Research article

Co-administration with *Tinospora cordifolia* attenuates drug induced nephrotoxicity – A histological and biochemical assessment

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ABSTRACT

Introduction and Aim: Various herbs have been prescribed as a cure for renal disorders by early literature. Nephroprotective herbs are protective against nephrotoxicity. *Tinospora cordifolia* is known for its role in treating diabetes and disorders of the kidney and metabolism. However, studying its protective effect on drug induced nephrotoxicity at different time periods is wanting. The aim is to study the nephroprotective effect of *Tinospora cordifolia* on drug induced nephrotoxic changes upon co-administration of the herb with nephrotoxicity induction by the drug.

Materials and Methods: Ethanolic extract of the stem of *Tinospora cordifolia* was prepared and evaluated for phytochemical constituents. Gentamicin induced nephrotoxicity model in adult, male, Wistar rats was utilised for the study. Gentamicin and the extract of *Tinospora cordifolia* were co-administered for 8 days. In the kidney, levels of superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione and lipid peroxidation were measured. Assessment of the renal tissue was carried out using histopathological severity grading.

Results: Phytochemicals like glycosides, flavonoids, saponins, steroids, quinone and coumarin were present in the stem of *Tinospora cordifolia*. Analysing the levels of oxidative stress and antioxidants in the kidney revealed the rise in the level of reduced glutathione (GSH). This correlated with the regenerative changes brought about by the herbal extract on the acute tubular necrosis in the renal tissue induced due to gentamicin nephrotoxicity, at the histological levels in terms of the reduction of enlargement and regeneration of the tubular epithelium.

Conclusion: *Tinospora cordifolia* extract constituted flavonoids which are known antioxidants. Co-administration of *Tinospora cordifolia* stem extract is protective in the nephrotoxic condition induced by drugs, hence, shall benefit the kidney when taken along while consuming potentially nephrotoxic drugs.

Keywords: Phytochemicals; gentamicin; acute tubular necrosis; oxidative stress; glutathione.

INTRODUCTION

Twenty per cent (approximately) of community and hospital acquired episodes of acute renal failure are known to be caused by drugs (1) through various pathogenic mechanisms exerting toxic effects.

Tinospora cordifolia (Family: *Menispermaceae*), is found throughout tropical India and certain parts of China. It is a climbing shrub known by the names Amrita and Guduchika (Sanskrit), Giloy (Hindi) and Seenthil kodi (Tamil). The herbal drug consists of dried, mature pieces of stem of *Tinospora cordifolia*, preferably collected in the month of May (2, 3). Terpenoids, Alkaloids (2) and Glycosides (3) are the major constituents of the medicinal herb. This bitter drug is used in churna, sattva, taila, kapacurak kudinir formulations and employed in the therapy for Mekam (Diabetes), Jvara/ Kaayccal (Fever) and also as a diuretic (Ciruneerperukki) (2, 3). Experimental, clinical, phytochemical and bioefficiency are some of the research areas that it is being worked upon. No significant toxicity or side effect of the herb has been reported and is traditionally considered safe (4).

Phytochemicals such as steroids, phenols, terpenoids, alkaloids, glycosides, tannins are secondary metabolites synthesized and stored by the plants and the bioactive molecules responsible for pharmacological activity of a medicinal plant. The phytochemical screening gives the chemical nature of these molecules. General qualitative screening is based on a colour reaction or precipitation in response to a particular reagent. It helps to determine the extraction procedure to be adopted to test the specific chemical class of phytoconstituents (5). Reviewing models of drug induced kidney injury, Gentamicin induced drug nephrotoxicity model mimicked the condition in humans, however, requiring higher dosage to achieve the changes in animals (6) while not harming the duodenal mucosa, especially in rats.

Pre-treatment with curcumin, taurine, the extract of garlic, lycopene16, and melatonin which are known antioxidants have been found to avert the oxidative stress and renal damage due to gentamicin (7). Sharma *et al*., (8) reported that the aqueous extract of *T. cordifolia*, upon pre-treatment, facilitated the serum biomarker levels to return to normal with the acute tubular necrosis due to toxicity induction showing marked recovery. Earlier studies with methanolic (9)

and aqueous extracts of *T. cordifolia* have exhibited modulation of the antioxidant levels in alloxan induced diabetic rat models.

In the kidney, few sources of oxidative stress are the metabolically active cells, cells of the renal tubules, vascular cells, mitochondria and granulocytes. Normally, the Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) generated during the cellular processes in these cells are balanced by the antioxidant enzymes cascade within. A disturbance in the ROS and the defence mechanisms, dynamically balanced within the cells, indicate the disease. Cellular components such as amino acids, lipids in membranes, DNA, basement membranes are majorly targeted by the oxidant molecules causing injury and result in dysfunction of the organ and promote pathways of cell death (10) making it important to quantify the antioxidant enzymes.

Partially reduced metabolites of oxygen are reported to cause ischemic, toxic and immune-mediated injury of the tissues. Molecules modified by the reactive oxygen species/ metabolites, antioxidant enzymes and molecules and transcription factors are the three categories of oxidative stress markers. Sources of exogenous antioxidants such as dietary antioxidants and naturally occurring antioxidants in medicinal plants are therapeutics that are being explored for their effectiveness in kidney diseases in humans (10).

Histopathological grading semi-quantitatively estimates the severity based on the percentage of tissue involvement and the magnitude of the various components present constituting the extent of the condition and allows tissue analysis and group comparisons (12).

To firmly establish *T. cordifolia* as a nephroprotective drug to be used in the clinics, the lacunae existed in further identifying and characterizing the phytoconstituents from *T. cordifolia* and exploring the exact mechanism of its nephro-protectivity (8). This can be achieved by assessing *T. cordifolia* with its extracts using other solvents, dose levels, extended test durations and new biochemical parameters.

The aim is to study the nephroprotective effect of the extract of *Tinospora cordifolia* on drug induced nephrotoxic changes upon co-administration. The phytochemistry of the ethanolic extract of *Tinospora cordifolia* will be assessed. Its protective effect upon co-administration on nephrotoxic changes induced by the nephrotoxic drug, Gentamicin, will be studied by measuring the oxidative stress and antioxidant levels and histological analysis.

MATERIALS AND METHODS

Procurement and authentication of the herb

The herb was procured from K. Ramaswamy Chetty Herbs, Chennai and authenticated at Plant Anatomy Research Centre [PARC/2018/3638], Chennai, as *Tinospora cordifolia* (Willd.) Miers ex Hook.F.& Thoms. The preparation and assessment of the herbal extract was carried out in the Department of Pharmacognosy, Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Chennai.

Preparation of ethanolic extract

The stem of the *Tinospora cordifolia* plant was dried and coarsely ground. 1000g of the ground herb was macerated for 72 hours in absolute ethanol (3L) of analytical grade and filtered. The filtrate was subjected to Rotary vacuum evaporation at 65°C -70°C. The crude extract was further concentrated using a mantle heater and was kept in a desiccator. The qualitative phytochemical evaluation was carried out by standard methods (5,13).

Experimentation using laboratory animals

Ethical consideration

The experiment was carried out in the Centre for Toxicology and Developmental Research (CEFT) and Department of Anatomy with the approval of Institutional Animal Ethics Committee (IAEC) of SRIHER (DU) (Approval No: IAEC/61/SRIHER/ 700/2020).

Fifteen male Wistar adult rats weighing 200-300 g were housed in polypropylene cages covered with stainless steel grid tops. The cages were lined with autoclaved paddy husks renewed every alternate day with relative humidity (30-70%) in a 12 hour artificial light/dark cycle at 19-23ºC. The animals were provided with standard rodent pellet feed and water *ad libitum*.

Experimental design

After five days of acclimatization, the animals were divided into three groups: Group I: Control (n=3), Group II: Nephrotoxicity (n=6) and Group III: Co-administration (n=6). Group I was administered with the vehicle, edible Olive Oil (1ml/100g b.wt.) by oral gavage. Nephrotoxicity was induced in Groups II and III using Gentamicin [G] [Genticyn (Abbott) 80 mg/2 ml vials] through the intra-peritoneal route at the dosage of 100mg/Kg for 8 days. Immediately following nephrotoxicity induction, Group III received ethanolic extract of *Tinospora cordifolia* (ETc) at the dosage of (400 mg/Kg) suspended freshly in Olive Oil through the oral route. On the 9th day, the animals were euthanized using Carbon dioxide asphyxia. The kidneys were harvested, cleared of surrounding fat, weighed and rinsed in normal saline for the following purposes: Left kidney - assessing oxidative stress parameters and Right kidney - fixed in 10% Neutral Buffered Formalin for histological assessment.

Measurement of oxidative stress markers

Upon harvesting and weighment, the left kidneys were stored at -80 degree Celsius for further analysis.

Normal saline 0.9% was added and homogenized. The kidney homogenate samples were taken for analysing (12, 14) the following parameters: Antioxidant Enzymes - Superoxide dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), non-enzymatic antioxidant, reduced Glutathione (GSH), and metabolites of lipid peroxidation (LPO).

Table 1: Severity grading - histopathology

Grade	Features	Percentage
Normal		<1%
Mild	Cortical damage	1-20%
	Tubular cast	
	Sloughing of epithelium	
	Eosinophilic cytoplasm	1 – 10%
	Prominent nuclei and nucleolus	
	Inflammation	
Moderate	Cortical damage	21-50%
	Tubular cast	
	Sloughing of epithelium	
	Eosinophilic cytoplasm	10 – 19%
	Prominent nuclei and nucleolus	
	Inflammation	
Severe	Cortical damage	51-100%
	Tubular cast	
	Sloughing of epithelium	
	Eosinophilic cytoplasm	20 – 40%
	Prominent nuclei and nucleolus	
	Inflammation	

Histopathological grading

The Haematoxylin and Eosin-stained tissue sections were observed using Labomed Vision 2000 microscope and photographed using Cilika digital microscope (MedPrime Technologies). Histopathological scoring system was developed based on the initial observation of the tissue sections (11). The tissue was graded as Normal, Mild, Moderate and Severe as per Table 1.

Statistical analysis

SPSS software (version 16.0) was employed for the statistical analysis. One-way ANOVA was used to analyse the difference between groups followed by Post-hoc tests which brought out the pairwise differences (Multiple comparisons). The data is expressed as Mean ± SD and is significant at $p \leq 0.05$.

RESULTS

Phytochemical evaluation

A dark brown, semi-solid ethanolic extract was obtained. Phytochemical evaluation revealed the following (Table 2).

Table 2: Phytoconstituents in the ethanolic extract of *Tinospora cordifolia* (Miers)

S. No.	Phytoconstituents	Results
1	Saponin	Presence
2	Tannin	Absence
3	Terpenoids	Absence
4	Steroids	Presence
5	Glycoside	Presence
6	Cardiac Glycoside	Presence
7	Flavonoids	Presence
8	Alkaloids	Absence
9	Quinone	Presence
10	Anthraquinone	Absence
11	Phenol	Absence
12	Coumarin	Presence

Animal experiment

The experimental animals neither exhibited any clinical signs nor was there mortality throughout the experimental period.

Oxidative stress and antioxidant levels

The difference in the levels of SOD, CAT, GPx and LPO was insignificant among the experimental groups (Table 3). A slight reduction in the level of SOD is seen in group II. CAT level showed a decrease in groups II and III. GPx level increased in group III. An increase was found in the LPO levels in group II. A lesser increase was recorded in group III. Compared to groups I and II, the GSH level in group III increased significantly ($p \leq 0.05$); the difference was insignificant between the groups I and II.

Histomorphology changes

In Group I, the cortex and medulla were normal. The glomeruli appeared normal and urinary space was uniform. The renal tubules were compactly arranged and the interstitium was not prominent. Slight peritubular capillary congestion was observed (Figs.1A & 1D; Table 4).

Table 3: Effect of co-administration with ETc on G induced nephrotoxic changes in levels of oxidative stress and antioxidants

Antioxidant levels – Co-administration (Mean ± SD)			
Treatment duration/ Group	Control (I) (n=3)	Nephrotoxicity (II) (n=6)	Co-administration (III) (n=6)
SOD (unit/ mg/mt)	7.35 ± 1.94	6.33 ± 1.19	7.27 ± 0.42
CAT (mcm/mt/mg ptn)	80.44 ± 14.64	57.56 ± 25.89	53.42 ± 11.07
GPx (nm/mt/mg ptn)	6.15 ± 1.06	5.79 ± 1.87	8.05 ± 3.14
GSH (mcm/g tissue)	5.20 ± 0.84	4.98 ± 0.31	6.82 ± 0.99*#
LPO (nm/g tissue)	118.84 ± 43.28	196.28 ± 100.05	162.76 ± 60.16

*Significant at $p \leq 0.05$ when compared to control; #Significant at $p \leq 0.05$ when compared to nephrotoxicity

Table 4: Effect of ETc co-administration on nephrotoxicity induced by G on the histopathological severity

Group	Grade - Histopathology
I - Control	Normal
II - Nephrotoxicity	Severe
III - Co-administration	Severe

Group II recorded almost 80% damage in the cortex exhibiting Acute Tubular Necrosis (ATN) of the Proximal Convoluted tubules (PCT), hence, severely affected (Table 4). PCT were selectively affected and appeared ghost-like with a predominantly granular and glassy hyaline cast. The brush border of the epithelium of PCT showed degeneration and the disintegration of the cytoplasm is seen. Glomeruli showed enlargement and slightly widened urinary space. Peritubular capillary congestion was also seen in the medulla. Inflammatory changes were seen. Interstitium was not prominent. PCT located nearer to the corticomedullary junction showed regenerative changes; the cytoplasm in these PCT was more eosinophilic. These PCT cells also showed prominent nucleoli (Figs.1 B&E). Group III showed enlarged glomeruli and acute tubular necrosis of the PCT. The brush border of PCT and the cytoplasm were degenerated; the histopathology grade was severe (Table 4). The granular and hyaline casts were less in the PCT. 40% regenerative changes were seen despite 60% degenerative changes (Figs. 1 C & F). Few tubules showed flattened nuclei in flattened epithelial cells. Inflammatory changes were seen with no adverse changes in the vasculature.

DISCUSSION

A dark brown, semi-solid ethanolic extract was obtained. An alcoholic extract similar in colour and nature has been reported (15).

Phytochemical screening of the herb during drug development research is always preferable to overcome crucial factors such as soil quality, season, etc., which might affect its quality and contents. The qualitative phytochemical analysis of ETc confirmed the presence of flavonoids, glycosides, cardiac glycosides, quinone, saponin, steroids, coumarin (Table 2). The phytoconstituents present in ETc in the present study have been compared with earlier studies in Table 5.

Glycosides, non-reducing organic substances, have shown consistent presence in the ethanolic extracts as mentioned in Tables 2 and 5 and rarely their absence. Glycosides exhibit biological activity such as immunomodulation and nitric oxide scavenging (16) and are said to lower the blood pressure. Tinosporaside, amritoside, diterpenoid furanolactone are some of the glycosides present in *T. cordifolia* (3).

Flavonoids (Table 2 and 5), a hydroxylated form of phenolic compounds, are known to have antioxidant, anti-inflammatory, anticancer, cardioprotective, hepatoprotective, antimicrobial, antiviral, anti-allergic, vasodilatory effects and also in the treatment of neurodegenerative diseases (17, 18). The extract of *T. cordifolia* prepared using methanol is reported to contain polyphenols and flavonoids which have good antioxidant potential (9).

Saponins, a subclass of terpenoids, have anti-inflammatory, anthelmintic, antitumor/ anticancer effects, antibacterial and are harmless when taken orally (17). Steroids from plants possess cardiotonic, insecticidal, antimicrobial and anthelmintic (5) properties and are employed in the fields of nutrition, herbal medicine and cosmetics. They form an important component of sex hormones. Similar to the current study, there are records of the presence of cardiac glycosides and absence of tannin and alkaloids in the methanolic extract of *T. cordifolia* (Table 5) (19). However, alkaloids have shown consistent presence in the ethanolic extracts (Table 5). Cardiac Glycosides are beneficial in treating supraventricular cardiac arrhythmias.

Quinone and coumarin are reported to be present, probably the first report, in the extract prepared in the current study (Table 2). Quinones are strongly coloured pigments found internally in the plants and are also formed as metabolites of drugs and dietary components (20). Quinones are derivatives of benzoquinone, naphthoquinone or anthraquinone structures. Many types of quinones are found in plants and they possess medicinal qualities such as antitumor, antibiotic, antiparasitic and antifungal (20). Coumarin is a benzo-α-pyrone derivative having flavouring properties (17); Few coumarins are used in anticoagulant therapy.

According to Ayurvedic texts, the kidney is one of the main targets of *T. cordifolia* along with liver and spleen (21).

Udupa *et al.*, (2019), as in the present experiment, did not report any clinical signs and lethality during the period of study (22). Tubular and Glomerular effects of gentamicin are mediated partly by ROS and the attenuation of kidney injury took place upon applying scavengers of ROS (23). An analysis also showed that the SOD, CAT and GSH levels were severely depressed and LPO increased in cortex and medulla. Therefore, the mitochondria, lysosomes, basolateral & brush border membranes and the internal organelles could have suffered major damage. A similar decrease in the SOD, GSH and GPx levels with an augmentation in the LPO concentration were reported. This was the pattern of changes in the oxidative stress levels observed in the nephrotoxicity induced group in this study as well.

The characteristic of *T. cordifolia* to scavenge free radicals has been established by earlier studies; Ethanolic extracts of *T. cordifolia* have exhibited antioxidant activity (4). Ayurveda states that *T. cordifolia* gives strength (balya) to tissues (2); its nature to destroy metabolic wastes in the cells could also be attributed to its antioxidant property (4). Methanolic, ethanolic and water extracts of *T. cordifolia* showed significant antioxidant potential compared to other solvents. The highest level of activity in scavenging of free radicals was seen in the ethanol extract of *T. cordifolia* in comparison with its Methanol extracts (4). Ethanolic extract of *T. cordifolia* with semi-polar antioxidant principles was protective against Cisplatin induced nephrotoxicity while treating malignancies (4).

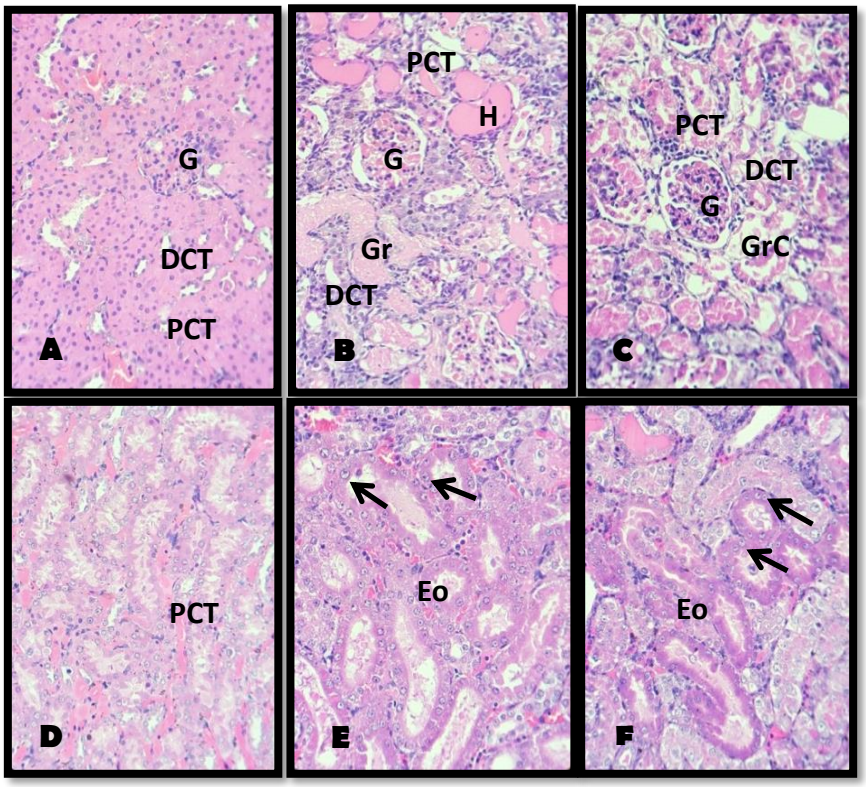

Fig. 1: Effect of co-administration of ETc upon nephrotoxicity induced by G: Photomicrograph of Histomorphological changes in the kidney, H&E, 400X: Kidney-Cortex :- A: Group I – Control; B: Group II- Nephrotoxicity; C: Group III – Co-administration; Kidney- Outer Medulla: D: Group I; E: Group II; F: Group III; G – Glomerulus, PCT – Proximal Convoluted Tubule, DCT – Distal Convoluted Tubule, Gr – Granular cast, GrC- Granular cast cleared off; H – Hyaline Cast; Eo – Eosinophilic cytoplasm; Arrows – Prominent nuclei and nucleoli

Table 5: Comparison - phytochemical constituents present in the ETc in the present study with earlier studies

Phytoconstituents	**Studies**	**Findings**
Glycoside	Tiwari *et al.*, 2011 (24)	Presence
	Yadav and Agarwala, 2011 (25)	Presence
	Mishra *et al.*, 2014 (25)	Presence
	Present Study	Presence
Flavonoids	Yadav and Agarwala , 2011 (25)	Presence
	Tiwari *et al.*, 2011 (24)	Presence
	Rani *et al.*, 2015 (27)	Presence
	Present study	Presence
Saponin	Tiwari *et al.*, 2011 (24)	Presence
	Yadav and Agarwala, 2011 (25)	Presence
	Present study	Presence
Steroids	Yadav and Agarwala, 2011 (25)	Presence
	Tiwari P *et al.*, 2011 (24)	Presence
	Present study	Presence
Phenol	Yadav and Agarwala, 2011 (25)	Presence
	Tiwari *et al.*, 2011 (24)	Presence
	Mishra *et al.*, 2014 (26)	Absence
	Rani *et al.*, 2015 (27)	Presence
	Present study	Absence
Tannin	Yadav and Agarwala, 2011 (25)	Presence

	Tiwari *et al.,* 2011 (24) Mishra *et al.,* 2014 (26) Rani *et al.,* 2015 (27) Present study	Presence Absence Presence Absence
Cardiac glycosides	Rani *et al.,* 2015 (27) Present study	Absence Presence
Terpenoids	Yadav and Agarwala, 2011 (25) Tiwari *et al.,* 2011 (24) Rani *et al.,* 2015 (27) Present study	Presence Presence Presence Absence
Alkaloids	Yadav and Agarwala, 2011 (25) Tiwari *et al.,* 2011 (24) Mishra *et al.,* 2014 (26) Rani *et al.,* 2015 (27) Present study	Presence Presence Presence Presence Absence

In the current study, Superoxide dismutase (SOD) enzyme level was decreased due to nephrotoxicity while its level was restored in the group treated with the herbal extract. Methanolic extract of *T. cordifolia* scavenged hydroxyl and superoxide radicals in vitro (29). Enzymatic antioxidants such as CAT and GPx did not seem to be significantly impacted by ETc co-administration.

As the glutathione turnover in the kidney is rapid, it requires ATP. Thus, a decrease in the production of ATP could render the tissue susceptible to damage due to the toxic injury. The reduction in the GSH concentration in toxicity group was probably due to the same. It is important to note that the GSH level in the group co-treated with the herb in the study increased significantly when compared to the Control and Nephrotoxicity induced groups. Similarly, *T. cordifolia* exerted a uroprotective role on cyclophosphamide induced toxicity by regulating GSH and pro-inflammatory levels of cytokine (5). Also, the hydroalcoholic extract of *T. cordifolia* was found to enhance the GSH levels and induce the critical detoxifying enzymes to alleviate oxidative stress which is through non-enzymatic pathways of anti-oxidant system. The epithelial cells of the renal tubules are conferred resistance to hydrogen peroxide induced damage by the enhancement of glutathione. Interestingly, a report states that supplementation with GSH did not influence the nephrotoxicity induced by Gentamicin notwithstanding the reduction in lipid peroxidation and increase in kidney GSH (glutathione) level (23).

Glutathione peroxidase destroys hydrogen peroxide and lipid hydroperoxides. However, the present study recorded only an insignificant variation in the GPx level.

Gentamicin induced nephrotoxic damage resulted in increased lipid peroxidation as indicated by the rise in the Malondialdehyde (one of the thiobarbituric acid reactive substances (TBARS) which are end-products of the damage due to lipid peroxidation) levels measured. Its increase was prevented by the co-treated group implying either decreased lipid peroxidation or quicker clearance of the metabolite. Alcoholic extract of *T. cordifolia* is reported to inhibit lipid peroxide formation (29) and reduce it.

The renal cortex was affected more than the medulla indicating that gentamicin, through the blood supply, reached the cortex at a comparatively higher concentration than the concentration entering the medulla (22). The current observations were similar. Interestingly, changes pointing towards regeneration were observed beginning from the medulla due to increased blood supply carrying less quantity of the toxic drug.

Acute tubular necrosis (ATN) indicated nephrotoxicity induced by gentamicin with multifocal areas of tubular dilatation & degeneration as reported in various studies (8, 22, 28) and PCT was selectively affected (30); 10-50% of PCT underwent ATN with cytoplasmic and nuclear debris filling up the tubular lumina; the corticomedullary junction also showed lymphocytic infiltration.

The tubules in the animals treated with ETc showed dilated lumen with cleared-off casts (cell debris) when compared to the nephrotoxicity group as the regenerating epithelium is immature consisting of flat elongated cells on the basement membrane with a pronounced increase in the rate of cell turnover in the cortex (30). This records the quickening in regeneration of the epithelium due to the treatment with the herbal extract following nephrotoxic damage. Yet, the severity grading shall only indicate severe changes. Significant reduction in renal tubular degeneration was seen due to the co-administration with methanolic extract of the stem and leaf of *T. cordifolia* with the leaf extract being more potent (31). Similarly, minimum tubular necrosis, granular tubular epithelial changes, congestion and haemorrhage was recorded upon co-administration with hydroethanolic stem extract of *T. cordifolia* on nephrotoxicity induced by gentamicin (32).

As in the present study, severe necrosis of the proximal tubules was reported upon administration of 40-60 mg/Kg of gentamicin per day in animals. However, mild to moderate degree of lesions due to

administration of 100 mg/Kg of Gentamicin for 14 days were also reported (28). This variation in the severity grading could be due to the difference in the methods followed and the criteria assessed.

Glomeruli appear enlarged due to tissue enlargement due to nephrotoxicity. However, there is a speculation that it could appear so due to the collapse of tubules or proliferation of the mesangial & endothelial cells in the glomeruli (33). Such glomerular structural alterations are seen to be decreased by ETc treatment in this study.

The present study adds more impetus to the influence of the ethanolic extract of *T. cordifolia* stem in modulating the level of GSH, the non-enzymatic antioxidant, in attenuating drug induced nephrotoxic alterations upon co-administration, hence, enhancing the recovery process from the nephrotoxic damage.

Limitations

Though the results of this study are indicative of the phytoconstituents of *T. cordifolia*, they are known to vary with the season, area of cultivation and type of support offered to this climber.

Scope of the study

The effect of higher doses of the ETc on different stages of nephrotoxicity induced based on the dose and duration of administration of gentamicin or in other models of drug induced nephrotoxicity can be studied. The ability of *T. cordifolia* to elevate the level of non-enzymatic antioxidant, reduced glutathione (GSH) can be explored further. Specific targets of the herb on the kidney can be explored.

CONCLUSION

The phytochemical screening reports the presence of quinone and coumarin and reaffirms the consistent presence of glycosides, flavonoids, saponins and steroids. The Antioxidants and Histological analysis revealed the positive changes brought about by *T. cordifolia* in the damaged renal tissue due to Gentamicin nephrotoxicity at the biochemical and histological levels in terms of the increase in GSH levels, reduction in the levels of SOD, CAT, GPx and LPO and enhancing regeneration of the tubular epithelium, respectively. These reflect the protective influence of the ethanolic extract of the stem of *T. cordifolia* on Acute Tubular Injury induced by Drug nephrotoxicity, upon co-administration. Therefore, *Tinospora cordifolia* extract may benefit the kidney when taken along with drugs that are potentially nephrotoxic.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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