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Protective Effect of *Tecoma stans* Flowers on Gentamicin-Induced Nephrotoxocity in Rats

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ABSTRACT

The present study was undertaken to evaluate the Ethanolic Extract of *Tecoma stans* (EETS) flowers for its protective effect on gentamicin-induced nephrotoxocity in rats. Nephrotoxocity was induced in rats by intraperitoneal administration of gentamicin 80 mg kg^{-1} day^{-1} for seven days. Effect of concurrent administration of EETS at a dose of 500 mg kg^{-1} day^{-1} given by intraperitoneal route was determined using serum and urinary creatinine and blood urea nitrogen as indicators of kidney damage. As nephrotoxocity of gentamicin is known to involve the induction of oxidative stress, so *in vitro* antioxidant activity, malondialdehyde (MDA) and glutathione (GSH) levels were also evaluated. Gentamicin-induced glomerular congestion and necrosis of kidney cells were found to be reduced in the group receiving EETS with gentamicin. Rats treated with gentamicin developed significant kidney dysfunction was observed from increased levels of urea, creatinine, sodium and decreased levels of protein, uric acid and potassium. *In vitro* studies revealed that the EETS posses significant antioxidant activity. The above results confirm that the flower extract acquire nephroprotective and regenerative capacity against gentamicin toxicity.

Key words: Nephroprotective, EETS, GC-MS, gentamicin, malondialdehyde, glutathione

INTRODUCTION

Nephrotoxocity is a major complication characterized by morphological destruction of intracellular organelles and cellular necrosis, followed by functional alterations including the depletion of the antioxidant defense system and mitochondrial damage (Ezejiofor *et al*., 2013). Gentamicin is an amino glycoside antibiotic that is still commonly used in the treatment of life-threatening infections. However, higher concentrations of these antibiotics are nephrotoxic. In some cases, it may give serious side effects are so severe that the use of the drug must be discontinued. Gentamicin generates free oxygen radicals, leading to tissue injury such as nephrotoxocity and ototoxicity (Rybak and Whitworth, 2005). Many plants have been used for the treatment of kidney failure in traditional system of medicine throughout the world (Kumar *et al*., 2013). Many research studies reported the protective effect of plant extracts on

gentamicin induced nephrotoxocity, which supports our current studies (Kanna *et al*., 2015; Manimala *et al*., 2015; Padmalochana and Rajan, 2015; Shalaby and Hammouda, 2014; Tavafi *et al*., 2012). *Tecoma stans* leaves, bark and roots contain many biologically active chemicals and extracts from those tissues have been used in traditional folk medicine to treat many diseases and conditions (Liogier, 1990). Perhaps the most promising compounds are monoterpine alkaloids which have been shown to effectively reduce the symptoms of diabetes mellitus in rats, dogs and mice (Aguilar *et al*., 1993; Lozoya-Meckes and Mellado-Campos, 1985; Hammouda and Amer, 1966). However, systematic and scientific reports on the investigation of *Tecoma stans* for its nephro protective activity is scarce. Therefore, the present study is an attempt to screen the *Tecoma stans* flowers for its nephroprotective activity. Gentamicin-induced nephrotoxocity is a model of acute renal failure caused by oxidative stress generated through the induction of superoxide anions (Maldonado *et al*., 2003). So, *in vitro* antioxidant activity of this extract has also been investigated.

MATERIALS AND METHODS

Chemicals: Thiobarbituric acid (TBA), 2,4, dinitrophenylhydrazine (DNPH), reduced glutathione and gentamicin were purchased from sigma Aldrich, Mumbai. All other chemicals and reagents used in this study were of analytical grade with high purity and were obtained from Glaxo Laboratories and Sisco Research laboratories, Mumbai, India.

Preparation of flower extract: *Tecoma stans* flowers were collected in the month of June-December, 2007 from Thanjavur district, Tamil Nadu and its authenticated by Dr. P. Jayaraman, Director Plant Anatomy Research Center (PARC), Tambaram, Chennai, India. A voucher specimen (No. PARC/2007/83) has been deposited in the herbarium of the department and this research project was conducted from 2014-2015. Flowers were dried under shade for 7 days. Dried flowers were subjected to grinding in Grinder to 20 mesh size and a homogenous yellow powder was obtained and stored in an air-conditioned room at 4°C before preparation of the extracts for analysis. The powder material was macerated with 70% ethanol at room temperature for 3 days. After 3 days, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45°C. A semi solid extract was obtained after complete elimination of alcohol. The residue was kept in the refrigerator for further use. The Ethanol Extract of *Tecoma stans* flowers (EETS) were prepared by dissolving known volume of distilled water just before the extract orally given to the rats.

Phytochemical analysis by GC-MS: The flowers of *Tecoma stans* were shade dried at room temperature. The dried material was then homogenized to obtain coarse powder and stored in airtight bottles for further analysis. Ten grams powdered flower material was soaked in 20 mL of ethanol overnight and then filtered through a Whatman No. 41 filter paper along with 2 g sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with methanol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and was concentrated to 1 mL. The extract contains both polar and non-polar phytocomponents. This ethanol extract is used for GC-MS analysis.

Programme:

Column	:	Elite-5MS (5% Diphenyl/95% Dimethyl poly siloxane), 30×0.25 mm×0.25 μm df
Equipment	:	GC Clarus 500 Perkin Elmer
Carrier gas	:	1 mL^{-1}, Split: 10:1
Detector	:	Mass detector Turbo mass gold-Perkin Elmer
Software	:	Turbomass 5.2
Sample injected	:	2 μL

Oven temperature programme: The 110°C-2 min hold, Up to 200°C at the rate of 10°C/min-No hold, Up to 280°C at the rate of 5°C/min-9 min hold, Injector temperature 250°C, total GC running time 36 min.

MS programme: Library used NIST Version-Year 2005, Inlet line temperature 200°C, Source temperature 200°C.

Electron energy	:	70 eV
Mass scan (m/z)	:	45-450
Solvent delay	:	0-2 min
Total MS running time	:	36 min

Experimental animals: Male albino rats of Wistar strain approximately weighing 125-150 g were used in this study. They were healthy animals from animal house, Annamalai University, Chidambaram. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2°C and 12 h light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The animal feed composition is crude protein (22.3%), crude oil (4.01%), crude fiber (4.02%), Ash (8.02%) and sand silica (1.02%).

Body weights of the animals were recorded and they were divided into 3 groups of 6 animals each as follows:

Group 1 : Normal control rats were fed with standard diet and received intraperitoneal injection of isotonic saline for 7 consecutive days

Group 2 : Rats were received daily i.p. injection of gentamicin (GM) (80 mg kg^{-1} b.wt.) for 7 consecutive days

Group 3 : Rats were treated with EETS (through intragastric tube) at the dose of 500 mg kg^{-1} b.wt., for every day in addition to injection of gentamicin for 7 consecutive days

Experiments were carried out in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) Guidelines and the study was approved by the Institutional Animal Ethical Committee (IAEC/CPCSEA/03/002/11).

Collection of samples: On completion of the experimental period, animals were anesthetized with thiopentone sodium (50 mg kg^{-1}). The blood was collected without EDTA as an anticoagulant, serum was separated by centrifugation. The kidney was excised immediately and immersed in physiological saline. The 10% homogenate was prepared by using phosphate buffer (pH 7.4).

Bio chemical estimation: Reduced glutathione (GSH) was estimated by the method of Moron *et al*. (1979). Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust (1978). Urea was estimated by the method of Natelson (1957). Serum sodium was estimated by colorimetric method of Maruna (1958) and Trinder (1951). Serum potassium was estimated by method of Sunderman and Sunderman (1958). Serum creatinine was carried out by alkaline picrate method of Bonsnes and Taussky (1945). Protein was estimated by the method of Lowry *et al*. (1951).

Evaluation of antioxidant activity

DPPH radical scavenging assay: DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity was determined by the method of Shimada *et al*. (1992).

Briefly, a 2 mL aliquot of the DPPH ethanol solution (25 mg mL^{-1}) was added to 0.5 mL sample solution at different concentrations (20, 40, 60, 80 and 100 μg mL^{-1}, respectively). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a spectrophotometer. The lower absorbance of the reaction mixture indicated higher free-radical scavenging activity. The scavenging activity of the sample was expressed as percentage:

$$\text{Radical scavenging activity (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

Histopathological examination: The kidney was excised and immediately fixed at 10% buffered formalin at the end of the experiment. The tissue specimen was embedded in paraffin after being dehydrated in alcohol and subsequently cleared with xylene. Five-micrometer thick serial histological sections were obtained from the paraffin blocks and stained with hematoxylin and eosin. The sections were examined under a light microscope to evaluate glomerular pathological changes and photomicrographs were taken.

Statistical analysis: The results were presented as Mean±SD. Data was statistically analyzed using student "t" test. The p-values set at lower than 0.001, 0.01 and 0.05 were considered as statistically significant. For the calculation of IC_{50}, linear regression analysis was done using Graph Pad prism statistical software.

RESULTS

The present study was carried out to evaluate the nephroprotective activity of EETS. The observations made on different groups of experimental and control animals were compared as follows.

Table 1 represents the levels of MDA and GSH in serum of normal and experimental rats. Group II gentamicin induced rats showed a significantly increased in the level of MDA when

Table 1: Effect of EETS on MDA and GSH in experimental rats

Parameters	Group I	Group II	Group III
MDA (nmol L^{-1})	2.13±0.92	6.64±2.01**	1.88±0.75*
GSH (mg dL^{-1})	3.96±0.35	2.04±0.24**	3.40±0.51*

Values were expressed as Mean±SD for six rats in each group, * Significantly different from Group II, ** Significantly different from Group I, MDA: Malondialdehyde, GSH: Glutathione, EETS, Ethanolic extract of *Tecoma stans*

Table 2: Effect of EETS on urea, creatinine, sodium and potassium in experimental rats

Parameters	Group I	Group II	Group III
Urea (mg dL^{-1})	41.33±2.19	78.33±4.51**	133.22±2.69*
Creatinine (mg dL^{-1})	0.47±0.01	1.84±0.03**	0.68±0.01*
Sodium (Meq dL^{-1})	92.76±4.01	172.58±15.53**	94.75±5.35*
Potassium (Meq dL^{-1})	8.85±0.8	4.00±0.43**	7.71±0.75*
Protein (g dL^{-1})	7.94±1.62	4.61±1.01**	7.22±1.40*
Uric acid (mg dL^{-1})	9.40±1.84	3.03±0.65**	6.16±1.36*

Values were expressed as Mean±SD for six rats in each group, * Significantly different from Group II, ** Significantly different from Group I, EETS: Ethanolic extract of *Tecoma stans*

compared to Group I rats. Group III gentamicin induced rats treated with EETS significantly decreased in the level of MDA when compared to group II. Group II gentamicin induced rats showed a significant decreased in the level of GSH when compared to Group I rats. Group III gentamicin induced rats treated with EETS significantly increased in the level of GSH as compared to group II.

Table 2 represents the levels of urea, creatinine, sodium and potassium in serum of normal and experimental rats. Group II gentamicin induced rats showed a significant increased in the level of urea when compared to group I rats. Group III gentamicin induced rats treated with EETS significantly decreased in the level of urea when compared to group II.

Group II gentamicin induced rats showed a significantly increased in the levels of creatinine and sodium when compared to group I rats. Group III gentamicin induced rats treated with EETS significantly decreased in the levels of creatinine and sodium as compared to group II. Group II gentamicin induced rats showed a significant decreased in the levels of potassium, protein and uric acid when compared to group I rats. Group III gentamicin induced rats treated with EETS significantly increased in the level of potassium, protein and uric acid as compared to group II.

Histopathological observation: In histopathological examination, normal architecture was observed in control animals (Fig. 1a) whereas renal lesions including marked tubular and focal area necrosis, inflammation and glomerular congestion changes in the kidney of gentamicin treated animals were observed (Fig. 1b). The lesions were reduced significantly in animals which received flower extract (500 mg kg^{-1}) to gentamicin treatment (Fig. 1c).

***In vitro* antioxidant activity**

DPPH method: IC_{50} values for the standard BHT was found to be 3.56 μg mL^{-1} where as IC_{50} values of ethanolic extract of flowers of the *Tecoma stans* was found to be 137.07 μg mL^{-1} (Table 3).

GC-MS analysis: GC-MS analyses indicated the presence of 14 phytocomponents (Table 4).

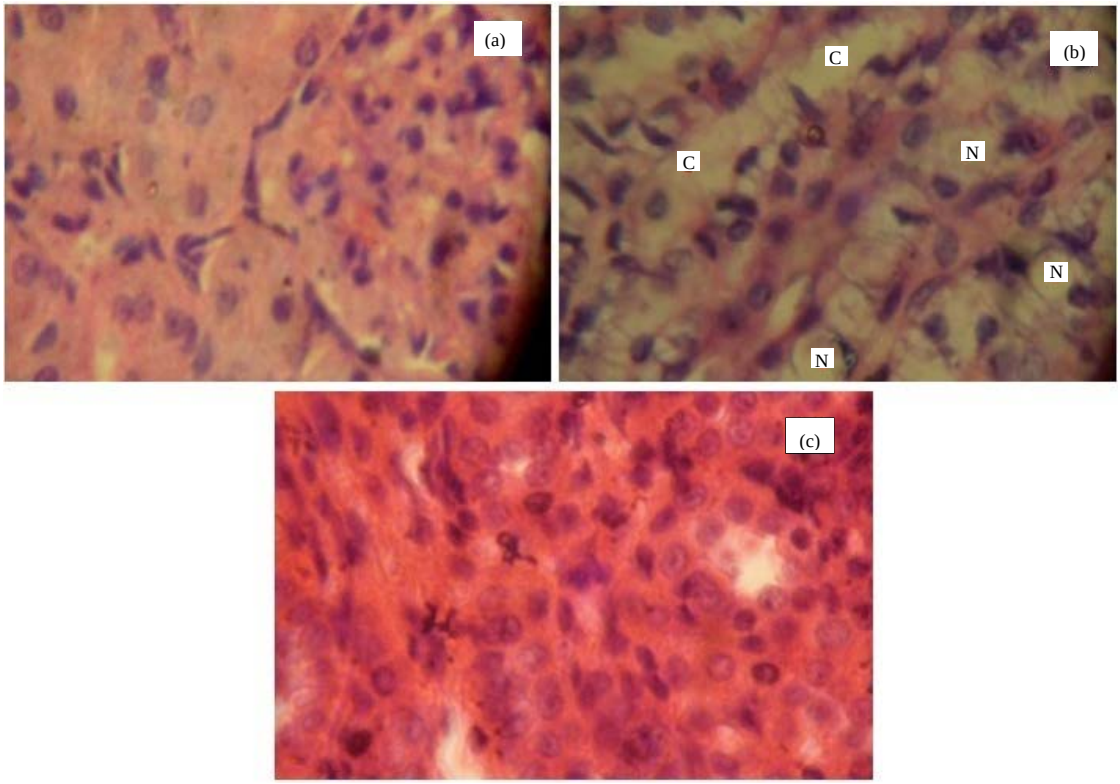

Fig. 1(a-c): (a) Kidney section of a control rat, (b) Representative images of kidneys of gentamicin treated rats with N: Necrosis and C: Congestion and (c) Tubular necrosis is less in flower extracts treated rats

Table 3: DPPH radical scavenging activity of *Tecoma stans* flower extract

Antioxidant activity	IC_{50} of standard BHT solution	IC_{50} of ethanolic extract of *Tecoma stans* flowers
DPPH free radical scavenging activity (μg mL^{-1})	3.56	137.06

Table 4: Phytocomponents identified in the ethanolic extract of *Tecoma stans* flowers by GC-MS

RT	Compounds	Molecular formula	MW	Peak area (%)
2.75	Propane,1,1,3-triethoxy	$C_9H_{20}O_3$	176	7.5
3.05	Thymine	$C_5H_6N_2O_2$	126	5.8
3.71	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144	5.3
4.57	1H-Azepoine, hexahydro-1-nitroso	$C_6H_{12}N_2O$	128	1.6
4.70	2-Furancarboxaldehyde, 5-(hydroxymethyl)	$C_6H_6O_3$	126	4.5
4.89	Hexyl n-valerate	$C_{11}H_{22}O_2$	186	3.4
6.11	Tetrahydrotecomine	$C_{11}H_{21}NO$	183	1.5
6.94	2-Cyclohexen-1-one, 2-methyl- 5-(1-methyle+hyl)	$C_7H_{10}O$	110	35.1
6.98	Furan, 2-(2-furanmethyl)-5-methyl	$C_{10}H_{10}O_2$	162	1.6
7.59	1,2,3,4,7,7*a*-Hexahydro-2,4,7-trimethyl-6H-2-pyridin-6-one (Synonyms: Tecomine)	$C_{11}H_{17}NO$	179	6.3
8.36	2,5-Furandione, dihydro-3-(2-methyl-2-propenyl)	$C_8H_{10}O_3$	154	4.5
8.64	Cyclobutanecarboxylic acid, decyl ester	$C_{15}H_{28}O_2$	240	4.1
10.01	Ethyl *α*-d-glucopyranoside	$C_8H_{16}O_6$	208	10.1
10.61	3-Deoxy-d-mannoic lactone	$C_6H_{10}O_5$	162	8.6
Total peak area (%)				99.99

MW: Molecular weight, RT: Retention time

DISCUSSION

Gentamicin (GM) is widely used aminoglycoside antibiotic, is recognized as possessing significant nephrotoxic potential in man and experimental animals (El Badwi *et al*., 2012). In this context a marked increase in the concentration of MDA was observed in gentamicin induced rats when compared to control rats. Administration of EETS significantly decreased the levels of MDA in gentamicin-induced rats. This view is supported by Barrouillet *et al*. (1999) studies. Glutathione status is a highly sensitive indicator of cell functionality and viability. The GSH depletion is linked to a number of disease states including cancer, neurodegenerative diseases, kidney and cardiovascular diseases. Kidneys are exposed to various cytotoxic agents before the elimination of these agents in urine. Thus the GSH concentrations in kidney cells are important (Pastore *et al*., 2003). In the present study, declined level of GSH was observed in gentamicin-induced rats when compared to control rats. The decrease in GSH level represents increased utilization for neutralizing free radicals generated from gentamicin. Supplementation of EETS to gentamicin induced rats, attained near normal level. The serum urea level is used as an index of kidney function (Stevens and Levey, 2005). Drugs that can increase urea levels include allopurinol, some diuretics, gentamicin and indometacin (Mason, 2004). In the present study also observed the increased level of urea in gentamicin intoxicated rats. Supplementation of EETS restored the increased level of urea in gentamicin-induced rats. The serum creatinine concentration is the most commonly used index of the kidney function. The level of creatinine is the blood rises if the kidney does not function properly (Stevens and Levey, 2005). Gentamicins have been reported to increase creatinine measurements (Mason, 2004). This result supports our findings. Administration of EETS restored the level of creatinine in gentamicin treated rats. The detection of proteinuria is necessary for the recognition of most kidney diseases (Cohen and Lemann Jr., 1991). In the present study, protein and uric acid were found to be decreased significantly in serum of gentamicin induced rats when compared to normal rats. The decreased level of protein and uric acid observed in gentamicin treated rats may be due to toxicity of free radical generated from gentamicin which damaged nephron and thereby loss of protein through urine. Administration of EETS normalized the level of protein and uric acid in serum of gentamicin treated rats. The imbalance of electrolytes can affects the homeostasis of the body. Gentamicin treated rats significantly lower in potassium levels and higher in sodium levels when compared with normal control rats. This due to antiport transport system of sodium and potassium i.e., the increased excretion of potassium is promoting the reabsorption of sodium (Cohen and Lemann Jr., 1991). Administration of EETS restored the normal level of sodium and potassium in gentamicin treated rats.

GC-MS analyses indicated the presence of 14 phytocomponents (Table 4) including 5-(hydroxymethy)-2-furancarboxaldehyde, tecomine, tetrahydrotecomine, that are probably involved in the antibacterial, antioxidant, antidiabetic and cytotoxicity activities (Al-Azzawi *et al*., 2012; Morales, 2008; Rigal and Gaset, 1983; Oskoueian *et al*., 2011). Nephroprotective activity of *Tecoma stans* flowers may be due to the presence of the phytocomponents found during GC-MS analysis. Moreover, the histopathology data has revealed that treatment with EETS has protected the renal tissues from necrosis induced by gentamicin. Thus, this study revealed that treatment with flower extract protected renal tissues against gentamicin induced nephrotoxicity.

CONCLUSION

Rats treated with gentamicin developed significant kidney dysfunction was observed from increased level of urea, creatinine, sodium and decreased levels of protein, uric acid and potassium. Supplementation of EETS to gentamicin intoxicated rats restored the altered above said

parameters. These findings can be further corroborated with histopathological studies. The above results confirm that flower extract acquire nephroprotective and regenerative capacity against gentamicin toxicity.

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