Nephroprotective and antioxidant activity of ethanol extract of whole plant *Biophytum sensitivum* (Linn.) dc on gentamicin-induced renal damage in rats

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Abstract
Present study was undertaken to investigate nephroprotective and antioxidant activity of whole plant ethanol extract of *Biophytum sensitivum* Linn. DC on gentamicin induced-nephrotoxicity in Wistar albino rats. Animals were divided into five groups, containing six animals in each. Gentamicin 100mg/kg/day; i.p., was given to all groups except normal control to induce nephrotoxicity for a period of 8 days in rats. Animals in Group I served as control and Group II as GM-treated or nephrotoxic control. Group III received standard quercetin; group IV received low dose of extract (250mg/kg) and group V received high dose of extract (50mg/kg). Serum levels of blood urea nitrogen, uric acid, creatinine, total protein, albumin, sodium, potassium, calcium, magnesium and antioxidant enzyme activities were determined. This study substantiated and confirmed the ethno-medical usefulness of *B. sensitivum* as nephroprotective and antioxidant agent.

Keywords: Antioxidant, *Biophytum sensitivum*, gentamicin, nephroprotection, quercetin

1. Introduction
Gentamicin (GM) is a typical aminoglycoside antibiotic which provides an effective treatment against life-threatening gram-negative infections [1]. However, 30% of patients treated with GM, for more than 7 days show some signs of nephrotoxicity [2]. It is still widely used in clinical practice because of its low cost and efficacy, even though less nephrotoxic antibiotics are available [3]. GM-induced renal damage is a widely used model for inducing nephrotoxicity in experimental animals [4]. It has been reported that GM induce apoptosis, necrosis, oxidative stress due to accumulation of drug at a concentration of 5–50 times higher than plasma in the tubular renal cell [5]. Lipid peroxidation (LPO) mediated by reactive oxygen species (ROS) has been suggested as a causative agent of cell death [6].

Medicinal plants have curative properties on drug induced nephrotoxicity due to the presence of various complex chemical substances. Ancient literature has prescribed that co-administration of various medicinal plants possessing nephroprotective activity along with different nephrotoxic agents may attenuate its toxicity [7].

*Biophytum sensitivum* Linn. DC (*B. sensitivum*; Vernacular names: Tamil-Nilaccurunki, Tintaanaalee; Malayalam-Mukkutti; Hindi-Lajalu, Lajjaalu, Lakshmana) belongs to family Oxalidaceae [8]. Phytochemical investigations of various extracts of *B. sensitivum* had revealed the presence of large amount of phenolic and poly phenolic compounds, saponins, polysaccharides, pectin and essential oils. The principle bioactive constituents are bioflavonoids like amentoflavone, with trace amounts of cupressoflavone, luteolin, isoorientin and isovitexin [9]. It has been used as a traditional folk medicine in various ailments. Grounded leaves shows diuretic activity and powdered form is indicated for urolithiasis [10]. Recently antioxidant [11], antibacterial [12], antidiabetic [13], antitumour [14], anti-inflammatory [15] and cardioprotective activities [16] were reported. Therefore, as the search for nephroprotective drug possessing significant antioxidant activities from natural sources has gained immense potential, this study was aimed to investigate the *in vivo* nephro-protective and antioxidant activity of ethanol extract of *B. sensitivum* (EEBS) in GM-induced nephrotoxicity in Wistar albino rats.

2. Materials and methods
2.1 Animals
Adult male albino rats of Wistar strain, weighing between 150-200g were housed under standard laboratory condition of 12:12 dark and light cycle, 50% humidity and temperature 25±2 °C.
They were allowed free access to standard commercial rat feed pellets (SAI Animal Feed Ltd., Bangalore, India) and were given water ad libitum. The animals were acclimatized to laboratory condition for 7 days before commencement of experiments. Ethical clearance (KMCET/Ph.D/12/2015-16) was obtained from Institutional Animals Ethics Committee (IAEC). The rats received human care according to the guideline of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.2 Drugs and Chemicals
Gentamicin was obtained from Central Drug House (CDH), New Delhi, India and quercetin from Microlabs (Pondicherry, India). All other chemicals and reagents used in this study were of analytical grade.

2.3 Animal grouping
GM-induced nephrotoxicity model was used to assess the nephroprotective and antioxidant activity in albino Wistar rats [17, 18]. Animals were divided into 5 groups, designated as Group I, II, III, IV and V, containing 6 animals each. Animals in Group I served as control normal saline for 8 days. GM (100 mg/kg/day; i.p.) was administered for a period of 8 days to animals in Group II to V for inducing nephrotoxicity. Group II served as GM-treated or nephrotoxic control and received vehicle (1% tween 80). Group III received standard quercetin (50 mg/kg, p.o.) for 8 days. Group IV received EEBS (250 mg/kg, p.o.) and Group V received EEBS (500 mg/kg, p.o.), for 8 days.

2.4 Plant source and identification
The whole plant, B. sensitivum was collected from Shevaroy Hills, Salem District, Tamil Nadu, India and was taxonomically identified and authenticated by Dr. A. Balasubramanian, Executive Director, ABS Botanical conservation, Research and Training Centre, Kaaripatti, Salem (Dt.) Tamil Nadu (Ref. No-AUT/JKK/095).

2.5 Preparation of Plant Extract
The whole plant was washed and shade dried for about 3 weeks. Dried plant was coarsely powdered, sieved (mesh size=40) and stored in air-tight container at room temperature. Powdered plant material (500 g) was sequentially extracted with petroleum ether (60-80 °C) for defatting and then with 70% ethanol using Soxhlation method. The extract was then filtered and evaporated to dryness in a water bath. After weighing, the dried extract was stored in air-tight container for further use.

2.6 Phytochemical investigation
The EEBS was tested for the presence of alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, terpenoids, steroids, protein, carbohydrates, and fixed oils using the standard procedures.

2.7 Acute Toxicity studies
Acute oral toxicity studies were not performed as studies of ethanolic extract of whole plant B. sensitivum have already been reported. Previous study by Anidya et al., [19] used 5000 mg/kg dose of EEBS as higher dose in albino rats. Hence, one tenth of this dose, 500 mg/kg (higher dose) and lower dose as 250 mg/kg have been selected for the present study.

2.8 Analysis of blood
After collecting the urine, the animals were anaesthetized with ketamine HCl and blood was collected from tail vein under mild anesthesia. Serum was separated by centrifugation at 10,000 rpm for 15 minutes and analyzed for total protein, albumin, sodium, potassium, calcium, magnesium, blood urea nitrogen (BUN), uric acid and creatinine.

2.9 Kidney homogenate analysis
The rats were sacrificed after administration of the last dose of gentamicin by euthanasia method. Both the kidneys were removed, isolated, removed extraneous tissues and washed with ice cold physiological solution. The separated left kidney was homogenized with a motor driven Teflon coated homogenizer with 0.1M Tris-HCl buffer (pH 7.4) to get 10% w/v homogenate. The homogenate was then centrifuged at 10,000 rpm for 10 min (4 °C) and the clear supernatant was collected and used for the estimation of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and malondialdehyde (MDA).

2.10 Statistical analysis
The results were expressed as mean ± SEM. Statistical significance between means was analyzed by one-way analysis of variance (ANOVA) followed by “Dunnett’s test.” P values <0.05 were considered statistically significant.

3. Results
The yields of petroleum ether and ethanol extracts were 4.92% w/w and 12.54% w/w respectively. Preliminary phytochemical investigation indicated that EEBS showed the presence of carbohydrates, alkaloids, steroids, saponins, proteins, aminoacids, falvonoids, tannins, phenolic compounds and fixed oils.

3.1 Effect of EEBS on serum biochemistry of control and experimental animals
The present study demonstrated a significant elevation in the serum levels of creatinine, uric acid and BUN (p<0.001) in GM-treated rats compared to control group [Table 1, group II]. Co-treatment with quercetinor EEBS (250 mg/kg and 500 mg/kg) significantly (p<0.01) decreased creatinine, uric acid and BUN, compared to animals in group II. Serum total protein, albumin (p<0.05), sodium, magnesium (p<0.01) and calcium levels (p<0.001) were found to be significantly decreased in GM-treated animals. Administration of 50 mg/kg of quercetin, or EEBS (250,500 mg/kg p.o.) significantly (p<0.01) attenuated changes in calcium and magnesium levels, compared to GM-treated group. [Table 1, group III, IV, V].
3.2 Effect of EEBS on markers of oxidation in control and experimental animals

Relationship between various drugs mainly GM-induced nephrotoxicity and oxidation stress has been confirmed in many experimental models. This study also revealed the association of GM-induced oxidative stress and nephrotoxicity. Antioxidant status in rats exposed to GM showed a significant diminution of activity of SOD (p<0.01), CAT (p<0.01), GSH (p<0.01) and GPx (p<0.01) compared to group I normal animals (Table 2, group II). Compared to animals in group II, administration of EEBS at dose level of 250 mg/kg to group IV and dose level of 500 mg/kg to group V significantly increased the catalase levels (3.50±0.02 to 3.62±0.010), GPx levels (0.29±0.02 to 0.28±0.02), GSH level (0.46±0.01 to 0.59±0.03) and SOD levels (3.68±0.19 to 3.69±0.16). A significant (p<0.01) increase in production of MDA was observed in group II toxic control animals compared to group I normal animals. Administration of EEBS to group IV and group V at dose level of 250 mg/kg, and 500 mg/kg significantly (p<0.01) decreased MDA levels to 0.15±0.03 and 0.14±0.001 respectively (Table 2, group IV & V). Kidney weight was significantly increased in GM-treated group (p<0.001) compared to normal control group. Significant (p<0.001) weight reduction of kidney was observed in animals co-treated with standard or high dose of EEBS.

### Table 1: Effect of EEBS on serum biochemistry of control and experimental animals

<table>
<thead>
<tr>
<th>Serum Parameters (Unit)</th>
<th>Group I Normal control</th>
<th>Group II GM 100mg/kg</th>
<th>Group III GM+QRTN 500mg/kg</th>
<th>Group IV GM+EEBS 250mg/kg</th>
<th>Group V GM+EEBS 500mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dL)</td>
<td>7.18±0.012</td>
<td>7.14±0.005</td>
<td>7.14±0.009</td>
<td>7.16±0.009</td>
<td>7.17±0.007</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.36±0.003</td>
<td>4.33±0.004</td>
<td>4.37±0.005</td>
<td>4.34±0.009</td>
<td>4.35±0.003</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>138.7±0.070</td>
<td>138.1±0.054</td>
<td>138.5±0.154</td>
<td>138.3±0.115</td>
<td>138.5±0.144</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>5.75±0.004</td>
<td>5.75±0.007</td>
<td>5.73±0.007</td>
<td>5.76±0.010</td>
<td>5.78±0.013</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>10.60±0.131</td>
<td>8.17±0.008</td>
<td>9.04±0.006</td>
<td>9.25±0.008</td>
<td>9.43±0.006</td>
</tr>
<tr>
<td>Magnesium (mg/dL)</td>
<td>2.44±0.014</td>
<td>2.23±0.027</td>
<td>2.54±0.011</td>
<td>2.34±0.009</td>
<td>2.44±0.016</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>15.83±0.196</td>
<td>28.89±0.202</td>
<td>12.13±0.207</td>
<td>18.92±0.014</td>
<td>14.74±0.014</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.68±0.001</td>
<td>1.98±0.020</td>
<td>0.81±0.003</td>
<td>1.51±0.014</td>
<td>0.94±0.010</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>2.13±0.088</td>
<td>3.22±0.14**</td>
<td>2.03±0.016</td>
<td>3.04±0.080</td>
<td>2.95±0.050</td>
</tr>
</tbody>
</table>

Values are expressed in mean ± SEM (n=6), *p<0.05; **p<0.01; ***p<0.001; **p<0.001; *significant compared with control group, **significant compared with GM-induced group, ***notsignificant

### Table 2: Effect of EEBS on markers of oxidation in control and experimental animals

<table>
<thead>
<tr>
<th>Parameters analyzed in Kidney homogenate</th>
<th>Group I Normal control</th>
<th>Group II GM 100mg/kg</th>
<th>Group III GM+QRTN 500mg/kg</th>
<th>Group IV GM+EEBS 250mg/kg</th>
<th>Group V GM+EEBS 500mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>0.64±0.007</td>
<td>0.97±0.050</td>
<td>0.70±0.080</td>
<td>0.73±0.005</td>
<td>0.71±0.008</td>
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<tr>
<td>SOD</td>
<td>4.67±0.050</td>
<td>3.28±0.091</td>
<td>4.02±0.010</td>
<td>3.68±0.192</td>
<td>3.69±0.162</td>
</tr>
<tr>
<td>Catalase</td>
<td>3.14±0.031</td>
<td>1.93±0.020</td>
<td>3.09±0.122</td>
<td>3.50±0.020</td>
<td>3.62±0.010</td>
</tr>
<tr>
<td>GPx</td>
<td>0.53±0.001</td>
<td>0.27±0.001</td>
<td>0.36±0.003</td>
<td>0.29±0.020</td>
<td>0.28±0.020</td>
</tr>
<tr>
<td>GSH</td>
<td>0.95±0.010</td>
<td>0.48±0.002</td>
<td>0.98±0.002</td>
<td>0.46±0.001</td>
<td>0.59±0.031</td>
</tr>
<tr>
<td>LPO</td>
<td>0.14±0.001</td>
<td>0.18±0.002</td>
<td>0.14±0.010</td>
<td>0.15±0.030</td>
<td>0.14±0.001</td>
</tr>
</tbody>
</table>

Units: Kidney weight (g); SOD (unit/min/mg protein); CAT (µmole of H₂O₂ consumed/min/mg protein); GPx (µmole of glutathione oxidized/min/mg protein); GSH (µg/mg protein); LPO (nmol of MDA formed/ mg protein). Values are expressed in mean ± SEM (n=6), *p<0.05; **p<0.01; ***p<0.001; **p<0.001; *significant compared with control group, **significant compared with GM-induced group.

### 4. Discussion

GM-induced nephrotoxicity has been mainly associated with fall in glomerular filtration rate. Disturbance of electrolyte homeostasis is a less well documented side-effect of aminoglycoside therapy. GM induced rise in urinary excretion of sodium and potassium has been reported previously [20]. Foster et al., [21] reported that acute GM infusion into rats induced hypercalciuria and hypermagnesemia. Hypomagnesemia, hypocalcaemia and hypokalaemia are most common syndrome in patients receiving aminoglycoside therapy [22]. This study demonstrated significant elevation of serum levels of creatinine, uric acid, BUN, and significant lowering of serum concentrations of total protein, albumin, sodium, calcium and magnesium in GM-treated rats compared to the rats in control group. This result clearly indicated the failure of kidney’s ability to filter waste product or to conserve cations adequately. Treatment with EEBS restored the elevated serum level of uric acid, BUN, creatinine and diminished calcium and magnesium levels. These results revealed that EEBS possessed significant protective effect against GM-induced nephrotoxicity in a dose dependent manner.

Oxidative stress is a condition associated with reduction in endogenous antioxidant enzymes like SOD, CAT, GPx and GST which catalyze neutralization of many types of free radicals. These enzymes play a significant role in destroying the peroxides and promote antioxidant defenses against ROS [23]. Lowering of their activities result in the accumulation of lipid peroxides and induces oxidative stress [24]. In this study, it was observed that treatment with EEBS significantly restored SOD, CAT, GSH and MDA levels thereby inhibited the changes associated with oxidative stress. Kidney weight was significantly increased in GM-treated group (p<0.001) compared to normal control group. Significant weight reduction of kidney was observed in animals treated with standard or EEBS.

### 5. Conclusion

The phytochemical screening of EEBS revealed the presence of carbohydrates, alkaloids, steroids, saponins, proteins, aminocids, flavonoids, tannins, phenolic compounds and fixed oils. It was clearly evident that co-administration of EEBS attenuated and restored the GM-induced elevation of serum uric acid, creatinine and BUN levels. EEBS treatment was also found to restored GM-induced disturbances in kidney weight, serum calcium and magnesium levels. Supplementation of EEBS at a dose of 250mg/kg and 500mg/kg markedly prevented renal oxidative stress induced
by GM in rats. These observations indicated that the nephroprotective activity of the extract might be due to presence of flavonoid content and its antioxidant nature. However, further studies are required to isolate the phytoconstituents and confirm these findings.

6. References.
19. Anindya B, Md Harun AR, Abdur R, Tapas KP. In-vivo screening of ethanolic extract of Biophytum sensitivum DC leaves on peptic ulcer induced by aspirin in Wistar Albino rats.