Nephroprotective and nephrocurative activity of *Alangium salvifolium* against Gentamicin induced nephrotoxicity in albino rats

Karra Geetha*1, Nadenla Ramarao 2

1CMR College of Pharmacy 1, Kandlakoya, Medchal, Hyderabad, Andhra Pradesh, India
2Chalapathi Institute of Pharmaceutical Sciences 2, Lam, Guntur, Andhra Pradesh, India

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ABSTRACT

**Objective:** To study the nephroprotective, nephrocurative effect of *Alangium salvifolium* ethanolic bark extract in gentamicin induced nephrotoxicity.

**Materials and methods:** Nephrotoxicity was induced in wistar male rats by intraperitoneal administration of gentamicin at 40mg/kg b.wt /day for 21 days. *Alangium salvifolium* was selected to check the effect by using ethanolic bark extract with different doses (250, 500, 750 mg/kg body weight respectively), was given by oral route. Serum parameters (serum creatinine, serum urea, serum proteins, and blood urea nitrogen (BUN)), urine parameters (urine creatinine and urine volume) and other parameters like body weight, in vivo antioxidants catalase, reduced glutathione (GSH) and Lipid peroxidase level were determined on 22nd day in wistar male rats. Histopathological study of kidney was studied.

**Results:** The three doses of the extracts produced significant nephroprotective, nephrocurative activities with increased doses. The increased actions of nephroprotective, nephrocurative activity in gentamicin induced nephrotoxicity models as evident by decrease in serum creatinine, serum urea, serum proteins, urine creatinine, BUN levels and lipid peroxidation (MDA). The increased glutathione (GSH), catalase (CAT) activities when compared to gentamicin control group which was further confirmed by histopathological study. **Conclusion:** The study revealed that ethanolic bark extract of *Alangium salvifolium* reversed the nephrotoxicity induced by gentamicin experimental animals. This indicates that *Alangium salvifolium* bark ethanolic extract can be used as an adjuvant with gentamicin to get the therapeutic benefit of these drugs without chances of its prominent nephrotoxicity side effect.

**KEYWORDS:** *Alangium salvifolium*, nephroprotective, nephrocurative, antioxidants, gentamicin.

1. INTRODUCTION

Drugs were shown to cause nephrotoxicity effects by one or more common pathogenic mechanisms. Drug-induced nephrotoxicity tends to be more common among certain patients and in specific clinical situations. Therefore, successful prevention requires knowledge of pathogenic mechanisms of kidney injury, drug-related risk factors, patient-related risk factors, and preventive measures, coupled with vigilance and early intervention [1, 2].

A number of antibiotics including the tetracyclines, cephalosporins, penicillins, as well as aminoglycosides and sulfonamides, are potential nephrotoxins. Gentamicin, a typical aminoglycoside antibiotic is widely used in clinical practices for the treatment of life threatening gram-negative infections. This antibiotic generally causes drug-induced dose-dependent nephrotoxicity in 10-20% of therapeutic courses [3]. Gentamicin generates hydrogen peroxide in rat renal cortex mitochondria and it can also enhance the generation of reactive oxygen species (ROS) [4]. Abnormal production of ROS may damage some macromolecules to induce cellular injury, direct tubular necrosis, without morphological changes in glomerular structures [5, 6]. Possible gentamicin mechanisms to induce nephrotoxicity are peroxidation of membrane lipids, protein denaturation and DNA damage [7-9]. Gentamicin also acts as an iron chelator and the iron-gentamicin complex is a potent catalyst of free radical generation.

*Alangium salvifolium* (L.f) Wang belongs to family Alangiaceae. Locally it called as Ankolam. Alangiaceae is a monogeneric family of trees and shrubs found in tropical and subtropical region. The plant is distributed in dry regions, plains and lower hills in India, Africa, Srilanka, and China.

*Alangium salvifolium* wang was medicinally used in India, China and Philippines. The parts of the plant are used to treat different diseases. Root bark was used as an antidote for rabies. Fruits are sweet and it’s...
used to treat burning sensation, constipation and haemorrhage. Stem bark exerts a biphasic action on the blood pressure in cats at lower doses and marked hypotension in higher doses.

The plant has been reported for its antitubercular, antispasmodic, antiarthritic, antibacterial, anti fungal and anticholinesterase activity. This plant was used as antirheumatic agent by the local people of Vellore and Tirupattur districts in Tamilnadu. Root bark was used as an anthelmintic, antiemetic, febrifuge, hypoglycemic, purgative, antileprotic agent and other skin diseases. The seed oil was used medicinally for externally and internally with palm jaggery for syphilitic ulcers and scabies, gonorrhea and internally for leprosy. Stem bark used to control vomiting and diarrhea. Previously ethanol extract has been reported to possess anti-microbial, analgesic and anti-inflammatory activities [10-17].

2. MATERIAL AND METHODS

2.1. Collection of plant materials:
Angulusalvifolium bark was collected from Hyderabad city and authenticated by P.V.Prasanna. (Scientist / officer In-charge) Botanical Survey of India bearing no. BSI/DRC/12-13/Tech./736.

2.2. Preparation of Angulusalvifolium extract:
The bark was allowed to dry under shade. The dried bark was powdered in a mill and extracted successively by using various solvents like n-hexane, ethyl acetate, ethanol, water.

Required quantity of ethanolic bark extract of Angulusalvifolium 250, 500, 750mg/kg body weight of the rat was weighed and administered.

2.3. Animals
Healthy wistar adult male albino rats weighing about 150-200gm were housed in polypropylene cages and maintained at 24± 2 °c under 12 hr light/dark and 60±5% humidity. They were fed with standard rat pellet diet and water ad libitum. Animals were acclimatized to our lab environment for about a week under laboratory conditions. All experiments were performed according to the ethical standards of animal handling and approved by Institutional Animal ethics committee (CPCSEA/1657/IAEAC/CMRCP/PhD-13/11-A).

2.4. Experimental Protocol
Fifty six male wistar rats were used for the study and animals were divided into seven groups containing eight animals in each.

**Group-I** (Normal control): received saline (1ml/kg b.wt, p.o).
**Group II** (Toxic control): received gentamicin (40mg/kg b.wt for 21 days i.p).
**Group III** (Plant control): received plant extract (200mg/kg b.wt for 21 days p.o).
**Group IV** (prophylactic): received gentamicin (40mg/kg b.wt, i.p) + 250mg/kg b.wt of plant extract (p.o).
**Group V** (prophylactic): received gentamicin (40mg/kg b.wt, i.p) + 500mg/kgb.wt of plant extract (p.o).
**Group VI** (prophylactic): received gentamicin (40mg/kg b.wt, i.p) + 750mg/kg b.wt of plant extract (p.o).

The IV, V, VI Prophylactic group animals were treated with gentamicin (40mg/kg b.wt, i.p),1 hr prior to administration of Angulusalvifolium bark ethanolic extract of respective doses for 13 days and only plant extract of respective doses will be given from 14th-21st day.

**Group VII** (Curative Group): received gentamicin (40mg/kg b.wt, i.p) for 13 days and best dose of the plant extract from the prophylactic treatment was selected and will be given from 14th day to 21st day.

2.5. Sample Collection:
Rats were placed in metabolic cages over a period of 24hr and urine volume collected was measured and it was used for estimation of creatinine.

Animals were sacrificed on 22nd day of the study after collection of blood by retro orbital puncture and were centrifuged to separate the serum. It was used for estimation of creatinine, blood urea nitrogen and total proteins.

2.6. Parameters assessed:

**Body weight:**
The weight of the animals (in grams) was noted on the 1st day and last day of the study and the difference in body weights was noted.

**Serum creatinine:**
Serum creatinine level was estimated by alkaline picrate method using creatinine kit and read absorbance at 520nm.

**Blood urea Nitrogen (BUN):**
BUN level in serum was estimated by kit of Auto span Pvt Ltd. and read the absorbance at 570nm.

**Total Proteins:**
Total proteins level in serum was estimated by kit of Auto span Pvt Ltd. and was read at 578nm.
Table 1: Effect of *Alangium salvifolium* bark ethanol extract on Body weights, BUN, Serum creatinine and Total proteins against Gentamicin induced nephrotoxicity

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>Difference in body weights (gm) (Mean±SEM)</th>
<th>BUN (mg/dl) (Mean±SEM)</th>
<th>Serum Creatinine (mg/dl) (Mean±SEM)</th>
<th>Total Protein (g/dl) (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal control</td>
<td>30.90±4.89</td>
<td>12.39±0.36</td>
<td>0.15±0.00</td>
<td>5.43±0.22</td>
</tr>
<tr>
<td>2</td>
<td>Plant control</td>
<td>42.80±2.63***</td>
<td>12.78±0.19***</td>
<td>0.17±0.00***</td>
<td>5.74±0.16***</td>
</tr>
<tr>
<td>3</td>
<td>Gentamicin control</td>
<td>-66.00±6.00***</td>
<td>37.47±0.40**</td>
<td>1.52±0.09***</td>
<td>11.95±0.48***</td>
</tr>
<tr>
<td>4</td>
<td>Prophylactic (250mg/kg)</td>
<td>4.60±5.53***</td>
<td>19.42±0.55***</td>
<td>1.29±0.02</td>
<td>8.60±0.28***</td>
</tr>
<tr>
<td>5</td>
<td>Prophylactic (500mg/kg)</td>
<td>5.80±3.33***</td>
<td>15.65±0.92***</td>
<td>0.88±0.03***</td>
<td>6.57±0.20***</td>
</tr>
<tr>
<td>6</td>
<td>Prophylactic (750mg/kg)</td>
<td>5.80±1.24***</td>
<td>13.37±0.33***</td>
<td>0.70±0.03***</td>
<td>5.95±0.22***</td>
</tr>
<tr>
<td>7</td>
<td>Curative (750mg/kg)</td>
<td>10.00±3.16***</td>
<td>14.92±13.66***</td>
<td>0.99±0.10***</td>
<td>9.05±0.61***</td>
</tr>
</tbody>
</table>

All of the data obtained from the experimental groups have been compared to gentamicin control group. The data was analyzed statistically by one way ANOVA followed by Dunnett test using graph pad prism 5.0 Software. Where Mean ± SEM (n=8) where *** (P<0.001) vs gentamicin control, ### (P<0.001) vs normal control.
Fig. 1: Effect of *Alangium salvifolium* bark ethanol extract on gentamicin induced nephrotoxicity in terms of changes in body weight.

Fig. 2: Effect of *Alangium salvifolium* bark ethanol extract on gentamicin induced nephrotoxicity in terms of changes in serum creatinine.

Fig. 3: Effect of *Alangium salvifolium* bark on gentamicin induced nephrotoxicity in terms of changes in BUN.

Fig. 4: Effect of *Alangium salvifolium* bark on gentamicin induced nephrotoxicity in terms of changes in total proteins.
3.3. Effect of *Alangium salvifolium* bark ethanol extract on urine creatinine in gentamicin induced nephrotoxicity.

Table 2 and Fig. 5 shows that urine creatinine was increased in gentamicin and plant extract in prophylactic (250 mg/kg, 500mg/kg and 750 mg/kg), curative (750 mg/kg) reduce the increased urine creatinine significantly with the probability of *** P<0.001. It was an indication of stabilization of plasma membrane as well as repair of kidney tissue damage caused by gentamicin.

**Table 2: Effect of *Alangium salvifolium* bark ethanol extract on Urine creatinine, Urine volume, GSH, MDA and Catalase against Gentamicin induced toxicity.**

<table>
<thead>
<tr>
<th>S No.</th>
<th>Treatment</th>
<th>Urine creatinine (mg/dl) (Mean±SEM)</th>
<th>Urine volume(ml) (Mean±SEM)</th>
<th>GSH (µmole/gm of tissue) (Mean±SEM)</th>
<th>MDA (µmole/gm of tissue) (Mean±SEM)</th>
<th>Catalase (u/mg of tissue) (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control</td>
<td>1.44±0.07</td>
<td>8.52±0.25</td>
<td>0.19±0.04</td>
<td>0.262±0.009</td>
<td>195.9±6.765</td>
</tr>
<tr>
<td>2</td>
<td>Plant control</td>
<td>1.478±0.09***</td>
<td>8.01±0.20</td>
<td>0.05±0.002***</td>
<td>0.227±0.002***</td>
<td>198.7±9.141</td>
</tr>
<tr>
<td>3</td>
<td>Gentamicin Control</td>
<td>4.613±0.09***</td>
<td>5.12±0.98</td>
<td>0.039±0.002***</td>
<td>0.411±0.008**</td>
<td>106.4±3.473</td>
</tr>
<tr>
<td>4</td>
<td>Prophylactic (250mg/Kg)</td>
<td>4.037±0.04***</td>
<td>6.04±0.48</td>
<td>0.071±0.002***</td>
<td>0.337±0.101***</td>
<td>144.5±4.266</td>
</tr>
<tr>
<td>5</td>
<td>Prophylactic (500mg/Kg)</td>
<td>2.763±0.08***</td>
<td>7.77±0.38</td>
<td>0.074±0.002**</td>
<td>0.288±0.008**</td>
<td>158.2±7.060**</td>
</tr>
<tr>
<td>6</td>
<td>Prophylactic (750mg/Kg)</td>
<td>1.606±0.08***</td>
<td>8.25±0.98</td>
<td>0.079±0.001***</td>
<td>0.250±0.006**</td>
<td>193.2±2.830</td>
</tr>
<tr>
<td>7</td>
<td>Curative (750mg/Kg)</td>
<td>3.073±0.07***</td>
<td>6.25±0.00</td>
<td>0.065±0.001**</td>
<td>0.164±0.008**</td>
<td>156.6±7.615**</td>
</tr>
</tbody>
</table>

All of the data obtained from the experimental groups have been compared to gentamicin control group. The data was analyzed statistically by one way ANOVA followed by Dunnett test using graph pad prism 5.0 Software. Where Mean ± SEM (n=8) where *** P<0.001, ** P<0.01, * P<0.05 compared to gentamicin control and ### P<0.001, ## P<0.01, # P<0.05 compared to normal control.

3.4. Effect of *Alangium salvifolium* bark ethanol extract on Urine volume:

Table 2 shows that urine volume was significantly decreased in gentamicin administrated group. However, it was significantly increased by administration of *Alangium salvifolium* bark ethanolic extract (250 mg/kg, 500mg/kg and 750 mg/kg) in dose related manner in prophylactic groups and curative group (750 mg/kg) also.

3.6. Effect of *Alangium salvifolium* bark ethanolic extract on MDA and Glutathione Level in gentamicin induced nephrotoxicity:

Table 2, Fig. 6 shows kidney tissue MDA levels were increased significantly (p<0.001) by gentamicin administration as compared to the normal control group but treatment of *Alangium salvifolium* bark ethanolic extract treated prophylactic groups (i.e 250mg/kg, 500mg/kg, 750mg/kg) and curative group (i.e.750mg/kg) reduced MDA significantly (p<0.001).

**Fig 5: Effect of *Alangium salvifolium* bark ethanol extract on gentamicin induced nephrotoxicity in terms of changes in Urine Creatinine.**

**Fig 6: Effect of *Alangium salvifolium* bark on gentamicin induced nephrotoxicity in terms of changes in MDA.**

significantly (p<0.001) as compared with gentamicin control. Table 2, Fig 7 shows that Glutathione were significantly (p<0.001) decreased in gentamicin treated rats compared to the normal control group. Whereas *Alangium salvifolium* bark ethanolic extract treated prophylactic groups (i.e. 250mg/kg, 500mg/kg, 750mg/kg) and curative group (i.e.750mg/kg) significantly increased (p<0.001) as compared with gentamicin control.
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Fig. 7: Effect of Alangium salvifolium bark ethanol extract on gentamicin induced nephrotoxicity in terms of changes in GSH.

3.7. Effect of Alangium salvifolium bark ethanolic extract on catalase activity in gentamicin induced nephrotoxicity:
Table 2, (Fig. 8) shows a significant decrease in renal catalase activity was observed in gentamicin control group compared to normal control. All the doses of Alangium salvifolium bark ethanolic extract treated groups showed significant increase in catalase levels compared to gentamicin control group.

![Graph showing GSH levels in different groups](image)

![Graph showing catalase levels in different groups](image)

Drug treatment

Fig. 8: Effect of Alangium salvifolium bark ethanol extract on gentamicin induced nephrotoxicity in terms of changes in Catalase.

3.8. Histopathological studies of rat kidneys:
Experimental animals were sacrificed and kidneys were identified and carefully dissected out for histopathological studies. After rinsing in normal saline, sections were taken from each harvested kidney. The tissue was fixed in 10% formal saline, dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into 4-5 microns thick sections stained with haematoxylin-eosin and observed under a photomicroscope (magnification power-200X). The sections of kidney treated with gentamicin showed degenerative tubular structures with vacuolization, necrosis in Fig. 10, where as sections of kidneys isolated from rats treated with 250mg/kg b. w. ethanol extract showed large degenerations in Fig. 12. In prophylactic medium dose 500mg/kg b.w. showed predominant normal kidney with minimal degenerations in Fig. 13. In prophylactic high dose 750mg/kg b.w. ethanol extract showed predominant normal kidney in prophylactic groups in Fig. 14. In curative group sections of kidneys isolated from rats treated with 750mg/kg b. w. showed predominant normal kidney in Fig. 15.

![Histopathological images](image)

Fig. 9. Control group showing normal glomeruli and tubules.
Fig. 10. Gentamicin control showing degenerative tubular structures with vacuolization, necrosis
Fig. 11. Plant control group showing normal glomeruli.
Fig. 12. Prophylactic group (250mg/kg) Showing large degenerations.
Fig. 13. Prophylactic group (500mg/kg) showing predominant normal kidney with minimal degenerating.
Fig. 14. Prophylactic group (750mg/kg) showing predominant normal kidney.
Fig. 15: Curative group (750mg/kg) showing predominant normal kidney.

6. REFERENCES


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