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Protective effect of ethanolic leaf extract of cleome gyanandra on gentamicin induced nephrotoxic rats

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Abstract
The aim of the study is to protective effect of ethanolic leaf extract of cleome gyanandra on gentamicin induced nephrotoxic rats. Nephrotoxicity is also known as toxic kidney disease. This is caused by aristolochic acid that is present in the body and causes injury or damage to the kidney. The model of gentamicin induced nephrotoxicity is considered as one of the most widely used experimental model to study the useful effects of many drugs and kidney function. The acute oral toxicity study was done according to OECD 423 guidelines. Wistar albino rats of either sex were selected randomly. The animals were fasted overnight, doses of 100, 250, 500, 1000, 2000, 3000 and 5000mg/kg body weight, were administered orally. The experimental animals were randomly divided in to 5 groups (n= 6). At the experiments: the pharmacological studies like acute toxicity studies, nephro protective activity like biochemical parameters in serum: i) renal biomarkers i.e., decrease of serum uric acid, urea, bun, creatinine levels, total serum proteins by increasing dose ii) non renal biomarkers i.e., higher dose is more effective in decreasing bilirubin, serum cholesterol, serum glucose level than lower dose. Bio-chemical parameters in urine like urea, uric acid, creatinine decreases as the dose increases. It seems that Cleome Gyanandra are able to improve kidney function against GM-induced nephrotoxicity. In this study it indicates that Cleome Gyanandra have shown a good nephroprotective activity by decreasing serum renal markers, serum non renal parameters and morphological parameters.

Keywords: Cleome gyanandra, gentamicin, nephrotoxicity

Introduction
Kidneys are responsible for the excretion of water and nitrogenous waste products produced from the body Kidneys maintain concentration of the blood and water balance in the body. The normal concentrating mechanism of kidney can increase concentration of toxins1. Drugs induced nephrotoxicity like aminoglyosides like gentamycin, antineoplastic agents like cisplatin, Antitumor antibiotics like Mitomycin, Antimicrobial agents like Tetracycline.Some examples of herbs used for nephrotoxicity is *Aerva lanata, Aerva Javanica, Acorus Calamus, Boerhaavia Diffusa, Crataeva Nurvula, Carica Papaya, Curcuma Longa.* The usage of herbs in nephrotoxicity is increased for being safe and alternative medicine [13, 14].
Multiple doses of gentamicin cause nephrotoxicity by inhibiting protein synthesis in renal cells [2]. This mechanism specifically causes necrosis of cells in the proximal tubule, resulting in acute tubular necrosis which can lead to acute renal failure [3].
Depending on the duration of drug exposure to animal's nephrotoxic logical studies may be done by urine or by serum samples parameters used are renal biomarker (uric acid, urea, creatinine, BUN, total proteins, albumins) and non-renal biomarkers (bilirubin, total cholesterol, total glucose). *Cleome gynandra* (Capparidaceae) is used as a medicinal plant and can be found in all over world. It grows as a weed in paddy fields and also in road sides and in open grass lands [14]. In India it is never cultivated but grows spontaneously everywhere [4]. This study aims to protective effect of ethanolic leaf extract of cleome gyanandra on gentamicin induced nephrotoxic rats.

Material and Methods
a) Plant collection and authentication

The whole leaves of *Cleome Gyanandra* were collected from gopalapuram forest, chitoor District, Andhra Pradesh.

b) Preparation of the extract

The whole leaves of *plant was* collected cleaned dried and powdered in a grinder –mixer to obtain a coarse powder and then passed through 40 mesh sieve. About 100 gm of powdered drug was extracted successively aqueous methanol and water by soxhlet apparatus. The dried extract thus obtained was kept in a desiccator and was used for further experiments [15].

Acute toxicity study

The acute oral toxicity study was done according to OECD 423 guidelines. Wistar albino rats of either sex were selected randomly. The animals were fasted overnight, doses of 100, 250, 500, 1000, 2000, 3000 and 5000mg/kg body weight, were administered orally. The animals were observed continuously for 2 hours, and then intermittently for 6 hours, the number of deaths was noted to determine LD50 of the extract [5].

Experimental Design

a) Nephroprotective activity

Albino rats (150-200gm) of either sex were used for the study. The experimental animals were randomly divided in to 5 groups (n=6) and treated with gentamicin for 8 days as per the treatment schedule. Plant extract of *Cleome Gyanandra* was administered to animals by oral feeding needle.

Group1 normal – vehicle (distilled water)

Group 2 control – Gentamicin (80mg/kg rat, I.P.) Group3 standard – EECG (500mg/kg, P.O.)

Group4 test 1(low dose) - Gentamicin (80mg/kg rat, i.p.) + EECG (250mg/kg, P.O.) Group5 test 2(high dose) - Gentamicin (80mg/kg rat, i.p.) + EECG (500mg/kg, P.O.) i.p = intra peritoneal, P.O = per oral, EECG = methanolic leaf extract of *Cleome Gyanandra.*

b) Collection of blood and urine samples

The blood samples were collected from the ret orbital venous plexus of rats without any coagulant for the separation of serum. After collecting the blood in effindraf tubes they were kept for 1 h at room temperature and serum was separated by centrifugation at 2000 rpm for 15 min and stored until analyzed for various biochemical parameters. Urine was collected over 24 hours on the 21st day by keeping the test animals in metabolic cages [6, 7].

The biochemical parameters in serum renal biomarkers are blood urea, creatinine, uric acid total protein, albumin, BUN non renal biomarkers are bilirubin, cholesterol glucose. Parameters in urine are uric acid, urea, and creatinine. Histopathologically studies used are body weight, kidney weight.

Statistical analysis

Results were analyzed using one way analysis of variance (ANOVA) followed by the Tukey's test by using statistical software package, Graph Pad Prism; version 5.3. Values were expressed as mean ± SEM and the $p < 0.05$ were considered as statistically significant.

Results

a) Acute Toxicity Studies

The plant extract of *Cleome Gyanandra* (EECG) was found to be safe since no animal died at the maximum single dose of 5000 mg/kg when administered orally and the animals did not show any gross behavioral changes. Hence 1/10th of this dose i.e. 500mg/kg were used as high dose and 250mg/kg were used as low dose in the subsequent study respectively.

b) Nephro protective activity biochemical parameters in serum

Renal Biomarkers

Table 1: Biochemical Parameters in Serum

Groups	Treatment	Uric Acid (Mg/Dl)	Urea (Mg/Dl)	Bun (Mg/Dl)	Creatinin E (Mg/Dl)	Total Protein (Mg/Dl)	Albumin (Mg/Dl)
1	Normal	1.42±0.88	29.67±0.33	13.86±0.15	95.21±0.38	49.0±0.05	3.03±0.08
2	Control Gentamicin (80mg/kg i.p)	2.27±0.10###	77.33±4.09###	33.38±1.30###	187.0±1.50###	###	###
3	Standard EECG (500mg/kg P.O).	1.24±0.02***	***	***	95.47±0.68***	5.40±0.11***	2.96±0.24***
4	Test-1 Gentamicin (80mg/k g i.p) +EECG (250mg/kg P.O)	1.53±0.16**	65.00±2.88*	27.77±0.84*	166.1±4.46**	3.23±0.14**	1.56±0.06*
5	Test-2 Gentamicin (80mg/k g i.p +EECG (500mg/kg P.O	1.40±0.10***	43.67±2.72***	18.19±1.13***	108.0±4.18***	***	***

All values are shown in mean ± SEM and n=6, ###, indicates $p < 0.001$, when compared with normal group, *** indicates $p < 0.001$, ** indicates $p < 0.01$, * indicates $p < 0.05$, when compared to control group.

Effects of renal biomarkers in serum

The effect of renal biomarker in serum shows that the treatment of EECG at a dose of 250mg/kg and 500mg/kg to treatment group of animals showed significance in decreasing the serum uric acid, urea, creatinine, bun, albumin, total protein levels when compared to control group of animal. This indicates that higher dose is more effective than the lower dose.

Non renal biomarkers

Table 2: Non renal biomarkers

Groups	Treatment	Total bilirubin (mg/dl)	Cholesterol (mg/dl)	Glucose (mg/dl)
1	Normal	0.610±0.015	36.67±0.88	125.3±1.33
2	Control Gentamicin(80mg/kg i.p)	###	###	###
3	Standard EECG (500mg/kg P.O).	***	***	***
4	Test-1 Gentamicin (80mg/kg i.p)+EECG (250mg/kg P.O)	*	**	***
5	Test-2 Gentamicin (80mg/kg i.p +EECG (500mg/kg P.O)	***	***	***

All values are shown in mean ± SEM and n=6, ###, indicates $p < 0.001$, when compared with normal group, *** indicates $p < 0.001$, **indicates $p < 0.01$, *indicates $p < 0.05$, when compared to control group.

Effects of non-renal biomarkers in serum

The effect of non-renal biomarker in serum shows that the treatment of EECG at a dose of 250mg/kg and 500mg/kg to treatment group of animals showed significance in decreasing the total bilirubin, cholesterol, glucose levels when compared to control group of animal. This indicates that higher dose is more effective than the lower dose.

Biochemical Parameters in Urine

Table 3: Biochemical Parameters

On 21ST day.				
Sr. No	**Treatment**	**Uric acid (mg/dl)**	**Urea (mg/dl)**	**Creatinine (mg/dl)**
1	Normal	1.76±0.14	45,33±1.76	29.67±0.33
2	Control Gentamicin(80mg/kg i.p)	3.77±0.13##	62.67±1.45###	76.67±0.33###
3	Standard EECG (500mg/kg P.O).	***	***	***
4	Test-1 Gentamicin(80mg/kg i.p)+EECG(250mg/kg P.O)	2.13±0.37**	52.00±1.52**	64.00±1.00**
5	Test-2 Gentamicin(80mg/kg i.p)+EECG(500mg/kg P.O)	***	***	***

All values are shown in mean ± SEM and n=6, ###, indicates $p < 0.001$, when compared with normal group, *** indicates p < 0.001, ** indicates $p < 0.01$, * indicates $p < 0.05$, when compared to control group

Effect of renal biomarkers in urine

The effect of renal biomarker in urine shows that the treatment of EECG at a dose of 250mg/kg and 500mg/kg to treatment group of animals showed significance in decreasing the serum uric acid, urea, creatinine, levels when compared to control group of animal. This indicates that higher dose is more effective than the lower dose.

Morphological Parameters

Effect of EECG on change in body weight

Table 4: Effect of EECG on change in body weight

Groups	**Treatment**	**% Body weight change (gms)**			
		1st Day	**7th Day**	**14th Day**	**21st Day**
I	Normal	0	7.8	9.3	11.0
II	Control GM (80mg/kg, I.P.)	0	-4.6	-15.5	-18.2
III	Standard EECG (500mg/kg, P.O.)	0	4.2	7.5	7.8
IV	Test-1 GM (80mg/kg, I.P.) + EECG (250mg/kg, P.O.)	0	-14.5	7.2	6.9
V	Test-2 GM (80mg/kg, i.p.) + EECG (500mg/kg, P.O.)	0	-15.6	10.0	12.2

All values are shown in mean and % body weight change, where n=6.

Effects of body weight

The body weight of control and test group were taken and percentage change in body weight is calculated. The weight of animal was slightly reduced in control treated group when compared with the normal group. So, the test 2 animal traded group shows significantly increased in body weight.

Effect of EECG on kidney weight

Table 5: Effect of EECG on kidney weight

Groups	**Treatment**	**21ST Day**
I	Normal	0.667±0.008
II	Control Gentamicin (80mg/kg rat, I.P.)	1.05±0.02###
III	Standard EECG (500mg/kg, P.O)	0.67±0.005***
IV	Test-1 Gentamicin (80mg/kg rat, i.p.)+ EECG (250 mg/kg, P.O)	0.967±0.008**
V	Test-2 Gentamicin (8mg/kg rat, i.p.) + EECG (500 mg/kg, P.O)	0.707±0.004***

All values are shown as mean ± SEM and n=6.
indicate $p < 0.001$ when compared to normal group
**indicate $p < 0.01$ when compared to control group.
*** indicate $p < 0.001$ when compared to control group.

Effect of kidney weight

The kidney weight of animal in control group is slight increase when compared to normal group of animals.

The animal of test 2 treated group that is higher dose of animal shows decrease of kidney weight when compared with control group.

Conclusion

In conclusion, the present study demonstrates that GM increases nephrotoxicity indices including plasma Creatinine and urea concentrations as well as renal tissue toxicity. It seems that *Cleome Gyanandra* are able to improve kidney function against GM-induced nephrotoxicity [8]. These beneficial effects of Cleome Gyanandra may work in a dose and time dependent style. Further investigations with different doses and time-courses are advised to elucidate Cleome Gyanandra action on GM induced nephrotoxicity.

The impairment in glomerular function was accompanied by an increase in blood urea [9]. The present study revealed that methanolic extracts of *Cleome Gyanandra* is a good source of phytochemicals with antioxidant properties [10]. The extracts also reversed the nephrotoxicity induced by gentamicin. This indicates that *Cleome Gyanandra* can be used as diaphoretic with Gentamicin. By this therapy, we can get the therapeutic benefit of the gentamicin without

botheration of its prominent side effect, nephrotoxicity. The phytoconstituents flavonoids, present in the extracts may be responsible for antioxidant activity [11, 12]. By the virtue of antioxidant activity, *Cleome Gyanandra* might have exhibited nephroprotective activity.

Hence the results obtained in this study indicates that *Cleome Gyanandra* have shown a good nephroprotective activity by decreasing serum renal markers, serum non renal parameters and morphological parameters. Histopathology of kidney also supported the protective effects of *Cleome Gyanandra*.

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