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## Floral extract of *Tecoma stans*: A potent inhibitor of gentamicin–induced nephrotoxicity *in vivo*

Raju S<sup>1\*</sup>, Kavimani S<sup>2</sup>, Uma Maheshwara rao V<sup>3</sup>, Sreeramulu Reddy K<sup>4</sup>, Vasanth Kumar G<sup>5</sup>

<sup>1</sup>Vijaya College of Pharmacy, Munaganoor, Ranga Reddy Dist, Andhra Pradesh, India–505511

<sup>2</sup>Mother Theresa Post Graduate Institute of Health Sciences, Gorimedu, Puducherry, India

<sup>3</sup>Nalla Narsimha Reddy College of Pharmacy, Korremula, Ranga Reddy Dist, Andhra Pradesh, India

<sup>4</sup>Assistant manager – Clinical R&D, Shantha Biotechnics Limited, Hyderabad, Andhra Pradesh, India –500004

<sup>5</sup>Health Management and Research Institute, Hyderabad, Andhra Pradesh, India

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### ABSTRACT

**Objective:** To highlight the nephroprotective activity of ethyl acetate extract of dried flowers of *Tecoma stans* for its protective effects on gentamicin–induced nephrotoxicity in albino rats.

**Methods:** For studying acute toxicity study, single oral dose of 5 000 mg ethyl acetate floral extract/kg body weight was administered to albino rats (five females, five males). Nephrotoxicity was induced in albino rats by intraperitoneal administration of gentamicin 80 mg/kg/day for eight days. Effect of concurrent administration of ethyl acetate floral extract of *Tecoma stans* at a dose of 100, 200 and 300 mg/kg/day given by oral route was determined using serum *creatinine*, serum uric acid, blood urea nitrogen and serum urea as indicators of kidney damage. The study groups contained six rats in each group. As nephrotoxicity of gentamicin is known to involve induction of oxidative stress, *in vitro* antioxidant activity and free radical–scavenging activity of this extract was also evaluated. **Results:** For acute toxicity testing both female and male rats administered with the extract at a dose of 5 000 mg/kg. The results showed no toxicity in terms of general behavior change, mortality, or change in gross appearance of internal organs (LD<sub>50</sub> > 5 000 mg/kg). It was observed that the ethyl acetate floral extract of *Tecoma stans* significantly protected rat kidneys from gentamicin–induced histopathological changes. Gentamicin–induced glomerular congestion, peritubular and blood vessel congestion, epithelial desquamation, accumulation of inflammatory cells and necrosis of the kidney cells were found to be reduced in the groups receiving the ethyl acetate floral extract of *Tecoma stans* along with gentamicin in a dose dependent manner. The floral extract also reduced the gentamicin–induced increase in serum creatinine, serum uric acid, blood urea nitrogen and serum urea levels ( $P > 0.01$ ). **Conclusions:** The present study indicates a very important role of reactive oxygen species (ROS) and the relation to renal dysfunction and point to the therapeutic potential of *Tecoma stans* in gentamicin induced nephrotoxicity.

### 1. 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<sup>[1]</sup>. The kidney is a common target for toxic xenobiotics, due to

its capacity to extract and concentrate toxic substances, and to its large blood flow share (about 20% of cardiac output). Experimental data suggest that both vascular, glomerular, and tubular targets are involved in drug–induced nephrotoxicity, as a result of mechanisms that disrupt normal cellular structures and functions (mitochondria, membrane integrity, etc.), induce renal injury through intratubular obstruction (crystal deposition), and promote cellular swelling and tubular luminal occlusion (through osmotic effects). Aminoglycoside antibiotics like gentamicin and streptomycin are the most commonly used antibiotics worldwide in the treatment of gram–negative bacterial

\*Corresponding author: Raju S, Assistant Professor, Vijaya College of Pharmacy, Munaganoor, Ranga Reddy Dist, Andhra Pradesh, India–505511.  
Tel: +919966164766  
E-mail: [rajenderreddysama@gmail.com](mailto:rajenderreddysama@gmail.com)

infections. However, aminoglycosides induce nephrotoxicity in 10%–20% of therapeutic courses. Aminoglycoside-induced nephrotoxicity is characterized by slow rises in serum creatinine, tubular necrosis and marked decreases in glomerular filtration rate and in the ultrafiltration coefficient[2]. Gentamicin, an aminoglycoside class of bactericidal antibiotic, in spite of inducing nephrotoxicity, is used clinically due to its wide spectrum of activities against gram-negative bacterial infections caused by *Pseudomonas*, *Proteus*, and *Serratia*. The gentamicin-induced nephrotoxicity occurs by selective accumulation of the drug in renal proximal convoluted tubules that leads to loss of its brush border integrity. The gentamicin-nephrotoxicity involves renal free radical generation, reduction in antioxidant defense mechanisms, acute tubular necrosis and glomerular congestion, resulting in diminished glomerular filtration rate and renal dysfunction. In past 2–3 decades, numerous pharmacological interventions have been demonstrated to prevent gentamicin induced nephrotoxicity [3]. Natural products and their active principles as sources for new drug discovery and treatment of diseases have attracted attention in recent years[4]. Antioxidants have been shown to ameliorate signs of gentamicin-induced nephrotoxicity[5]. Several approaches, utilizing different mechanisms, have been attempted to reduce the nephrotoxicity of GM and related aminoglycoside antibiotics[6]. Among these agents, extract of medicinal plants like garlic[7], have been reported to possess properties to ameliorate gentamicin-induced nephrotoxicity. A potential therapeutic approach to protect or reverse gentamicin-induced oxidative stress and nephrotoxicity would have more importance for clinical consequences[8]. However, systematic and scientific reports on the investigation of *Tecoma stans* (*T. stans*) for its effects on renal function are scarce. Therefore, the present study was designed to investigate the possible protective effects of ethyl acetate floral extract of *T. stans* against gentamicin-induced renal damage in rats.

## 2. Materials and methods

### 2.1. Preparation of flower extract

*T. stans* flowers (6.8 kg) were collected from the Munaganoor region of Ranga Reddy district, Andhra Pradesh in the month of August 2009 and its identity was confirmed by Department of Botany, Osmania University, Hyderabad. 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. 500 g of the powder was initially extracted with methanol by using Soxhlet apparatus for 8–10 h. The methanol was completely evaporated under vacuum to get dried extract (26.5 g). Then the fractionation was carried out using liquid-liquid extraction. Dried methanolic extract was dissolved in water

and partitioned with ethyl acetate by vigorously shaking the separating funnel for 2 h. Then the ethyl acetate extract was separated and completely evaporated at 40 °C using a vacuum evaporator (10.6 g) [9]. Oral suspensions containing 25 mg/mL of the ethyl acetate extract was prepared in 1% w/w gum acacia.

### 2.2. Chemicals

Assay kits for the estimation of serum creatinine, serum uric acid, blood urea nitrogen (BUN) and serum urea were purchased from Excel Diagnostics Ltd., Hyderabad, India. All other chemicals used were of analytical grade.

### 2.3. Preliminary phytochemical investigation

The preliminary phytochemical screening was carried out for qualitative identification of type of phytoconstituents present[10,11].

### 2.4. Experimental animals

Albino rats of either sex weighing between 170–220 g obtained from M/S. Mahaveer Enterprises, Hyderabad, India were used in the study. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at  $(25\pm 3)$  °C and 35–60% humidity). Standard pelletized feed (Gold Mohar, Lipton India) and tap water were provided ad libitum. The Institutional Animal Ethical Committee of Vijaya College of Pharmacy, Hyderabad, approved the study.

### 2.5. Acute toxicity study

#### 2.5.1. Experimental animals and administration

For determination of the dose of LD<sub>50</sub>, Seven weeks old, male and female albino rats, weighing within 200–220 g were used. Animals were randomly assigned to control and treatment groups (5 rats/sex group). Animals were deprived of food except water 16–18 hour prior to dosing on day 0. Then the extract at the dose of 5 000 mg/kg was given orally to test group of rats, while the control group received water in the same volume by gavage using a ball-tipped stainless steel feeding needle.

#### 2.5.2. Observation of toxicity signs

Body weight, signs of toxicity (general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes, and change in skin and fur) and mortality were observed after the administration at the first, second, fourth and sixth hour and once daily for next 14 days. On the 15th day, all rats were kept fasted overnight, and then anesthetized with thiopental sodium (50 mg/kg). Rats were sacrificed for necropsy examination. The internal organs were excised and weighed. The gross pathological observations of the tissues were performed by histopathological examination[12].

## 2.6. Evaluation of nephroprotective activity

Thirty male albino rats were randomly divided into five groups of six animals each. Group-I was kept as normal control receiving isotonic saline (0.5 mL, i.p.) for 8 consecutive days, and animals of groups II, III, IV and V were administered gentamicin, [(Gentaryl, Alkem Laboratories Ltd, India) 80 mg/kg/day, i.p.]] for 8 consecutive days, which is well known to produce significant nephrotoxicity in rats. Injections of gentamicin were made daily at 08:00 hours to minimize the circadian variation in nephrotoxicity. Groups I and II received vehicle (0.5 mL gum acacia 1% w/v, p.o.) for 8 days and group III, IV and V were orally administered with ethyl acetate extract of *T. stans* suspended in 1% w/v gum acacia at a dose of 100 mg/kg, 200 mg/kg/day and 300 mg/kg/day for 8 days.

Group-I: Kept as normal control receiving isotonic saline (0.5 mL, i.p.) for 8 days.

Group-II: Gentamicin-treated group, (80 mg/kg/day, i.p.) for 8 consecutive days.

Group-III: Gentamicin (80 mg/kg/day, i.p.) as well as ethyl acetate floral extract of *T. stans* at a dose of 100 mg/kg/day, p.o for 8 consecutive days.

Group-IV: Gentamicin (80 mg/kg/day, i.p.) as well as ethyl acetate floral extract of *T. stans* at a dose of 200 mg/kg/day, p.o for 8 consecutive days.

Group-V: Gentamicin (80 mg/kg/day, i.p.) as well as ethyl acetate floral extract of *T. stans* at a dose of 300 mg/kg/day, p.o for 8 consecutive days.

After dosing on the 8th day, the rats were anaesthetized with a combination of ketamine (60 mg/kg) and xylazine (5 mg/kg) given intraperitoneally. Blood samples were collected via retro-orbital puncture in plain plastic tubes, left to stand at 4 °C for 1 hour, and centrifuged (900 g for 15 min at 5 °C) to separate serum. The serum obtained was stored at -5 °C until analysis<sup>[13]</sup>. Then the serum was processed for determination of serum creatinine, serum uric acid, blood urea nitrogen (BUN) and serum urea using commercially available kits of Excel Diagnostics Ltd., Hyderabad, India<sup>[14]</sup>. Three rats per group were sacrificed and both kidneys were isolated from each rat. The kidneys were processed for histopathological examination.

## 2.7. Histopathological examination

The kidneys were sectioned longitudinally in two halves and were kept in 10% neutral formalin solution<sup>[15]</sup>. Both kidneys were processed and embedded in paraffin wax and sections were taken using a microtome. The sections were stained with hematoxylin and eosin and were observed under a computerized light microscope. They were evaluated for various histopathological features and assigned<sup>[16]</sup> scores as follows: Score 0=normal; 1= areas of focal granulovacuolar epithelial cell degeneration and granular debris in tubular lumens with or without evidence of tubular epithelial cell desquamation of small foci (< 1% of total tubule population); 2= tubular epithelial necrosis and desquamation easily seen but involving less than half of cortical tubules; 3= more than half of proximal tubules showing desquamation of necrosis

but involved tubules easily found; 4= complete or almost complete tubular necrosis.

## 2.8. Statistical analysis

The data obtained was analyzed using one-way ANOVA followed by Dunnett's multiple comparison tests. The  $P < 0.01$  was considered significant.

## 3. Results

### 3.1. Phytochemical investigation

Preliminary phytochemical studies revealed that the floral extract contains flavonoids, carbohydrates, saponins, tannins, glycosides, where as steroids, proteins and alkaloids were found to be absent.

### 3.2. Acute toxicity study

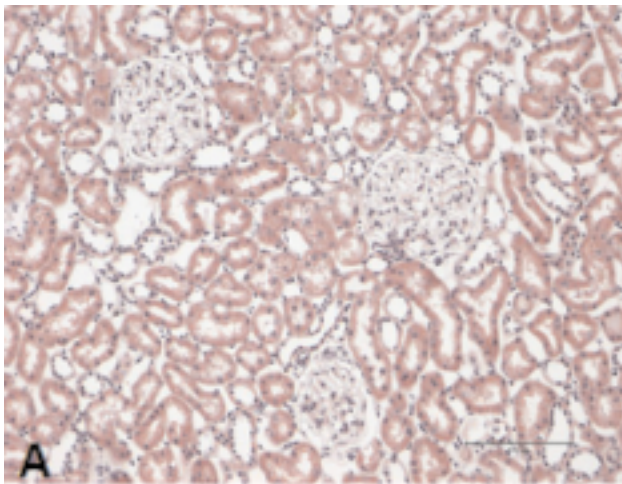
Both female and male rats administered with the ethyl acetate floral extract at a dose of 5 000 mg/kg did not show any toxicity during the experimentation period. In both sexes of rats, body weight gain of treatment rats was not changed significantly relative to that of control. The internal organs of treatment rats such as brain, lung, heart, liver, spleen, pancreas, adrenal gland, kidney, and sex organ showed no pathological abnormality relative to these organs of the control (data considered not necessary to be included). Thus, the ethyl acetate floral extract of *T. stans*, with an  $LD_{50} > 5\ 000$  mg/kg is considered to be non-toxic through acute exposure in rats.

### 3.3. Effect on biochemical parameters

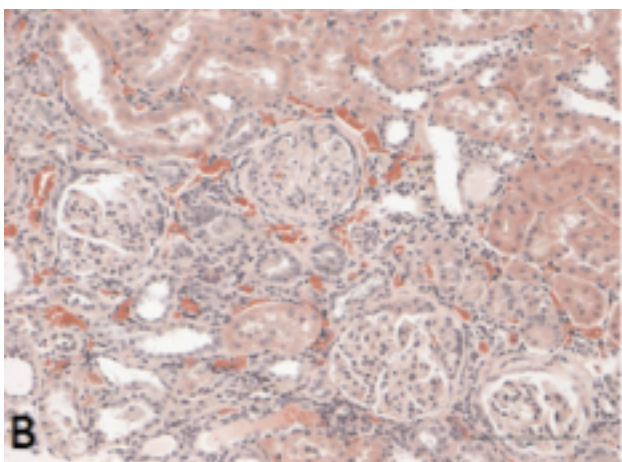
Administration of gentamicin, 80 mg/kg/day, i.p for 8 consecutive days, produced significant nephrotoxicity in rats. As shown in Table 1 serum levels of creatinine, uric acid, blood urea nitrogen and urea were significantly ( $P < 0.01$ ) higher in the gentamicin treated animals when compared with the control group [(0.85±0.03) mg/dL versus (3.22±0.12) mg/dL; (3.65±0.08) mg/dL versus (5.11±0.15) mg/dL; (17.84±0.23) mg/dL versus (46.53±0.35) mg/dL; (35.99±0.6) mg/dL versus (60.65±0.41) mg/dL respectively]. It is also evidenced by histopathological studies. Control rats showed normal glomeruli with an intact Bowman's capsule and proximal convoluted capsule (Figure 1). Rats treated with gentamicin, showed tubular epithelial loss with intense granular degeneration involving >50% renal cortex. In addition to the tubular epithelial loss, some of the tubular epithelium contains tubular casts and blood vessel congestion and result in the presence of inflammatory cells in kidney sections (Figure 2). Whereas co-treatment with the *T. stans* floral extract (TSFE) at a dose of 100, 200 and 300 mg/kg/day was found to significantly reduced the serum levels of creatinine, uric acid, blood urea nitrogen and urea when compared to gentamicin treated group.

### 3.4. Histopathology

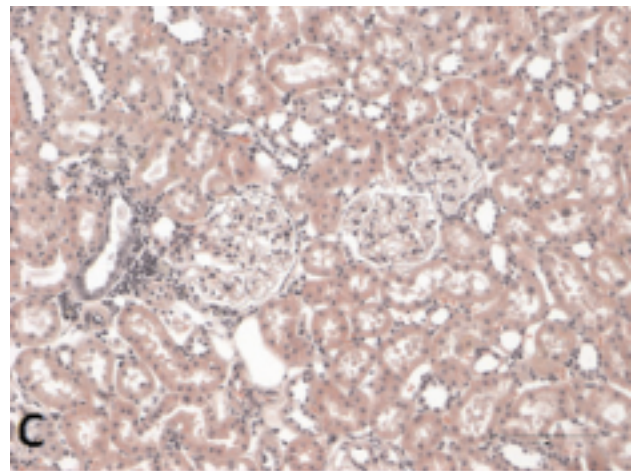
On the other hand, simultaneous administration of TSFE plus gentamicin protected kidney tissues against nephrotoxic effects of gentamicin as evidenced from amelioration of histopathological changes. (Figure 3–5). Rats treated with gentamicin, showed tubular epithelial loss with intense granular degeneration involving > 50% renal cortex. In addition to the tubular epithelial loss, some of the tubular epithelium contains tubular casts with complete or almost complete tubular necrosis. (Score 4+). The histomorphology of rats treated with TSFE 100 mg/kg, p.o. plus gentamicin showed moderate tubular epithelial degeneration with desquamation in patchy areas of the renal cortex (score 2+). Whereas, the histomorphology of rats treated with TSFE 200 and 300 mg/kg, p.o. plus gentamicin showed mild tubular epithelial degeneration with or without evidence of tubular epithelial cell desquamation of small foci (< 1% of total tubule population) respectively (score 1+).



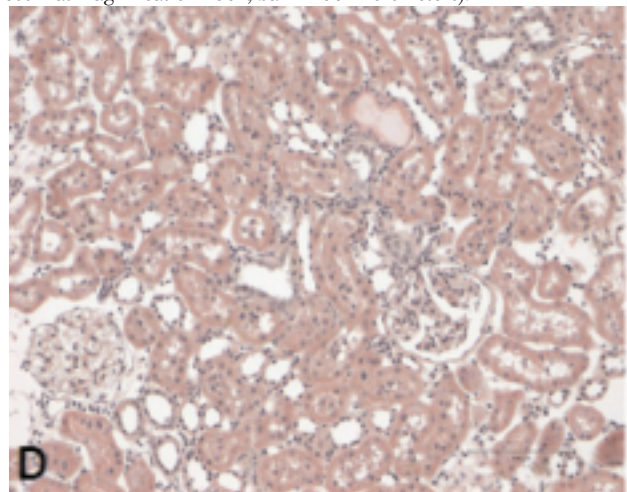
**Figure 1.** Kidney section of normal control rat with normal glomeruli an intact Bowman's capsule and proximal convoluted tubule and normal architecture (Haematoxylin and eosin at magnification 200x; bar = 100 micrometers).



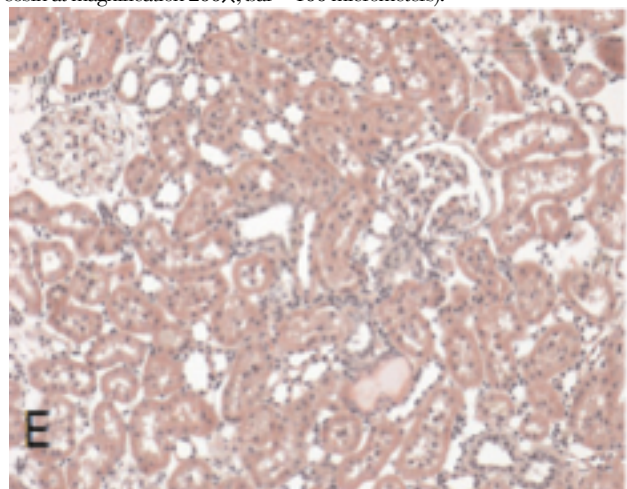
**Figure 2.** Kidney section of gentamicin treated rat demonstrating tubular epithelial damage with intense granular degeneration, glomerular congestion, inflammatory cells, complete tubular necrosis and tubular casts (Stain haematoxylin and eosin at magnification 200x; bar = 100 micrometers).



**Figure 3.** Kidney section of gentamicin + TSFE 100 mg/kg treated rat demonstrating moderate glomerular congestion, blood vessel congestion, interstitial edema and moderate tubular casts (Stain haematoxylin and eosin at magnification 200x; bar = 100 micrometers).



**Figure 4.** Kidney section of gentamicin + TSFE 200 mg/kg treated rat demonstrating normal arrangement of cells with mild glomerular congestion, blood vessel congestion and epithelial cell degeneration without evidence of tubular epithelial cell desquamation of small foci (Stain haematoxylin and eosin at magnification 200x; bar = 100 micrometers).



**Figure 5.** Kidney section of gentamicin + TSFE 300 mg/kg treated rat demonstrating normal arrangement of cells with mild blood vessel congestion, epithelial cell degeneration without evidence of tubular epithelial cell desquamation of small foci (Stain haematoxylin and eosin at magnification 200x; bar = 100 micrometers).

**Table 1**

Effect of floral extract on various biochemical parameters (mg/dL).

Group	Treatment	Serum creatinine	Serum uric acid	Blood urea nitrogen	Serum urea
Group –I	Normal saline	0.85±0.03	3.65±0.08	17.84±0.23	35.99±0.6
Group –II	Gentamicin 80 mg/kg. I.P	3.22±0.12**	5.11±0.15**	46.53±0.35**	60.65 ± 0.41**
Group – III	Gentamicin +TSFE 100 mg/kg. P.O	1.85±0.03**	4.30±0.04**	28.55± 0.49**	51.72 ± 0.50**
Group – IV	Gentamicin +TSFE 200 mg/kg. P.O	1.71±0.37**	3.89±0.05 ns	24.45±0.27**	46.16 ± 0.38**
Group –V	Gentamicin +TSFE 300 mg/kg. P.O	1.56±0.01**	3.66±0.04 ns	21.45±0.37**	41.67±0.48**

Values are mean ± SEM; n=6 in each group; \*\*significantly different at P&lt;0.01, ns=not significant.

**Table 2**

Histopathological features of the kidneys of rats of different treatment group.

Histopathological features	Ctrl	Gentamicin 80 mg/kg. I.P.	Gentamicin + TSFE 100 mg/kg	Gentamicin + TSFE 200 mg/kg	Gentamicin + TSFE 300 mg/kg
Glomerular congestion	–	+++	+++	+	+
Blood vessel congestion	–	++	+	+	–
Interstitial edema	–	++	+	–	–
Inflammatory cells	–	++	+	–	–
Necrosis	–	++	–	–	–
Tubular casts	–	+++	+	+	+

+: Presence, --: Absence

#### 4. Discussion

In this study, we hypothesized that ethyl acetate floral extract of *T. stans* would effectively protect kidneys by its antioxidant effect on gentamicin-induced injury. To the best of our knowledge, this is the first study to evaluate these effects of ethyl acetate floral extract of *T. stans* in an attempt to prevent kidney damage from gentamicin. Our results demonstrate that *T. stans* will be able to reduce the damage to the rat kidney induced by gentamicin. This was verified by both biochemical and histopathological observations. Results of this study confirmed that gentamicin at a dose of 80 mg/kg produces significant nephrotoxicity. The nephrotoxicity induced by gentamicin has been found to be a complex phenomenon characterized by an increase in serum creatinine, urea, uric acid and BUN levels and severe proximal renal tubular necrosis followed by renal failure[17]. *T. stans* treatment suppressed the gentamicin-induced increases in serum creatinine, urea, uric acid and BUN levels which are dose dependent. The reduction in glomerular filtration rate, which is indicated by the increase in serum creatinine level, would be accompanied by an increase in serum urea, uric acid and BUN levels when a marked renal parenchymal injury occurs[18]. A relationship between oxidative stress and nephrotoxicity has been well demonstrated in many experimental animal models. Administration of superoxide dismutase and vitamin E significantly reduced the nephrotoxic symptoms produced by Adriamycin[19, 20]. A huge body of experimental evidence has indicated that Reactive Oxygen Species (ROS) are involved in the pathogenesis of gentamicin nephrotoxicity. The impairment of renal mitochondrial antioxidant system by gentamicin intoxication supports the role of ROS in gentamicin-induced renal damage given the crucial role

of maintenance of mitochondrial antioxidant status in cell survival[21]. Gentamicin also activates phospholipases and alters the lysosomal membrane in addition to oxidative stress[22]. Hence natural and synthetic antioxidants and free radical scavengers are claimed to provide nephroprotection in gentamicin renal injury. Vitamin C, Vitamin E, selenium, etc. among the natural free radical scavengers and glycine, Probuco, etc[23–28], amongst the synthetic molecules, have been shown to possess partial protection against gentamicin-induced oxidative damages. The preliminary phytochemical investigation showed presence of flavonoids. The best-described property of almost every group of flavonoids is their capacity to act as antioxidants or free radical scavengers. Flavonoids can prevent injury caused by free radicals by their significant free radical scavenging activity[29].

Taking into account that flavonoids from other nephroprotective medicinal plants have been reported of inhibiting xenobiotic-induced nephrotoxicity in experimental animal models due to their potent antioxidant or free radicals scavenging effects, it is possible for this biological principle and other active principles contained in *T. stans* flowers to be responsible for the nephroprotective activity of the extract. The probable mechanism of nephroprotection by ethyl acetate floral extract of *T. stans* may be attributed to its antioxidant and free radical scavenging property which enhance renal mitochondrial antioxidant system[30], thereby protecting against gentamicin induced nephrotoxicity. To conclude, the *T. stans* ethyl acetate floral extract may have a promising role in the treatment of acute renal injury induced by nephrotoxins, especially gentamicin. Further isolation of active components and its nephroprotective activity in chronic renal failure are in progress.

## Conflict of interest statement

We declare that we have no conflict of interest.

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