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EVALUATION OF NEPHROPROTECTIVE POTENTIALS OF AQUEOUS EXTRACT OF *Trigonella foenum-greacum* Linn. AGAINST GENTAMICIN INDUCED RENAL DAMAGE IN ALBINO RATS

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ABSTRACT

Nephrotoxicity is considered to be one of the most important clinical ailments of the human society. The existing line of treatments has more side effects and caters less to the requirement of the poor people. Need of the hour is to explore an alternate therapeutic intervention that is safe & cost effective. Drugs rather than synthetic substance but of natural source are found to be more secure. In the present study, attempts were made to evaluate the Nephroprotective potential of aqueous extract of plant *Trigonella foenum-graecum* Linn. against gentamicin induced experimental renal damage. Wistar strains of albino rats were selected for the study and were divided into 6 groups. Group I served as normal control, Group II, III, IV & VI were induced with Gentamicin (80mg/kg bw intraperitoneally). Group II kept as Disease control, Group III & IV served as plant drug treated groups at the dose level of 150mg/kgbw, 300mg/kgbw and Group VI act as a standard drug control and Group V served as plant drug control. Various physical, biochemical and antioxidant parameters were studied using serum and kidney homogenate of experimental animals. Histopathological sectioning of kidney was also studied. From the data of the results obtained it was clearly evident that the group II animals showed symptoms of nephrotoxicity. The aqueous extract at the dose of 300 mg/kgbw showed potent nephroprotective activity which is comparable to that of standard drug treated group V.Hence, the results obtained in the present study, indicates that ***Trigonella foenum-graecum*** **Linn.** aqueous extract has the capability to treat nephrotoxicity induced by Gentamicin.

KEYWORDS: ***Trigonella foenum-graecum*** **Linn., Nephrotoxicity, Gentamicin, biochemical and antioxidant parameters.**

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INTRODUCTION

A number of environmental contaminants, chemicals and drugs including antibiotics dramatically alter the structure and function of various tissues and produce multiple adverse effects in the liver, kidney, heart and intestine[1]. Nephrotoxicity is a poisonous effect of substances such as toxic chemicals and medication on the kidneys. A number of antibiotics including penicillin, cephalosporins, tetracyclines, aminoglycosides and sulfonamides are potent nephrotoxins. Aminoglycoside nephrotoxicity is manifested functionally by decreased urine concentrating capacity, tubular proteinuria, lysosomal enzymuria, mild glucosuria, decreased ammonium excretion and lowering of glomerular filtration rate (GFR). Approximately 8% to 26% of patients who receive aminoglycosides for more than 7-10 days develop mild renal impairment[2]. Gentamicin is a very effective antibiotic in treating gram-negative bacterial infection in both humans and animals. Gentamicin induced nephrotoxicity is an experimental model of acute renal failure caused by oxidative stress generated through the induction of superoxide[3]. In recent decades, a resurgent interest has been observed in traditional plant treatment for nephroprotective activity. As plants often contain substantial amounts of antioxidants, it has been suggested that an antioxidant action may be an important property of plant medicines associated with nephroprotective activity[4]. Therefore, in the present study a common medicinal plant *Trigonella foenum-graecum* Linn. was selected and investigated for its nephroprotective effect on gentamicin induced nephrotoxicity in Wistar rats.

MATERIALS & METHODS

COLLECTION OF PLANT MATERIAL

Plant chosen for the present study is *Trigonella foenum-graecum* Linn. and was collected from the herbal garden of Srimad Andavan Arts and Science college, Trichy – 5, identified with the help of Flora of Presidency of Madras[5] and confirmed by comparing with the Herbarium specimen deposited at Royal Botanics Garden, Kew **(Voucher specimen number K001122691)**. The whole plant was shade dried and coarsely powered.

EXPERIMENTAL ANIMALS

Healthy adult wistar strain of albino rats of both sexes of two to three months old and weighing 150g-200g were obtained from Tamilnadu Veterinary and Animal Sciences University, Chennai. The animals were allowed to be acclimatized under laboratory conditions for a period of 5 days prior to the experiment. Rats were housed in standard polypropylene cages. Six rats were housed per cage, to provide them with sufficient space, and to avoid unnecessary morbidity and mortality. Animals were maintained under standard condition of 12: 12- hour's light/ dark cycle and at an ambient temperature at 23 ± 1°C, with 65 ± 5 % humidity. Animals were fed with standard rat chow pellet obtained from Sai Durga Foods and Feeds, Bangalore, India and water *ad libitum*. All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee (Approval No: 790/03/ac/CPCSEA).

EXPERIMENTAL DESIGN

A total of 36 rats were randomly divided into eight groups contains five animals each. Group I served as untreated control and received regular rat feed and drinking water. Group II served as disease control and received Gentamicin (80mg/kgbw/day IP) for 15 days. Group III, IV and VI also received Gentamicin as Group II animals and treated with the plant drug at the dose level of 150mg/kgbw, 300mg/kgbw and standard drug Norfloxacin at the dose of 200mg/kgbw for 15 days respectively. Group V was plant drug control and received plant drug at the dose of 300mg/kgbw throughout the experimental period. At the end of the experimental period, animals were sacrificed by cervical dislocation. Blood was collected and used for various biochemical estimations. The Kidneys were dissected out and washed in ice-cold saline. They were homogenized, in 0.1 M phosphate buffer, pH 7.4 and used for analyzing various biochemical parameters.

STATISTICAL ANALYSIS

All the results were expressed as mean ± S.E.M. The data were statistically analyzed by one – way analysis of variance (ANOVA) and P values <0.05 were considered significant.

EVALUATION OF BODY WEIGHT

The body weight of the experimental animals was measured by using the rough tabletop balance. The body weight of the animals were weighed before the induction of nephritis using Gentamicin and during the treatment with plant drug sources at an interval of 3 days till the end of the experimental period.

BIOCHEMICAL STUDIES

The Following biochemical parameters such as urea[6], uric acid[7], creatinine[8] and enzymatic, non-enzymatic antioxidant like LPO[9], GSH[10], SOD[11] and Catalase[12] has been studied to evaluate nephrotoxicity.

HISTOPATHOLOGICAL STUDIES

For histopathological study, the tissues were fixed in Bouin's fluid. The classical paraffin sectioning and haematoxylin eosin staining techniques were used for histopathological studies**.** The various steps involved in the preparation of tissues for histopathological studies were Fixation, Dehydration, Clearing, Impregnation, Embedding, Section cutting, Staining and Mounting[13].

RESULTS & DISCUSSION

Medicinal plants are used from time immemorial and were found to have more efficacy in treating various ailments including nephrotoxicity. This study has been carried out to scientifically validate one such drug source ***Trigonella foenum-graecum Linn***. The results obtained were presented in the sequel.

Table 1
Effect of Trigonella foenum-graecum Linn. aqueous extract on Body weight of Animals

S. No	Groups	Body Weight in gm	
		Initial	Final
1.	Group I	123 ± 4.47	124 ± 4.18
2.	Group II	244 ± 5.47	219 ± 14.31*
3.	Group III	141 ± 0.89	149 ± 4.18
4.	Group IV	178 ± 10.83	204 ± 5.47**
5.	Group V	125 ± 5.22	132 ± 5.70
6.	Group VI	154 ± 6.51	164 ± 5.47

Values were expressed as Mean± S.E.M (n=6)
P<0.05 Statistically significant when Group II compared with Group I
**** P<0.05 Statistically significant when Group IV compared with Group II***

Table 1 depicted changes in the body weight of the experimental animals. The body weight is reduced significantly in the group II animals due to the decreased water intake and food consumption when compared to the Group I normal control animals[14,15]. In contrast, the experimental animals that received plant extract at different dosages. Standard drug treatment also showed significant increase in body weight. It could be due to the alterations in nutrient absorption and metabolic utilization. Meanwhile, no significant alteration of body weight was recorded from the rats treated with the 300mg/kg plant extract only (Group VI).

Table 2
Effect of Trigonella foenum-graecum Linn. aqueous extract on Biochemical Parameters in Gentamicin induced nephrotoxicity

S. No	Groups	Total Protein (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)
1.	Group I	7.65 ± 0.016	1.33 ± 0.022	35.44 ± 0.027	3.44 ± 0.025
2.	Group II	3.86 ± 0.032*	4.76 ± 0.014*	98.73 ± 0.021*	6.78 ± 0.016*
3.	Group III	4.95 ± 0.011	2.96 ± 0.010	61.22 ± 0.019	5.13 ± 0.021
4.	Group IV	7.32 ± 0.017**	1.76 ± 0.012**	39.13 ± 0.016**	4.24 ± 0.019**
5.	Group V	7.41 ± 0.012	1.65 ± 0.018	36.42 ± 0.019	3.95 ± 0.014
6.	Group VI	7.43 ± 0.015	1.43 ± 0.017	35.87 ± 0.011	3.75 ± 0.025

Values were expressed as Mean ± S.E.M (n=6)
P<0.05 Statistically significant when Group II compared with Group I
**** P<0.05 Statistically significant when Group IV compared with Group II***

The renal dysfunction due to Gentamicin treatment was manifested by significant increase in serum creatinine, urea and uric acid levels as compared to the normal group of rats[16,17]. It was reported that treatment with Gentamicin produce nephrotoxicity and reduces renal functions by impairment in glomerular functions which was characterized by an increase in serum creatinine, urea and uric acid level. Tables 2 also show the efficacy of plant extract in reducing serum Creatinine, urea & uric acid level. From the results obtained it was clearly noted that the plant extract under study has the ability to protect the kidney from chemical toxins like gentamicin. The effect was found to be dose dependent manner. The plant extract treated group V animals showed no significant changes in the serum creatinine, urea and uric acid levels[18,19].

Table 3
Effect of Trigonella foenum-graecum Linn. aqueous extract on Antioxidant Analysis in Gentamicin induced nephrotoxicity

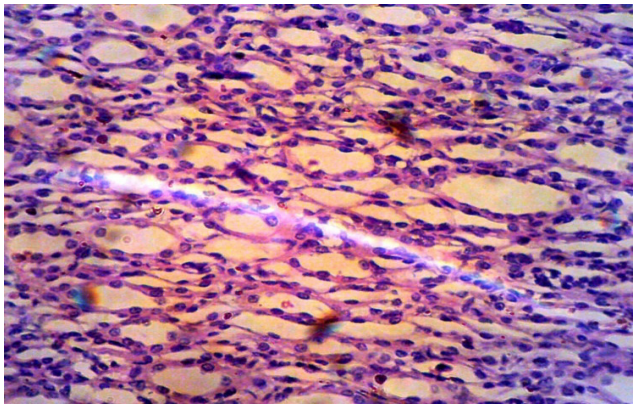
S. No	Groups	Lipid Peroxidation (nmole/g tissue)	Reduced Glutathione (µmol/g tissue)	Superoxide Dismutase (Unit/g tissue)	Catalase (µmole of H_2O_2/g tissue)
1.	Group I	12.23 ± 0.014	65.56 ± 0.015	22.14 ± 0.019	325.43 ± 0.02
2.	Group II	28.75 ± 0.010*	42.14 ± 0.010*	09.44 ± 0.023*	205.24 ± 0.023*
3.	Group III	22.18 ± 0.010	56.64 ± 0.025	14.23 ± 0.018	247.76 ± 0.016
4.	Group IV	15.24 ± 0.015**	62.36 ± 0.016**	21.13 ± 0.021**	317.44 ± 0.017**
5.	Group V	13.20 ± 0.019	64.46 ± 0.012	21.43 ± 0.014	323.26 ± 0.019
6.	Group VI	12.94 ± 0.007	64.92 ± 0.021	22.05 ± 0.010	323.92 ± 0.020

Values were expressed as Mean ± S.E.M (n=6)
P<0.05 Statistically significant when Group II compared with Group I
**** P<0.05 Statistically significant when Group IV compared with Group II***

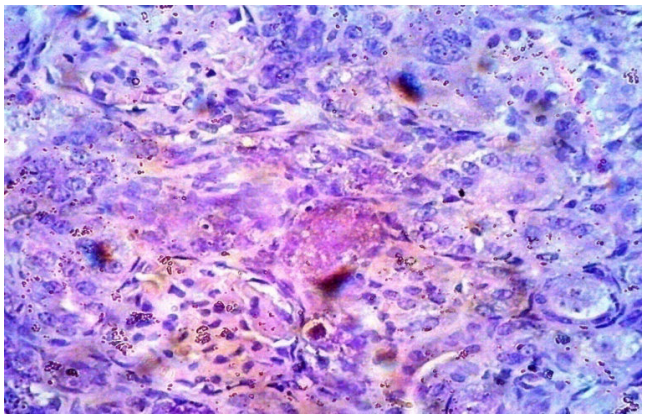
The aminoglycoside antibiotic Gentamicin elicits renal tubular toxicity and cell death through the formation of reactive oxygen species and causes proximal tubular necrosis and acute renal failure. It was found that Gentamicin administration increases the production of superoxide anions & thereby enhances the production of

H_2O_2 in renal cortical mitochondria. Superoxide anion and H_2O_2 may interact to form a reactive and unstable hydroxyl radical[20,21]. Gentamicin nephrotoxicity causes accumulation of oxygen radicals. Normal renal cells exhibit a defensive mechanism by using various antioxidant enzymes such as Superoxide dismutase and Catalase[22]. Reduced activity of one or more antioxidant systems, due to the direct toxic effect of gentamicin or depletion due to gentamicin administration, leads to an increase in lipid peroxidation. The decreased amount of intracellular glutathione and the accumulation of H_2O_2 and hydroxyl radicals are the triggering factors in Gentamicin nephrotoxicity[23]. Results of the present study have clearly substantiated the ability of Gentamicin to induce oxidative stress in rat kidney, as evidenced by the significant increase in lipid peroxidation and significant decline of endogenous antioxidants such as reduced Glutathione, superoxide dismutase and Catalase (Group II, Table 3)[17,24,25]. Plant extract chosen for the present investigation was found to have a profound ability to reduce the oxidative stress in rat kidney. Significant decrease in lipid peroxidation and significant rise in endogenous antioxidants status reduced Glutathione, superoxide dismutase and Catalase was observed to be in a dose dependent manner[26,27]. The histological changes in the kidney sections of different groups and the photomicrographs of kidney sections are presented. The histopathology of tissue sections suggested that the disease control group had encountered many histological damages (Group II, Figure 4 (B)). Inflammatory cells were also seen in kidney section of the Gentamicin treated animals. The infiltrated oedema of mononuclear cells was described in Gentamicin treated group. Hyaline changes, vacuolization and necrosis in the proximal tubular epithelial cells were also seen. Concurrent treatment with the plant aqueous extract were found to reduce such changes in the kidney histology induced by Gentamicin. However, aqueous extract of ***Trigonella foenum-graeum*** **Linn.** at a level of 300mg/kgbw showed almost normal glomerular and tubular arrangements with minimal blood vessel congestion, epithelial cell desquamation and presence of tubular cast with very few inflammatory cells.

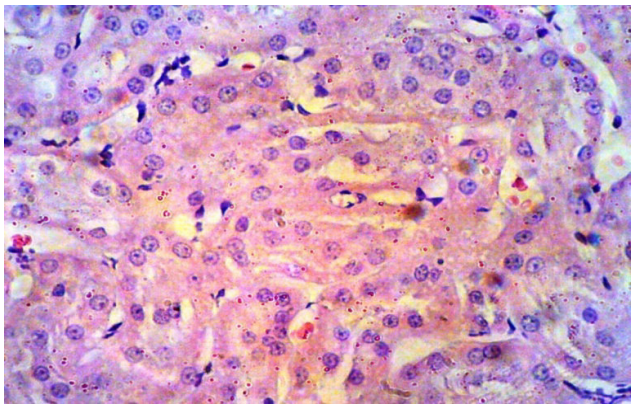
Figure 4
Histological study of kidney tissue in control and experimental groups of rats



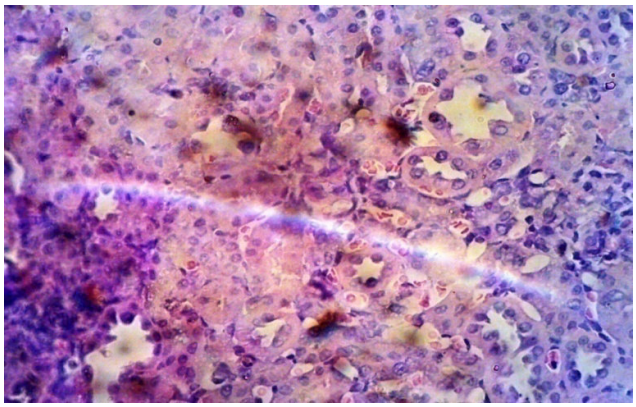
(A) Normal rats showing normal glomerulus and renal tubules



(B) Disease Control showing degeneration of glomerular cells and acute tubular necrosis



(C) Plant drug treated (300mg/kgbw) nephrotoxic rats showing regeneration of glomerular cells and tubules indicate recovery



(D) Standard drug treated nephrotoxic rats showing regeneration of glomerular cells and tubules indicate recovery

(A) Normal morphological view of renal sections in control group. (B) Histopathological view of renal sections in GM treated group showing degeneration, desquamation, necrosis in tubules, and swelling in glomerulus as compared to control group. (C) Animal treated with TFGL 300 mg/kg and slight tubular degenerative and necrotic changes (D) Animal treated with standard drug 200 mg/kg showed regeneration in tubular epithelial cells. The histological changes from the kidney sections of different groups and the photomicrographs of kidney sections are presented. The histopathology of tissue sections suggests that the disease control group had encountered many histological damages. Inflammatory cells were also seen in kidney section of the Gentamicin treated animals. Concurrent treatment with the plant aqueous extract was found to reduce such changes in kidney histology induced by Gentamicin. However, aqueous extract of ***Trigonella foenum-graeum*** **Linn.** at a level of 300mg/kgbw showed almost normal glomerular and tubular arrangements and presence of tubular cast with very few inflammatory cells.

CONCLUSION

Nephroprotective potentials of aqueous extract of ***Trigonella foenum-graecum*** **Linn.** was studied against gentamicin induced renal damage in wistar strains of albino rats. Data obtained from the study revealed that the gentamicin treated group showed significant changes in the level of biochemical markers such as creatinine, urea, uric acid and protein when compared to the normal control group of animals. But in the plant drug treated groups, levels of biochemical parameters were reverted back to near normal in a dose dependent manner. The antioxidant study also revealed that the group II animals showed marked changes which were reverted back to near normal in the plant drug treated groups and are also in dose dependent manner. From the study it was proved that the aqueous extract of ***Trigonella foenum-graecum*** **Linn.** at maximum dose level exhibits good protection against experimental renal damage and is comparable with that of the standard drug norfloxacin treated animals. Such scientific studies can promote international recognition and global acceptance for herbal drugs and contribute significantly to the healthcare of the human society.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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