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Research Article

Nephroprotective effects of *Tinospora cordifolia* against carboplatin-induced renal toxicity through oxidative stress modulation and GC-MS-based identification of bioactive compounds

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ABSTRACT

Carboplatin induced nephrotoxicity though life threatening with patients being treated symptomatically, has no specific drugs available for management. The current research was conducted to compare the nephroprotective potential of different doses of ethanolic leaf extract of *Tinospora cordifolia* (TC) against carboplatin-induced nephrotoxicity. 36 rats were divided into 6 groups (n=6). Groups 1 and 2 were given 1% sodium carboxymethylcellulose (CMC) 1ml/kg/day orally and TC extract, 400 mg/kg/day orally for 14 days respectively. Carboplatin 90 mg/kg intraperitoneal single dose was administered to group 3 on day 5. TC extract, 200, 400, and 600 mg/kg/day were administered to groups 4, 5, and 6 respectively for 14 days along with carboplatin on day 5. Serum was collected on days 0, 7, and 14 to evaluate renal function. Renal tissue was used to estimate antioxidant levels and for histopathological investigations. Results were analyzed using ANOVA followed by Tukey's test and paired t-test. A significant ($p<0.05$) increase was seen in serum urea, creatinine and oxidative stress markers and reduction in antioxidants, catalase, total thiol, in the carboplatin-treated group as compared to control. All three doses of the ethanolic leaf extract of TC exhibited significant nephroprotective effects against carboplatin-induced nephrotoxicity, likely through antioxidant and anti-inflammatory mechanisms. This was supported by decreased levels of blood urea and serum creatinine, along with histopathological evidence of renal tissue recovery.

Keywords:
Antineoplastic agents; Antioxidants; Nephroprotective; Oxidative stress; *Tinospora cordifolia*

1. INTRODUCTION

One in six fatalities is attributable to cancer, making it one of the second leading causes for nearly nine million deaths globally. These patients now have greater control and survival rates owing to modern cancer care developments such as targeted drug therapy, radiotherapy, immunotherapy, and aggressive and minimally invasive surgery. Nevertheless, with these advancements, a major issue restricting the efficacy of cancer treatment is the toxicity of anticancer medications[1]. Among the various anticancer drug toxicities, damage to the kidneys can alter blood electrolyte levels and affect the process of urine production. Patients with renal insufficiency are particularly susceptible to the nephrotoxic effects of medicines. Though they have been insensitive in identifying early renal impairment, renal serum markers

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such as blood urea nitrogen and serum creatinine are indicators of renal toxicity and dysfunction[2].

Doxorubicin, vinblastine, carboplatin, cisplatin, and methotrexate are known for causing nephrotoxicity in treated patients. High blood pressure, tubulopathies brought on by electrolyte imbalances, proteinuria from glomerulopathy, acute kidney disease (AKD) due to renal tubule necrosis, and chronic kidney disease (CKD) are some of the most common clinical nephrotoxic side effects of anticancer drugs[1]. Even with the recent advances in our understanding of the mechanisms underlying this toxicity, drug dose adjustments and symptom relief continue to be the primary components of treatment. Data on medications used to treat nephrotoxicity induced by chemotherapy is scarce. Therefore, it is necessary to actively assess individuals on anticancer medications for renal impairment[3].

Currently used to treat ovarian, head, neck, and lung cancers, carboplatin is a second-generation platinum-containing antineoplastic drug[4, 5]. Because of increased reactive oxygen species influx, impaired renal antioxidant enzyme activity, and decreased protein and glutathione expression in the kidneys, high dosages of carboplatin can be damaging to the renal system[6]. They can also lower the glomerular filtration rate and cause substantial quantities of platinum–DNA adduct to persist within the kidneys[7].

In the classical ayurveda medical system, *Tinospora Cordifolia* (TC), commonly referred to as Guduchi, has been used therapeutically to treat several ailments, such as pyrexia, jaundice, cancer, snake bites, fractures, pains, asthma, dermatological diseases, poisonous insect bites, chronic diarrhoea, and eye disorders[8,9]. Previous investigations indicate that the secondary metabolites of this plant block the SARS-CoV2 major protease, perhaps serving as an antidote. During the COVID-19 epidemic, TC was widely used due to the newly acquired knowledge of its uses[10]. TC is an extensively studied medicinal plant recognized for its diverse pharmacological activities. Extracts derived from the whole plant have been reported to exhibit antiulcer, antidiarrhoeal, analgesic, immunomodulatory, antioxidant, gastroprotective, nootropic, cardioprotective, and hepatoprotective properties. The stem extract has demonstrated antidyslipidemic, anti-inflammatory, radioprotective, hypoglycemic, osteoprotective, antifertility, antiasthmatic, neuroprotective, and antimalarial effects. Additionally, extracts from the aerial parts have shown potential neuroprotective and antineoplastic activities. Although the root extract has been shown to confer nephroprotective benefits, the leaf extract remains relatively unexplored in this regard[10,11]. Considering its ease of harvest and wider availability, phytochemical and pharmacological properties the current study aims to evaluate the nephroprotective potential of TC ethanolic leaf extract on carboplatin-induced kidney damage in male Wistar rats.

2. MATERIALS AND METHODS

2.1. Animals, reagents and study design

The study was conducted following approval by the Institutional Animal Ethics Committee (IAEC/KMC/92/2022). The Central Animal House of the Institution housed the animals as per the CCSEA guidelines. Male Wistar rats (n=36) weighing 240 g, aged around 8 weeks were taken from the Central animal research facility, Manipal. The animals were maintained under well-ventilated conditions with 25±3 °C temperature, 12-hour light and 12-hour dark cycles, 50% humidity. Rats were fed with standard food pellets and water. Three animals per group were kept in polypropylene cages comprising sterile paddy husk (which was procured locally) used as bedding during the study and standard laboratory food granules (VRK Nutritional Solutions, Pune, India) were provided along with water ad libitum.

2.2. Drugs and reagents

Carboplatin (Kemocarb®) 150 mg/15 ml two vials, manufactured by Fresenius Kabi Oncology Ltd. was purchased from Radha Medicals, Manipal. The reagents were purchased from Med source (Medsource Ozone Biomedicals Pvt. Ltd.). 1% sodium CMC was used as vehicle. The experimental TC leaves were obtained, and the plant was authenticated by a botanist at Mahatma Gandhi Memorial College, Udupi.

2.3. Preparation of plant extract

TC leaf powder was continuously extracted along with ethanol using a Soxhlet apparatus. Filter paper was used to pack 50 g of dried leaf powder before it was kept at the thimble of the Soxhlet apparatus. After setting up the equipment, 300 ml of 70% ethanol was put into the flask and the process was carried out at 30 ºC±1 ºC temperature. The resulting ethanolic extract was dried under reduced pressure at a room temperature of not more than 40 ºC and it was stored in a desiccator for further use[12].

$$\text{Percentage yield} = \frac{\text{Weight of TC extract} - \text{weight of empty china dish}}{\text{Weight of powdered drug}} \times 100$$

The percentage yield obtained using the above formula was 44% (w/w)[13].

2.4. Gas chromatography–mass spectrometry (GC-MS) analysis

The sample of the ethanolic extract was analyzed using gas chromatography (GC) coupled with Shimadzu Mass Spectrometer (MS), (GC/MS—QP2020 NX

SHIMADZU, ShimadzuCorp. Tokyo, Japan). The GC equipped with capillary column SH-I-5Sil MS Capillary, 30 mm x 0.25 mm x 0.25 mm was used for chromatographic analysis. The injector temperature was 250 ºC (split less injector mode), with a column flow of 1.69 mL/min (Helium gas), and total flow is 50 mL/min. The oven temperature program was set at an initial temperature of 50 °C held for 1 min, followed by an increase up to 140 °C at the rate of 20 °C/min, which was further increased at the rate of 10 ºC up to 280 ºC with a hold time of 3 min[14].

2.4.1 MS Parameters:

- Library used NIST Version-2011
- Interface temperature 290 °C
- Ion Source temperature 170 °C
- Mass scan (m/z) 60-350 amu, with scan speed 625
- Solvent cut time 4 min
- Total MS running time: 22.50 min.

2.4.2 Experimental procedure

A total of 36 rats were used in the experiment, and they were split up into 6 groups of 6 rats each. All the groups were treated for 14 days as shown in Table 1. The doses of the extract are based on the previous studies[3,7]

2.4.3 Collection of kidney tissues and blood samples:

Retro orbital blood collection from inner canthus of the eye using capillary tubes was done and the serum was collected for estimation of kidney function tests using suitable kits on day 0, 7 and 14. Both the kidneys were collected through dissection, on day 14 for weighing, biochemical estimation, and histopathological analysis. The dissected kidneys were rinsed in physiological saline and homogenized in Phosphate buffered solution (pH 7.4). The homogenates were centrifuged (2000 rpm for 20 min), and the supernatants were decanted and used for antioxidant estimation[15].

2.4.4 Parameters measured:

Body weights of rats belonging to different groups were measured on day 0 and 14. On day 14, rats were euthanized, kidneys dissected, and the weight of both kidneys were measured. Kidney weight to body weight ratio was measured by the formula-

Ratio=Sum of both the kidney weights ×100/Body weight[16]

Table 1. Animal groups, drugs, and dose schedules

Group	Drugs	Dose and route
1	Control	1% sodium CMC vehicle 1ml/kg/day orally for 14 days
2	Drug control (TC)	TC:400mg/kg/day extract orally for 14 days
3	Carboplatin	Carboplatin:90 mg/kg i.p single dose on day 5
4	Carboplatin+ low dose TC	TC:200mg/kg/day extract orally for 14 days Carboplatin:90mg/kg i.p single dose on day 5
5	Carboplatin+ mid dose TC	TC:400mg/kg/day extract orally for 14 days Carboplatin:90mg/kg i.p single dose on day 5
6	Carboplatin+ high dose TC	TC:600mg/kg/day extract orally for 14 days Carboplatin:90 mg/kg i.p single dose on day 5

2.5. Biochemical estimations

2.5.1 Serum urea estimation

In the Berthelot Method the samples were pipetted into the test tubes labelled Blank, Standard and Test. For the blank test tube, add 1ml of working reagent mix and incubate it for 3 minutes at 37 ºC followed by 1 ml of alkaline buffer. For the test tube labelled standard, add 1ml of working reagent and 10 microlitre of urea standard and incubate it for 3 minutes at 37 ºC followed by 1ml of alkaline buffer. For the test tube labelled test, add 1ml of working reagent and 10 microlitre of serum samples and incubate it for 3 minutes at 37 ºC followed by 1 ml of alkaline buffer. After adding the above reagents to test tubes respectively, mix and incubate it for five min at 37 ºC. Mix and read the absorbance of Standard and Test against reagent Blank at 578 nm (Berthelot's kit method).

2.5.2 Serum Creatinine estimation

The Modified Jaffe's Kinetic Method was employed in which the samples were pipetted into the test tubes labelled Standard and Test. Creatinine reagent

1 ml and creatinine standard 100 microlitre were added to the test tube labelled standard. For the test tube labelled test, add 1 ml of creatinine reagent and 100 microlitre of serum samples. Mix well and keep at room temperature for 5 min. Mix well and read the absorbance of Standard against distilled water at 520 nm[17].

2.6. Oxidative stress markers determination:

2.6.1. Nitric oxide

Naphthyl-ethylenediamine to produce a chromophore, which is measured at 540 nm. The Griess Reagent assay involves addition of 500 microlitre of Griess reagent in the test tube labelled test along with 50 microlitre of tissue supernatant and 450 microlitre of distilled water. For the test tube labelled blank, add 500 microlitre of Griess reagent with 500 microlitre of distilled water. Optical density was taken at 550nm[18].

2.6.2. Malondialdehyde

Take 100 microlitre of test sample into a test tube, add 1000 microlitre of 0.67% Thiobarbituric acid, 500 microlitre 20% Trichloroacetic acid to it. Incubate at 100 °C for 20 min; use marble while incubation and cool under running tap water. Transfer the content to an Eppendorf tube, centrifuge at 12,000 rpm for 5 min. The absorbance of supernatant was taken at 532 nm against water blank[19].

2.7. Antioxidant markers

2.7.1. Catalase

2 ml of phosphate buffer was pipetted into a cuvette, at 240 nm. 50 microlitre of catalase solution were added, mixed and 100 microlitre of 3% hydrogen peroxide added. For every 10 seconds the value was noted at 240 nm[20].

2.7.2. Total Thiol

In the Ellman's method**,** pre-incubate PBS (Phosphate Buffer Solution), DTNB and 0.2 M Na_2HPO_4 (Disodium hydrogen phosphate) containing 2 mM EDTA (Ethylenediaminetetraacetic acid) at 37 ºC before the assay. Take 3 tubes for Reagent Blank (RB), Test (T) and Sample Blank (SB). For the test tube labelled RB, pipette out 900 microlitre of 0.2 M Na_2HPO_4 containing 2 mM EDTA, 100 microlitre PBS, 20 microlitre 10 mM DTNB. For the test tube labelled SB, add 920 microlitre of 0.2 M Na_2HPO_4 containing 2 mM EDTA, 100 microlitre of serum sample. For the test tube labelled T, add 900 microlitre of 0.2 M Na_2HPO_4 containing 2 mM EDTA, 20 microlitre 10 mM DTNB, 100 microlitre of serum sample. The contents in each of the tubes labelled as RB, SB and T were mixed in a vortex mixture, warmed to 37 ºC for 5 min and transferred to a cuvette and absorbance was measured at the end of 5 min at 412 nm. The reaction was completed in 5 min during which there was an increase in the absorbance. The maximum value was recorded[21].

2.8. Histopathological analysis:

Rats were euthanized by ketamine overdose after the fourteenth day. Following perfusion with buffered saline to remove blood, kidneys were fixed using 10% neutralized buffered formalin for histopathological analysis[22].

The kidney tissue was examined using specimens embedded in paraffin and qualitative analysis was done. The qualitative analysis involved noting the presence or absence and the nature of certain features: Normal Kidney Structure: Observing if elements like glomeruli, Bowman's capsules, and tubules appeared normal.

Signs of Damage: Identifying specific indicators of injury in the carboplatin-treated group, such as:

- Casts within the tubular lumen.
- Desquamated tubular epithelium (shedding of cells lining the tubules).
- Granular debris in the tubular lumen.
- Renal glomeruli atrophy (wasting away of the kidney's filtering units).

Signs of Improvement: Noting the reduction or absence of these damaging features in the TC-treated groups, for example, observing "nil cast/desquamation/debris in tubular lumen". We also qualitatively assessed "slight increase," "moderate increase," or "significant increase in cellularity in the glomeruli"[23].

Using the conventional technique, sections were stained with haematoxylin and eosin (H&E). Following incubation, tissue sections were allowed to cool before being immersed in xylene for 30 min in order to separate out wax. Sections were hydrated using graded series of decreasing alcohol concentrations (e.g., 100%, 90%, 70%, and 50% ethanol for 5 min each) prior to washing in distilled water. Haematoxylin was used for staining after 5 min of washing in distilled water. For bluing, sections were cleaned with tap water. Eosin staining was done. Dehydration was accomplished using a graded series of increasing alcohol concentrations (e.g., 50%, 70%, 90%, and 100% ethanol for 5 min each). After employing xylol for 5 to 10 min, DPX (dibutyl phthalate polystyrene xylene) was employed to mount it beneath a cover slip. H&E-stained tissues were examined using a light microscope with a 10x magnification after the slides had dried.

2.9. Statistical analysis

The statistical analysis was performed using the SPSS Version 20 software and analyzed with One Way ANOVA between the groups followed by Post hoc Tukey's Multiple comparison test. Paired t-test was used to compare the results between day 0 and day 7 or 14 of each group. Probability values $p \leq 0.05$ were considered significant. The data was expressed as Mean± SD.

3. RESULTS

3.1. Phytochemical screening and GCMS analysis

The results of preliminary phytochemical screening of ethanolic leaf extract of TC showed presence of tannins, saponins, alkaloids, flavonoids, carbohydrates, and proteins. On GC-MS analysis, a total of 137 compounds were identified by the library (detail peak integration) (available as supplementary document). Further, automatic integration of top 25 peaks (based on height) was extracted. Data of the compounds showing the highest peak intensity in the ethanolic extract of TC as depicted in Table 2.

The corresponding chromatogram is depicted in Figure 1.

Figure 2 represents the most important phytochemical derivatives identified from the methanolic extract. The present analysis showed the highest areas and peaks for hexadecenoic acid or palmitic acid, its methyl ester form and octadecanoic acid. This is in concordance with previous reports[24, 25].

Among the identified compounds, the retention time and highest peaks were seen with n-hexadecanoic acid (23.06%, 13.507) , DL-proline 5-oxo-methyl ester (0.91%, 8.173), 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione Benzothiazole, 2-(2-hydroxyethylthio) (3.51%,12.946), Hexadecanoic acid, ethyl ester (4%, 13.762), 2,4-Di-tert-butylphenol (0.19%,18.717), phytol (1.81%,14.874) , 9,12,15-Octadecatrienoic acid (6.43%,15.126), Octadecanoic acid (13.54%, 15.360), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl (6.21%, 18.298), and Hexatriacontane (0.47%,18.531).

Table 2. List of 20 compounds showing highest peak

Sl No	Compound Name	RT	m/z	Area	Height
1	5-Amino-1-methyl-1H-pyrazole-4-carboxamide, 3TMS	6.789	341	1049248	610351
2	Cetene	7.692	69	354872	191642
3	DL-Proline, 5-oxo-, ethyl ester	8.172	84	5529993	2807411
4	2,4-Di-tert-butylphenol	8.829	191	10001328	5560377
5	Tetradecanoic acid	11.405	73	1138027	377542
6	2-Phenyl-1,2-propanediol, 2TMS derivative	11.65	73	920376	452833
7	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	12.945	205	3096033	1433942
8	Benzothiazole, 2-(2-hydroxyethylthio)-	13.327	167	1906655	886597
9	**n-Hexadecanoic acid**	**13.502**	**73**	**40760782**	**8075192**
10	3,4,4'-Triaminodiphenylsulfone	13.682	263	1008819	352354
11	**Hexadecanoic acid, ethyl ester**	**13.763**	**88**	**6785493**	**3424938**
12	Phytol	14.874	71	3996385	1780612
13	9,12,15-Octadecatrienoic acid, (Z, Z, Z)-	15.126	79	6111034	2052376
14	**Octadecanoic acid**	**15.359**	**73**	**14162840**	**4868121**
15	Octadecanoic acid, ethyl ester	15.621	88	1635070	782508
16	Glutarimide, N-(2-octyl)-	15.896	114	3814626	1754353
17	N-Isopropyl-2-methyl-1-(2-methylbutyl)-4-(methylsulfonyl)-6-oxopiperazine-2-carboxamide	16.849	179	1417009	691047
18	2-Methyl-4-trihexylsilyloxyoct-5-yne	17.414	253	915266	439037
19	**Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester**	**18.299**	**98**	**7499658**	**2607833**
20	4,4'-((p-Phenylene) diisopropylidene) diphenol	19.454	331	1547519	705515
21	Octadecanoic acid, 2,3-dihydroxypropyl ester	19.902	98	2418575	1002030

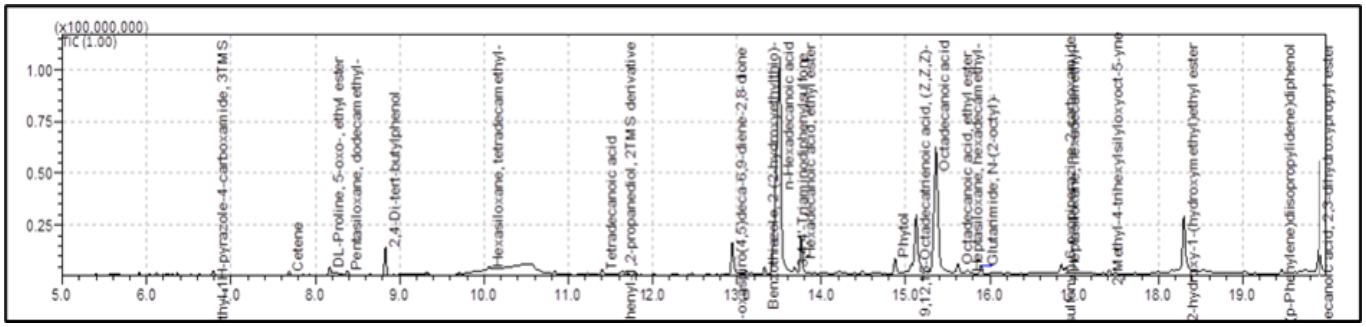

Figure 1. Chromatogram of ethanolic extract of *T. cordifolia*.

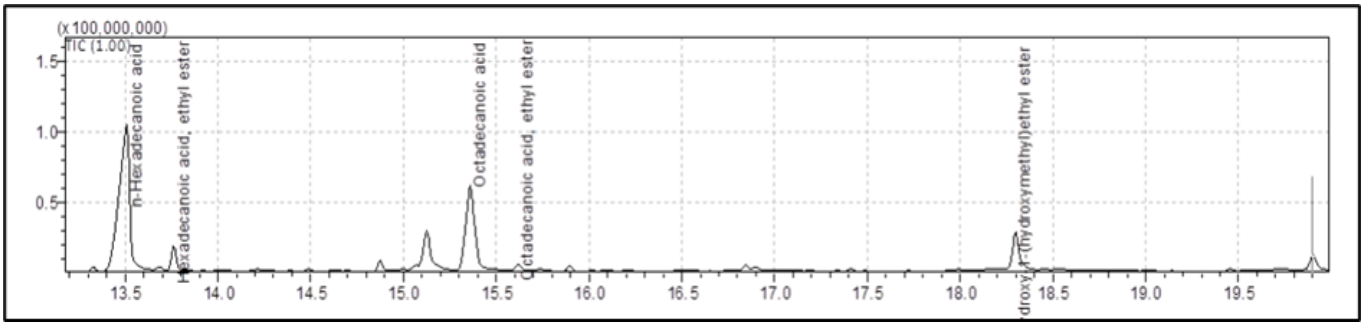

Figure 2. Important phytochemical derivatives from ethanolic extract of *T. cordifolia.*

3.2. Body weight and kidney weight to body weight ratio of rats

There was no significant difference in the weight of animals between the groups on day 0. On day 14, there was a decrease in body weight in the carboplatin-treated group when compared to the control, but the decrease was statistically not significant. There was a significant increase in body weight in Car + TC 200 mg ($p<0.05$) and Car + TC 600 mg ($p<0.05$) groups as compared to carboplatin treated group on day 14 (Table 3).

There is a significant increase in kidney weight to body weight ratio in carboplatin-treated group when compared to the control ($p<0.05$). Administration of TC 200 mg, TC 400 mg, and TC 600 mg caused a significant decrease in kidney weight/body weight ratio compared to carboplatin treated group ($p<0.01$) as shown in Table 3.

3.3. Biochemical estimation:

3.3.1 Serum urea and creatinine

On day 14, carboplatin treated group confirmed significant increase in serum urea and creatinine values as compared to the control ($p<0.05$) as shown in Table 4. Also, on day 7 the carboplatin treated group showed significant increase in serum creatinine as compared to day 0 ($p<0.01$).

On day 14, Car + TC 200 mg and Car+ TC 400 mg groups showed significant reduction in serum urea and creatinine levels as compared to the carboplatin treated group ($p<0.01$). The Car + TC 600 mg group has shown significant decrease in serum creatinine when compared to carboplatin treated group ($p<0.0001$). On day 14 the Car + TC 200 mg group showed a significant decrease in serum creatinine as compared to day 0 ($p<0.01$) as shown in Table 4.

3.4. Oxidative Stress Markers Determination

3.4.1 Nitric oxide and Malondialdehyde

Carboplatin treated group showed a significant increase in nitric oxide and malondialdehyde levels as compared to the control ($p<0.01$). Car + TC 200 mg and Car + TC 400 mg groups showed significant decrease in nitric oxide and malondialdehyde levels as compared to control ($p<0.01$). All the treatment groups except Car+ TC 200 mg group showed a significant decrease in nitric oxide and malondialdehyde levels when compared to carboplatin treated group ($p<0.0001$). Car + TC 400 mg and Car + TC 600 mg groups showed a significant decrease in nitric oxide and malondialdehyde levels when compared to Car + TC 200 mg group ($p<0.01$) as shown in Table 5.

3.5. Antioxidants

3.5.1 Catalase and Thiol levels

Thiol levels and catalase activity were significantly lower in the carboplatin-treated group than

in the control group ($p<0.05$). Car + TC 200 mg, Car + TC 400 mg, and Car + TC 600 mg groups showed a significant increase in thiol levels and catalase activity as compared to the carboplatin treated group ($p<0.05$). There is a significant increase in catalase activity in the Car + TC 200 mg group as compared to the control ($p<0.0001$). There is also a significant increase in catalase activity in the Car + TC 400 mg and Car + TC 600 mg groups as compared to the Car + TC 200 mg group ($p<0.01$). The total thiol levels in the Car + TC 600 mg group show a significant increase as compared to Car + TC 200 mg group ($p<0.01$) as shown in Table 6.

Table 3. Body weight of rats of different groups on day 0 and day 14; Kidney weight to body weight ratio of different groups on day 14.

GROUPS	Body Weight(g) on day 0	Body Weight (g) on day 14	Kidney Weight to Body Weight Ratio
1.Control	210.8 ±18.31	259.2 ± 23.15	0.39 ± 0.030
2.Drug control (TC)	198.7 ± 17.68	236.3 ± 24.91	$0.36 \pm 0.033^{\#\#}$
3.Carboplatin	228.5 ± 33.82	214.2 ± 35.64	$0.51 \pm 0.073^{*}$
4.Car + TC 200mg	257.0 ± 24.84	$263.3 \pm 25.87^{\#}$	$0.36 \pm 0.059^{\#\#}$
5.Car + TC 400mg	228.8 ± 34.71	246.5 ± 24.96	$0.35 \pm 0.081^{\#\#}$
6.Car + TC 600mg	258.0 ± 25.06	$271.8 \pm 20.92^{\#}$	$0.34 \pm 0.037^{\#\#}$

One way ANOVA on day 14, $p<0.01$; post hoc Tukey test, $^{*}p<0.05$ vs control $^{\#}p<0.05$ vs carboplatin, $^{\#\#}p<0.01$ vs carboplatin. All the data expressed as Mean±SD

Table 4. Serum urea and creatinine levels in different groups on day 0, 7 and 14.

GROUPS	Urea(mg/dL)			Creatinine (mg/dL)		
	day 0	day 7	day 14	day 0	day 7	day 14
1. Control	54.57 ± 14.66	50.76 ± 8.32	48.13 ± 13.05	0.58 ± 0.12	0.57 ± 0.08	0.56 ± 0.16
2. Drug control (TC)	55.41 ± 11.98	53.27 ± 10.94	60.40 ± 7.80	0.50 ± 0.06	0.42 ± 0.08	0.44 ± 0.15
3. Carboplatin	50.25 ± 10.06	58.00 ± 13.37	$78.54 \pm 19.27^{*b}$	0.25 ± 0.12	0.44 ± 0.09^{b}	$0.84 \pm 0.10^{**b}$
4. Car + TC 200mg	42.45 ± 5.38	60.27 ± 17.93	$42.62 \pm 8.41^{\#}$	0.51 ± 0.08	0.57 ± 0.11	$0.34 \pm 0.09^{\#\#b}$
5. Car + TC 400mg	47.56 ± 10.01	50.96 ± 4.55	$45.68 \pm 10.47^{\#}$	0.48 ± 0.12	0.60 ± 0.10	$0.35 \pm 0.14^{\#\#}$
6. Car + TC 600mg	44.87 ± 6.62	67.95 ± 12.77	73.28 ± 30.22	0.47 ± 0.14	0.49 ± 0.16	$0.27 \pm 0.06^{\#\#}$

One way ANOVA on day 14, $p<0.05$; Post Hoc Tuckey test, $^{*}p<0.05$ vs control, $^{\#}p<0.01$ vs carboplatin group, $^{**}p<0.001$ vs control, $^{\#\#}p<0.01$ vs carboplatin. Paired t test $^{b}p<0.05$ vs day 0. All the data expressed as Mean±SD. Paired t test $^{b}p<0.01$ vs day 0. All the data expressed as Mean±SD.

Table 5. Nitric Oxide and Malondialdehyde levels in kidney tissues of different groups

	GROUPS	Nitric Oxide (µM/mg of protein)	Malondialdehyde (µM/L)
1.	Control	1.01± 0.098	0.20± 0.046
2.	Drug control (TC)	$0.94 \pm 0.015^{\#}$	$0.23 \pm 0.035^{\#}$
3.	Carboplatin	$1.52 \pm 0.030^{*}$	$0.72 \pm 0.052^{*}$
4.	Car + TC 200mg	$1.37 \pm 0.061^{*}$	$0.65 \pm 0.045^{*}$
5.	Car + TC 400mg	$1.13 \pm 0.116^{\#a}$	$0.33 \pm 0.057^{*\#a}$
6.	Car + TC 600mg	$1.09 \pm 0.071^{a\#}$	$0.27 \pm 0.059^{a\#}$

One way ANOVA, $p<0.0001$; post hoc Tukey test, $^{*}p<0.01$ vs control, $^{\#}p<0.0001$ vs carboplatin, $^{a}p<0.01$ vs carboplatin + TC 200mg. All the data expressed as Mean±SD.

Table 6. Catalase and Thiol levels in kidney tissues of different groups

	GROUPS	Catalase (Unit/mg protein)	Total Thiol (µM/L)
1.	Control	5.18 ± 0.208	607.5 ± 15.42
2.	Drug control (TC)	4.90 ± 0.095$^{\#}$	603.8 ± 13.33$^{\#}$
3.	Carboplatin	2.59 ± 0.150*	387.8± 18.30*
4.	Car + TC 200mg	4.26 ± 0.360$^{\#*}$	481.8± 48.93$^{\#*}$
5.	Car + TC 400mg	4.83 ± 0.052$^{\#a}$	538.5± 37.36$^{\#*}$
6.	Car + TC 600mg	5.04 ± 0.051$^{\#a}$	569.8± 24.97$^{\#a}$

One way ANOVA, $p<0.05$; post hoc Tukey test, $^{*}p<0.05$ vs control, $^{\#}p<0.05$ vs carboplatin, $^{a}p<0.01$ vs carboplatin + TC 200mg, $^{b}p<0.01$ vs carboplatin + TC 400mg. All the data expressed as Mean±SD.

3.6. Histopathological examinations

In Group 1 (Control) rats as seen in Figure 3, H&E-stained renal cortical sections revealed cut sections of glomeruli, Bowman's capsules, collecting tubules, proximal convoluted tubules, and distal convoluted tubules that appeared normal (blue arrow) with no defective renal cells. In Group 2 drug control (TC) rats as seen in Figure 4, normal kidney structure was visualized (blue arrow), at par with the control (group 1). H&E-stained renal cortical sections revealed normal cut sections of glomeruli, Bowman's capsules, collecting tubules, proximal convoluted tubules, and distal convoluted tubules that appeared normal (blue arrow) with no defective renal cells. In Group 3 Carboplatin-treated group rats as seen in Figure 5, there were signs of nephrotoxicity, including atrophy of renal glomeruli noted (black arrow) and desquamated tubular epithelium forming cast (red arrow). In Group 4 Car + TC 200 mg rats as seen in Figure 6, a slight increase in cellularity is observed in the glomeruli, which is considered a sign of improvement (black arrow). In Group 5 Car + TC 400 mg rats as seen in Figure 7, a moderate increase in cellularity in the glomeruli was observed, marking a sign of improvement (black arrow). In Group 6 Car + TC 600 mg rats as seen in Figure 8, a significant increase in cellularity in the glomeruli was observed, indicating a sign of improvement (blue arrow). This group also showed a normal kidney structure, comparable to the control (group 1). The treatment groups, Car + TC 200 mg showed slight increase, Car + TC 400 mg showed moderate increase, Car + TC 600 mg showed significant increase in cellularity in the glomeruli alongside nil cast/desquamation/debris in tubular lumen in groups 4, 5 and 6 which is a mark of improvement.

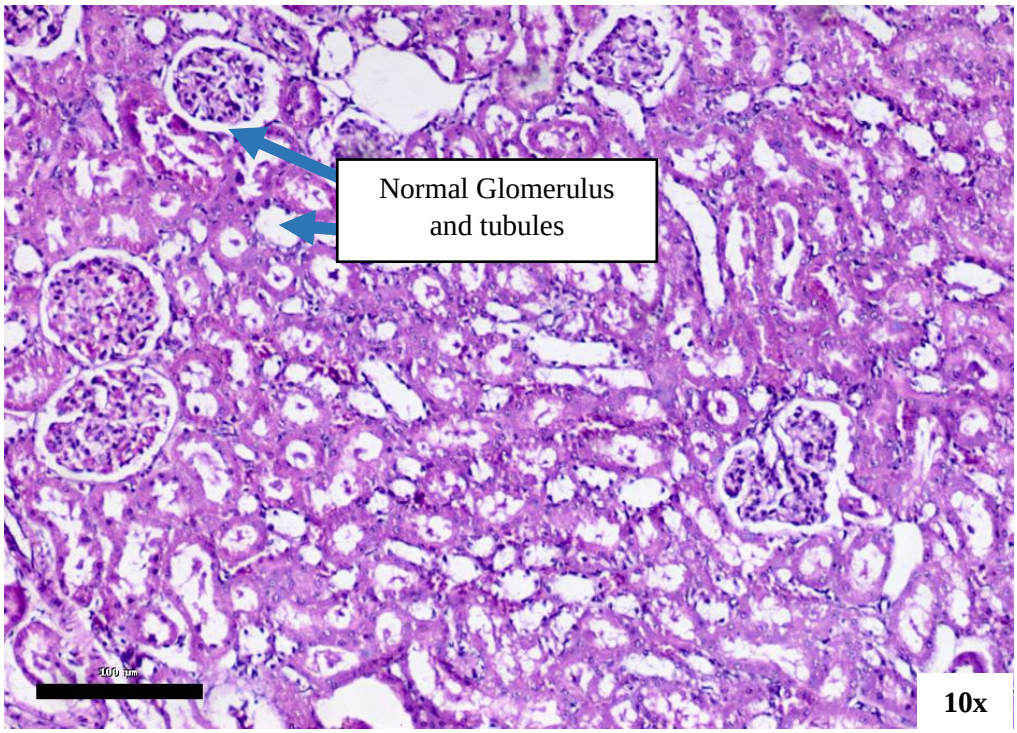

Figure 3. Microphotograph of rat kidney stained by hematoxylin and eosin [Magnification: 10x] Group 1 (Control)

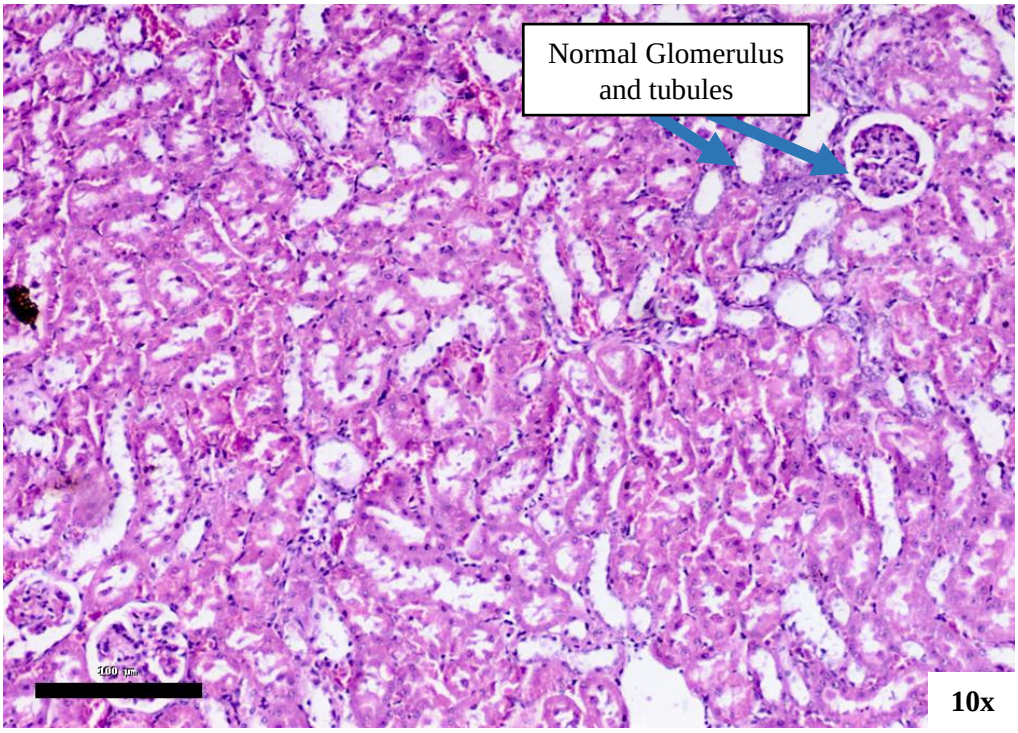

Figure 4. Microphotograph of rat kidney stained by hematoxylin and eosin [Magnification: 10x] Group 2 - Drug control (TC)

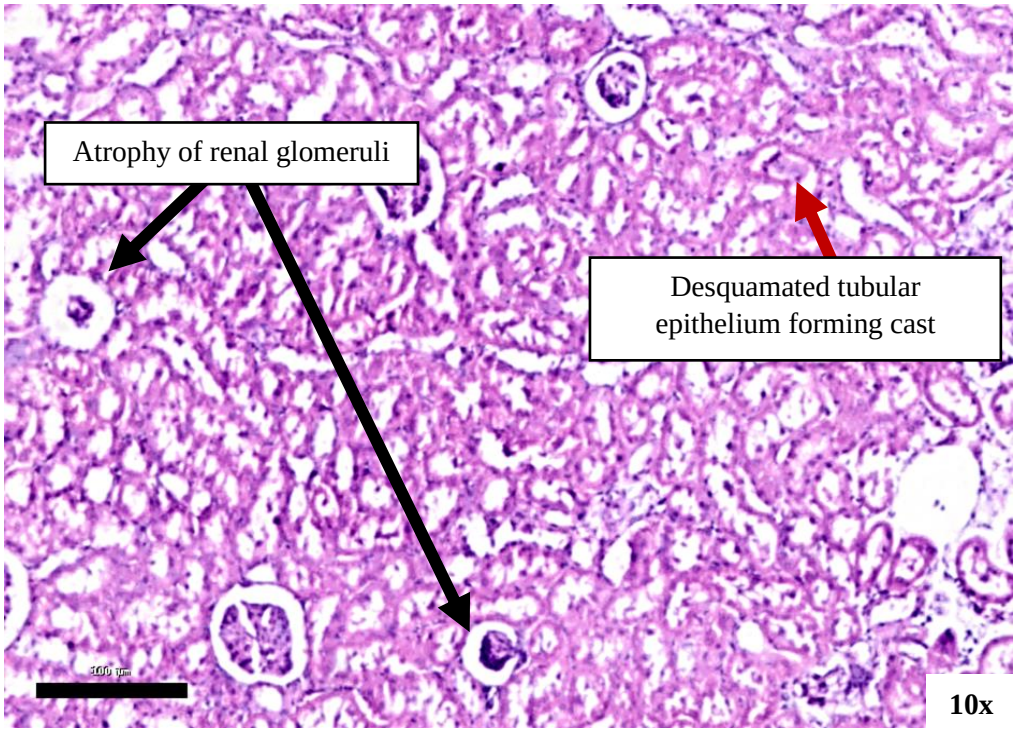

Figure 5. Microphotograph of rat kidney stained by hematoxylin and eosin [Magnification: 10x] Group 3 - Carboplatin

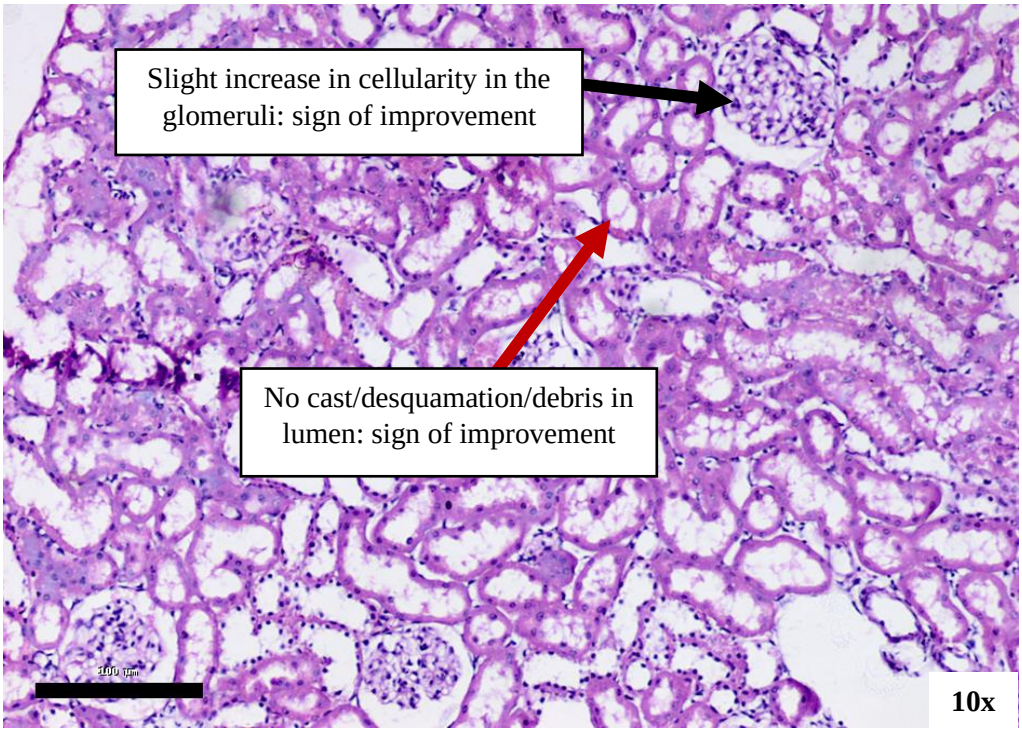

Figure 6. Microphotograph of rat kidney stained by hematoxylin and eosin [Magnification: 10x] Group 4 – Car + TC 200 mg

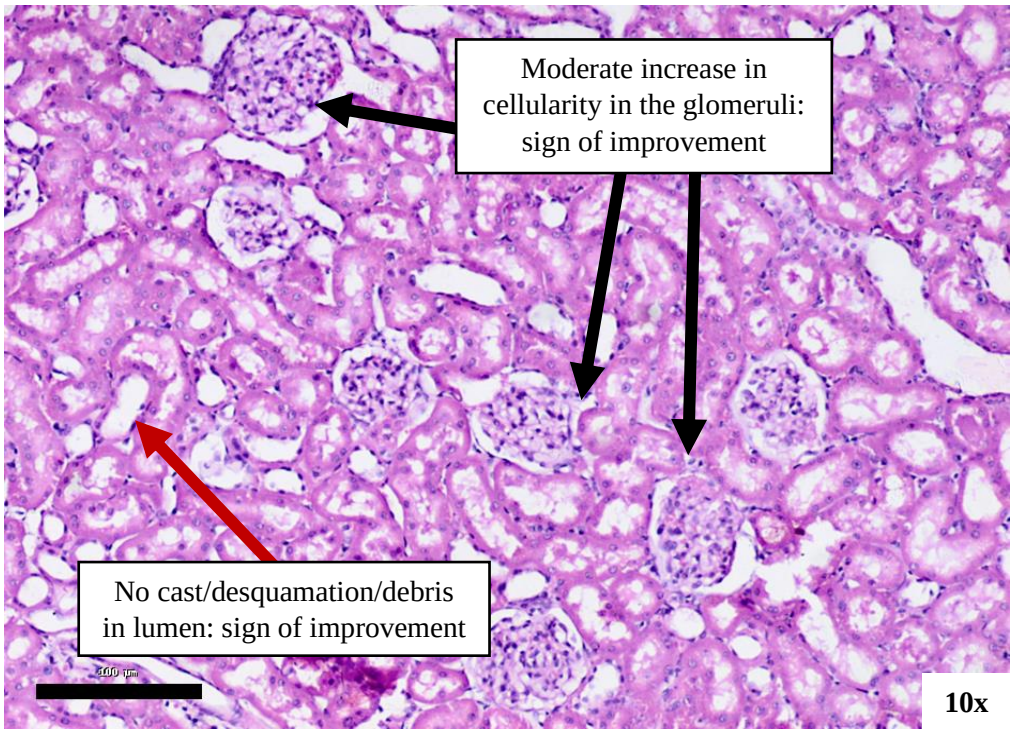

Figure 7. Microphotograph of rat kidney stained by hematoxylin and eosin [Magnification: 10x] Group 5 – Car + TC 400 mg

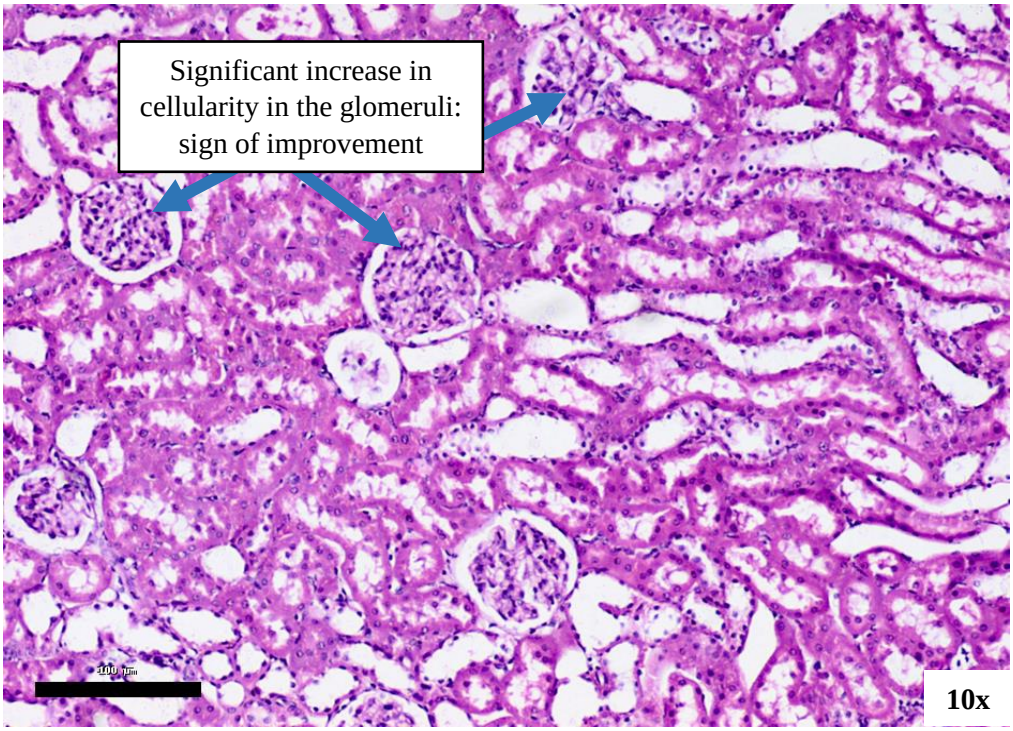

Figure 8. Microphotograph of rat kidney stained by hematoxylin and eosin [Magnification: 10x] Group 6 - Car + TC 600 mg

4. DISCUSSION

In the current investigation, carboplatin caused nephrotoxicity by raising the levels of serum creatinine and blood urea in male Wistar rats on days 7 and 14. This was consistent with a prior study, where there was an increase in the renal indicators, creatinine and urea, after being induced with carboplatin[5]. Treatment with 200 and 400 mg/kg of the ethanolic leaf extract of TC decreased serum urea level and doses of 200, 400 and 600 mg/kg, also reduced serum creatinine levels relative to the group receiving carboplatin. Ethanolic leaf extract of TC may offer protection against carboplatin-induced nephrotoxicity, as evidenced by the decrease in urea and creatinine in the current study. Similar results were also seen with the aqueous stem extract of TC conducted by Sharma *et al*[26]. The current study depicted that there was a rise in kidney weight to body weight ratio after carboplatin administration suggestive of nephrotoxicity owing to carboplatin-induced tubular damage which was similar to the study conducted by Kabel Ahmed M *et al* [27]. Treatment with TC extract caused a reduction in the kidney weight to body weight ratio indicating its nephroprotective effect.

The administration of carboplatin in this study resulted in a decrease in antioxidants such as catalase and total thiols and an increase in pro-oxidants such as malondialdehyde and nitric oxide. This suggests that carboplatin is causing oxidative stress. Similar findings of carboplatin-induced oxidative stress have been reported in the past, probably because of the enhanced consumption of glutathione by carboplatin metabolites. Additionally, carboplatin causes mitochondrial dysfunction, and the reactive oxygen species it produces activate the proteins p53, p21, and mitogen-activated protein kinase, which leads to tubular damage [28].

Treatment with the ethanolic leaf extract of TC at the dose of 400 and 600 mg/kg demonstrated a decrease in oxidative stress markers malondialdehyde and nitric oxide levels in kidney tissue. TC showed dose dependent increase in activity of antioxidants catalase and thiols in the doses of 200, 400 and 600 mg/kg indicating their antioxidant mechanism in the current study. Previous research on TC showed that the root extracts have an antioxidant effect by raising GSH and catalase levels in kidney tissues while lowering malondialdehyde [3,29]. This is explained by the TC extract's flavonoid and alkaloid content. Tannins and phenolic compounds present in the extract also have free radical scavenging activity which can explain the decrease in nitric oxide and malondialdehyde levels. The extract's proteins and amino acids have an indirect impact on the antioxidant defense system and oxidative damage. All these components can explain the reversal of oxidative stress caused by carboplatin[30].

According to Yusef *et al*. the carboplatin treatment had increased the nitric oxide levels in the kidney tissues due to increase in the lipid peroxidation reaction[31]. Similar findings in this study showed that the carboplatin induction of nephrotoxicity raised the amount of nitric oxide in the renal tissues. Nitric oxide is also a marker of inflammation and hence reduction in levels of nitric oxide by TC is suggestive of its anti-inflammatory effect[32]. Thiols are compounds with

sulfhydryl(-SH) groups which scavenge reactive oxygen species and prevent them from oxidizing the proteins, lipids, and DNA[33]. In the present study carboplatin decreased the total thiols and treatment with the TC ethanolic leaf extract reversed this effect which is comparable to a study conducted on its root extracts with similar findings[34]. The present study also confirmed the higher dose of 600 mg/kg TC extract was significant compared to lower doses of 200 and 400 mg/kg in its antioxidant potential.

Among the identified phytoconstituents by GC-MS, evidence from various literature shows that n-hexadecanoic acid has anti-inflammatory effect[35], DL-proline 5-oxo-methyl ester, 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, Benzothiazole, 2-(2-hydroxyethylthio), Hexadecanoic acid, ethyl ester have antioxidant activity[36,37,38,39], 2,4-Di-tert-butylphenol, phytol, 9,12,15-Octadecatrienoic acid, Octadecanoic acid Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl, Hexatriacontane has both anti-inflammatory and antioxidant activity[39,40,41]. This implies that the reno-protective activity of TC is facilitated by these phytoconstituents.

From Gas column mass spectrometry analysis, it can be reported that inositol, 1-deoxy-, trans-sinapyl alcohol, n-hexadecanoic acid were present in the major amount in methanol stem extract. The findings from this study reveal *Tinospora cordifolia* contains an adequate amount of phenolic and flavonoids content, vital bioactive antioxidant compounds, and a good source of carbohydrates and fibers which potentially adds to the overall value of the plant[42].

Histopathological examinations revealed that there were no defective renal cells in the normal 1% sodium CMC treated group and Car + TC 400 mg group rats. But the administration of carboplatin produced casts, desquamated tubular epithelium and granular debris in the tubular lumen. Renal glomeruli atrophy was also found in the histopathological interpretation, these agreed with an earlier study on carboplatin induced nephrotoxicity by Yousef *et.al* [31]. Treatment with all three doses caused absence of debris or cast in the tubular lumen as compared to the carboplatin group which was predominant at the dose of 600 mg/kg. Nitric oxide and malondialdehyde levels increased while catalase and total thiol levels decreased, indicating oxidative damage as the reason for carboplatin-induced nephrotoxicity. This was reversed by administration of ethanolic leaf extract of TC which was also supported by the histopathological evaluation.

5. CONCLUSION

The ethanolic extract of TC proved nephroprotective against carboplatin induced nephrotoxicity in the current study because of its antioxidant plus anti-inflammatory properties. Histopathology reports confirmed the reversal of oxidative damage by TC which was induced by carboplatin. All three doses of TC 200, 400, 600 mg/kg of ethanolic leaf extract were shown to be effective in correcting the elevated serum indicators urea and creatinine levels that were induced by carboplatin.

TC restored the oxidative stress induced by carboplatin by reducing nitric oxide and malondialdehyde and increasing the antioxidants catalase and total thiols. Research is necessary to determine whether the phytoconstituents in the ethanolic extract of TC possess anti-inflammatory and antioxidant properties. To conclude its nephroprotective efficacy, additional studies including these specific phytoconstituents for longer treatment durations along with investigating their molecular mechanisms of action is needed.

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Data analysis / interpretation – PN, VN, RSP, SS, SV, NH
Drafting manuscript: PN, VN, RSP, SNH, VRB, SS, SV
Critical revision of manuscript: PN, VN, RSP
Statistical analysis: PN, VN, RSP
Admin, technical or material support: VN, RSP
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