>
<td>1.295 ± 0.30**</td>
<td>1.286 ± 0.30**</td>
<td>1.26 ± 0.30**</td>
</tr>
<tr>
<td>C-ta</td>
<td>1.258 ± 0.95</td>
<td>1.252 ± 0.95</td>
<td>1.246 ± 0.95</td>
<td>0.95 ± 0.67***</td>
</tr>
</tbody>
</table>

Blood urea nitrogen (BUN)

Blood urea nitrogen was elevated in gentamicin-treated animals on day 11 (37.78 ± 2.14 mg/dl compared with control 13.75 ± 1.04 mg/dl, p < 0.0001) and on day 21 (54.18 ± 2.6 mg/dl compared with control 14.14 ± 1.12 mg/dl, p < 0.0001). Further, group GC-ta and C-ta was significantly different compared with gentamicin treated animals (p < 0.0001) as given in Table 3.

Serum creatinine

Serum creatinine increased significantly in gentamicin treated animals on day 11 (1.96 ± 0.14 mg/dl compared with control 0.71 ± 0.10 mg/dl, p < 0.0001) and further increased on day 21 (4.02 ± 0.14 mg/dl compared with control 0.80 ± 0.10 mg/dl, p < 0.0001). Group GC-ta and C-ta was significantly different compared with gentamicin treated animals (p < 0.0001) as given in Table 3.

Creatinine clearance

Creatinine clearance decreased significantly in gentamicin treated animals on day 11 (2.08 ± 0.25 ml/min compared with control 5.08 ± 0.82 ml/min, p = 0.0058) and further decreased on day 21 (0.76 ± 0.09 ml/min compared with control 4.99±1.16 ml/min, p = 0.0047). Group GC-ta and C-ta was significantly different compared with gentamicin treated animals on day 21 (p < 0.0001) as given in Table 3.

Serum uric acid

Serum uric acid was significantly elevated in gentamicin treated animals on day 21 (2.34 ± 0.12 mg/dl compared with control 1.51 ± 0.02 mg/dl, p = 0.0058). Further, serum uric acid of group GC-ta and C-ta was significantly different compared with gentamicin treated animals (p = 0.0013 and < 0.0001, respectively) as given in Table 3.

Table 3: Serum BUN, creatinine, creatinine clearance and uric acid on day 21 for all animal groups

<table>
<thead>
<tr>
<th>Group</th>
<th>BUN (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
<th>Creatinine clearance (ml/min)</th>
<th>Serum uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>14.14 ± 1.12</td>
<td>0.80 ± 0.10**</td>
<td>4.99±1.16</td>
<td>1.51±0.0***</td>
</tr>
<tr>
<td>G</td>
<td>54.18±2.60</td>
<td>4.02±0.14</td>
<td>0.76±0.09</td>
<td>2.34±0.1***</td>
</tr>
<tr>
<td>GC-ta</td>
<td>16.75±2.58</td>
<td>1.21±0.09**</td>
<td>3.53±0.43</td>
<td>1.56±0.1***</td>
</tr>
<tr>
<td>C-ta</td>
<td>13.26±0.89</td>
<td>0.83±0.05**</td>
<td>4.71±0.52</td>
<td>1.23±0.1***</td>
</tr>
</tbody>
</table>

Urinary protein

Urinary protein increased significantly in gentamicin treated animals on day 11 (2.51 ± 0.30 mg/dl compared with control 1.64 ± 0.17 mg/dl, p = 0.0329), and further increased on day 21 (3.86 ± 0.32 mg/dl compared with control 1.81 ± 0.22 mg/dl, p = 0.0004). Further, Group GC-ta and C-ta was significantly different from gentamicin treated animals on day 21 of study period (p = 0.0002 and 0.0013 respectively) as shown in Fig 1.

Urinary volume

Urinary volume of gentamicin treated animals was statistically unchanged on day 11 (168 ± 11.96 ml compared with control 200 ± 9.16 ml, P = 0.0596). However on day 21 it was significantly decreased (126 ± 9.09 ml compared with control 217 ± 19.77 ml, P = 0.0019). Further, urinary volume of group GC-ta and C-ta was...
significantly different from gentamicin treated animals on day 21 ($P = 0.0003$ and 0.0002 respectively) as shown in Fig 2.

**DISCUSSION**

The present study was aimed to investigate the nephroprotective potentials of *C. tamala* against gentamicin-induced toxicity. Renal damage caused by gentamicin depends upon the dose and duration of the treatment. Different researchers used different doses, ranging between 8 and 80mg/kg/day to produce renal damage [11]. However, these toxic effects are only associated if the drug is taken five to ten times normal doses [12, 13]. Therefore, in the current study we used a daily dose of 80 mg/kg of gentamicin for a period of 21 days to produce significant nephrotoxic effects.

Rise in serum creatinine and necrosis of tubules have been reported in animals given daily doses of 30 – 60 mg/kg gentamicin for 5 – 10 days [12-14]. Elevation in BUN and serum creatinine and significant fall in creatinine clearance has been reported with the toxic use of gentamicin [14] which was in agreement with the current findings for gentamicin treated animals. The protective role of *C. tamala* extract can easily be concluded from current results.

Gentamicin treated animals significantly lost their body weight compared with control group. However, extract-treated animals did not show significant loss in their body weight when compared with gentamicin treated animals. The blocking of tubules by necrotic debris and leaking of filtrate through the ruptured tubules might be responsible for the rise in serum creatinine [15]. However, it has also been reported that tubular necrosis and alteration in renal function parameters are independent of each other [14,16].

A significant fall in urinary volume and rise in urinary protein was observed in gentamicin-treated animals which were significantly different from those of control and extract-treated groups; this is suggestive of the protection afforded by *C. tamala*. In the current study, histopathological examination of kidney revealed the presence of regenerating cells, necrosis and hydropic changes in gentamicin-treated animals as reported previously [13,17,18]. Elevation of protein excretion may due to cellular degeneration caused by the accumulation of hyaline and granular casts in the proximal tubules. The blockade of tubules by cast cells may be a reasonable cause for the induction of nephrotoxicity associated with gentamicin [15,18]. The fact that there were no significant necrosis, derangement of cells, hydropic changes and hyaline were detected in control
and extract-treated animals confirms the protective role of plant extract.

CONCLUSION

Increase in serum creatinine, blood urea nitrogen and serum uric acid, and fall in creatinine clearance with morphological changes, usually induced by gentamycin, can be prevented by co-administration of *C. tamala* and gentamicin.

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COMPETING INTERESTS

The authors declare no conflict of interest.

REFERENCES