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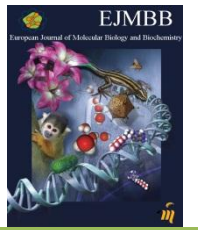

TRIANTHEMA DECANDRA (L.): A NEPHROPROTECTOR DURING GENTAMICIN-INDUCED NEPHROTOXICITY IN MALE WISTAR RATS

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ABSTRACT

The purpose of this study was to evaluate the nephroprotective effect of *Trianthema decandra* (L.) on gentamicin induced nephrotoxicity in male Wistar rats. Methods: Gentamicin (80 mg/kg, b.w., i.p) was administered for 8 days to induce toxicity in the toxic group. The ethanol extract of the roots of *Trianthema decandra* (L.) (150mg/kg and 300 mg/kg) was administered orally to treatment groups rats for 3 days before gentamicin treatment and thereafter with gentamicin for 8 days. Gentamicin alone treated rats shown significant increases in urea, creatinine, uric acid, thiobarbituric acid reactive substances, followed by a significant reduction in glutathione peroxidase, superoxide dismutase, catalase, and reduced glutathione in plasma, liver and kidney tissues. Further, gentamicin induced nephrotoxicity was evidenced through renal histological examination as tubular necrosis. *Trianthema decandra* (L.) pretreatment altered the antioxidant status and significantly reversed the levels to near normal. Histopathological changes were in accordance with the biochemical findings. The results show that the root extract of *Trianthema decandra* (L.) with the presence of natural antioxidants, bioflavanoids, and other bioactive compounds scavenged the free radicals generated by gentamicin and ameliorated the severity of gentamicin induced nephrotoxicity by enhancing the antioxidant system and protecting the cellular integrity of kidney tissues. In conclusion, the present study provides the corroborative scientific evidence for the folklore use of *Trianthema decandra* (L.) in urinary troubles.

INTRODUCTION

A lot of herbal remedies independently or in combination have been suggested in various medical treatises for the therapy of different diseases. *Trianthema decandra* L. (TDL) belonging to the Aizoaceae family, commonly known as gadabani (Hindi) and vellai sharuni (Tamil) is a prostrate herb distributed in the tropical and sub-tropical regions of the world, and also found abundantly in India [1].

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TDL has been used in different parts of Asia, Africa, Australia and South America as remedial for various diseases and ailments of human beings. In African countries the plant has been accepted use for skin diseases, wound healing, fever and tooth aches [2]. The juice of leaves is used to treat the black quarter. The leaves contain vast amount of vitamin C which is used in the treatment of oedema. A decoction of the herb is used as a vermifuge and is useful in rheumatitis. It is also an antidote to alcoholic poison [3]. The root of the plant is well known as an aperient and reported to be useful in hepatitis, asthma and in orchitis [4,5]. The bitter roots are used for curing bacterial infections and it is also given in combination with ginger as a cathartic [6]. The plant has diverse medicinal

properties and has been reported to have antimicrobial, hepatoprotective, analgesic, anti- inflammatory, anti-hyperglycaemic and antioxidant properties [7, 8]. One of the related species *Trianthema portulacastrum* L. has been reported to possess nephroprotective effect [9]. Though TDL is widely used in the traditional systems of medicine such as Ayurveda and Unani, siddha medicine for various disorders of eye, stomach indigestion, inflammation, convulsions [10] but the nephroprotective effect has not yet evaluated. A wide range of phytochemical compounds including terpenoid, alkaloid and flavanoids have been isolated from this species and but its nephroprotective mechanism against nephrotoxicity has never been explored.

Gentamicin, an amino-glycoside antibiotic, is used as an effective agent against Gram-negative infections [11]. Its chemical stability and rapid bactericidal action has made it a first-line drug in a variety of clinical situations. However, nephrotoxicity has always been a limiting factor in the therapeutic application of gentamicin. It imposes a ceiling on the total dose as well as the total length of the treatment and even necessitates substantial reduction in the dose in patients with compromised renal function. Studies have also shown that 30% of the patients treated with gentamicin for more than seven days show signs of nephrotoxicity [12]. Nephrotoxicity has been traced to be due to marked accumulation and retention of aminoglycosides in the proximal convoluted tubular cells [13].

There is a continuous search for agents which provide nephroprotection against the renal impairment caused by drugs like cisplatin and gentamicin. Thus, it is imperative that mankind turns towards alternative systems of medicine for treatment. Hence, the present study was an attempt to screen TDL root extract for its nephroprotective and curative activities to fill the lacuna in this regard and will provides the corroborative scientific evidence for the folklore use of *Trianthema decandra* L. in urinary troubles.

MATERIALS AND METHODS

Drugs and chemicals

Gentamicin was purchased from Sigma-Aldrich Chemical Pvt. Ltd. (St. Louis, MO, USA). All other chemicals used were of analytical grade, purchased from Hi-media Laboratories Pvt, Ltd., Mumbai, India.

Preparation of *Trianthema decandra* (L.) root extract (TDL)

Fresh roots of *Trianthema decandra* L. were collected in and around Thanjavur and were botanically identified. Voucher specimen of the plant was deposited for future reference. The roots were separated and washed with running tap water to remove any adherent impurities and shade dried. The dried roots were powdered and extracted with ethanol 90% as the solvent by cold maceration process. The extract was reduced to a molten mass by rotatory vacuum evaporator at 40°C.

Animals

Adult male Wistar rats weighing 150 g – 200 g were used in the study. The animals were housed in polypropylene cages and were provided with standard pellet diet (Amrut laboratory Mysore Feeds Limited, Bangalore, Karnataka, India) and water *ad libitum*. The animals were maintained under controlled conditions of temperature (23 $\pm$ 2ºC) and humidity (65-70%) with a 12 h light/dark cycle. The study was approved by Institutional Animal Ethical Committee (IAEC) constituted for the purpose of CPCSEA.

Experimental Protocol

The animals were divided into five equal groups randomly, each group comprising 6 rats. The treatment schedule was as follows:

Group 1 rats served as a control and were received carboxy methyl cellulose (2ml/kg b.w. *p.o*) orally for 11 days.

Group 2 rats were administered with gentamicin (80mg/kg/day *i.p.*) for eight days from 4th day onwards; this dose has already been shown to induce nephrotoxicity [14].

Group 3 and 4 rats were received gentamicin (80mg/kg/day *i.p.*) for eight days from 4th day onwards. In addition to this they also received 150mg/kg and 300mg/kg.*p.o.*, of TDL root extract in CMC respectively, which was started three days prior to the gentamicin injection and continued for 11 days.

Group 5 rats were received 300mg/kg TDL root extract in CMC orally throughout the experimental period.

Sample Collection

At the end of treatment, the rats were kept in individual metabolic cages for 24 hr urine collection. Blood samples were collected by retro – orbital puncture at the end of these 24 hours. The blood samples were taken in plain as well as heparinized bulbs for biochemical estimation. The kidneys were dissected out and processed for the histopathological study.

Biochemical Estimations

Markers of renal impairment

Estimation of urinary sodium and potassium was done by using Flame photometer. Blood urea was estimated by Berthlot method [15]. Creatinine clearance was calculated after estimating the serum and urinary creatinine. Uric acid in serum was estimated by the method of Brown [16]. The biochemical estimations were done using a semi auto analyser (Secomam, France).

Markers for oxidative stress

Portion of kidney tissues from control and experimental animals were washed with ice-cold saline and homogenised using appropriate buffer (TBARS, 0.025 M Tris-HCl buffer, pH 7.5; reduced glutathione [GSH] and glutathione peroxidase [GPx], 0.4 M phosphate buffer, pH-

7.0; superoxide dismutase [SOD], 0.025 M sodium pyrophosphate buffer, pH 8.3; catalase [CAT], 0.01 M phosphate buffer, pH 7.0) in an all-glass homogeniser with Teflon pestle and used for biochemical estimations. Lipid peroxidation was estimated as evidenced by the formation of thiobarbituric acid reactive substances (TBARS). Tissue lipid peroxidation was done by the method of Ohkawa et al., [17]. Superoxide dismutase activity in plasma and kidney tissue was assayed by the method of Kakkar et al., [18]. The activity of glutathione peroxidase (GPx) in plasma and kidney was determined using the method of Rotruck et al., [19]. The activity of catalase in plasma and kidney was assayed by the method of Sinha [20]. The reduced glutathione level in plasma and kidney was determined by the method of Beutler and Kelley [21].

Histopathological examination

Histopathological investigations were performed on kidney tissues of the control and experimental animals in each group. Tissues were fixed in 10% buffered formalin and routinely processed and embedded with paraffin; 2–3 mm sections were cut in a rotary microtome, fixed on glass slides, and stained with hematoxylin and eosin.

Statistical analysis

The data are expressed as mean ± S.D. Statistical comparisons were performed by one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test (DMRT) using SPSS version 12.0 for windows (SPSS Inc., Chicago, IL, USA; http:// www.spss.com). The results were considered statistically significant if the p values were 0.05 or less.

RESULTS

Evaluation of kidney function markers

The present study estimated the nephroprotective activity of TDL root extract against gentamicin induced nephrotoxicity in Wistar rats. Table 1 show that marked increase in blood urea, serum creatinine and uric acid in gentamicin alone treated group as compared to control. Pre administration of TDL root extract decreased the rise in blood urea, serum creatinine and uric acid to near normal (Group 3&4).

Table 2 shows the levels of creatinine clearance, urinary sodium and urinary potassium in control and experimental rats. The concentrations were significantly regulated in TDL root extract treated groups as compared to control group.

Estimation of TBARS and antioxidant activity

Tables 3 and 4 show the status of TBARS, enzymatic (SOD, CAT, GPX) and non-enzymatic (GSH) antioxidants in plasma and kidney of control and experimental rats in each group. An increase in TBARS and decrease in antioxidant activity were noticed in plasma and kidney tissue of rats treated with gentamicin alone (Group 2). Oral administration of TDL root extract at dose of 150mg/kg (Group 3) and 300mg/kg (Group 4) to gentamicin treated rats brought back the concentration of TBARS and antioxidants to near normal range. No significant difference in TBARS and antioxidant levels were noticed in rats treated with TDL root extract alone (Group 5) as compared to control rats (Group 1).

Histopathological observations

Kidney sections from control and TDL root extract alone treated rats showed normal glomerulus and tubules with regular morphology (Figure 1a and e). Histopathological findings of the gentamicin alone treated rats (Figure 1b) showed degenerating tubular structures with vacuolization, varying degree of necrosis and loss of tubular architecture. All the preventive and curative groups showed signs of recovery.

DISCUSSION

Gentamicin has still maintained a leading role among the aminoglycosides antibiotics in clinical practice because of their bactericidal efficacy against gram-negative bacterial infections, synergism with b-lactam agents, low cost and limited bacterial resistance. In recent report, about 30% of patients treated with gentamicin for more than 7 days show some signs of nephrotoxicity [22]. The serious complications resulting from gentamicin induced nephropathy are limiting factors for its clinical usage. Gentamicin demonstrates trough-concentration-dependant nephrotoxicity owing to elevated concentration of gentamicin reabsorption in proximal tubules of kidneys [23]. Various studies have revealed that gentamicin induces renal injury by high free radical production. Gentamicin in addition to oxidative stress also alters the lysosomal membranes and activates phospholipases in adding together with severe tissue damage [24]. Though ROS generated during normal cellular functions, are eliminated by intrinsic antioxidant enzyme systems like superoxide dismutase, catalase and glutathione peroxidase. Therefore, synthetic and natural antioxidants and free radical scavengers could be claimed to provide nephroprotection and have the capacity to partially reduce or eliminate the deleterious effects induced by gentamicin.

Natural antioxidants viz., quercetin, ascorbic acid and alpha-tocopherol have already been found as nephroprotective in experimental animal model [25]. Much of the work has been done on different parts of the plant *Trianthema decandra* (L.) in the field of pharmacognostic and phytochemical screening and the presence of secondary metabolites has been documented. Previously, its species *Trianthema portulacastrum* (L.) has been reported for its nephroprotective activity due to its free radical scavenging activity [8]. Although a lot of studies have been carried out on *Trianthema decandra* (L.) for its pharmacological properties, the study regarding its renoprotective effects was lacking and the present study is a positive step in this direction. Our group also established

the presence of flavonoids in TDL root extract [26] and Balamurugan and Muthusamy [27] reported the hepatoprotective activity of the ethanolic extract of TDL in previous studies. Hepatoprotective activity was also reported in the aqueous-ethanolic extract of TDL by Sengottuvelu *et al*., [28]. Hence, the probable mechanism of nephroprotection by *Trianthema decandra* (L.) may be attributed to its inherent antioxidant nature and free radical scavenging property.

Results from several studies have shown that gentamicin created an elevation in the concentrations of biochemical indicators of kidney function such as blood urea, serum creatinine and uric acid. Consistent with the data from the study of Lakshmi et al., [29] we observed in our study that creatinine clearance, urinary sodium and potassium were also altered after gentamicin injection signifying tubular dysfunction. On the other hand, blood urea, serum creatinine and uric acid levels were augmented representing glomerular damage. However, pre-treatment of TDL root extract to rats injected with gentamicin resulted in significant reduction in the elevated levels of blood urea, serum creatinine and uric acid. These results could be in accord with a number of other researches, which reported that, plant extracts with antioxidant properties such as *Aerva lenata*, *Trianthema portulacastrum*, *Bauhinia purpurea*, *Withania somnifera* and *Cardiospermum helicacabum* [29-32] inhibited the alterations induced by gentamicin in rats.

In the present study, the role of ROS in gentamicin-induced nephrotoxicity was assessed by the treatment of TDL root extract and further assessment of alterations in the biochemical indicators of oxidative stress mainly TBARS levels, GSH, GPx, SOD and CAT activities beside histological changes. It has been reported that, TDL exerts its antioxidant effects by scavenging free radicals and inhibiting lipid peroxidation. Glutathione (GSH) has a key role in protecting against oxygen free radical damage by providing reducing equivalents for numerous enzymes; GSH is also a scavenger of hydroxyl radicals and singlet oxygen. The enzyme GPx is selenium dependant enzyme and its main function is removal of H_2O_2 and it prevents formation of highly reactive hydroxyl (OH-) radical [33]. In the present study, the levels of GSH and GPx in rat kidney tissues were significantly reduced after gentamicin injection compared with control group. An explanation to plasma GSH depletion after gentamicin treatment is increased utilization of GSH in non-enzymatic removal of oxygen-radicals. GSSG is of great biological importance, since it allows fine-tuning for the cellular redox background under normal conditions and upon the onset of stress, and provides the basis for GSH stress signalling [34]. Pre-treatment with TDL root extract significantly increases the GSH level in renal tissues. As a result, under oxidative stress reaction, the levels of GSH are higher in TDL root extract treated group than in other groups which may due to recycling of GSSG back to GSH.

We have observed elevated lipid peroxide levels (TBARS) in the gentamicin treated group, consistent with prior studies mentioned. On the other hand, the activities of SOD and CAT enzymes were greatly reduced in gentamicin -treated rats compared with control group. Gentamicin causes vast changes in membrane lipid composition may be induced by free radical-initiated lipid peroxidation. This observation is supported by increased TBARS levels; one of the products of lipid peroxidation, in gentamicin treated rats kidney leads to tissue damage which results in acute renal failure. The scavenging of superoxide radicals is achieved through SOD, which catalyses the dismutation of superoxide to H_2O_2. Reduction in SOD and CAT activities after gentamicin injection in the present study has been corroborated with previous records, suggesting that oxidative stress is one of the causes of gentamicin-induced renal damage. Interestingly, the pre oral administration of TDL root extract to gentamicin treated rats reverted all of these alterations.

We have noticed tubular necrosis as a sign of irreversible injury in most sections of kidney tissue examined from gentamicin group. Biochemical results were concordant with pathological findings since TDL root extract acts as an antioxidant inhibits lipid peroxidation and prevents renal cell injury manifested as swelling of endothelium lining the glomerular tufts, tubular vacuolization, tubular degeneration and necrosis.

Table 1. Effect of gentamicin and TDL on serum creatinine, blood urea and uric acid of control and experimental rats in each group

Groups	Treatment	Serum Creatinine (mg/dl)	Blood Urea (mg/dl)	Uric acid (mg/dl)
I	None (control)	0.71 ± 0.04	25.3 ± 1.2	1.14 ± 0.02
II	Gentamicin (80 mg/kg, i.p.,)	2.54 ± 0.16$^{\#}$	86.0 ± 9.5$^{\#}$	4.88 ± 0.33$^{\#}$
III	Gentamicin (80 mg/kg, i.p.,) +TDL (150 mg/kg. *p.o*)	1.41 ± 0.05*	59.7 ± 6.4*	3.37 ± 0. 18*
IV	Gentamicin (80 mg/kg, i.p.,) +TDL (300 mg/kg. *p.o*)	0.87± 0.03**	32.5 ± 1.9**	1.77 ± 0.12**
V	TDL (300 mg/kg. *p.o*)	0.70 ± 0.04	24.7 ± 1.5	1.22 ± 0.06

Values are given as mean ± SD from six rats in each group. Values not sharing a common superscript differ significantly at $p<0.05$ (DMRT).

Table 2. Effect of gentamicin and TDL on renal parameters of control and experimental rats in each group

Groups	Treatment	Creatinine clearance (ml/min)	Urinary sodium (meq/day)	Urinary potassium (meq/day)
I	None (Control)	0.42 ± 0.02	0.86 ± 0.06	0.89 ± 0.06
II	Gentamicin (80 mg/kg, i.p.,)	0.063 ± 0.005$^{\#}$	0.53 ± 0.04$^{\#}$	0.59 ± 0.04$^{\#}$
III	Gentamicin (80 mg/kg, i.p.,) + TDL (150 mg/kg. *p.o*)	0.19 ± 0.01*	0.73 ± 0.05*	0.67 ± 0.05*
IV	Gentamicin (80 mg/kg, i.p.,) + TDL (300 mg/kg. *p.o*)	0.32 ± 0.02**	0.65 ± 0.04**	0.76 ± 0.04**
V	TDL (300 mg/kg. *p.o*)	0.43 ± 0.03	0.87 ± 0.06	0.90 ± 0.08

Values are given as mean ± SD from six rats in each group. Values not sharing a common superscript differ significantly at p<0.05 (DMRT).

Table 3. Status of TBARS, enzymatic and non-enzymatic antioxidants in plasma of control and experimental rats in each group

Groups	Treatment	TBARS (nmol/ml)	SOD (U^A/ml)	CAT (U^B/ml)	GSH (mg/dl)	GPX (U^C/L)
I	None (Control)	2.45 ± 0.15	6.42 ± 0.52	0.96 ± 0.07	29 ± 1.08	49.1 ± 3.6
II	Gentamicin (80 mg/kg, i.p.,)	3.89 ± 0.31$^{\#}$	3.06 ± 0.35$^{\#}$	0.43 ± 0.04$^{\#}$	15 ± 1.17$^{\#}$	19.8 ± 1.5$^{\#}$
III	Gentamicin(80 mg/kg,i.p.,) + TDL (150 mg/kg. *p.o*)	3.38 ± 0.29*	4.19 ± 0.48*	0.73 ± 0.03*	21 ± 1.43*	27.6 ± 2.6*
IV	Gentamicin (80 mg/kg, i.p.,) + TDL (300 mg/kg. *p.o*)	2.99 ± 0.28**	5.38 ± 0.56**	0.81 ± 0.08**	25 ± 1.54**	38.4 ± 4.4**
V	TDL (300 mg/kg. *p.o*)	2.43 ± 0.14	6.43 ± 0.64	0.97 ± 0.05	30 ± 1.48	48.2 ± 4.1

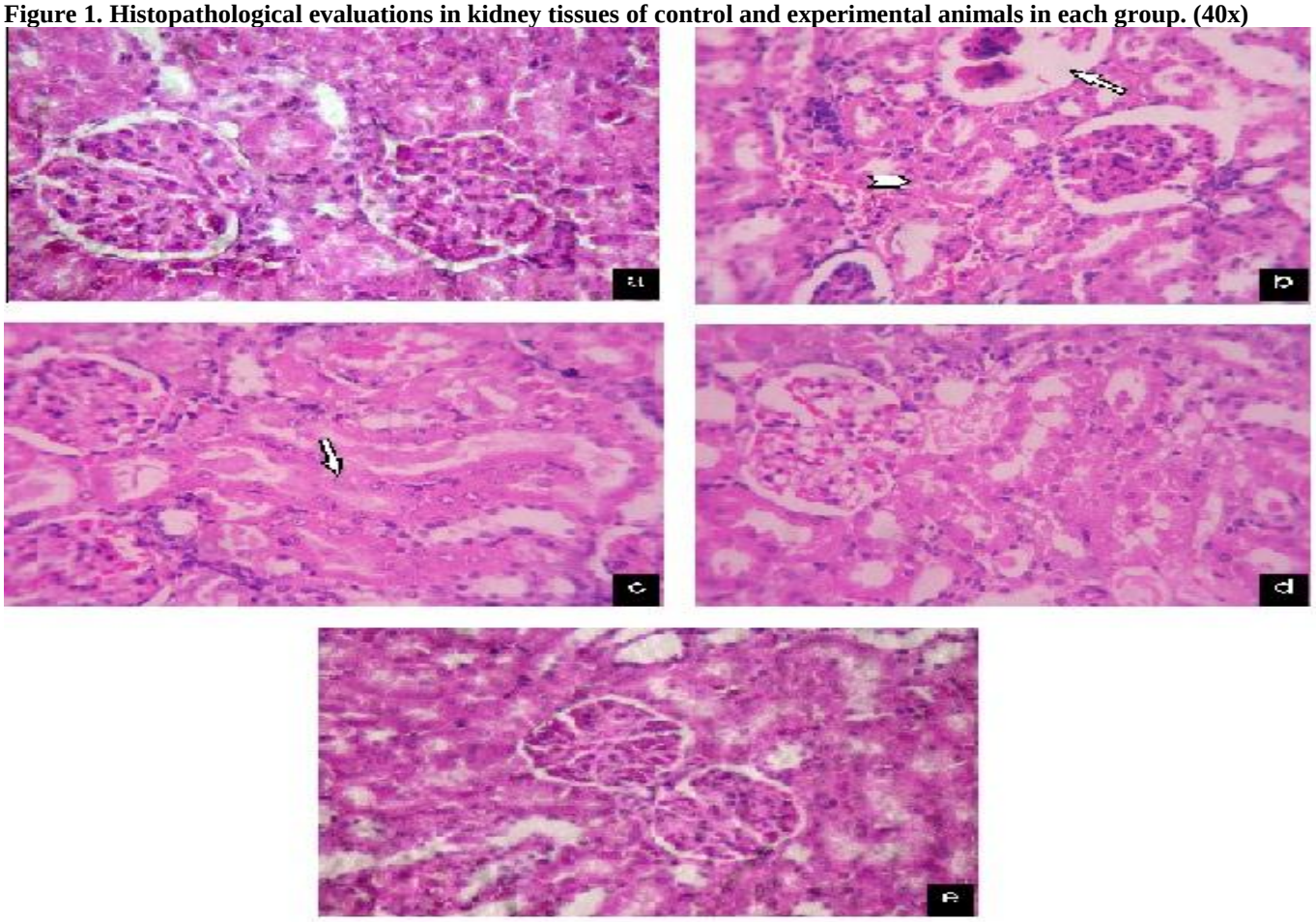
Values are given as mean ± SD from six rats in each group. Values not sharing a common superscript differ significantly at p<0.05 (DMRT). U^A - The amount of enzymes required to inhibit 50% NBT reduction; U^B - µm of H_2O_2 utilized per second; U^C - µm of glutathione utilized per minute.

Table 4. Status of TBARS, enzymatic and non-enzymatic antioxidants in kidney of control and experimental rats in each group

Groups	Treatment	TBARS (mmol/100g tissue)	SOD (U^A/mg protein)	CAT (U^B/mg protein)	GPX (U^C/g protein)	GSH (mg/100g tissues)
I	None (Control)	65.9 ± 3.35	6.42 ± 0.4	4.76 ± 0.3	8.9 ± 0.6	35.7± 1.96
II	Gentamicin (80 mg/kg, i.p.,)	96.2 ± 8.96$^{\#}$	3.06 ± 0.2$^{\#}$	1.53 ± 0.1$^{\#}$	5.5 ± 0.5$^{\#}$	21.2± 1.92$^{\#}$
III	Gentamicin (80 mg/kg, i.p.,) + TDL (150 mg/kg. *p.o*)	85.6 ± 5.79*	4.29 ± 0.3*	2.73 ± 0.3*	6.9 ± 0.6*	28.6± 1.88*
IV	Gentamicin (80 mg/kg, i.p.,) + TDL (300 mg/kg. *p.o*)	76.8 ± 5.28**	5.38 ± 0.4**	3.91 ± 0.4**	7.7 ± 0.7**	31.7± 2.43**
V	TDL (300 mg/kg. *p.o*)	66.1 ± 3.36	6.43 ± 0.4	4.77 ± 0.4	9.0 ± 0.8	36.5±2.25

Values are given as mean ± SD from six rats in each group. Values not sharing a common superscript differ significantly at p<0.05 (DMRT). U^A - The amount of enzyme required to inhibit 50% NBT reduction; U^B - µm of H_20_2 utilized / sec; U^C- µm of glutathione utilized / min.

Figure 1. Histopathological evaluations in kidney tissues of control and experimental animals in each group. (40x)



(a) & (e) Representative photomicrograph showing normal glomerulus and renal tubules in control and TDL alone treated rats. (b) Gentamicin alone treated animals showing glomerular retraction (arrow) and loss of tubular architecture (arrow head). (c) TDL root extract (150 mg/kg) and gentamicin treated animals showing mild tubular degeneration (arrow). (d) TDL root extract (300mg/kg) and gentamicin treated animals showing normal morphological view.

CONCLUSION

The present study revealed the nephrotoxic effects of gentamicin. Oxidative stress reactions and ROS may be one of the mechanisms of gentamicin-induced nephrotoxicity as indicated from alterations in oxidative stress biomarkers. The rectification of oxidative stress biomarkers by TDL was consistent with amelioration of the histopathological changes induced by gentamicin. The use of TDL during gentamicin treatment minimized its toxicity also revealed from restoration of renal function markers. The ameliorative effect of TDL against gentamicin-induced renal damage may be at least in part due to its antioxidant and free radicals scavenger properties of TDL due to the presence of flavonoids and phenolic compounds. Further isolation of active components and its nephroprotective activity in such situations have to be evaluated.

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