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# Ethanolic extract of *Alangium salvifolium* stem bark attenuates gentamicin-induced nephrotoxicity in rats

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**ABSTRACT**

**Objective**: To study the nephroprotective, nephrocurative effect of *Alangium salvifolium* ethanolic stem 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 7 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 proteins and blood urea nitrogen (BUN)), other parameters like body weight, in vivo antioxidants catalase, Superoxide dismutase (SOD), reduced glutathione (GSH) and Lipid peroxidase level were determined on 22$^{nd}$ 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, 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* (EBAS) recovered the nephrotoxicity induced by gentamicin experimental animals.

**KEYWORDS**: *Alangiumsalvifolium*, nephroprotective, nephrocurative, antioxidants, gentamicin.

## INTRODUCTION

Drug-induced renal disease is a common problem. Drugs cause several renal syndromes, such as prerenal azotemia, fluid and electrolyte abnormalities, acute tubular necrosis, acute interstitial nephritis, and chronic interstitial nephritis. Acute renal failure due to acute tubular necrosis is the most common syndrome and is most frequently caused by aminoglycoside antibiotics, radiographic contrast agents, and amphotericin B. 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, preventive measures coupled with vigilance and early intervention [1, 2].

A number of antibiotics including the tetracyclines, cephalosporins, penicillins, as well as aminoglycoside antibiotics 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. It 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 is used to treat burning sensation, constipation and haemorrhage.

The plant has been reported for its antitubercular, antispasmodic, antiarthritis, 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 .**Materials**

Gentamicin- 80mg/2ml was purchased from Ranbaxy, n-hexane, ethyl acetate, ethanol from SD Fine Chemicals Ltd; Hyderabad, India.

2.2. **Plant materials**: *Alangium salvifolium* stem 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.

2.3. **Preparation of *Alangium salvifolium* extract**: The bark was allowed to dry under shade. The dried bark was powered in a mill and extracted successively by using various solvents like n-hexane, ethyl acetate, ethanol, water. Required quantity of ethanolic bark extract of *Alangium salvifolium (*EBAS) 250, 500,750mg/kg body weight of the rat was weighed and administered.

2.4. **Animals:** Healthy wistar adult male albino rats weighing about 150-200gm were housed in polypropylene cages and maintained at 24± 2 ºc 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/IAEC/ CMRCP/PhD-l3/11-A).

2.5. **Experimental design**: Forty two male wistar rats were used for the study and animals were divided into seven groups containing six animals in each.

Group-I (Normal control): received vehicle (1ml/kg b.wt, *p.o*).

Group II (Plant control): received plant extract (250mg/kg b.wt for 14 days *p.o*).

Group III (Disease control): received gentamicin (40mg/kg b.wt for 7days *i.p*).

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/kg b.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 EBAS extract for first 14 days. gentamicin (40mg/kg b.wt, *i.p*), was administrated from $15^{th}$ to $21^{st}$ day.

Group VII (Curative Group): received gentamicin (40mg/kg b.wt, *i.p*) from $1^{st}$ to $7^{th}$ day and best dose of the plant extract from the prophylactic treatment was selected and will be given from $8^{th}$ to $21^{st}$day.

2.6. **Sample Collection**: Animals were sacrificed on $22^{nd}$ 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. The kidney tissues were homogenized in phosphate buffer pH 7.4. The homogenates obtained were centrifuged at 800 rpm for 5 minutes at 4°C (REMI CM-12) to separate the molecular debris. The supernatant so obtained was centrifuged at 10,500 rpm for 20 minutes at 4°C to get the post-mitochondrial supernatant (PMS) .

2.7. **Parameters assessed:**

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

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

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

2.7.4. **Total Proteins**: Total proteins level in serum was estimated by kit of Auto span Pvt Ltd. and was read at 578nm.

2.7.5. **GSH**: GSH level in the kidney homogenate was estimated by the method of Ellman.et al.1959. [18]

2.7.6. **Catalase**: Catalase activity in kidney tissue was determined by measuring the rate of decomposition of hydrogen peroxide at 240 nm, according to the method given by Aebi et al 1974. [19]

2.7.7. **Lipid peroxidation**: The concentration of MDA in kidney homogenate was determined according to the method of Ohkawa et al. 1979. [20]

2.7.8. **Histopathological studies**: Kidney of sacrificed rats was carefully dissected out. After rinsing in normal saline the tissue was fixed in 10% formalin-saline dehydrated with 100% ethanol solution and embedded in paraffin. Then it was cut into 4-5µm thick sections stained with haematoxylin-eosin and observed under microscope (magnification power- 100X).

**2.8. STATISTICAL ANALYSIS**

All the data was expressed as mean ± S.E.M. Statistical significance between more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad,version:5.04). Statistical significance was set at $p<0.05$

**3. RESULTS**

3.1. **Effect of EBAS extract on body weight in gentamicin induced nephrotoxicity**: Gentamicin treatment caused a significant reduction in body weight in disease control (group III), When compared with the normal animals (group I), which is an indication of renal impairment ($p < 0.05$). Animals pretreated with EBAS (group IV, V, VI) showed significant increase in body weight when compared to the disease control ($p < 0.05$). Similarly rats treated with plant extract alone (group II), when compared with control group (II) had also produced significant increase in body weight. Treatment with ERAV in curative group (VII), has shown significant increase in the body weight when compared with control group, reflecting preservation of renal function ($p < 0.05$).

3.2. **Effect EBAS extract on serum creatinine, blood urea nitrogen and total proteins**: Table 2 shows the levels of serum creatinine and blood urea nitrogen increased significantly in gentamicin control animals when compared to normal animals. The level of serum creatinine and BUN was reduced significantly upon administration of EBAS extracts (i.e.250mg/kg, 500mg/ kg and 750mg/kg *p.o*.). When compared to gentamicin control. The curative group dose was selected based on the dose dependent activity of plant extract (i.e. 750mg/kg *p.o*.) Significantly ($p<0.05$) reduced the increased level in serum creatinine and BUN when compared with the gentamicin control.

A significant decrease in the serum total protein was noted in disease control group (III) when compared to the normal group (I). There was significant increase in the serum total proteins in EBAS alone treated group (II) in comparison with control rats ($p<0.05$).A significant increase in the levels of serum total proteins was observed in both prophylactic treatment (IV, V, VI) and curative treatment (VII) groups when compared to the disease control group (III) indicating that the EBAS had reduced the urinary leakage of proteins by ameliorating the renal injury ($p<0.05$).

3.3. **Effect of EBAS extract on MDA, SOD,GSH and Catalase in gentamicin induced nephrotoxicity**

Table 3 shows kidney tissue MDA levels were increased significantly ($p<0.05$ ) by gentamicin administration as compared to the normal control group but treatment of EBAS extract treated prophylactic groups (i.e 250mg/kg, 500mg/kg, 750mg/kg.) and curative group (i.e.750mg/kg) reduced the MDA.

In prophylactic (IV, V, VI) and curative groups (V), treatment with ethanolic extract of *A. salvifolium* significant increase in the levels of SOD, glutathione and catalase was observed when compared to the control group (group III). In the like manner the plant extract alone treated group (II) has also significantly increased the SOD, glutathione, catalase levels indicating that the plant extracts selected for the present study can possess good antioxidant activity ($p<0.05$). After inducing nephrotoxicity by treating the rats with gentamicin, SOD, glutathione, catalas levels decreased significantly in disease control group III when compared to the normal group (I) ($p<0.05$).

**3.4. Histopathalogical studies:** Kidney of sacrificed rats was carefully dissected out. After rinsing in normal saline the tissue was fixed in 10% formalin-saline dehydrated with 100% ethanol solution and embedded in paraffin. Then it was cut into 4-5µ thick sections stained with hematoxylin-eosin and observed under microscope (magnification power-100X).

The sections of kidney treated with gentamicin showed degenerative tubular structures with vacuolization, necrosis in Fig. A, where as sections of kidneys isolated from rats treated with 250mg/kg b. wt ethanol extract showed large degenerations in Fig D. In prophylactic medium dose 500mg/kgb.wt showed predominant normal kidney with minimal degenerations in Fig.E, In prophylactic high dose 750 mg/kgb.wt ethanol extract showed predominant normal kidney in prophylactic groups in Fig. F. In curative group sections of kidneys isolated from rats treated with 750mg/kg b. wt showed predominant normal kidney in Fig .G.

## 4. DISCUSSION

Gentamicin induces nephrotoxicity by the formation of ROS which causes renal phospholipidosis through inhibition of lysosomal hydrolases. ROS also accumulates in renal cortex and causes renal damage [21, 22].

The renal toxicity induced by gentamicin (40mg/kg, *i.p.*) in rats was evidenced by the alteration in the serum biomarkers of glomerular and tubular damage. The changes in the biochemical parameters were well correlated with the renal histological score of gentamicin treated rats.

Biochemical parameters like serum creatinine, blood urea nitrogen, total proteins and enzymatic parameters like SOD, GSH, Catalase and MDA level were studied. Gentamicin caused significant ($p < 0.05$) elevation of serum createnine, BUN and MDA levels and it also decreased GSH, catalase and SOD when compared to the normal group. However, these changes were attenuated by EBAS extract in dose related fashion. The plant extract had more significant effect at 500mg/kg, 750mg/kg when compared to 250mg/kgb.wt. The curative group dose was selected after biochemical result analysis of prophylactic groups. EBAS extract had significant curative effect when given at a dose of 750 mg/kgb.wt. The results of the study disclosed that the EBAS extract had significant nephroprotective and nephrocurative effect against gentamicin induced nephrotoxicity in a dose dependent fashion. These findings correlated with renal histological examination which revealed the degenerative tubular structures with vacuolization, necrosis in gentamicin induced toxicity and recovered by treating with EBAS extract.

**5. CONCLUSION**: In conclusion, the present study provided convincing evidence that oxidative stress remains the corner stone in nephrotoxicity caused by gentamicin. Our results suggested that the ethanolic extracts of *Alangium salvifolium* bark, has a significant therapeutic benefit when administered along with gentamicin therapy by attenuating oxidative stress and lipid peroxidation. Hence, EBAS extract may find role in preventing the complications of nephrotoxicity induced by gentamicin.

**Table 1: Effect of EBAS extract on body weight in gentamicin induced nephrotoxicity**

| S. No | Group | Initial body weights (gms) | Final body weights (gms) | Difference in body weights (gms) |
|---|---|---|---|---|
| 1 | Normal | 178.2±9.254 | 198.2±6.535 | 19.5±2.65 |
| 2 | Plant control (250 mg/kg; *p.o*) | 198.5±6.569 [a] | 218.5±9.561 [a] | 18.2±1.365 [a] |
| 3 | Disease control (Gentamicin 40mg/kg; *i.p*) | 145.73±2.342 [b] | 152.0±4.721 [b] | 6.27±2.379 [b] |
| 4 | Prophylactic (250 mg/kg; *p.o*) | 182.0±3.851 [c] | 192.5±5.050 [c] | 10.5±0.809 [c] |
| 5 | Prophylactic (500 mg/kg; *p.o*) | 178.4±2.981 [c] | 189.1±4.012 [c] | 11.7±1.031 [c] |
| 6 | Prophylactic (750 mg/kg; *p.o*) | 180.2±2.894 [c] | 193.7±3.897 [c] | 13.5±0.745 [c] |
| 7 | Curative (750mg/kg; *p.o*) | 175.5±4.245 [c] | 190.0±5.356 [c] | 12.5±0.874 [c] |

All values are expressed as Mean ± SEM

b indicates $p<0.05$ when compared gentamicin control with normal group

a and c indicates $p<0.05$ when compared plant control test groups with disease control respectively.

**Table 2: Effect of EBAS extract on total proteins, serum creatinine and BUN in gentamicin induced nephrotoxicity**

| S. No | Groups | Total proteins (gm/dl) | Serum Creatinine (mg/dl) | BUN (mg/dl) |
|---|---|---|---|---|
| 1 | Normal | 6.22±0.040 | 0.195±0.00211 | 13.25±0.214 |
| 2 | Plant control | 5.56± 0.003[a] | 0.153±0.00114 [a] | 11.54±0.145 [a] |
| 3 | Disease control (Gentamicin 40mg/kg; *i.p*) | 10.71 ± 0.004 [b] | 1.043±0.03801 [b] | 45.189±0.417 [b] |
| 4 | Prophylactic( 250mg/kg; *p.o)* | 9.38± 0.036[c] | 0.741±0.00987 [c] | 20.459±0.791 [c] |
| 5 | Prophylactic(500mg/kg; *p.o)* | 8.35 ± 0.006[c] | 0.566±0.03214 [c] | 19.881±0.4385 [c] |
| 6 | Prophylactic (750mg/kg; *p.o)* | 7.47 ± 0.049 [c] | 0.361±0.00947 [c] | 17.321±0.8974 [c] |
| 7 | Curative (750mg/kg; *p.o)* | 7.96 ± 0.012[c] | 0.465±0.00674 [c] | 18.098±0.328 [c] |

All values are expressed as Mean ± SEM
b indicates p<0.05 when compared gentamicin control with normal group
a and c indicates p<0.05 when compared plant control test groups with disease control respectively.

**Table 3:Effect of EBAS extract on Superoxide Dismutase, Catalase,GSH and MDA in gentamicin induced nephrotoxicity**

| S.No | Groups | SOD (U/mg Tissue ) | CAT (µmoles of $H_2O_2$ consumed/min/mg protein) | GSH µmole/mg Tissue) | MDA(nmoles/mg protein) |
|---|---|---|---|---|---|
| 1 | Normal | 17.23 ± 1.025 | 195.92± 16.76 | 0.555 ± 0.017 | 2.012 ± 0.01 |
| 2 | Plant control | 15.01 ± 2.32 [a] | 189.25 ± 14.32 [a] | 0.499 ± 0.102 [a] | 1.89 ± 0.001 [a] |
| 3 | Disease control (Gentamicin 40mg/kg; *i.p*) | 8.43 ± 1.12 [b] | 139.25 ± 15.98 [b] | 0.302 ± 0.013 [b] | 4.12 ± 1.45 [b] |
| 4 | Prophylactic( 250mg/kg; *p.o)* | 13.92± 2.325 [c] | 183.81± 16.99 [c] | 0.373± 0.044 [c] | 1.45± 0.092 [c] |
| 5 | Prophylactic(500 mg/kg; *p.o)* | 14.05± 2.867 [c] | 185.35± 17.002 [c] | 0.399± 0.056 [c] | 1.75± 0.086 [c] |
| 6 | Prophylactic (750mg/kg; *p.o)* | 15.80± 3.431 [c] | 186.65± 16.88 [c] | 0.425± 0.010 [c] | 1.87± 0.029 [c] |
| 7 | Curative (750mg/kg; *p.o)* | 14.79± 3.921 [c] | 189.05± 16.98 [c] | 0.396± 0.023 [c] | 2.01± 0.032 [c] |

All values are expressed as Mean ± SEM
b indicates p<0.05 when compared gentamicin control with normal group
a and c indicates p<0.05 when compared plant control test groups with disease control respectively.

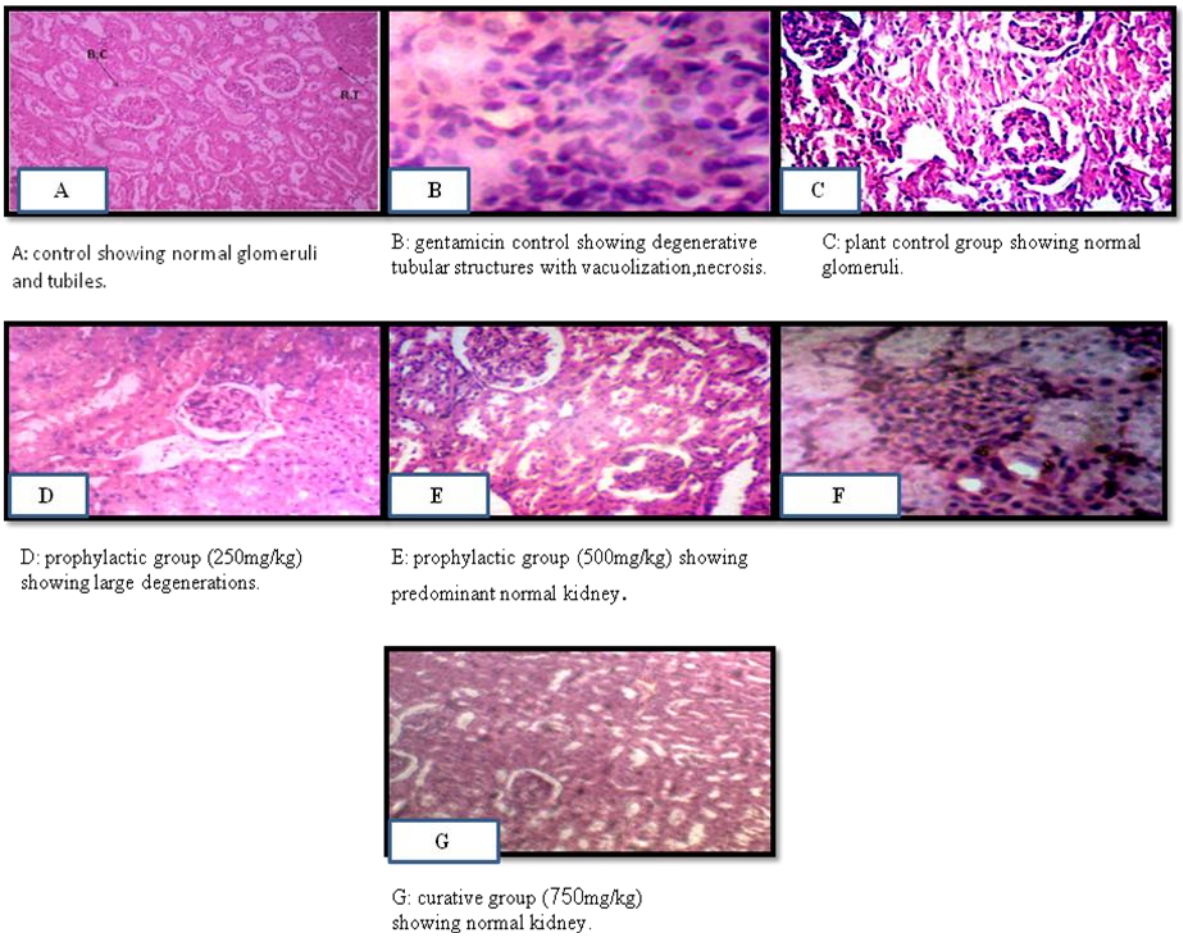


**Figure 1: Effect of EBAS on histomorphology of kidney rat in gentamicin induced nephrotoxicity.**

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