EVALUATION OF NEPHROPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF BAUHINIA PURPUREA IN GENTAMICIN INDUCED NEPHROTOXICITY IN RATS

M. A. Rana *, M. Nasiruddin 1, R. A. Khan 1 and A. A. Khan 2

Department of Pharmacology 1 & Department of Anatomy, J.N. Medical College, Aligarh Muslim University, Aligarh, India.

ABSTRACT: Objective: To study the nephroprotective activity of Bauhinia purpurea unripe pods and bark against gentamicin induced nephrotoxicity. Materials and Methods: Twenty four healthy adult albino rats of either sex (150-200 g) were randomly divided in four groups of six animals each. Group I served as vehicle control and Group II as negative control (gentamicin 80 mg/kg/d, i.p). Groups III and IV (test groups) were administered ethanolic extract of Bauhinia purpurea bark (BBE) and unripe pods (BPE) in the dose of 400 mg/kg/day, one hour prior to gentamicin administration. All the treatments were given for a period of 8 days. On 9th day, blood and urine samples were used for biochemical analysis and kidneys for histology and assessing anti-oxidant activity. Results: Gentamicin caused nephrotoxicity as evidenced by highly significant (p<0.001) elevation of blood urea, serum creatinine and urine glucose in Group II as compared to Group I. Also administration of Gentamicin in group II caused oxidative stress in rats suggested by significant increase in MDA level and significant decrease in Catalase and Reduced GSH level. While administration of BBE and BPE in group III and IV respectively caused a significant decrease (p<0.01) of blood urea, serum creatinine, and urine glucose as compared to group II. In case of antioxidant status there is significant increase (p<0.01) in Catalase and Reduced GSH level while there is significant decrease (p<0.01) in MDA level was observed in group III and IV. Conclusion: Ethanolic extract of Bauhinia purpurea unripe pods and bark has nephroprotective activity against gentamicin induced nephrotoxicity in rats.

INTRODUCTION: Gentamicin, a commonly used wide spectrum antibiotic is highly effective against severe gram negative bacterial infections 1. Unfortunately its high efficacy is associated with the side effect of nephrotoxicity.
Abnormal ROS production leads to proximal tubular necrosis via lipid peroxidation of membrane lipids, protein denaturation and DNA damage. From the last few years, tremendous interest has been shown on the role of herbal substances as an antioxidant for the management of various diseases.

*Bauhinia purpurea*, medium sized deciduous tree belongs to family caesalpiniaceae, found throughout India predominantly in sub-himalayan forests. It is commonly known as orchid tree, lalkachnar and kaniar. It has been used to treat various human ailments in folklore medicine such as pain, dropsey, rheumatism, wound healing, delirium, and septicaemia. Based on its folklore uses various pharmacological activities have been reported like analgesic and anti-inflammatory, antimalarial, antitubercular, antifungal and cytotoxic cardiotoxic, hypolipidaemic, antioxidant, hepatoprotective and antidiabetic. There is single study, where nephroprotective activity of ethanolic extracts of unripe pods at the dose of 300 mg/kg against gentamicin induced nephrotoxicity has been studied. In this study we have evaluated the nephroprotective and antioxidant activities of unripe pods as well as bark of *Bauhinia purpurea* against gentamicin induced nephrotoxicity.

**MATERIAL AND METHODS:**

**Plant Materials:** The unripe pods and bark of *bauhinia purpurea* were collected from garden of Aligarh Muslim University, Aligarh during the month of April-May. Collected unripe pods and bark were identified and authenticated by Dr. Athar Ahmed, Assistant professor, Department of Botany, AMU., Aligarh and a voucher specimen with voucher no.DWS/VS/01 was submitted for future reference. Freshly collected unripe pods and bark in bulk were shade dried and pulvORIZED to coarse powder by mechanical grinder. Powder was then subjected to soxhlet extraction with absolute ethanol. Extract thus obtained was evaporated to dryness. Thus the yield obtained was 4.88 % (w/v).

**Experimental Animals:** Adult albino rats of either sex were procured from central animal house, J.N Medical College, AMU., Aligarh (Registration no. 401/CPCSEA). They were housed in polypropylene cages and maintained according to CPCSEA guidelines i.e at room temperature of 27 ± 2°C under 12 hour light and dark cycle. Rats were acclimatized for one week prior to experimentation. Ethical clearance for the study was obtained from Institutional Animal Ethics Committee of Jawaharlal Nehru medical college, A.M.U, Aligarh.

**Experimental Design:** Twenty-four adult healthy albino rats of either sex were divided into four groups, each group containing six rats.

- **Group I** served as vehicle control group received normal saline intraperitoneally (1ml/kg) and distilled water per orum (1ml/kg) daily.
- **Group II** served as negative control group received gentamicin at the dose of 80 mg/kg intraperitoneally and distilled water per orum daily (1ml/kg).
- **Group III (BBE)** and **Group IV (BPE)** were administered *Bauhinia purpurea* unripe pods and bark extract in a dose of 400 mg/kg per orum respectively daily along with same dose of gentamicin as in group II. Extract were given one hour prior to administration of gentamicin in the treatment group. All the groups were given treatment over a period of 8 consecutive days. Following last dose of treatment animals were housed individually in separate metabolic cages to collect 24 hour urine. Twenty four hours after last dosing i.e on 9th day rats were anaesthetized with ketamine and sacrificed. Blood samples were collected by cardiac puncture method. Kidneys were dissected out and weighed. One kidney was preserved in 10 % formalin for histological examination and the other kidney was homogenized for biochemical analysis for oxidative stress and antioxidant activity.

**Biochemical analysis and Tissue Studies:** Serum urea and creatinine levels as well as urine glucose levels were determined using diagnostic kits from span diagnostics, Hyderabad. For estimation of oxidative stress the kidney tissue was cut into small pieces and homogenized in 10 % phosphate buffer, by using homogenizer. Tissue lipid peroxidation level (MDA) was determined by method described by Buege and Aust. MDA condense with two equivalents of thiobarbituric acid to give a fluorescent red derivative which was assayed spectrophotometrically at 532nm. The results were expressed as nmol/g wet tissue weight. Catalase, an antioxidant enzyme level was estimated by method described by Sinha and was expressed as Units.
of \( \text{H}_2\text{O}_2 \) consumed/ min/ g wet tissue. Reduced glutathione level estimation was performed according to the protocol of Ellman.\(^{22}\)

**Histological Examinations:** Formalin preserved kidney tissue were embedded in paraffin and 5-6 \( \mu \text{m} \) sections were cut using a rotary microtome and stained with hematoxylin and eosin (H&E).\(^{23}\) All sections were examined with light microscope for tubular degeneration, tubular necrosis, mononuclear cell infiltration, and hyaline casts.

**Statistical analysis:** Results were expressed as the mean ± SD. Statistical significant difference was determined by one-way analysis of variance (ANOVA) followed by post-hoc dunnets test for multiple comparisons. Probability values (\( P \)) less than 0.05 were considered to be statistically significant.

**RESULTS AND DISCUSSION:**

The nephrotoxicity of aminoglycoside antibiotics especially that of the most commonly used antibiotic, gentamicin (GM) is well documented.\(^{24-25}\) Several studies have reported that oxygen-free radicals are considered to be important mediators of GM-induced acute renal failure.\(^{26-27}\) Therefore agents with antioxidant property can be used for amelioration of GM induced nephrotoxicity. Results summarized in Table 1 show the effect of GM alone and in simultaneous treatment with bauhinia purpurea bark (BBE400) and unripe pods (BPE400) extract in the dose of 400 mg/kg on the physical parameters.

**TABLE 1: EFFECT OF ETHANOLIC EXTRACTS OF UNRIPE PODS AND BARK OF **\textit{B. PURPUREA}** ON PHYSICAL PARAMETERS IN GENTAMICIN INDUCED NEPHROTOXICITY.**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Groups</th>
<th>% Change in body weight</th>
<th>Kidney weight per 100g B. wt (g)</th>
<th>Urine volume/day (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Vehicle Control</td>
<td>(+) 3.76±0.54</td>
<td>0.40±0.017</td>
<td>9.83±0.77</td>
</tr>
<tr>
<td>II.</td>
<td>Negative Control</td>
<td>(-) 9.51±0.47***</td>
<td>0.60±0.007***</td>
<td>18.75±1.13***</td>
</tr>
<tr>
<td>III.</td>
<td>BBE 400</td>
<td>(-) 7.32±1.02</td>
<td>0.55±0.014*</td>
<td>15.00±0.28</td>
</tr>
<tr>
<td>IV.</td>
<td>BPE 400</td>
<td>(-) 6.54±0.89</td>
<td>0.55±0.016*</td>
<td>16.00±0.76</td>
</tr>
</tbody>
</table>

BBE: Bauhinia purpurea bark extract, BPE: Bauhinia purpurea unripe pods extract; 400 denotes doses in mg/kg; Data were expressed in mean ± SEM (n=6 rats/group). Negative control group was compared with Normal control group and all other groups were compared to Negative control group, ***\( p <0.001, **\( p <0.01, *\( p <0.05 \) was considered significant.

GM treatment resulted in significant decrease in % change in BW while there is significant increase in kidney weight and urine volume was observed compared to vehicle control group. Decrease body weight resulted from the increase catabolism seen in acute renal failure accompanied by decrease in food intake.\(^{28}\) Administration of ethanolic extract of Bauhinia purpurea bark (BBE) and unripe pods (BPE) in dose of 400 mg/kg for 8 days caused mild protection from deleterious effect of GM on above physical parameters. There was less reduction in body weight in group III and group IV as compared to negative control group. But the improvement was less than in normal control group, suggesting that supplementary energy must be added to diet. The significant increase in normalised kidney weight of gentamicin treated negative control group (Group II) probably resulted from the oedema due to drug induced tubular necrosis. Also, the significant increase in urine volume per day in gentamicin treated negative control group (Group II) compared to normal control group (Group I) confirms the gentamicin induced nonoliguric acute renal failure. However, the increase in kidney weight and urine volume in \textit{B. purpurea} treated groups (Group III and Group IV) was significantly less as compared to negative control group (Group II). But the \textit{B. purpurea} treatment failed to completely prevent the edema caused by gentamicin administration. Probably, it might be due to shorter duration of study.

Nephrotoxicity in the form of acute renal failure was induced in the negative control group (Group II) according to the method described by Singh P et al.,2009.\(^{19}\) Gentamicin administration in the dose of 80 mg/kg/d for 8 days in negative control group (Group II) significantly elevated blood urea, serum creatinine and urine glucose (\( p<0.001 \)) as shown in Table 2. Eight days treatment of Ethanolic extract of \textit{B. purpurea} bark and unripe pods in the dose of 400 mg/kg along with gentamicin, produced moderate protection compared to negative control group. While \textit{B. purpurea} bark extract (BBE400) reduced blood urea, serum creatinine, urine glucose by 22.42%, 27.80% and 22.10 % to a level of 156.8 ±2.83, 2.57 ± 0.052, 74.77 ± 2.13 mg/dl
respectively. *B. purpurea* unripe pods extract (BPE400) reduced the blood urea, serum creatinine and urine glucose by 23.29%, 28.25% and 21.22% to a level of 155.63 ± 2.47, 2.56 ± 0.055 and 75.44 ± 2.38 respectively. Our experimental results indicate that BBE and BPE at the dose of 400 mg/kg has exerted protection against GM nephrotoxicity. The mechanism of this protective effect is not certain. However, BBE and BPE may have antagonized the oxidative stress of GMs explained subsequently.

### TABLE 2: EFFECT OF ETHANOLIC EXTRACTS OF UNRIPE PODS AND BARK OF *B. PURPUREA* ON BIOCHEMICAL PARAMETERS OF GENTAMICIN INDUCED NEPHROTOXICITY

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Blood urea (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
<th>Urine glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Vehicle Control</td>
<td>55.8±3.92</td>
<td>0.96±0.076</td>
<td>15.72±1.17</td>
</tr>
<tr>
<td>II.</td>
<td>Negative Control</td>
<td>186.00±14.45***</td>
<td>3.19±0.267***</td>
<td>91.53±5.19***</td>
</tr>
<tr>
<td>III.</td>
<td>BBE 400</td>
<td>156.8±2.83**</td>
<td>2.57±0.052**</td>
<td>74.77±2.13**</td>
</tr>
<tr>
<td>IV.</td>
<td>BPE 400</td>
<td>155.63±2.47***</td>
<td>2.56±0.055**</td>
<td>75.44±2.38**</td>
</tr>
</tbody>
</table>

BBE: Bauhinia purpurea bark extract, BPE: Bauhinia purpurea unripe pods extract; 400 denotes doses in mg/kg; Data were expressed in mean ± SEM (n=6 rats/group). Negative control group was compared with Normal control group and all other groups were compared to Negative control group, ***p <0.001, **p<.01* p<0.05 was considered significant.

Significant increase in lipid peroxidation indicated by elevated MDA level (p<0.001) and reduction in GSH and CAT activity (p<0.01) after the treatment of gentamicin in negative control group indicated the production of free radicals and involvement of oxidative stress to nephrotoxicity caused by gentamicin treatment (Table 3). The same results have been reported earlier by Walker et al., 1999. 26. Administration of Ethanolic extract of *B. purpurea* bark and unripe pods produced protection against lipid peroxidation (p<0.01) and increased the activity of antioxidant reduced GSH and Catalase (p<0.01) against gentamicin induced oxidative stress (Table 3). *B. purpurea* contains major class of secondary metabolites like glycosides, flavonoids, saponins, triterpenoids, phenolic compounds, oxepins, fatty acids and phytosterols. Therefore the nephroprotection observed with the treatment of BBE and BPE 400 mg/kg might be due to the presence of these polyphenolic compounds.

### TABLE 3: EFFECT OF ETHANOLIC EXTRACTS OF PODS AND BARK OF *BAUHINIA PURPUREA* ON GENTAMICIN INDUCED OXIDATIVE STRESS

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>MDA (nmoles/g wet tissue wt.)</th>
<th>Catalase (nmoles H2O2consumed/min/mg protein)</th>
<th>GSH (nmoles/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Normal Control</td>
<td>58.69 ± 1.94</td>
<td>124.81±2.20</td>
<td>20.62± 0.25</td>
</tr>
<tr>
<td>II.</td>
<td>Vehicle Control</td>
<td>117.19±1.83***</td>
<td>75.09± 2.06***</td>
<td>10.16±0.22***</td>
</tr>
<tr>
<td>III.</td>
<td>BBE 400</td>
<td>108.18±0.79**</td>
<td>84.79±1.31***</td>
<td>10.97±0.11***</td>
</tr>
<tr>
<td>IV.</td>
<td>BPE 400</td>
<td>107.80±1.15**</td>
<td>85.04±1.67**</td>
<td>11.00±0.16**</td>
</tr>
</tbody>
</table>

BBE: Bauhinia purpurea bark extract, BPE: Bauhinia purpurea unripe pods extract; 400 denotes doses in mg/kg; Data were expressed in mean ± SEM (n=6 rats/group). Negative control group was compared with Normal control group and all other groups were compared to Negative control group, ***p <0.001, **p<.01* p<0.05 was considered significant.

These findings correlated with the histological examination (Table 4).

### TABLE 4: GRADING OF HISTOLOGICAL FEATURES OF RAT KIDNEY TISSUE SECTIONS IN DIFFERENT GROUPS IN GENTAMICIN INDUCED NEPHROTOXICITY

<table>
<thead>
<tr>
<th>Histopathological features</th>
<th>Vehicle control</th>
<th>Negative control</th>
<th>BBE400</th>
<th>BPE400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular congestion</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Mononuclear cells infiltration</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Tubular necrosis</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Tubular hyaline casts</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Light microscopic examination of stained kidney tissue slices from vehicle control group (Group I) showed normal histological features with no structural alterations in glomeruli and tubules.
Figure 3(A)), but the GM-treated group (Group II) revealed more extensive and marked tubular necrosis and numerous hyaline casts (Figure 3(B)). There were mononuclear infiltrations which are considered to be the response of the body tissue facing any injurious agent. These alterations could be due to the accumulation of free radicals resulting from an increased lipid peroxidation in the renal tissues of the GM-treated group. Renal lesions were also characterized by vascular congestion as well as tubular obstruction. Similar changes were also reported by Yaman et al. 31 and Abdel-Raheem et al. 32 who demonstrated structural changes in renal tissue of GM-treated animals and its reversal by various agents. Glomerular and tubular epithelial changes were considerably mild in the group treated with both GM along with B. purpurea unripe pods and bark extract (Figure 3(C&D)), thus showing preventive effect of BPE400 and BBE400 against GM-induced tissue damage.

**CONCLUSION:** The results of our study showed that cotreatment with Bauhinia purpurea unripe pods and bark afforded significant protection against nephrotoxicity induced by gentamicin treatment. The beneficial effect of Bauhinia purpurea as suggested by biochemical findings and supported by histological evidence in gentamicin toxicity might be due to scavenging effect of extract. These findings indicate that Bauhinia purpurea ethanolic extract supplementation may reduce gentamicin induced nephrotoxicity.

**REFERENCES:**


