



Evaluation of Nephro-Protective and Antioxidant Potential of *Sterculia Foetida* Linn. Leaves in Gentamicin Induced Acute Renal Failure

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Abstract

Gentamicin is a aminoglycoside antibiotic and it is toxic to the kidney. It induces inflammation, vascular damage and cause death to the cells of tubular epithelium. Our investigation study is to evaluate the potential of *sterculia foetida* linn leaves on gentamicin induced nephrotoxicity. Studies covered the etiopathology of gentamicin induced toxicity and the effects of anti-fibrotic by MESF (Methonolic extract of *sterculia foetida*). In this experiment Rats were divided into five group's control, alone gentamicin, gentamicin + MESF low dose, gentamicin + MESF high dose, and standard drug. In this group gentamicin is administered 100mg/kg through i.p. MESF of low dose 200mg/kg and high dose of 400mg/kg are administered orally in a specific time. The kidneys were removed for analysis. Gentamicin transiently increased serum creatinine, urea, total protein and urine creatinine with abnormal rise and MESF prevents such rises. Quantitative analysis of histological lesions representing marked structural damage apoptosis in the gentamicin group, with the lesions being reduced by MESF treatment. MESF significantly reduced renal fibrosis compared with the gentamicin group. MESF provided protection against gentamicin-induced acute nephrotoxicity and subsequent fibrosis by reduced apoptosis and inflammation.

Keywords

Gentamicin, *sterculia foetida*, nephro-toxicity, anti-biotic, fibrosis.

INTRODUCTION:

Kidneys have sensitive tasks that they eliminate toxic substances from the body. Therefore, it is critical when kidneys stop functioning, some drugs eliminate from the body only through kidneys, and their dose is reduced when there is improper functioning of kidneys. Nephro-toxins cause damage to nephrons. Severe drugs and one such gentamicin cause nephro-

toxicity. Gentamicin uptake by OCT and results in damage of tubular cells. Nephrotoxicity results in several ways, along with general and local vascular effects, direct effect on renal tubules, tubular obstruction, and acute interstitial nephritis. Acute glomerulonephritis can also occur. Most drugs found to cause nephrotoxicity to exert toxic effects in many ways of pathophysiology. These include altered

intraglomerular hemodynamics, tubular cell toxicity, inflammation, crystal nephropathy, rhabdomyolysis, and thrombotic microangiopathy. Knowledge of offending drugs and their particular pathogenic mechanisms of renal injury is critical to recognizing and preventing drug-induced renal impairment.

MATERIALS AND METHODS:

Materials:

List of equipment's:

Auto analyzer, (Qualisystems AR-601, Glaxo Smithkline Pharmaceutical Ltd, Mumbai), Micro centrifuge, Homogenizer (Remi motors, Mumbai). Digital pH meter (Microprolabmete) UV/Vis Spectrophotometer (Lab India UV-3000) Electronic balance Metabolic cages.

List of chemicals:

DTNB, Gentamicin, Formalin, Hydroxylamine, Nitro blue tetrazolium, Hydrogen peroxide, Petroleum ether, Estimation kits (Erba and Swan), Methanol, Sodium chloride, Sodium hydroxide, Sodium metabisulphate, Sulphosalicylic acid, Thiobarbituric acid. Hydrogen peroxide, Potassium dihydrogen ortho phosphate, $\text{Na}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ($\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$), $\text{Na}_2\text{H}_2\text{PO}_4$, Himedia Labs Pvt. Ltd. Mumbai. Sodium carbonate (Na_2CO_3), Formaldehyde solution- S.D. Fine chemicals Pvt Ltd. Mumbai.

Collection of Plant material:

The leaves of *Sterculia foetida* were collected from Thirupathi forest in Andhra Pradesh, in India. The collected leaves were identified and authenticated by a qualified Botanist of Dr. Madhava chetty, Asst. Prof. in Sri Venkateswara University, Thirupathi, Andhra Pradesh, with plant voucher no: 1525

Method:

In this process leaves powder of *Sterculia foetida* 500gms soaked in 2.5 litres of methanolic solvent in a closed container. Leaving the mixture for seven days with occasional shaking/ stirring. The process is repeated from plant powder by Stirring. Process is

done once or twice with fresh solvent. At last the last residue is centrifuged or done it by mechanically to squeeze extract and filter the residue in a glass container, for evaporation under reduced pressure at 30°C to get a dried solid product and was stored in dry air tight bottles for the pharmacological studies. The portion of the extract which is non-soluble remains in the thimble and it was discarded.

DESCRIPTION OF THE METHOD

Selection of animal species

Young adult and healthy animals used in laboratory strains employed. Females are nulliparous and non-pregnant. Individual animal, f it's dosing, between 8- and 12-weeks age and its weight within $\pm 20\%$ of the average weight of previously any dosed animals.

Experimental design:

Methodology:

Thirty male Wister rats were assigned to for five groups, each group consists of 6 animals. Group I was the control group administered distilled water or saline solution, II group is gentamicin-treated, group III -gentamycin as well as plant extract low dose-treated group & IV group gentamicin- as well as plant extract of high dose-treated group V was the Standard as well as Gentamicin induced group.

Each group consisted of six rats. The II group gentamicin-treated received 100 mg/kg/day gentamicin by the intraperitoneal route, III Group given 100 mg/kg/d gentamicin i.p. and plant extract low dose for oral in eight days and IV group given 100 mg/kg/d gentamicin i.p. and high dose of plant extract, group V was received gentamycin 100 mg/kg. i.p and standard drugs for oral in eight days. Control group rats are just saline solution sterile is given in oral. On 8th day after dosing, blood samples collected through retro orbital puncture method at the end of 24 h. The serum rapidly separated and processed for serum determination; urine parameters and kidneys were isolated for antioxidants studies and histopathology.

The protective effects of Methanolic extract of *Sterculia foetida* leaf (MESF) in gentamicin induced nephrotoxicity in rats.

Table No: 1- Grouping of animals

Group	Treatment, dose and route of administration.	Schedule	Studied parameters
I	Normal (vehicle treated)	7 days	Kidney weight, Serum parameters: Urea, Creatinine, Total protein Urine parameters: Urine volume, sodium, Potassium, Creatinine.
II	Vehicle-Gentamicin (100mg/k.g.b.w) i.p.	7days	Kidney homogenate: Lipid peroxidation Reduced glutathione, Catalase
III	MESF (200mg/k.g.b.w) p.o. Gentamicin (100mg/k.g.b.w) i.p.	7days	Kidney homogenate: Lipid peroxidation, Reduced glutathione Catalase Histopathology
IV	MESF (400mg/k.g.b.w) p.o. Gentamicin (100mg/k.g.b.w) i.p.	7days	Kidney homogenate: Lipid peroxidation, Reduced glutathione Catalase
V	STD+Gentamicin (100mg/kg)	7days	Histopathology:

RESULTS:

The protective effects of methanolic extract of *Sterculia foetida* (MESF) in gentamicin induced nephrotoxicity in rats.

Table No: 2 The effects on kidney weights, urine creatinine, urine Na⁺, urine K⁺ in normal, gentamicin, MESF and vit. E, treated rats.

Gps	Treatment	Wt. of kidney (g)	Urine creatinine (mg/dl)	Urine Na (mEq/day)	Urine K (mEq/day)
I-N.C	Vehicle-2ml saline oral	0.54 ± 0.014	93±0.028	0.735±0.035	0.860±0.023
II-D.C	Gentamicin- 100mg/kg.i.p	0.838± 0.048*	221±0.048*	0.681±0.046	0.723±0.030***
III-L. D	Gentamicin 100mg/kg. i.p+MESF-200mg/kg.oral	0.562± 0.028**	179±0.045**	0.740±0.027	0.735± 0.039**
IV-H. D	Gentamicin 100mg/kg. i.p+MESF-400mg/kg.oral	0.553±0.022***	134±0.036***	0.753±0.021	0.766± 0.05*
V-STD	Gentamicin- 100mg/kg.i.p +Vitamine-E-250mg/kg	0.548±0.018	125±0.033	0.739±0.038	0.798 ±0.027

The values are shown in mean ±SEM Data was analyzed by one-way ANOVA followed by Tukey's test. Values of p (0.01<0.05), p(0.02<0.05), p(0.02<0.05),p(0.02<0.05)were considered as significant. Mean percentage ± SEM; n = 6 animals in each group.

Effect of saline oral, gentamicin 100mg/kg.i.p, MESF low dose 200mg/kg.oral, MESF high dose 400mg/kg.oral and Std drug of vit.E , oral on nephrotoxic rats kidney weights.

The protective effects of methanolic extract of *Sterculia foetida* (MESF) in gentamicin induced nephrotoxicity in rats.

Table No: 3: The effects on serum creatinine, serum urea, serum total protein in normal, gentamicin, Std and MESF treated rats.

Gps	Treatment	Urine volume (ml/day)	Serum creatinine (mg/dl)	Serum urea(mg/dl)	Serum total protein
I-N.C	Vehicle-2ml saline oral	2.85 $\pm$ 0.171	0.894 $\pm$ 0.020	44.50 $\pm$ 0.031	6.6 $\pm$ 0.026
I-D.C	Gentamicin- 100mg/kg.i.p	2.661 $\pm$ 0.131*	1.121 $\pm$ 0.046*	94.170 $\pm$ 0.17*	9.2 $\pm$ 0.048*
III-L.D	Gentamicin 100mg/kg. i.p+MESF-200mg/kg.oral	2.717 $\pm$ 0.045**	0.983 $\pm$ 0.034**	63.830 $\pm$ 0.060**	8.1 $\pm$ 0.036**
IV-H. D	Gentamicin 100mg/kg. i.p+MESF-400mg/kg.oral	2.750 $\pm$ 0.021***	0.931 $\pm$ 0.026***	57.17 $\pm$ 0.042***	7.3 $\pm$ 0.029***
V-STD	Gentamicin- 100mg/kg.i.p+Vitamine-E- 250mg/kg	2.80 $\pm$ 0.016	0.912 $\pm$ 0.018**	48.31 $\pm$ 0.033	6.9 $\pm$ 0.028**

The values are shown in mean $\pm$ SEM Data was analyzed by one-way ANOVA followed by Tukey's test. Values of p(0.01<0.05), p(0.02<0.05), p(0.04<0.05), p(0.02<0.05)were considered as significant. Mean percentage $\pm$ SEM; n = 6 animals in each group.

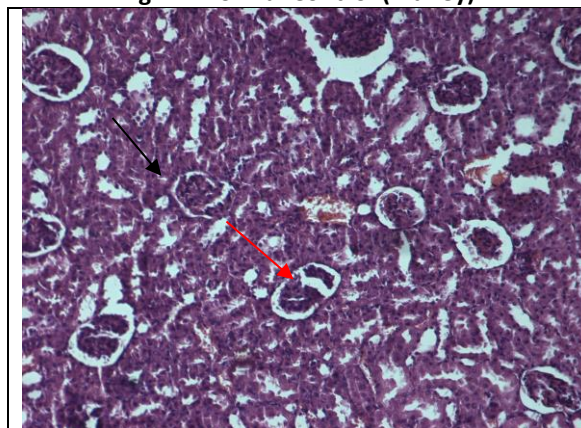
Table No: 4 the effect of LPO, GSH, Catalase in normal, gentamicin, msfl and std on gentamicin induced rats.

Gps	Treatment	LPO -(nmol/mg protein)	GSH-(nmol/mg proteinn)	Catalase - (Units/mg protein)
I-N.C	Vehicle-2ml saline oral	0.032 $\pm$ 0.0033	24 $\pm$ 0.05	0.49 $\pm$ 0.021
II-D.C	Gentamicin- (100mg/kg.i.p)	0.068 $\pm$ 0.0051****	8.8 $\pm$ 0.022	0.26 $\pm$ 0.02
III-L.D	Gentamicin (100mg/kg). i.p+MESF-(200mg/kg) oral	0.052 $\pm$ 0.0041**	14 $\pm$ 0.041***	0.32 $\pm$ 0.014**
IV-H.D	Gentamicin (100mg/kg. i.p)+MESF-(400mg/kg)oral	0.043 $\pm$ 0.035*	19 $\pm$ 0.048**	0.41 $\pm$ 0.019**
V-STD	Gentamicin- (100mg/kg.i.p)+Vitamine-E- (250mg/kg)oral	0.041 $\pm$ 0.033*	22 $\pm$ 0.0.049*	0.45 $\pm$ 0.021*

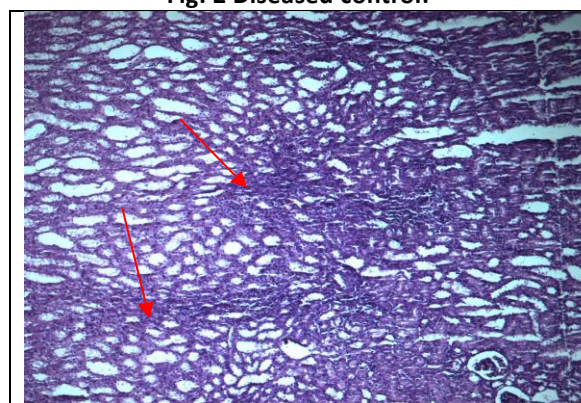
The values are shown in mean $\pm$ SEM Data was analysed by one-way ANOVA followed by Tukey's test. Values of p(0.03<0.05), p(0.02<0.05), p(0.01<0.05) were considered as significant. Mean percentage $\pm$ SEM; n = 6 animals in each group.

Fig:

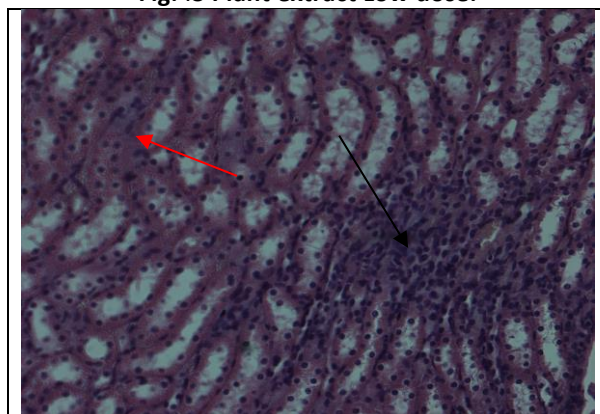
Effect of saline oral, gentamicin 100mg/kg.i.p, MESF low dose 200mg/kg.oral, MESF high dose 400mg/kg.oral and Std drug of vit.E , oral on nephrotoxic rats of Lpo levels.

Histopathology:
Fig: 1 - Normal Control (Kidney)


Glomerulus appeared normal – Red arrow
Tubular region appeared normal – Black arrow
No degeneration, necrosis/ inflammation noticed in kidney.
Entire cortex and medullary region appeared normal.

Fig: 2 Diseased control:


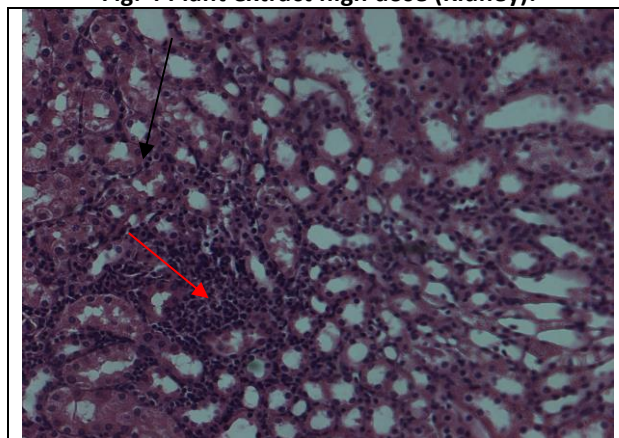
Multifocal, moderate tubular inflammation along with infiltration of inflammatory cells noticed in the tubular region of kidney – Red arrow
Moderate tubulonephritis

Fig: .3 Plant extract Low dose:


Multifocal moderate tubular degeneration noticed in the tubular region of kidney – Red arrow

Also multifocal moderate Tubulonephritis in which inflammation along with infiltration of inflammatory cells noticed in the tubular region of kidney. Black arrow

Fig: 4 Plant extract high dose (Kidney):

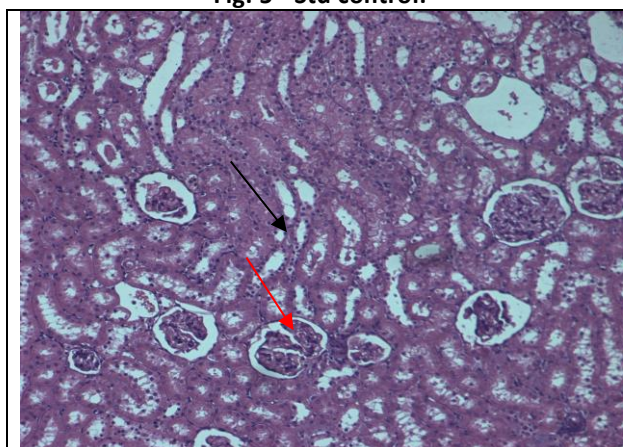


Mild tubular inflammation in which infiltration of inflammatory cells noticed in the tubular region of kidney. -Red arrow

Also interstitium between the tubular regions also showed inflammation with infiltration of inflammatory cells. – Red arrow

Above condition called as mild interstitial or tubular nephritis

Fig: 5 - Std control:



Glomerulus appeared as normal – Red arrow

Tubular and interstitial region of between tubules appeared normal – Black arrow

SUMMARY:

Gentamycin induced nephrotoxicity shows a similar pattern of injury to I/R, being primarily associated with pathological alterations of proximal tubular cells, & disruption of cell adhesions & apoptosis. In a model of gentamycin -induced renal cell injury, MESF protected rat proximal tubular epithelial cells against gentamycin -induced cytotoxicity via activation of the Epac-Rap signaling.

The preservation of the intercellular junctions and anti-apoptotic effects were both abrogated by silencing Epac-Rap signalling and were independent of protein kinase A. Therefore, activation of Epac-Rap signalling pathway has potential to protect against nephrotoxicity. These data identify Epac-Rap

signalling is cAMP dependent pathway & the activation of Epac-Rap signalling pathway represents a potential strategy for reducing nephrotoxicity associated with cisplatin treatment. ATP depletion is the central biochemical event during renal ischemic injury.

Sterculia foetida L leaf containing the anti-inflammatory compound, 2 flavonoids, phenylpropanoid glucose ester, glycosides.

This investigation of each step offers the possibility of identifying the preventive treatment of *sterculia foetida* plant high dose (400mg/kg) reducing the nephrotoxicity compared to gentamicin induced nephrotoxic rats.

CONCLUSION:

To sum it all up, the results of this study have shown methanolic leaf extract of *Sterculia foetida*, (MESF) present protection against the damaging renal side effects of gentamicin.

The organic cation transporters (OCTs) are involved in the uptake of gentamicin. The gentamycin induced tubular injury may be related to basolateral OCTs. This is evident as it is reported that an inhibitor of OCTs, like cimetidine could partially avert gentamicin-induced cytotoxicity and over expression of OCT2 in tubular cells leads to the increased nephrotoxicity because of increased uptake of gentamicin. The nephroprotective activity shown by MESF, they may be due to their possible role in the inhibition of OCT2, thereby prevents the uptake of gentamicin into the tubular cells. In the near future, the of *Sterculia foetida* could lead us to discover a new drug for preventing the drug-induced nephrotoxicity.

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