DOI: 10.7860/JCDR/2022/57133.17039

Original Article

Anatomy Section

Effect of *Tinospora cordifolia* on Oxidative Stress Level due to Drug Induced Nephrotoxicity: An Experimental Study

JANANI MAHESHWARI V VYAS[1], SENTHIL KUMAR SAMPATH KUMAR[2], LEENA DENNIS JOSEPH[3]

ABSTRACT

Introduction: Various herbs are known to confer nephroprotectivity against renal damage of different origins. *Tinospora cordifolia* (Willd.) Miers is known to treat kidney disorders. Yet, a lacuna of studying the protective effect of the herb on drug induced nephrotoxicity at different time periods exists.

Aim: To determine the protective effect of *T. cordifolia* (Willd.) Miers on drug induced nephrotoxic biochemical changes upon post-treatment with the herb.

Materials and Methods: An experimental study was carried out at the Centre for Toxicology and Developmental Research, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India, from September 2020 to October 2020. Gentamicin induced drug nephrotoxicity model was employed for the study. The ethanolic extract of *T. cordifolia* was prepared. Total 51 adult male Wistar rats were housed under standard animal laboratory conditions for 30 days and were assigned to four groups: Control group (Olive oil, per os), Nephrotoxicity group (Gentamicin), Toxicity cessation group (Gentamicin-Olive oil) and Post-treatment group (Gentamicin *T. cordifolia* ethanolic extract). To induce nephrotoxicity, gentamicin (100 mg/kg) was administered through the Intraperitoneal (IP) route for 8 days. The ethanolic extract of the stem of *T. cordifolia* (Willd.) Miers (400 mg/kg) was administered orally for one, two and three weeks following nephrotoxicity induction. Levels of activity of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Reduced Glutathione (GSH) content and Lipid peroxidation in the kidney were measured. Statistical analysis was performed using One-way Analysis of Variance (ANOVA) and Post-hoc tests.

Results: The reduction in SOD (6.33±1.19 unit/ mg/mt), CAT (57.56±25.89 mcm/mt/mg ptn), GPx (5.79±1.87 nm/mt/mg ptn) activity, and GSH (4.98±0.31mcm/g tissue) levels and increase in the lipid peroxidation (196.28±100.05 nm/g tissue) in the kidney due to gentamicin nephrotoxicity was reversed upon post-treatment with *T. cordifolia* (Willd.) Miers extract for 1 week (7.06±0.25 unit/mg/mt, 119.69±22.79 mcm/mt/mg ptn, 7.08±1.73 nm/mt/mg ptn, 6.19±0.99 mcm/g tissue and 93.10±9.11 nm/g tissue), 2 weeks (7.03±0.43 unit/ mg/mt, 181.79±39.00 mcm/mt/mg ptn, 5.07±0.81 nm/mt/mg ptn, 7.58±0.73 mcm/g tissue and 188.31±54.89 nm/g tissue) and 3 weeks (7.81±0.47 unit/mg/mt, 165.83±37.48 mcm/mt/mg ptn, 3.92±1.87 nm/mt/mg ptn, 7.03±1.28 mcm/g tissue, 214.40±72.93 nm/g tissue).

Conclusion: Post-treatment with *T. cordifolia* (Willd.) Miers stem extract is protective in drug induced nephrotoxic condition, even upon 1 week of administration. Its therapeutic influence on the alterations in the oxidative stress markers and antioxidant levels produced due to gentamicin nephrotoxicity has been demonstrated.

Keywords: Antioxidants, Gentamicin, Lipid peroxidation, Rats, Therapeutics, Wistar

INTRODUCTION

Among events of acute renal failure in the community and hospitals, drugs are known to cause around 20% of them [1,2]. Antioxidants such as curcumin, extract of garlic, lycopene16, taurine and melatonin [3,4] and different extracts of *T. cordifolia* [5,6] have been able to prevent drug-induced renal damage, especially, upon pretreatment. The dried and mature stem *of T. cordifolia,* a climber found throughout India, consists of phytochemicals such as terpenoids, alkaloids [7] and glycosides [8]. It is used in the form of churna, sattva, taila, kapacurakkudinir and is known to be antidiabetic, antipyretic and also is a diuretic [7,8]. This traditional medicinal herb is considered safe [9] and various phytochemical and clinical research is being carried out using this herb [10].

Drug induced nephrotoxicity model in rats using gentamicin can be employed to study the effect of post-treatment with *T. cordifolia*, as it is found to resemble the condition seen in humans [11,12]. Nephrotoxicity occurs as the proximal convoluted tubules concentrate gentamicin, which binds to the lysosomes forming myeloid bodies, impairing the mitochondrial function and tubular transport, thus, increasing the generation of free radicals and lipid peroxidation and decreasing the level of antioxidants [13,14]. It manifests as Acute Tubular Necrosis (ATN) brought about by Reactive Oxygen Species (ROS) [15,16]. In addition to the renal tubular cells, vascular cells and the granulocytes which are metabolically active are few of the sources of oxidative stress in the renal tissue. The amino acids, lipids, Deoxyribonucleic Acid (DNA) are some of the major targets of the ROS [17]. The ROS generated during various normal cellular processes is normally balanced by the antioxidant enzymes cascade [18] within these cells. An imbalance in this mechanism reflects diseases, hence, making it significant to measure the levels of the antioxidants [19].

Humes HD recommends a careful observation of the recovery phase of acute renal failure from a biochemical approach [20]. Though the recovery from acute nephrotoxic damage occurs naturally, antioxidants can also be supplied exogenously, through diet and as medicines from plant sources to help in the process, the beneficial potential of which is being explored with regard to diseases in the kidney [21]. Exploring the mechanisms of nephroprotectivity of *T. cordifolia*, assessing its extracts at various dose levels and at various test durations shall be vital in establishing it as a nephroprotective drug [6]. Since, it remains to be explored whether treatment with *T. cordifolia* extract after an episode of drug induced renal failure shall benefit the kidney or not, the present study was conducted with an aim to study the nephroprotective effect of *T. cordifolia* extract

on drug induced nephrotoxic changes upon its post-administration following the administration of the nephrotoxic drug, gentamicin, by determining the levels of oxidative stress and antioxidants.

MATERIALS AND METHODS

An experimental study was carried out at the Centre for Toxicology and Developmental Research, SRIHER (DU), Porur, Chennai, Tamil Nadu, India. Gentamicin induced drug nephrotoxicity model was employed for the study. Total 51 adult male Wistar rats (200-250 g) were housed in the animal facility, and the experiment was carried out with the approval of the Institutional Animal Ethics Committee (IAEC) (IAEC/61/SRIHER/700/2020). Throughout the study duration of 30 days (September 2020 to October 2020), polypropylene cages lined with autoclaved paddy husk housed the animals; 12 hour artificial light/dark cycle with relative humidity (30-70%) at 19-23°C was maintained and access to standard rodent feed and water were allowed ad libitum.

Inclusion criteria: Healthy male Wistar rats, 8-9 weeks of age (adult) were included in the study.

Exclusion criteria: Unhealthy male and female Wistar rats aged less than 8 weeks were excluded from the study.

Preparation of Ethanolic Extract of *T. cordifolia* [ETc]

Authenticated stem of *T. cordifolia* (Willd.) Miers ex Hook.F.&Thoms (PARC/2018/3638) was used in the preparation of herbal extract, in the Department of Pharmacognosy, Faculty of Pharmacy, SRIHER (DU), Chennai. Dried, coarsely ground stem of *T. cordifolia* was macerated in 99.9% (absolute) ethanol and filtered. The filtrate was concentrated using rotary evaporator, mantle heater and desiccator. Later, it was stored in an air-tight glass container at room temperature, in a cool, dry place, away from sunlight, till further use.

Total 51 rats were assigned to four groups:

- **Control group (Group I):** Rats were administered with edible olive oil, the vehicle used to suspend the ethanolic extract of *T. cordifolia*, at 1 mL/100 g body weight.
- **Nephrotoxicity (Group II):** Nephrotoxic damage in the kidney was achieved in the group with only administering gentamicin {Genticyn (Abbott) 80 mg/2 mL vials}, IP, at 100 mg/kg.
- **Toxicity cessation group (Group III):** Group III represented the natural reversal from the nephrotoxic condition for the same duration 1, 2 and 3 weeks as the post-treatment group; it was hence administered 100 mg/kg gentamicin for 8 days followed by the vehicle, 1 mL/100 g body weight edible olive oil. [Table/Fig-1] shows the design of the experiment, treatment, dosing period, duration of intervention and the day of euthanasia of the animals. The animals were euthanised using carbon dioxide asphyxia.
- **Post-treatment (Group IV):** Group IV determined the effect of the post-treatment with ETc following the nephrotoxicity induction; it was hence administered 100 mg/kg gentamicin for 8 days followed by the ETc, orally, at 400 mg/kg dosage for 1, 2 and 3 weeks respectively, therefore, referred to as the Post-treatment group.

No. of animals (n)	Groups		Sub-groups	No. of animals treated	Treatment	Dosing period (Day) (Week)	Euthanasia (day)
9	Control	I	I A	3	Olive oil: 1 mL/100 g b.wt	9-15 (1)	16
			I B	3		9-22 (2)	23
			I C	3		9-29 (3)	30
6	Nephrotoxicity; *GEN	II	-	6	GEN:100 mg/kg	1-8	9
18	Toxicity cessation; GEN-Olive oil	III	-	18	GEN:100 mg/kg	1-8	-
			III A	6	Olive Oil: 1 mL/100 g b.wt	9-15 (1)	16
			III B	6		9-22 (2)	23
			III C	6		9-29 (3)	30
18	Post-treatment; GEN-**ETc	IV	-	18	GEN:100 mg/kg	1-8	-
			IV A	6	ETc (400 mg/kg)	9-15 (1)	16
			IV B	6		9-22 (2)	23
			IV C	6		9-29 (3)	30

[Table/Fig-1]: Experiment - Grouping, Treatment, Dosing and Euthanasia schedule. *GEN: Gentamicin; **ETc: Ethanolic extract of *T. cordifolia*; b.wt: body weight

Study Procedure

Estimation of oxidative stress markers: The harvested left kidneys were stored at -80ºC for further analysis after being weighed. Normal saline 0.9% was added and homogenised. The kidney homogenate samples were then taken for further analysis of the enzymatic activity of Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) and the contents of reduced Glutathione (GSH), a non enzymatic antioxidant and Malondialdehyde (MDA), a metabolite of lipid peroxidation.

Superoxide Dismutase (SOD): SOD was assayed by taking 0.05 mL of 10% kidney homogenate followed by addition of sodium pyrophosphate buffer (pH 8.3), Phenazine Methosulphate (PMS) (186 µM) and Nitroblue Tetrazolium (NBT) (300 µM). The reaction was initiated by addition of reduced Nicotinamide Adenine Dinucleotide (NADH) (780 µM). The reaction was stopped by addition of glacial acetic acid. Then the reaction mixture was stirred vigorously and shaken with n-butanol. The colour intensity of the chromogen was read at 560 nm using a spectrophotometer (Multiskan spectrum, v1.2, USA) [22].

Lipid peroxidation (LPO)-Thiobarbituric Acid Reactive Substances (TBARS): A 10% kidney homogenate was boiled with 0.8 mL saline, Butylated Hydroxytoluene (BHT) and Thiobarbituric Acid (TBA) reagent for 90 min in water bath. After cooling, the solution was centrifuged at 2,000 rpm for 10 min and the precipitate obtained was removed. The absorbance of the supernatant was determined at 532 nm using a spectrophotometer (Multiskan spectrum, v1.2, USA) [23].

Reduced Glutathione (GSH): Glutathione content was estimated according to the method of Moron MS et al., [24]. A 10% of kidney enzyme preparation was added to equal volume of ice cold 5% Trichloroacetic Acid (TCA). To aliquot of supernatant, 0.2 M phosphate buffer (pH 8.0) and 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) (0.6 mM) was added and mixed well. The absorbance was read at 412 nm using a spectrophotometer (Multiskan spectrum, v1.2, USA).

Total protein by biuret method: Estimation of protein was assayed by taking 0.2 ml saline, 10% homogenate followed by addition of 1.25 ml of biuret reagent working solution. Following incubation at room temperature for 15 min, the colour intensity was read at 540 nm in a spectrophotometer (Multiskan spectrum, v1.2, USA) [25].

Glutathione Peroxidase (GPx): The activity of GPx was assayed by taking Tris-HCl buffer, Ethylenediaminetetraacetic Acid (EDTA) along with 100 µL of sodium azide and kidney enzyme preparation and mixed well. Thereafter, 200 µL of GSH solution, followed by 0.1 mL H_2O_2 were added. The overall reaction was arrested by adding 10% TCA. The non enzymatic reaction rate was correspondingly assessed, by replacing the enzyme sample by buffer. The remaining GSH in the supernatant was determined by adding 1.0 mL of DTNB. The absorbance was read at 412 nm using an UV/Visible spectrophotometer (Perkin Elmer, Lambda 25, USA) [26].

Catalase (CAT): The CAT assay reaction mixture contained H_2O_2 (2 mM), 0.2 mL of the homogenate in a final volume of 1 mL in phosphate buffer (pH 7.4). It was incubated at 37ºC for 5 min and then Dichromate acetic acid reagent was added and absorbance

was taken at 570 nm. Dichromate acetic acid reagent acted as blank whereas the reaction mixture without homogenate acted as control [27].

STATISTICAL ANALYSIS

Statistical analysis was performed by using the Statistical Package for the Social Sciences (SPSS) software version 16.0. Difference between groups was analysed using One-way Analysis of Variance (ANOVA) and Pair-wise differences were identified (multiple comparisons) using Post-hoc tests. Data was expressed in mean±standard deviation and was considered as statistically significant at p≤0.05.

RESULTS

All animals survived the experimental period except three animals in Group III and one in Group IV that died within a week following nephrotoxicity induction.

Effect of 1 week of post-treatment with ETc on the markers of oxidative stress: A reduction in the levels of SOD, CAT, GPx activity and GSH content and an increase in the Lipid Peroxidation (LPO) level was seen in the Group II compared to the Group I A. Group III A showed increase in the level of SOD and GSH and a decrease in LPO when compared to the Group II; it showed a reduction in CAT and GPx (significant at p≤0.05) activity levels when compared to Group IA akin to Group II. Group IVA showed an increase in the SOD level compared to the Group III A and in the GPx level when compared to Groups II and III A. The levels of CAT and GSH in Group IV A significantly increased (p≤0.05) and significantly decreased (p≤0.05) the LPO level in comparison to Group II [Table/Fig-2-6].

Treatment duration/ Group	Sub-groups	Superoxide dismutase (unit/mg/mt)			
		Group I (Mean±SD, n)	Group II (Mean±SD, n)	Group III (Mean±SD, n)	Group IV (Mean±SD, n)
Week 1	A	7.16±1.13 (3)	6.33±1.19 (6)	6.71±0.39 (5)	7.06±0.25 (5)
Week 2	B	6.59±0.42 (3)	6.33±1.19 (6)	5.85±0.67 (5)	7.03±0.43 (6)
Week 3	C	6.70±0.75 (3)	6.33±1.19 (6)	5.60±1.36 (5)	7.81±0.47$ (6)

[Table/Fig-2]: Effect of post-treatment with ETc on SOD activity levels in gentamicin induced nephrotoxicity.
$Significant at p≤0.05 when compared to toxicity cessation

Catalase (mcm/mt/mg ptn)					
Treatment duration/ Group	Sub-groups	Group I (Mean±SD, n)	Group II (Mean±SD, n)	Group III (Mean±SD, n)	Group IV (Mean±SD, n)
Week 1	A	131.64±50.62 (3)	57.56±25.89* (6)	65.51±26.98* (5)	119.69±22.79# (5)
Week 2	B	183.49±2.92 (3)	57.56±25.89* (6)	156.46±23.57# (5)	181.79±39.00# (6)
Week 3	C	176.71±19.35 (3)	57.56±25.89* (6)	125.20±12.55# (5)	165.83±37.48# (6)

[Table/Fig-3]: Effect of post-treatment with ETc on CAT activity levels in gentamicin induced nephrotoxicity.
*Significant at p≤0.05 when compared to control
#Significant at p≤0.05 when compared to nephrotoxicity

Glutathione peroxidase (nm/mt/mg ptn)					
Treatment duration/ Group	Sub-groups	Group I (Mean±SD, n)	Group II (Mean±SD, n)	Group III (Mean±SD, n)	Group IV (Mean±SD, n)
Week 1	A	9.35±2.17 (3)	5.79±1.87 (6)	5.35±1.88* (5)	7.08±1.73 (5)
Week 2	B	6.27±0.52 (3)	5.79±1.87 (6)	4.01±1.40 (5)	5.07±0.81 (6)
Week 3	C	4.84±1.10 (3)	5.79±1.87 (6)	4.63±4.62 (5)	3.92±1.87 (6)

[Table/Fig-4]: Effect of post-treatment with ETc on GPx activity levels in gentamicin induced nephrotoxicity.
*Significant at p≤0.05 when compared to control

Reduced Glutathione (mcm/g tissue)					
Treatment duration/ Group	Sub-groups	Group I (Mean±SD, n)	Group II (Mean±SD, n)	Group III (Mean±SD, n)	Group IV (Mean±SD, n)
Week 1	A	6.19±0.73 (3)	4.98±0.31 (6)	5.50±0.36 (5)	6.19±0.99# (5)
Week 2	B	8.93±0.11 (3)	4.98±0.31* (6)	6.30±0.73*# (5)	7.58±0.73*#$ (6)
Week 3	C	8.57±0.81 (3)	4.98±0.31* (6)	6.47±0.88* (5)	7.03±1.28# (6)

[Table/Fig-5]: Effect of post-treatment with ETc on GSH levels in gentamicin induced nephrotoxicity.
*Significant at p≤0.05 when compared to control; #Significant at p≤0.05 when compared to nephrotoxicity; $Significant at p≤0.05 when compared to toxicity cessation

Lipid peroxidation (nm/g tissue)					
Treatment duration/ Group	Sub-Groups	Group I (Mean±SD, n)	Group II (Mean±SD, n)	Group III (Mean±SD, n)	Group IV (Mean±SD, n)
Week 1	A	97.58±4.91 (3)	196.28±100.05 (6)	106.84±14.26 (5)	93.10±9.11# (5)
Week 2	B	104.30±3.39 (3)	196.28±100.05 (6)	232.86±3.69 (5)	188.31±54.89 (6)
Week 3	C	183.48±16.26 (3)	196.28±100.05 (6)	297.27±47.84 (5)	214.40±72.93 (6)

[Table/Fig-6]: Effect of post-treatment with ETc on LPO levels in gentamicin induced nephrotoxicity.
#Significant at p≤0.05 when compared to nephrotoxicity

Effect of 2 weeks of post-treatment with ETc on the markers of oxidative stress: A decrease in the activity of SOD, CAT (significant at p≤0.05), GPx, GSH content (significant at p≤0.05) and an increase in LPO levels was seen in Group II when compared to Group I B. Group III B showed reduction in SOD, GPx and GSH (significant at p≤0.05) and an increase in LPO levels when compared to Group I B; the CAT level increased significantly (p≤0.05) when compared to Group II. Group IV B showed an increase in SOD, CAT (significant at p≤0.05) and GSH (significant at p≤0.05) compared to the Group II; CAT and GPx levels increased in comparison to the Group III B; GSH level increased significantly (p≤0.05) and LPO reduced in comparison to Groups II and III B [Table/Fig-2-6].

Effect of 3 weeks of post-treatment with ETc on the markers of oxidative stress: In comparison to Group I C, levels of SOD, CAT (significant at p≤0.05) and GSH (significant at p≤0.05) reduced and GPx and LPO increased in Group II. Group III C showed decrease in SOD, GPx and GSH (significant at p≤0.05) levels and an increase in the LPO level when compared to Group I C; the CAT level significantly increased (p≤0.05) compared to Group II. Group IV C showed a decrease in the LPO and GPx levels; the SOD level increased significantly (p≤0.05) compared to Group III C; the CAT level significantly increased (p≤0.05) compared to Group II and was higher than Group III C and closer to Group I C value; the GSH level increased significantly (p≤0.05) when compared with Group II [Table/Fig-2-6].

DISCUSSION

The post-treatment with ETc has certainly helped, to curb the imbalance in the oxidative stress and antioxidant levels aiding quicker recovery of the kidney when compared to the untreated group in its recovery phase. It could be observed from the oxidative stress markers and the antioxidant levels, that the enzymatic and non enzymatic antioxidants work together, complementing the activity of one another. Importantly, the post-treatment with ETc restored near normal values, right in the end of the first week of treatment compared to the untreated toxicity cessation. This further improved upon herbal extract treatment for the next two weeks, which could be due to the nature of *T. cordifolia* to mediate the strengthening of the antioxidant pool against oxidative insult [28,29].

The present study is probably the first, to explore the effect of post-treatment with the ETc on drug induced nephrotoxicity employing gentamicin. ROS trigger antioxidant response, as a cytoprotective adaptation. While the pre and co-treatment studies bring out the preventive and protective effect of *T. cordifolia* on drug nephrotoxicity, the current study with post-treatment explored the therapeutic effect. The utilisation of ROS by the growth promoters as signalling aids gives rise to the speculation, that ROS could be playing a role in the recovery phase post ATN [30]. In this study, the toxicity cessation group with untreated recovery would reflect this natural response of the kidney, to the increase in oxidative stress which reverses the injury and repairs the kidney. The oxidative stress markers and antioxidant levels upon natural recovery are also probably reported for the first time.

The ETc was insoluble in water and suspended well in edible olive oil, hence, it was used as the vehicle. To eliminate the possibility of its confounding antioxidant effect, olive oil was also administered to control (Group I) and toxicity cessation (Group III) groups. Therefore, comparison between post-treatment (Group IV), control and toxicity cessation groups clearly brought out the effect of the herbal extract on the nephrotoxic damage.

As recorded by earlier studies, nephrotoxicity induction in this study also decreased SOD [31-34], CAT [33,34], GSH [31-34], GPx [34,35] and increased LPO [31-34]. Such an increase in free radical generation and peroxidation of lipids and decrease in the level of antioxidants due to gentamicin induced nephrotoxicity [13,14] is known to result in ATN [15,16] which signifies the loss of the tubular epithelium. Renal vasculature, haemodynamics, glomerular filtration and tubular reabsorption and secretion are directly or indirectly affected by oxidative stress, during an injury or disease [21] due to major damage to the mitochondria, lysosomes, basolateral and brush border membrane and internal organelles.

Male rats were utilised in this experimental study, as they manifest more severe necrosis and degeneration [36] than the females, upon gentamicin administration. Also, more number of Differentially Expressed Genes (DEGs) of different time points, induced by gentamicin, co-regulates the process in males when compared to females [37].

No clinical signs or mortality was observed throughout the period of nephrotoxicity induction, similar to earlier studies [34,38]. However, during the recovery phase, the animals treated with the herbal extract (Group IV) withstood better the effect of toxicity induction in comparison to the animals that were allowed untreated recovery (Group III) in terms of mortality, which was observed only in the first week upon post-treatment while it was observed in all the three weeks upon toxicity cessation. The routine cage side observation of clinical signs, which were not quantified, also indicated the same.

The superoxide/oxygen radical impacts the renal vasculature, by mediating the mesangial cell contraction,induced by gentamicin [39]. SOD is an antioxidant enzyme found in the cytosol, mitochondria and extracellular spaces that scavenges the superoxide anion and alters the renal blood flow. In this study, the level of SOD activity, reduced due to the nephrotoxicity (6.33±1.19 unit/mg/mt) in comparison to the control (7.16±1.13 unit/mg/mt). A level similar to nephrotoxicity group was seen in the toxicity cessation group (6.71±0.39 unit/mg/mt) at the end of the first week, indicating less improvement. However, the SOD activity in the post-treatment group (7.06±0.25 unit/mg/mt) was similar to the control group and higher than the toxicity cessation group. At the end of week 2, the results were similar whereas at the end of the third week, the SOD level upon post-treatment was significantly higher than the toxicity cessation group and closer to the control group [Table/Fig-2]. Therefore, the level of SOD activity had been restored to near normal levels when observed at the end of all 3 weeks upon post-treatment with ETc in comparison to the toxicity cessation, possibly ensuring normal blood flow in the kidney. Scavenging of superoxide radicals by the methanolic extract of *T. cordifolia* has been reported earlier [40].

In the kidney, the Proximal Convoluted Tubule (PCT) cells exclusively localise CAT in their cytoplasm which makes assessing its level important and interesting as gentamicin induces ATN involving the PCT [41]. Nephrotoxicity induced by gentamicin reduced the CAT activity (57.56±25.89 mcm/mt/mg ptn) significantly compared to the control groups. Catalase, in its role as an antioxidant, catalyzes the decomposition of hydrogen peroxide, often resulting from the action of SOD, into oxygen and water, without the involvement of cofactors. Therefore, the reduction of CAT activity lead to the rise in hydrogen peroxide, a free radical. At the end of week 1, CAT was significantly less upon toxicity cessation (65.51±26.98 mcm/mt/mg ptn) when compared to the control (131.64±50.62 mcm/mt/mg ptn) and showed only a slight increase compared to the nephrotoxicity group. However, the CAT level upon post-treatment with ETc (119.69±22.79 mcm/mt/mg ptn) was higher than nephrotoxicity and significantly higher than the control groups. The toxicity cessation and post-treatment groups showed significant increase in the CAT level at the end of weeks 2 and 3 compared to the nephrotoxicity induced group, however, the CAT levels upon post-treatment was higher than upon toxicity cessation [Table/Fig-3]. Hence, post-treatment with ETc helped restoration of CAT activity levels significantly even after a week of treatment. The study, that there was increased catalase activity in the membrane of erythrocytes in diabetic rats brought about by the methanolic extract of *T. cordifolia* adds strength to the present outcome [42]. It is also noteworthy that the apoptosis induced by gentamicin was prevented by the overexpression of CAT by reducing ROS generation [41].

The improvement in the GPx activity upon post-treatment with ETc was seen right in the first week. Nephrotoxicity reduced GPx levels though not significantly, in the present study. GPx, located in the cytoplasm and mitochondria, catalyses the destruction of the oxidising hydrogen peroxide molecule and other organic peroxides. At the end of week 1, the post-treatment group (7.08±1.73 nm/mt/mg ptn) showed good improvement with GPx activity increasing closer to the control group (9.35±2.17 nm/mt/mg ptn) while toxicity cessation (5.35±1.88 nm/mt/mg ptn) showed significant reduction compared to the control and was closer to the nephrotoxicity group (5.79±1.87 nm/mt/mg ptn). The GPx level did not differ significantly across the four groups at the end of weeks 2 and 3. The level of GPx activity of the post-treatment group were higher than the toxicity cessation group and closer to the control group whereas the toxicity cessation group had GPx level lesser than the nephrotoxicity group [Table/Fig-4]. Supportive of the present outcome, co-treatment with the methanolic extract of *T. cordifolia* is known to increase the level of GPx [28].

ETc post-treatment, helps in faster restoration of GSH levels compared to natural recovery. GSH is the major thiol antioxidant in tissues which along with GPx, provides a critical mechanism for the detoxification of peroxides. The non enzymatic antioxidant, GSH content was reduced upon nephrotoxicity (4.98±0.31 mcm/g tissue); it was significantly lesser than the control in the weeks 2 and 3. One week of post-treatment with ETc (6.19±0.99 mcm/g tissue) significantly increased the GSH level, compared to the nephrotoxicity group which was higher as compared to toxicity cessation group (5.50±0.36 mcm/g tissue) as well. Generally, the turnover of GSH in the kidney is rapid, hence, requiring ATP [43]; however, a reduction in the ATP increases susceptibility to toxic injury induced damage. This probably explains the decrease in GSH concentration in the nephrotoxicity group. Two weeks of post-treatment with ETc showed significant increase (7.58±0.73 mcm/g tissue) compared to nephrotoxicity and control groups. GSH level upon toxicity cessation (6.30±0.73 mcm/g tissue) was significantly lower than the control group (8.93±0.11 mcm/g tissue), yet, significantly higher than the nephrotoxicity induced indicating slight improvement. At the end of week 3, the GSH content upon toxicity cessation remained almost the same as week 2 and significantly lower than control group while upon post-treatment, the GSH level was significantly higher than nephrotoxicity induced group though similar to week 2 values

[Table/Fig-5]. Modulating and enhancing GSH has been shown to confer nephroprotectivity [10,44,45]. Priya LB et al., reported, that the methanolic extract of Tc decreased the utilisation of GSH by cadmium intoxication in the heart tissue of rats upon co-treatment, may be, due to its free radical scavenging activity [28]. These reports are supportive of the present observations of the groups III and IV. Torres AM et al., demonstrated that the replenishment of GSH level was quicker than the restoration of renal functions with time, which could indicate that restoration of the renal GSH pool is necessary before the restoration of renal function, following a rise in oxidative stress; based on the present analysis, such a concept of restoration of normal levels of antioxidants with time seems extensible to all the parameters observed [29]. While Fauconneau B et al., [46] did not observe much fluctuation in the GSH level due to gentamicin induced nephrotoxicity, few researchers also reported that supplementation with GSH did not have an impact on it, immaterial of the decrease in lipid peroxidation and increase in level of GSH in the kidney [47]. These contradictions to the impact of ETc observed on the GSH levels presently do pave the way for prospective research in this area.

One of the first targets for ROS is generally the membranes of cells and subcellular organelles producing MDA, one of the TBARS as a metabolite due to LPO, which is an indirect marker of cell oxidation. Secondly, the interaction of ROS with Poly Unsaturated Fatty Acids (PUFA) leads to the generation of hydroperoxides and their metabolites, which are additional radicals resulting in a chain reaction of ROS production. The LPO levels increased upon nephrotoxicity (196.28±100.05 nm/g tissue) though not significantly. While toxicity cessation (106.84±14.26 nm/g tissue) did show a decrease in LPO level at the end of week 1, post-treatment with ETc (97.58±4.91 nm/g tissue) decreased LPO significantly compared to nephrotoxicity induced group. No significant difference between any of the groups was seen at the end of weeks 2 and 3, however, the LPO levels upon post-treatment was lesser than upon toxicity cessation [Table/Fig-6]. The reduction of MDA upon post-treatment with ETc implies, either lesser lipid peroxidation or faster clearance of the metabolite, which is indicative of the curtailing of the damage. Alcoholic extract of *T. cordifolia* inhibited lipid peroxide formation [40] and reduces it [48]. Ramasammy LS et al., opined that LPO is a consequence of gentamicin nephrotoxicity and also found that pretreatment with antioxidant vitamin E prevented nephrotoxicity, by preventing an increase in esterified acids [33]. But, Fauconneau B et al., did not report much fluctuation in the level of LPO [46] while, Martinez-Salgado C et al., reported that gentamicin does not cause any change in fluidity or LPO in the plasma membrane [39].

The observed beneficial effect of the ETc could be probably due to its antioxidant phytoconstituents. The free radical scavenging property of *T. cordifolia* [40] and its extracts have been established in vitro and in vivo [10,45] where its ethanolic extract showed the highest activity compared to aqueous or methanolic extracts [45]. It has been found to be protective against cisplatin and aflatoxin induced nephrotoxicity, due to its semi-polar antioxidant principles [49,50] and free-radical scavengers such as alkaloids, flavonoids and glycosides [9,10]. Though *T. cordifolia* is considered safe for consumption [9], it is only after confirmation in relevant models of diseases, that such antioxidant therapy shall be used in ATN in humans for recovery after an episode of ATN [30].

Further, determining the effect of a higher dosage of ETc on the various stages of the nephrotoxicity depending on the dosage and duration of gentamicin may be beneficial. The impact of ETc on CAT and GSH levels is worthy of exploration.

Limitation(s)

Experimenting with a larger sample size, would provide a clearer picture of the impact of the herbal extract on the oxidative stress and antioxidant levels due to drug induced nephrotoxicity. Comparing these levels with the tissue changes observed at the gross and histological levels, shall help to understand the implication better. In addition to scientific leads, practical difficulties in conducting the experiment, such as manpower and resource intensive aspects of housing, maintaining the animals and carrying out the procedures limited the inclusion of female rats in the present study.

CONCLUSION(S)

The present study showed that the negative impact on oxidative stress markers and antioxidants rendered by gentamicin, i.e. reduction in SOD, CAT, GPx activity and GSH levels and increase in the lipid peroxidation reversed upon post-treatment with *T. cordifolia* stem ethanolic extract; this occurred at the end of the first week of post-treatment with ETc than during the untreated recovery following toxicity cessation, reflecting its therapeutic influence. Hence, *T. cordifolia* (Willd.) Miers extract shall be beneficial to the kidney, even when taken after the consumption of potentially nephrotoxic drugs.

Conflict of interest: The authors have no conflict of interest to declare. The current study is a part of the PhD work carried out by Ms. Janani Maheshwari V Vyas (Primary researcher) under the guidance of Dr. Senthil Kumar Sampath Kumar (Supervisor) and Dr. Leena Dennis Joseph (Co-Supervisor).

Acknowledgement

The study was carried out under the Founder Chancellor Shri.N.P.V. Ramasamy Udayar Research Fellowship provided by Sri Ramachandra Institute of Higher Education and Research.

REFERENCES

[1] Kaufman J, Dhakal M, Patel B, Hamburger R. Community-acquired acute renal failure. Am J Kidney Dis. 1991;17(2):191-98.
[2] Nash K, Hafeez A, Hou S. Hospital acquired renal insufficiency. Am J Kidney Dis. 2002;39(5):930-36.
[3] Maldonado PD, Barrera D, Medina-Campos ON, Hernández-Pando R, Ibarra-Rubio ME, Pedraza-Chaverrı J, et al. Aged garlic extract attenuates gentamicin induced renal damage and oxidative stress in rats. Life Sci. 2003;73(20):2543-56. Available from: https://linkinghub.elsevier.com/retrieve/pii/S002432050300609X.
[4] Ali BH, Al-Wabel N, Mahmoud O, Mousa HM, Hashad M. Curcumin has a palliative action on gentamicin-induced nephrotoxicity in rats. Fundam Clin Pharmacol. 2005;19(4):473-77. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1472-8206.2005.00343.x.
[5] Koorse KG, Reni J, Surya S, Sujith S, John B, John P, et al. Indian Society of Veterinary Pharmacology and Toxicology (ISVPT). XVI Annual conference of Indian Society of Veterinary Pharmacology and Toxicology (ISVPT) and National symposium on "Animal Health and Production: Challenges & Opportunities in Veterinary P. In: Nephroprotective Potential of Tinospora Cordifolia on Gentamicin Induced Nephrotoxicity in Rats. Navsari. Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science & Animal Husbandry, Navsari Agricultural University. 2016;82.
[6] Sharma M, Pundir J, Vishwakarma P, Goel RK, Saini M, Saxena K. Evaluation of nephrotoxicity activity of Tinospora cordifolia against gentamicin induced nephrotoxicity in albino rats: An experimental study. Int J Basic Clin Pharmacol. 2019;8(6):1179-84.
[7] Department of Ayurveda, Yoga & Naturopathy, Unani S and H (AYUSH). The Ayurvedic Pharmacopoeia of India. Part-I, Vol.1. Ministry of Health and Family Welfare, Government of India. 2001;53-55.
[8] Department of Ayurveda, Yoga & Naturopathy, Unani S and H (AYUSH). The Siddha Pharmacopoeia of India. Part-I, Vol.1. Ministry of Health and Family Welfare, Government of India. 2008;40-42.
[9] Singh B, Nathawat S, Sharma RA. Ethnopharmacological and phytochemical attributes of Indian Tinospora species: A comprehensive review. Arab J Chem. 2021;14(10):103381. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1878535221003968.
[10] Panchabhai TS, Kulkarni UP, Rege NN. Validation of therapeutic claims of Tinospora cordifolia: A review. Phytother Res. 2008;22(4):425-41. Available from: https://onlinelibrary.wiley.com/doi/10.1002/ptr.2347.
[11] Bao YW, Yuan Y, Chen JH, Lin WQ. Kidney disease models: Tools to identify mechanisms and potential therapeutic targets. Zool Res. 2018;39(2):72-86. Available from: http://www.zoores.ac.cn/EN/10.24272/j.issn.2095-8137.2017.055.
[12] Suzuki S, Takamura S, Yoshida J, Shinzawa Y, Niwa O, Tamatani R, et al. Comparison of gentamicin nephrotoxicity between rats and mice. Comp Biochem Physiol Part C Pharmacol Toxicol Endocrinol. 1995;112(1):15-28. Available from: https://linkinghub.elsevier.com/retrieve/pii/0742841395000755.
[13] Perazella MA. Drug-induced nephropathy: An update. Expert Opin Drug Saf. 2005;4(4):689-06.

[14] Banday AA, Farooq N, Priyamvada S, Yusufi ANK, Khan F. Time dependent effects of gentamicin on the enzymes of carbohydrate metabolism, brush border membrane and oxidative stress in rat kidney tissues. Life Sci. 2008;82(9-10):450-59. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0024320507008478.

[15] Baud L, Ardaillou R. Reactive oxygen species: Production and role in the kidney. Am J Physiol Renal Physiol. 1986;251(5):F765-76. Available from: https://www.physiology.org/doi/10.1152/ajprenal.1986.251.5.F765.

[16] Walker PD, Shah SV. Evidence suggesting a role for hydroxyl radical in gentamicin-induced acute renal failure in rats. J Clin Invest. 1988;81(2):334-41. Available from: http://www.jci.org/articles/view/113325.

[17] Freeman BA, Crapo JD. Biology of disease: Free radicals and tissue injury. Lab Invest. 1982;47(5):412-26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6290784.

[18] Grisham MB, McCord JM. Chemistry and cytotoxicity of reactive oxygen metabolites. In: Taylor AE, Matalon S, Ward P B, editor. Physiology of Oxygen Radicals. American Physiological Society. 1986;01-18.

[19] Andreoli SP. Reactive oxygen molecules, oxidant injury and renal disease. Pediatr Nephrol. 1991;5(6):733-42. Available from: http://link.springer.com/10.1007/BF00857888.

[20] Humes HD. Aminoglycoside nephrotoxicity. Kidney Int. 1988;33(4):900-11. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0085253815342563.

[21] Sheridan AM, Fitzpatrick S, Wang C, Wheeler DC, Lieberthal W. Lipid peroxidation contributes to hydrogen peroxide induced cytotoxicity in renal epithelial cells. Kidney Int. 1996;49(1):88-93. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0085253815592971.

[22] Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984;21:130-32. Available from: http://nopr.niscair.res.in/handle/123456789/19932.

[23] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-58. Available from: https://linkinghub.elsevier.com/retrieve/pii/0003269779907383.

[24] Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta Gen Subj. 1979;582(1):67-78. Available from: https://linkinghub.elsevier.com/retrieve/pii/0304416579902897.

[25] Robinson T. The determination of proteins in plant extracts that contain polyphenols. Plant Sci Lett. 1979;15(3):211-16. Available from: https://linkinghub.elsevier.com/retrieve/pii/0304421179901123.

[26] Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG, et al. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 1973;179(4073):588-90. Available from: https://www.science.org/doi/10.1126/science.179.4073.588.

[27] Sinha AK. Colorimetric assay of catalase. Anal Biochem. 1972;47(2):389-94. Available from: https://linkinghub.elsevier.com/retrieve/pii/0003269772901327.

[28] Priya LB, Baskaran R, Elangovan P, Dhivya V, Huang CY, Padma VV, et al. Tinospora cordifolia extract attenuates cadmium-induced biochemical and histological alterations in the heart of male Wistar rats. Biomed Pharmacother. 2017;87:280-87. Available from: https://linkinghub.elsevier.com/retrieve/pii/S075333221632162X.

[29] Torres AM, Rodriguez JV, Ochoa JE, Elias MM. Rat kidney function related to tissue glutathione levels. Biochem Pharmacol. 1986;35(19):3355-58. Available from: https://www.sciencedirect.com/science/article/abs/pii/0006295286904351.

[30] Nath KA, Norby SM. Reactive oxygen species and acute renal failure. Am J Med. 2000;109(8):665-78. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0002934300006124.

[31] Adil M, Kandhare AD, Dalvi G, Ghosh P, Venkata S, Raygude KS, et al. Ameliorative effect of berberine against gentamicin-induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation, apoptosis and mitochondrial dysfunction. Ren Fail. 2016;38(6):996-1006. Available from: http://www.tandfonline.com/doi/full/10.3109/0886022X.2016.1165120.

[32] Ali BH, Mousa HM. Effect of dimethyl sulfoxide on gentamicin-induced nephrotoxicity in rats. Hum Exp Toxicol. 2001;20(4):199-03. Available from: http://journals.sagepub.com/doi/10.1191/096032701678766859.

[33] Ramsammy LS, Josepovitz C, Ling KY, Lane BP, Kaloyanides GJ. Failure of inhibition of lipid peroxidation by vitamin E to protect against gentamicin nephrotoxicity in the rat. Biochem Pharmacol. 1987;36(13):2125-32. Available from: https://linkinghub.elsevier.com/retrieve/pii/0006295287901407.

[34] Sathyanarayana B. Evaluation of nephroprotective and anti-nephrotoxic properties of muniprabha plus tablet. Int J Res Ayurveda Pharm. 2018;9(6):64-70.

[35] Acharya C, Thakar H, Vajpeyee S. A study of oxidative stress in gentamicin induced nephrotoxicity and effect of antioxidant vitamin C in Wistar rats. Natl J Physiol Pharm Pharmacol. 2013;3(1):14-20. Available from: http://www.scopemed.org/fulltextpdf.php?mno=22332.

[36] Ozer JS, Dieterle F, Troth S, Perentes E, Cordier A, Verdes P, et al. A panel of urinary biomarkers to monitor reversibility of renal injury and a serum marker with improved potential to assess renal function. Nat Biotechnol. 2010;28(5):486-94. Available from: https://www.nature.com/articles/nbt.1627.

[37] Qiu Y, Hong M, Li H, Tang N, Ma J, Hsu CH, et al. Time-series pattern of gene expression profile in gentamycin-induced nephrotoxicity. Toxicol Mech Methods. 2014;24(2):142-50. Available from: https://doi.org/10.3109/15376516.2013.869780.

[38] Udupa V, Prakash V. Gentamicin induced acute renal damage and its evaluation using urinary biomarkers in rats. Toxicol Rep. 2019;6:91-99. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2214750018304165.

[39] Martínez-Salgado C, Eleno N, Tavares P, Rodríguez-Barbero A, García-Criado J, Bolaños JP, et al. Involvement of reactive oxygen species on gentamicin-induced mesangial cell activation. Kidney Int. 2002;62(5):1682-92. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0085253815487241.

[40] Mathew S, Kuttan G. Antioxidant activity of Tinospora cordifolia and its usefulness in the amelioration of cyclophosphamide induced toxicity. J Exp Clin Cancer Res. 1997;16(4):407-11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9505214.

[41] Shamsuyarova A. Overexpression of catalase prevents gentamicin – induced apoptosis of renal proximal tubular cells in transgenic mice. Université de Montréal. 2015. Available from: http://hdl.handle.net/1866/12058.

[42] Sivakumar V, Rajan MSD. Antioxidant effect of Tinospora cordifolia extract in alloxan-induced diabetic rats. Indian J Pharm Sci. 2010;72(6):795-98. Available from: http://www.ijpsonline.com/text.asp?2010/72/6/795/84600.

[43] Griffith OW, Meister A. Glutathione: Interorgan translocation, turnover, and metabolism. Proc Natl Acad Sci USA. 1979;76(11):5606-10. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.76.11.5606.

[44] Goligorsky MS, Morgan MA, Lyubsky S, Gross RW, Adams DT, Spitz DR, et al. Establishment of a hydrogen peroxide resistant variant of renal tubular epithelial cells: Role of calcium-independent phospholipase A2 in cell damage. Arch Biochem Biophys. 1993;301(1):119-28. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0003986183711239.

[45] Reddy N, Reddy RN. Tinospora cordifolia chemical constituents and medicinal properties: A review. Sch Acad J Pharm. 2015;4(8):364-69. Available from: https://www.saspublishers.com/media/articles/SAJP-48364-369.pdf.

[46] Fauconneau B, Tallineau C, Huguet F, Piriou A. Gentamicin-induced kidney damage and lipid peroxidation in rats. Toxicol Lett. 1995;76(2):127-34. Available from: https://linkinghub.elsevier.com/retrieve/pii/037842749403205L.

[47] Randjelovic P, Veljkovic S, Stojiljkovic N, Sokolovic D, Ilic I. Gentamicin nephrotoxicity in animals: Current knowledge and future perspectives. EXCLI J. 2017;16:388-99. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28507482.

[48] Reddy SS, Ramatholisamma P, Karuna R, Saralakumari D. Preventive effect of Tinospora cordifolia against high-fructose diet-induced insulin resistance and oxidative stress in male Wistar rats. Food Chem Toxicol. 2009;47(9):22242-29. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0278691509002828.

[49] Khanam S, Mohan NP, Devi K, Sultana R. Protective role of Tinospora cordifolia against cisplatin- induced nephrotoxicity. Int J Pharm Pharmaceut Sci. 2011;3(4):16-18.

[50] Uppuluri S, Ali SL, Nirmala T, Shanthi M, Sipay B, Uppuluri KB, et al. Nephroprotector activity of hydro alcoholic extract of Tinospora cordifolia roots on cisplatin induced nephrotoxicity in rats. Drug Invent Today. 2013;5(4):281-87. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0975761913000719.

PARTICULARS OF CONTRIBUTORS:

1. PhD Scholar, Department of Anatomy, Sri Ramachandra Medical College, Sri Ramachandra Institute of Higher Education and Research (DU), Chennai, Tamil Nadu, India.
2. Professor, Department of Anatomy, Sri Ramachandra Medical College, Sri Ramachandra Institute of Higher Education and Research (DU), Chennai, Tamil Nadu, India.
3. Professor, Department of Pathology, Sri Ramachandra Medical College, Sri Ramachandra Institute of Higher Education and Research (DU), Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Senthil Kumar Sampath Kumar,
Professor, Department of Anatomy, Sri Ramachandra Medical College, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai-600116, Tamil Nadu, India.
E-mail: senthilkumar.s@sriramachandra.edu.in

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Apr 21, 2022
- Manual Googling: Aug 02, 2022
- iThenticate Software: Aug 04, 2022 (13%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? No
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Apr 16, 2022**
Date of Peer Review: **May 22, 2022**
Date of Acceptance: **Aug 05, 2022**
Date of Publishing: **Oct 01, 2022**