

Study of Nephroprotective Activities of Stem Bark Extracts of *Ficus Racemosa* in Gentamicin Induced Acute Renal Failure in Rats

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ABSTRACT

Nephrotoxicity induced by aminoglycosides manifests clinically as nonoliguric renal failure, with a slow rise in serum creatinine and a hypoosmolar urinary output developing after several days of treatment. . Gentamicin has been shown to enhance the generation of superoxide anion and hydrogen peroxide by renal cortical mitochondria. Drug-induced nephrotoxicity tends to be more common among certain patients and in specific clinical situations. The primary goal of this research is to present the scientific evidence for the use of herbs like *Ficus racemosa* as a complimentary for acute renal failure. Albino wistar rats were divided into 6 groups, 6 rats each. Control group, gentamicin (80mg/kg,i.p.) and ethanolic stem bark extracts (200 and 400mg/kg,p.o.) treated groups. The nephrotoxicity in rat was induced by the administration of gentamicin (80mg/kg.i.p. for 8 days). The degree of nephroprotection was measured by measuring kidney weight, urine volume, serum urea, serum creatinine and antioxidant parameters- lipid peroxidation, catalase and glutathione estimation. The signs of nephrotoxicity in rats significantly alleviate by ethanol extracts of *Ficus racemosa*. Hence the ethanol extract of this plant can be as a neuroprotective agent.

Keywords: Aminoglycosides, *Ficus racemosa*, gentamicin, stem bark extract.

INTRODUCTION

Aminoglycosides are nephrotoxic because a small but sizable proportion of the administered dose (≈5%) is retained in the epithelial cells lining the S1 and S2 segments of the proximal tubules after glomerular filtration. Aminoglycosides accumulated by these cells are mainly localized with endosomal and lysosomal vacuoles but are also localized with the Golgi complex. After only a few days of administration of aminoglycosides to the laboratory rats induce conspicuous and characteristic changes in lysosomes and proximal tubular cells consistent with the accumulation of polar lipids. These changes are preceded and accompanied by signs of tubular dysfunctions or alterations (release of brush-border and lysosomal enzymes; decreased reabsorption of filtered proteins; wasting of K⁺, Mg²⁺, Ca²⁺, and glucose; phospholipiduria; and cast excretion. In animals, tubular alterations have clearly

been associated with the development of focal necroses and apoptoses in the tubular epithelium, together with an extensive tubular and peritubular cell proliferation.¹ Acute renal failure is a major complication of aminoglycoside antibiotics, which are widely used in the treatment of gram-negative infections. Gentamicin has been shown to enhance the generation of superoxide anion and hydrogen peroxide by renal cortical mitochondria. The interaction between superoxide anion and hydrogen peroxide in the presence of metal catalyst can lead to the generation of hydroxyl radical.² *Ficus racemosa* (syn. *Ficus glomerata* Roxb.) is a species of plant in the Moraceae family. Popularly known as the Cluster Fig Tree or Goolar (Gular) Fig, this is native to Australasia, South-East Asia and the Indian Subcontinent. It is unusual in that its figs grow on or close to the tree trunk. In India

the tree and its fruit are called gular in the north and atti in the south. The fruits are a favorite staple of the common Indian macaque. In Vietnam, it is called sung³. This plant is a popular medicinal plant in India, which has long been used in ayurveda, the ancient system of Indian medicine for various diseases/disorders including diabetes, liver disorders, diarrhoea, inflammatory conditions, and hemorrhoids, respiratory and urinary diseases. As a part of this concept survey of locally available medicinal plants was undertaken. It was observed that the plant *Ficus racemosa* Linn is grown widely and abundantly. In addition, a native practitioner has claimed that this plant is very useful nephroprotective agent. The stem bark and fruits are used in India for the treatment of various diseases. Methanol extracts of *Ficus racemosa* contained relatively higher levels of total phenolics than the other extract⁴. Keeping all these facts in view the present study is aimed at giving a scientific basis for the native claims and traditional knowledge.

Materials and methods

1. Collection and extraction

The stem barks of *Ficus racemosa* were collected and identified and authenticated by a qualified Botanist. The shade dried and powdered stem bark (1 kg) was extracted with 95% ethanol in a soxhlet apparatus (55°C; 25–30 cycles). The ethanol extract was concentrated to a small volume and then evaporated to dryness. The ethanolic bark extract of *Ficus racemosa* (EBFR) was kept in air tight containers and used for the this study.⁵

2. Animals

Adult male albino rats (150-200g) were used in this study. The animal care and experimental protocols were made in accordance with CPCSEA/ IAEC. The rat doses of EBFR was selected as 200, 400mg/kg b.w, p.o. respectively. The rats were acclimatized to the laboratory environment for one week. The study was conducted after obtaining clearance certificate from IAEC.

3. Methods

The rats were divided into six groups consisting of 6 rats in each group. Group I rats (control) were given normal saline daily for orally for 11 days and intraperitoneally for 8 days. Group II rats were given gentamicin (80mg/day) for 8 days and normal saline for 11 days. Group III and IV rats were given gentamicin (80mg/k.g.i.p) for 8days and EBFR (200 and 400mg/kg p.o.) respectively for 11 days. The EBFR treatment was started 3 days prior to the gentamicin injections and continued along with the gentamicin treatment for 8days⁶. On the last day of treatment the rats were housed in individual metabolic cages. On the day 9, urine was collected with the help of metabolic cages and the urine samples were subjected for estimation of urinary functional parameters like creatinine and urea. Twelve hour urines were collected. Urine volumes were determined and the samples were diluted with purified water. Aliquots of urine were immediately frozen until analyzed. A drop of conc. HCl was added to the collected urine. This prevents the growth of microbes and also prevents metal hydrolysis. The collected urine was measured and transferred to a cleaned airtight container. After 24h of the last administration, the rats were anesthetized with ketamine (60mg/kg) and xylazine (5mg/kg) given intraperitoneally and blood samples were collected by retro orbital sinus puncture. The blood was allowed to clot at 37°C for 40 min and the clot was separated. The separated serum was transferred into centrifuge tube. The serum was centrifuged at 3000 RPM for 10min. The clear serum was stored refrigerator. The kidneys were removed and washed with ice-cold saline and weighed and then they were homogenized in ice-cold 0.1 M Tris-HCl buffer (pH 7.4). The resultant homogenate was centrifuged using cold centrifuge. The supernatant was used for assay of lipid peroxidation⁷, catalase⁸ and glutathione⁹. The serum was used for the estimation of serum creatinine and urea. The urine and serum were analyzed for creatinine and urea. From the urine samples the urea

and creatinine were estimated using semi autoanalyzer and erba diagnostic kits.

Statistical analysis

Results were expressed as mean + SEM. The results were analyzed statistically using ANOVA followed by Tukey's test. Values of $p < 0.05$ were considered significant.

Results

There is significant increase in the urine volume in rats administered with gentamicin alone (group II), when compared with normal control rats (group I). There is significant decrease in the urine volume in rats (group III and IV) treated with gentamicin and EBRF (200 and 400mg/kg) when compared with gentamicin alone treated rats. The significant increase of the kidney weight in gentamicin alone administered rats when compared to the normal control rats. There is insignificant decrease in the kidney weights in rats treated with gentamicin and EBRF (200 and 400mg/kg). There is significant increase in urine urea, urine creatinine, serum urea and serum creatinine in rats administered with gentamicin alone for 8 days (group II) when compared with normal control rats (group I). The rats (group III and IV) treated with gentamicin and EBRF (200 and 400mg /kg) has shown significant decrease in urine urea, urine creatinine, serum urea and serum creatinine when compared to the gentamicin alone administered group (group I), suggesting the nephroprotection. There is significant increase in the lipid peroxides- the end product of lipidperoxidation ($p < 0.05$) in the kidney tissues of gentamicin alone treated rats (group II) as compared to normal control rats (group I). There is insignificant decrease (table-II) in the lipid peroxides in rats (group III and IV) administered with gentamicin and EBRF (200 and 400mg/kg) when compared to gentamicin control rats (group II). The gentamicin control rats also showed a significant ($p < 0.05$) decrease in catalase and glutathione levels in the kidney tissue. The rats treated with gentamicin and

EBFR (200 and 400mg/kg) has shown dose dependent significant increase ($p < 0.05$) (table II) increase in catalase and glutathione in the kidney tissue as compared to the gentamicin control rats (group II.) when compared to the normal control rats (group I).

Discussions

Gentamicin is an aminoglycoside antibiotic used to treat many types of bacterial infections, particularly those caused by gram negative organisms. Various environmental toxicants and clinically used aminoglycoside antibiotics like kanamycin, gentamicin can cause severe organ toxicities through the metabolic activation to highly reactive free radicals including the superoxides and oxygen reactive species. Gentamicin is also ototoxic and nephrotoxic, with this toxicity remaining a major problem in clinical use. The gentamicin may accumulate in epithelial tubular cells causing a range of effects starting with loss of the brush border in epithelial cells and ending in overt tubular necrosis, activation of apoptosis and massive proteolysis. GM also causes cell death by generation of free radicals, phospholipidosis, extracellular calcium-sensing receptors stimulation and energetic catastrophe, reduced renal blood flow and inflammation.¹⁰ Administration of gentamicin (80mg/kg i.p.) for 8 days reported to causes nephrotoxicity⁶. The significant increase in serum urea and serum creatinine was observed in GM administered rats. The kidney tissues of these rats also showed insignificant decrease in lipid peroxide and significant decrease in the catalase and glutathione levels. The creatinine is freely filtered by the renal glomerulus. Tubular reabsorption does not occur. Creatinine is freely filtered by the renal glomerulus. Tubular reabsorption does not occur. The elevation of the serum creatinine is produced by kidney damage, which leads to a decreasing GFR and serum creatinine filtration. The increase in the serum creatinine levels in the GM treated group is due to decreased GFR caused by the

gentamicin.¹¹ In renal disease, the serum urea accumulates because the rate of serum production exceeds the rate of clearance.¹² Elevation of urea and creatinine was taken as the index of nephrotoxicity. It was reported that EBRF has powerful antioxidant activity and contains steroids, triterpenoids, polyphenols, coumarins, flavonoids and tannins.¹³ The nephroprotective effects of

EBFR in gentamicin induced nephrotoxicity may be due to flavonoids and tannins present in the extract. In conclusion, the findings of the present study, gives the experimental evidence for supporting the nephroprotective effects of ethanol stem bark of *Ficus racemosa* in gentamicin induced acute renal failure in rats.

Table I: Table showing the effects on urine volume, kidney weight, serum urea and serum creatinine, in normal control, gentamicin, EBRF treated rats

Gs	Treatment	Urine volume ml/12h	Kidney weight in g	Serum urea (mg/dL)	Serum creatinine (mg/dL)
I	Control (vehicle)	3.28 ± 0.43	1.28 ± 0.29	42.50 ± 2.590	0.69 ± 0.010
II	Gentamicin+ vehicle	9.45 ± 0.31*	2.09 ± 0.19*	94.170 ± 3.71*	1.023 ± 0.086*
III	Gentamicin+ EBRF(200mg/kg)	6.88 ± 0.54**	1.99 ± 0.35	63.830 ± 5.040**	0.790 ± 0.079**
IV	Gentamicin+ EBRF(400mg/kg)	5.89 ± 0.16**	1.93 ± 0.29	57.17 ± 2.930**	0.833 ± 0.044**

Each value is mean ± SEM (n=6)

* Significantly different from normal control, p<0.05

** Significantly different from gentamicin control, p <0.05

Results of group II were compared with group I. The results of group III and IV were compared with group II. The values are shown in mean ±SEM. Data was

analyzed by one way ANOVA followed by Tukey's test.

Table II: Table showing the effects on lipid peroxidation, SOD, catalase and GSH in normal, and gentamicin, EBRF treated rats

Gs	Treatment	Lipid peroxidation mM/100g tissue	Catalase (U/mg protein)	GSH nM/mg protein
I	Control (vehicle)	27.705 ± 0.795	19.773 ± 0.596	21.338 ± 1.018
II	Gentamicin	36.590 ± 0.913*	10.300 ± 0.848*	10.170 ± 0.372*
III	Gentamicin+ EBRF(200mg/kg)	34.713 ± 0.802	14.601 ± 0.600**	18.596 ± 0.671**
IV	Gentamicin+ EBRF (400mg/kg)	34.846 ± 0.668	13.843 ± 0.572**	17.815 ± 0.714**

Each value is mean ± SEM (n=6)

* Significantly different from normal control, p<0.05

** Significantly different from gentamicin control, p <0.05

Results of group II were compared with group I. The results of group III and IV were compared with group II. The values are shown in mean ±SEM. Data was analyzed by one way ANOVA followed by Tukey's test.

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