Research in Pharmaceutical Sciences, August 2024; 19(4): 376-386
Received: 09-05-2023
Peer Reviewed: 10-07-2023
Revised: 28-07-2023
Accepted: 24-04-2024
Published: 19-08-2024

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Antioxidant and anti-inflammatory activity by modulating IL-6 as a potential mechanism in the nephroprotective and hepatoprotective properties of *Tribulus terrestris*

Neha Shetty[1], Sadhana Holla[2,*], Veena Nayak[2], Vijetha B Shenoy[3], and Rao KG Mohandas[4]

[1]*Global Investigator Support, Clinical Trial Training Services, 29, MSR Vaishnavi, Union Street, Bangalore, India-560001.*
[2]*Department of Pharmacology, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India-576104.*
[3]*Department of Biochemistry, Kasturba Medical College, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India-576104.*
[4]*Department of Basic Medical Sciences, Manipal, Manipal Academy of Higher Education, Manipal, Karnataka, India-576104.*

Abstract

Background and purpose: Carboplatin, a second-generation platinum-containing compound is associated with renal tubular injury and hepatic damage in cancer patients. *Tribulus terrestris* (TT) is widely used in Indian traditional medicine for its anti-inflammatory properties. The present study aimed to evaluate TT's beneficial effects against liver and kidney damage induced by carboplatin.
Experimental approach: *An in-vivo* study was conducted on thirty rats. All the groups, except the control, received intraperitoneal carboplatin 90 mg/kg on day 5; the three treatment groups received TT extract (1 g/kg, 1.25, and 1.5 g/kg) for 14 days. Serum and tissue parameters for liver functions, kidney functions, oxidative stress, and inflammatory marker interleukin 6 were measured along with histopathological assessment.
Findings/Results: TT at 1.5 g/kg on day 14 significantly reduced creatinine and aspartate transaminase levels compared to the carboplatin group. The increase in malondialdehyde levels and decrease in glutathione levels was significantly reversed in the groups treated with TT 1.25 and 1.5 g/kg. Interleukin 6 showed a significant decrease in treatment groups when compared to the carboplatin group. Carboplatin distorted hepatic architecture and caused diffused inflammatory cell infiltration in the peritubular interstitial spaces in the kidney. The histopathological evaluation confirmed that TT extract ameliorated hepatic and kidney damage by restoring to normal architecture.
Conclusion and implications: Aqueous extract of TT demonstrated a therapeutic effect against nephrotoxicity and hepatotoxicity caused by carboplatin. The observed benefits can be attributed to its anti-inflammatory action and antioxidant properties.

Keywords: Interleukin; Liver damage; Nephrotoxicity; Oxidative damage; *Tribulus terrestris*.

INTRODUCTION

Carboplatin is a second-generation platinum coordination complex, used to treat cancers of the head and neck, brain, ovarian cancer, non-small cell carcinoma of the lungs, and seminoma. The efficacy of carboplatin-based chemotherapy for patients with gynecological malignancies is equally effective as cisplatin. It is preferred over cisplatin in cancer therapy treatments due to its lesser adverse effect profile (1). The predominant adverse effects of carboplatin include bone marrow suppression, hypersensitivity, and ototoxicity. There are reports of increased alkaline transferase in 37% and nephrotoxicity in 22-27% of patients treated with carboplatin (2).

*Corresponding author: S. Holla
Tel: +91-8746828049, Fax: +91-8202571927
Email: sadhana.holla@manipal.edu

Access this article online	
	Website: http://rps.mui.ac.ir
	DOI: 10.4103/RPS.RPS_66_23

Hepatotoxicity and nephrotoxicity are attributed to the formation of reactive oxygen species and free radicals causing oxidative damage. Management of carboplatin-induced nephrotoxicity and hepatotoxicity has no specific medication regimen and is managed symptomatically in patients. Carboplatin-induced nephrotoxic side effects are mostly seen with higher dosage regimens. A rat model was used to show carboplatin-induced oxidative renal injury, increased lipid peroxidation, and altered plasma creatinine and blood urea nitrogen levels (3).

Carboplatin causes a mild and temporary increase in blood aminotransferase levels in 50% of patients. Carboplatin-induced liver damage can range in severity from a mild, reversible enzyme rise to sinusoidal obstruction syndrome to acute liver failure. The toxicity results from the chemotherapy-induced effects on tissue necrosis, apoptosis, and inflammation (4). Carboplatin significantly increased liver enzymes and produced marked histopathological changes in the liver in a rat model compared to the control group (5).

As carboplatin is mainly excreted by the renal route, a change in dosage regimen is required in patients with impaired kidney function tests. Hydration, hypertonic saline, diuretics, and the use of alternative chemotherapeutic agents render protection against carboplatin-induced nephrotoxicity to some extent (6). Thus, there is a need for drugs that can prevent carboplatin-induced nephrotoxicity and hepatotoxicity.

Tribulus terrestris (TT) is also referred to as Gokshur (Sanskrit) or Puncture vine (English). It belongs to the family Zygophyllaceae and has been frequently used in Chinese and Indian traditional medicine. The effects of TT include diuretics, anti-hypertensive, antioxidant, anti-inflammatory, and anti-urolithiatic properties. For kidney-related ailments, it is one of the main components of traditional systems of medicine in India. TT has been reported to have a prophylactic effect against oxidative damage in the renal parenchyma and plays an important role in reducing vascular endothelial dysfunction and impaired enzyme activity (7).

The plant extract also exhibits diuretic, aphrodisiac, anti-urolithic, immunomodulatory, antidiabetic, hypolipidemic, hepatoprotective, anti-inflammatory, analgesic, antispasmodic, antibacterial, and anti-cariogenic properties (8). Given the above beneficial effects of the herb, we planned to investigate the effects of TT on carboplatin-induced nephrotoxicity and hepatotoxicity in the rats.

MATERIALS AND METHODS

Experimental animals

The study was conducted after approval by the Institutional Animal Ethics Committee. Animals were obtained from the Central Animal House of the institute in accordance with the Committee for Control and Supervision of Experimentation on Animals (CPCSEA) guidelines. The study was conducted after approval by the Institutional Animal Ethics Committee under Ethic No. IAEC/KMC/63/2021.

For the study, 30 male albino Wistar rats were taken, each weighing between 150 and 250 g and 8 to 10 weeks old. Standard housing conditions for animals included a 12/12-h light/dark cycle, 50% humidity, and 28 °C temperature with food and water *ad libitum*. Rats were sacrificed with an overdose of ketamine at the end of the study.

Drugs and chemicals

Carboplatin was purchased from a local pharmacy (Manufactured by Fresenius Kabi Oncology Ltd). TT was obtained as powdered extract (Gokshura Churna) from an Ayurveda Pharmacy, in Karnataka, India. (ISO 9001: 2015 & GMP CERTIFIED).

Method of TT administration

From the Churna, 50 g of powdered extract was mixed with 200 mL of distilled water, boiled, and reduced to one-fourth. In the end, the 50 mL of extract was cooled and filtered. The human dose was converted to animal dose by using the Paget and Barnes table (9). Up to 20 g of TT Churna extract is used in traditional medicine in humans. Even though up to doses of 5 g/kg of TT are safe in rats, a dose of more than 2 g/kg body weight in rats has been reported to cause diuresis. Hence the doses were selected below 2 g/kg body weight (10).

Experimental design

A total of 30 rats in the experiment, were divided into 5 groups (n = 6). All groups were treated for 14 days as follows: group 1: the control group received distilled water: 1 mL/kg/day orally for 14 days; group 2: The carboplatin group received carboplatin 90 mg/kg on the 5th day (intraperitoneal); group 3: the carboplatin + TT group received TT extract (1 g/kg) orally from day 0 to day 14 and carboplatin (90 mg/kg) on the 5th day (intraperitoneal); group 4: the carboplatin + TT group received TT extract (1.25 g/kg) orally from day 0 to day 14 and carboplatin (90 mg/kg) on the 5th day (intraperitoneal); group 5: the carboplatin + TT group received TT extract (1.5 g/kg) orally from day 0 to day 14 and carboplatin (90 mg/kg) on the 5th day (intraperitoneal).

Blood withdrawal and tissue sample collection

Retro orbital blood collection was done from the inner canthus of the eye using capillary tubes for biochemical estimation using suitable kits on days 0, 7, and 14. After the 14th day, the kidneys and livers were dissected and weighed for histopathological analysis.

Kidney and liver weights

The weight of rats belonging to different groups was measured on days 0 and 14. On day 14 the rats were sacrificed, and the kidney and liver weights were measured.

Kidney function tests

Blood urea estimation

Urea was estimated by the glutamate dehydrogenase (GLDH) kinetic method (11). Urease breaks down urea to produce ammonia and carbon dioxide. The following product, ammonia reacts with ketoglutarate and NADH to create glutamate and NAD^+. A reduction in absorbance over a predetermined period of time was used to calculate the rate of oxidation of NADH to NAD^+. This decline was proportional to the sample's urea concentration. The samples were pipetted into test tubes and labeled as standard (S) or test (T). The enzymes and starter reagents were added and mixed well. The absorbance A_1 and A_2 were recorded at 30 and 60 s. Change in absorbance was calculated.

Creatinine estimation

Creatinine was estimated by the modified Jaffe's Kinetic method (12). Creatinine forms a red-colored complex with alkaline picrate. The rate of formation of this complex was directly proportional to the creatinine concentration. The procedure was similar to urea estimation. Change in absorbance was calculated.

Liver function tests

Aspartate aminotransferase estimation

Aspartate aminotransferase (AST, IU/L) was estimated according to the IFCC (International Federation of Clinical Chemistry) method (13). The amino group transfers between L-aspartate and ketoglutarate were catalyzed by AST to form oxaloacetate and glutamate. Malate dehydrogenase facilitates the reaction between the produced oxaloacetate and NADH to convert to NAD^+. The rate of oxidation of NADH to NAD^+ was analyzed by measuring a reduction in absorbance, proportional to the level of AST in the sample. The samples were pipetted in a test tube and labeled. Enzyme reagent and sample were added, incubated for 1 min and starter reagent was added. The reagents and sample were mixed, the initial absorbance (A_0) was read after 1 min, and the readings were repeated every 1, 2, and 3 min. The absorbance per minute was calculated.

Alanine aminotransferase estimation

Alanine aminotransferase (ALT, IU/L) was estimated using a modified IFCC method (14). L-alanine and ketoglutarate undergo an amino group transfer that is catalyzed by ALT to produce pyruvate and glutamate. In the presence of lactate dehydrogenase, the produced pyruvate interacts with NADH to convert to NAD^+. The rate of oxidation of NADH to NAD^+ was analyzed by measuring a reduction in absorbance, proportional to the level of ALT in the sample. The absorbance per minute was calculated using a similar procedure to AST estimation.

Serum interleukin-6

This assay was based on the principle of enzyme-linked immunosorbent assay (ELISA). The standard's interleukin (IL)-6 binds to the coated antibodies on the wells. Afterward, a biotinylated human IL-6 antibody was added,

and it bound to IL-6 in the sample as well. Streptavidin-horse radish peroxidase (HRP) was subsequently added, which bound the biotinylated IL-6 antibody. After incubation, unbound streptavidin-HRP was removed by adding streptavidin peroxidase enzyme. When the substrate solution was added, a yellow color that was inversely correlated with the concentration of human IL-6 appeared. Acid (100 µL) was added to stop the process. Finally, a microplate reader was used to measure the absorbance at 450 nm (15).

Antioxidant estimation

Glutathione assay

Glutathione (GSH) reductase is a non-protein compound containing the sulfhydryl group. The Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid), DTNB) reacts with the reduced GSH to produce a yellow-colored compound which was directly proportional to the concentration of the GSH. This was measured at an absorbance of 412 nm (16). Tissue homogenate (200 µL) was mixed with 200 µL of 5% trichloroacetic acid solution which was then centrifuged, and the supernatant was collected. In 96-well plates, 25 µL of supernatant with 25 µL of 1 mM DTNB reagent and 150 µL of phosphate-buffered saline were added in replicate and incubated for 10 min at room temperature. The homogenization buffer was kept as blank. Absorbance was taken at 412 nm using an ELISA plate reader. Concentration was calculated using a standard curve. GSH standard (6.1 mg/mL, 20 mM stock) was prepared. One hundred µM of working standards were used. It is expressed as µmol/mg of total protein.

Lipid peroxidase by malondialdehyde assay

Malondialdehyde (MDA) is an important oxidation product and marker for lipid peroxidation which reacts with thiobarbituric acid resulting in the production of a colored compound. This was measured at 523 nm (17,18). A volume of 2.5 mL of thiobarbituric acid, trichloroacetic acid, and hydrochloric acid was combined with 0.5 mL of tissue homogenate (HCL). The mixture was heated at 90 °C for 10 min. The mixture was centrifuged at 2000 rpm for 5 min and absorbance was taken at 525 nm. Lipid peroxidation was expressed as nM of MDA per mg of tissue.

Histopathological examination:

The histological examination of kidney and liver tissues was done using paraffin-embedded specimens. Qualitative analysis was performed. Sections were stained with hematoxylin and eosin (H&E) according to the standard procedure (19). After incubation, tissue sections were cooled and kept in xylene for 30 min to remove wax. Hydration of sections was done through different alcohol series (90%, 70%, and 50%). Distilled water was used for washing for 5 min and staining was done with hematoxylin. Sections were washed in tap water for blueing. Staining with eosin was done. Dehydration through different alcohol series was performed. Xylol was added for 5-10 min and then mounted using DPX under a cover slip. Once the slides were dried, H&E-stained tissues were observed under the light microscope (resolution: 40 ×, 10×).

Statistical analysis

Statistical comparisons were performed using Graph Pad Prism 8.0.1 Demo Version. One-way analysis of variance (ANOVA) followed by the post hoc Tukey test was done for intergroup comparison. Repeated measures ANOVA followed by post hoc Bonferroni's test was used to compare data within each group at different periods. Results were expressed as mean ± SD, and *P*-values ≤ 0.05 were considered significant.

RESULTS

Body, kidney, and liver weight changes

The present study investigated the impact of carboplatin and TT on the body, kidney, and liver weight of experimental subjects indicating a decrease in body weight in groups treated with carboplatin and TT at 1, 1.25, and 1.5 g/kg; however, it was not significant compared to the control on day 0 and day 14 (Table 1).

The kidney weight of carboplatin-treated animals notably increased compared to the control group. In contrast, the kidney weight of TT-treated groups decreased which was not significant compared to the carboplatin group (Table 2).

Table 1. Changes in body weight of rats in different groups on days 0 and 14. Data are expressed as mean ± SD.

Groups	Body weight at day 0 (g)	Body weight at day 14 (g)
Control	220 ± 28.68	222.83 ± 30.14
Carboplatin	232 ± 19.33	210.83 ± 21.31
Carboplatin + TT (1 g/kg)	220 ± 23.85	197.5 ± 21.31
Carboplatin + TT (1.25 g/kg)	210.83 ± 22.25	194.16 ± 22.79
Carboplatin + TT (1.5 g/kg)	209.33 ± 11.32	194.83 ± 22.79

TT, Tribulus terrestris.

Table 2. Changes in kidney and liver weight of rats in different groups. Data are expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ indicate significant differences in comparison with the control group; $^{\#}P < 0.05$ versus carboplatin group.

Groups	Kidney weight (g)	Liver weight (g)
Control	1.14 ± 0.028	9.36 ± 1.204
Carboplatin	1.43 ± 0.117*	8.31 ± 0.287
Carboplatin + TT (1 g/kg)	1.26 ± 0.004	7.18 ± 0.457$^{***,\#}$
Carboplatin + TT (1.25 g/kg)	1.24 ± 0.252	7.11 ± 0.492$^{***,\#}$
Carboplatin + TT (1.5 g/kg)	1.34 ± 0.207	7.78 ± 0.430**

TT, *Tribulus terrestris.*

Table 3. Blood urea and serum creatinine levels in different groups on days 0,7, and 14. Data are expressed as mean ± SD. $^{*}P < 0.05$ and $^{**}P < 0.01$ indicate significant differences in comparison with the control group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus carboplatin group; $^{a}P < 0.05$ and $^{aaa}P < 0.001$ versus day 0; $^{b}P < 0.05$ and $^{bbb}P < 0.001$ against day 7.

Groups	Urea (mg/dL)			Creatinine (mg/dL)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Control	35.05 ± 11.854	35.18 ± 8.060	29.38 ± 6.792	0.24 ± 0.031	0.27 ± 0.040	0.25 ± 0.023
Carboplatin	36.35 ± 5.930	48.26 ± 6.357	35.76 ± 4.798^{b}	0.25 ± 0.023	1.81 ± 0.483^{**,a}	1.66 ± 0.273^{**,a}
Carboplatin + TT (1 g/kg)	35.44 ± 4.587	39.99 ± 6.526	34.43 ± 7.918	0.26 ± 0.044	1.12 ± 0.466	0.81 ± 0.265$^{\#\#}$
Carboplatin + TT (1.25 g/kg)	36.74 ± 6.295	43.95 ± 10.505	31.51 ± 3.415^{b}	0.24 ± 0.037	0.88 ± 0.404	0.82 ± 0.375$^{\#}$
Carboplatin + TT (1.5 g/kg)	36.98 ±7.331	57.54 ± 7.781^{*,aaa}	31.88 ± 3.475^{bbb}	0.23 ± 0.034	0.90 ± 0.544	0.70 ± 0.094$^{**,\#\#,aa}$

TT, *Tribulus terrestris.*

The results indicate a notable decline in liver weight across the treatment groups (carboplatin in combination with TT at 1, 1.25, and 1.5 g/kg) when compared to the control group. Furthermore, the administration of carboplatin in combination with TT at 1 and 1.25 g/kg led to a decrease in liver weight compared to the carboplatin-only group (Table 2).

The effect of TT on kidney function

On day 14, carboplatin and treatment groups (except for the carboplatin + TT at 1 g/kg) showed a significant decrease in urea levels when compared to day 7. On day 7, urea levels in the treatment group (carboplatin + TT 1.5 g/kg) significantly increased compared to day 0. Also, the group treated with carboplatin + TT 1.5 g/kg showed a significant increase in urea levels compared to the control. Creatinine levels on days 7 and 14 in the carboplatin group increased significantly compared to day 0. On day 14, there was a significant decrease in creatinine levels in the treatment groups when compared to the carboplatin group (Table 3).

The effect of TT on liver function

AST levels of the carboplatin group increased significantly on days 7 and 14 compared to day 0. AST levels in the carboplatin group on both days 7 and 14 showed elevation in comparison with the control group. On days 7 and 14, AST levels

significantly decreased in the treatment groups compared to the carboplatin group. On day 14, AST levels significantly decreased in the treatment groups (except for the combination of carboplatin and TT 1.5 g/kg) compared to those on day 7 (Table 4).

ALT levels of the carboplatin group on days 7 and 14 significantly increased compared to the control group. The groups treated with carboplatin and TT (1 and 1.25 g/kg) on day 14 showed a significant decrease in ALT levels compared to day 7 (Table 4).

Antioxidant effects of TT

There was a significant increase in MDA levels of kidney tissue in the carboplatin group compared to the control group. In the treatment groups, MDA levels of kidney tissue considerably decreased compared to the carboplatin group (Table 5).

All groups have shown a significant increase in MDA of liver tissue levels compared to the control group. The levels of MDA in liver tissue notably decreased in the groups treated with various amounts of TT compared to the carboplatin group (Table 5).

The carboplatin group significantly reduced the GSH levels compared to the control group. The group treated with the combination of carboplatin and + TT (1.25 g/kg) significantly increased GSH levels compared to the carboplatin group (Table 6).

Table 4. Serum aspartate transaminase and alanine transaminase levels in different groups on days 0, 7, and 14. Data are expressed as mean ± SD. $^{*}P < 0.05$ and $^{***}P < 0.001$ indicate significant differences in comparison with the control group; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.01$ versus carboplatin group; $^{a}P < 0.05$, $^{aa}P < 0.01$, and $^{aaa}P < 0.001$ versus day 0; $^{b}P < 0.05$ against day 7.

Groups	Aspartate transaminase (IU/L)			Alanine transaminase (IU/L)		
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14
Control	118.43 ± 27.24	115.80 ± 26.85	118.48 ± 26.49	26.79 ± 7.205	25.41 ± 9.044	25.16 ± 11.11
Carboplatin	116.85 ± 25.63	182.36 ± 4.93$^{***, aaa}$	167.18 ± 8.08$^{*, aaa}$	27.21 ± 6.565	96.15 ± 28.959$^{**, aa}$	86.65 ± 18.971$^{**, aa}$
Carboplatin + TT (1 g/kg)	117.8 ± 22.43	125.14 ± 27.30$^{\#\#}$	91.52 ± 30.67$^{\#\#, b}$	26.79 ± 7.205	92.58 ± 34.616^{aa}	62.67 ± 14.488$^{*, a, b}$
Carboplatin + TT (1.25 g/kg)	125.6 ± 28.34	126.07 ± 29.18$^{\#\#\#}$	85.58 ± 32.13^{b}	27.74 ± 6.601	71.69 ± 14.119*	40.64 ± 10.373$^{\#\#, b}$
Carboplatin + TT (1.5 g/kg)	118.45 ± 27.24	115.66 ± 27.12$^{\#\#\#}$	85.94 ± 19.53$^{\#\#\#}$	27.21 ± 6.333	79.46 ± 10.547$^{*, aa}$	70.82 ± 51.166^{a}

TT, *Tribulus terrestris.*

Table 5. Malondialdehyde levels in kidney and liver tissue of rats. Data are expressed as mean ± SD. $^{*}P < 0.05$ and $^{***}P < 0.001$ indicate significant differences in comparison with the control group; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.01$ versus carboplatin group.

Groups	Malondialdehyde levels (nM/mg)	
	Kidney tissue	Liver tissue
Control	51.34 ± 1.333	25.21 ± 2.513
Carboplatin	72.10 ± 1.759***	42.60 ± 1.932***
Carboplatin + TT (1 g/kg)	63.47 ± 2.113$^{***, \#\#}$	33.12 ± 1.898$^{***, \#\#\#}$
Carboplatin + TT (1.25 g/kg)	53.85 ± 4.602$^{\#\#\#}$	29.47 ± 2.015$^{*, \#\#\#}$
Carboplatin + TT (1.5 g/kg)	51.47 ± 6.016$^{\#\#\#}$	32.34 ± 2.524$^{***, \#\#\#}$

TT, *Tribulus terrestris.*

Table 6. Glutathione levels in kidney tissue of rats. Data are expressed as mean ± SD. $^{*}P < 0.05$ indicates significant differences in comparison with the control group; $^{\#\#}P < 0.01$ versus carboplatin group.

Groups	Glutathione levels (µM/mg tissue)
Control	1.25 ± 0.1228
Carboplatin	0.85 ± 0.4086*
Carboplatin + TT (1 g/kg)	0.98 ± 0.1599
Carboplatin + TT (1.25 g/kg)	1.30 ± 0.1513$^{\#}$
Carboplatin + TT (1.5 g/kg)	1.23 ± 0.1967

TT, *Tribulus terrestris.*

Immunomodulatory effects of TT

IL 6 levels significantly increased in the carboplatin group on day 7 compared to the control group. On day 14, IL 6 levels of the carboplatin group significantly increased compared to day 0. On day 7, the treatment group (carboplatin + TT 1 g/kg) showed a significant decrease in IL 6 levels compared to the carboplatin group. On day 14, treatment groups (carboplatin + TT 1.25 g/kg and carboplatin + TT 1.5 g/kg) showed a significant decrease in IL 6 levels compared to the carboplatin group (Table 7).

Histopathological examinations

H&E-stained sections of the cortical region of the kidney of rats belonging to the normal control group showed normal-looking cut sections of glomeruli, Bowman's capsules, proximal convoluted tubules, distal convoluted tubules, and collecting tubules. The cortex of carboplatin control group rats (group 2) had some areas of diffused inflammatory cell infiltration into the peritubular interstitial spaces (indicated by the red arrow in Fig. 1) which could indicate interstitial nephritis. Groups 3, 4, and 5 exhibited normal kidney structure similar to that of group 1 (Fig. 1).

Table 7. Interleukin 6 levels on day 0, day 7, and day 14. Data are expressed as mean ± SD. $^{*}P < 0.05$ indicates significant differences in comparison with the control group; $^{\#}P < 0.05$ versus carboplatin group; $^{a}P < 0.05$, versus day 0.

Groups	Interleukin (pg/mL)		
	Day 0	Day 7	Day 14
Control	75.11 ±52.04	92.01 ± 43.56	98.49 ± 45.105
Carboplatin	49.31 ±5.35	178.44 ± 27.69*	127.98 ± 54.72^{a}
Carboplatin + TT (1 g/kg)	60.99 ±21.44	93.63 ± 16.92$^{\#, a}$	73.07 ± 55.81
Carboplatin + TT (1.25 g/kg)	87.95 ±7.180	98.45 ± 36.23	64.89 ± 19.06$^{\#}$
Carboplatin + TT (1.5 g/kg)	71.77 ±44.34	97.91 ± 25.12	103.65 ± 13.81$^{\#}$

TT, *Tribulus terrestris.*

.

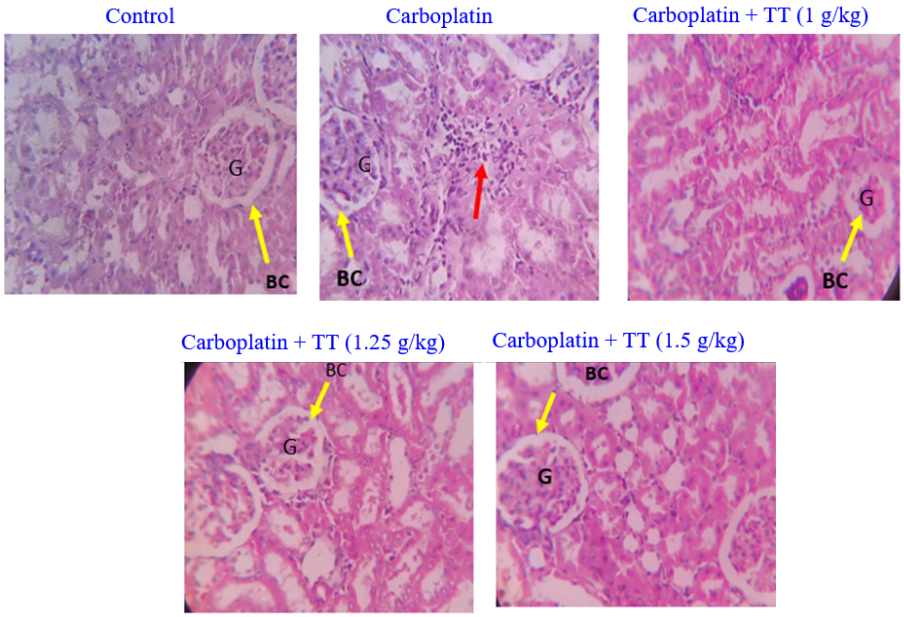

Fig. 1. Microphotographs of rat kidneys stained by H&E, magnification 40×, 10×. Representative H&E-stained sections of the cortical region of the kidney of rats treated with carboplatin and different doses of TT. It can be noted that the groups treated with TT at 1, 1.25, and 1.5 g/kg showed almost normal kidney structure with that of control. However, the carboplatin-treated group showed some areas of diffused inflammatory cell infiltration into the peritubular interstitial spaces (indicated by red arrow). TT, *Tribulus terrestris;* G, glomerulus; BC, Bowman's capsule; H&E, hematoxylin and eosin.

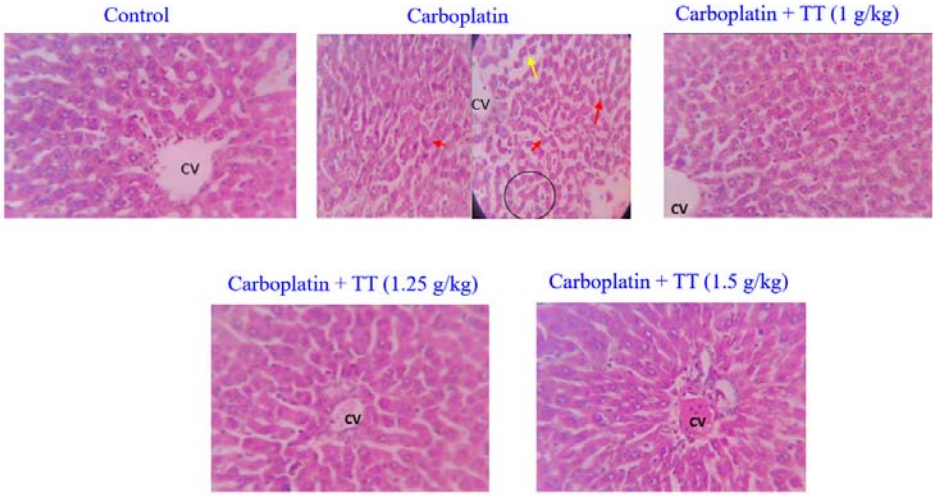

Fig. 2. Microphotographs of rat livers stained by H&E, magnification 40×. Representative H&E-stained sections of the liver of rats treated with carboplatin and different doses of TT. It can be noted that the groups treated with TT at 1, 1.25, and 1.5 g/kg showed almost normal liver structure with that of the control group. However, in some areas of the carboplatin-treated group, the hepatocytes have lost their normal polygonal shape (indicated by the red arrow). In addition, in some places, hepatic cords looked irregular (indicated by a black circle) and hepatic sinusoids appeared to be expanded (indicated by a yellow arrow). TT, *Tribulus terrestris*; H&E, hematoxylin and eosin

H&E-stained liver sections were observed under the light microscope at 40 × magnifications. Group 1 (normal control) showed a normal liver structure with hexagonal hepatic lobules, a central vein, and a typical arrangement of hepatic cords with polygonal-shaped hepatocytes radiating from the central vein. Portal triads were also normal in their location and pattern. In group 2, though the liver structure broadly looked normal, there were some areas where the hepatocytes had lost their normal polygonal shape (indicated by the red arrow in Fig. 2). Further, in group 2, in some places, hepatic cords looked irregular (indicated by a black circle in Fig. 2) in their arrangement, and hepatic sinusoids appeared to be expanded (indicated by a yellow arrow in Fig. 2). Groups 3, 4, and 5 exhibited normal hepatic architecture similar to that of group 1.

DISCUSSION

In the present study, carboplatin has induced nephrotoxicity and hepatotoxicity in male Wistar rats. The study drug, an aqueous extract of TT intermediate dose (1.25 g/kg) and high dose (1.5 g/kg) was able to demonstrate nephroprotective and hepatoprotective properties.

Nephrotoxicity was induced by carboplatin which elevated blood urea and serum creatinine levels in male Wistar rats on days 7 and 14. Carboplatin increased MDA levels and significantly reduced GSH levels indicating oxidative injury. This outcome is consistent with studies that showed compromised kidney function with carboplatin (20,21). In our study, an aqueous extract of TT (1.5 g/kg) decreased serum creatinine levels in comparison to the carboplatin group. It has been reported TT prevented renal injury induced by mercuric chloride in a rat model by decreasing creatinine and blood urea levels (22). Elevated renal parameters are consistent with platinum compounds as kidneys are the major route of elimination and cytotoxicity to somatic cells occurs due to upregulation of inflammatory genes, generation of free radicals, and release of reactive oxygen species (23). Extracts with antioxidant potential have a beneficial role in ameliorating these symptoms. TT extract significantly inhibited lipid peroxidation and attenuated renal oxidative stress parameters. Along with molecular mechanisms, histopathological observations were done in the present study for the nephroprotective effect. Evaluation of the groups treated with the combination of carboplatin with TT showed a

decrease in the inflammation and restoration of the kidney structure with the control group. A similar study with hydroalcoholic extract of TT showed a nephroprotective effect by diminishing histological alteration induced by cisplatin in male mice (24).

Carboplatin caused hepatotoxicity by increasing AST and ALT on days 7 and 14. Though the therapeutic index of carboplatin is considered better than cisplatin and there are limited studies in favor of carboplatin-induced hepatic injury, some cases of carboplatin-induced hepatotoxicity have been reported (25). The liver injury induced by carboplatin varies from mild hepatic enzyme elevation to obstruction of sinusoids causing hepatotoxicity. Acute liver necrosis and failure have been observed when carboplatin compounds have been incorporated into the chemotherapy regimen (26).

The hepatoprotective properties of TT aqueous extract were evaluated in the current study in male Wistar rats. TT extracts were significantly able to reduce liver weight in the treatment groups in comparison with the control and carboplatin groups. TT also significantly reduced the levels of AST and ALT enzymes in the treatment group compared to the carboplatin group on days 7 and 14. A similar finding was observed in a study, where the TT extract ameliorated hepatic damage, by improving the results of liver function tests and reducing histopathological changes (27). This could be explained by the presence of flavonoid contents in extracts of TT which increased cell viability and prevented hepatocyte enzyme leakage (28).

Histopathological evaluation of liver tissue in the TT-treated group in the study showed an improvement in hepatocellular architecture concerning hepatic sinusoids and the biliary system. In addition to histological evaluation of the liver, hepatic antioxidant markers were studied. Carboplatin elevated liver MDA levels in this study which is a significant marker of oxidative stress. TT was able to significantly reduce liver MDA levels which is comparable to findings in studies conducted on TT extract where it demonstrated the hepatoprotective antioxidant effect by decreasing hepatic biomarkers and minimizing histological alterations in hepatotoxicity model in rats (29,30). IL-6 is a cytokine that controls multiple genomic expressions and modulates the inflammatory response along with immunomodulation, hematopoiesis, metabolism, and organ development (29). Carboplatin administration significantly raised the levels of inflammatory marker IL-6 in carboplatin-treated animals. IL-6 is an indicator of renal and hepatocyte injury. TT reduced the levels of IL-6 in high doses suggesting their anti-inflammatory activity.

The findings of the present study demonstrated an equi-efficacious nephroprotective and hepatoprotective effect of TT mediated by anti-inflammatory and antioxidant properties at three different doses. While the aforementioned findings provide valuable insights, it is imperative to be corroborated through extensive, long-term clinical investigations.

CONCLUSION

Aqueous extract of TT demonstrated a therapeutic effect against nephrotoxicity and hepatotoxicity caused by carboplatin. The aqueous extract of TT could produce nephroprotective and hepatoprotective effects by improving biochemical markers, oxidative parameters, and histopathological alterations. TT contains several phytoconstituents, which are the key factors in the medicinal value of this herb. More studies are required to clinically correlate the mechanism with therapeutic effects.

Conflict of interest statement

All authors confirmed no conflict of interest in this study.

Authors' contribution

N. Shetty collected and analyzed the data, reviewed the literature, and prepared the original draft of the article; S. Holla conceptualized the idea, edited and reviewed the manuscript; V. Nayak reviewed the article, supervised and provided inputs; V. Shenoy provided resources and reviewed the manuscript; M Rao did the formal analysis and provided scientific inputs. The finalized article was read and approved by all authors.

REFERENCES

1. Azar I, Yazdanpanah O, Jang H, Austin A, Kim S, Chi J, *et al*. Comparison of carboplatin with cisplatin in small cell lung cancer in US veterans. *JAMA Netw Open*. 2022;5(10):e2237699,1-11. DOI: 10.1001/jamanetworkopen.2022.37699.
2. Fotopoulou C. Limitations to the use of carboplatin-based therapy in advanced ovarian cancer. EJC Suppl. 2014;12(2):13-16. DOI: 10.1016/S1359-6349(15)70005-4.
3. Husain K, Jagannathan R, Hasan Z, Trammell GL, Rybak LP, Hazelrigg SR, *et al* Dose response of carboplatin-induced nephrotoxicity in rats. Pharmacol Toxicol. 2002;91(2):83-99. DOI: 10.1034/j.1600-0773.2002.910207.x.
4. Zhang BY, Wang YM, Gong H, Zhao H, Lv XY, Yuan GH, *et al*. Isorhamnetin flavonoid synergistically enhances the anticancer activity and apoptosis induction by cisplatin and carboplatin in non-small cell lung carcinoma (NSCLC). Int J Clin Exp Pathol. 2015; 8(1):25-37. PMID: 25755690.
5. Hassan ES, Majeed SA, Mohammad AR, Gaen KK. The protective effect of the N acetylcysteine on acute liver toxicity induced by carboplatin in rat model. Int J Pharm Res. 2019;11(3):356-364.
6. Kintzel PE. Anticancer drug-induced kidney disorders. Drug Saf. 2001;24(1):19-38. DOI: 10.2165/00002018-200124010-00003.
7. Yadav HN, Sharma US, Singh S, Gupta YK. Effect of combination of *Tribulus terrestris*, *Boerhavia diffusa* and *Terminalia chebula* reverses mercuric chloride-induced nephrotoxicity and renal accumulation of mercury in rat. Orient Pharm Exp Med. 2019;19(4):497-507. DOI: 10.1007/s13596-019-00381-1.
8. Abdel-Kader MS, Al-Qutaym A, Saeedan ASB, Hamad AM, Alkharfy KM. Nephroprotective and hepatoprotective effects of *Tribulus terrestris* L. growing in Saudi Arabia. J Pharm Pharmacogn Res. 2016;4(4):144-152. DOI: 10.56499/jppres16.112_4.4.144.
9. Lawrence DR, Bacharach AL. Evaluation of drug activities, pharmacometrics. Vol 2. London and New York: Academic Press; 1964. p.161.
10. Chhatre S, Nesari T, Somani G, Kanchan D, Sathaye S. Phytopharmacological overview of Tribulus terrestris. Pharmacogn Rev. 2014;8(15):45-51. DOI: 10.4103/0973-7847.125530.
11. Kedar K, Patil J, Nimkar S. Urea and creatinine levels in vaginal fluid-a reliable marker for prelabour rupture of membranes. J Evol Med Dent Sci. 2018;7(20):2456-60. DOI:10.14260/jemds/2018/553.
12. Marakala V, Avinash SS, Ramachandrayya SA, Malathi M, Kumar A. Serum creatinine assay: enzymatic vs kinetic Jaffe's method. J Evol Med Dent Sci. 2012;1(4):258-264. DOI: 10.14260/jemds/54.
13. SGOT ASAT Kit Reitman Frankel method- For determination of SGOT (ASAT) activity in serum https://www.scribd.com/doc/47717927/SGOT-ASAT-Kit-Reitman-Frankel-method/2021.
14. SGPT- range, causes, symptoms and IFCC method of measurement https://www.tec2med.com/sgpt-range-cause-symptom-ifcc/2021.
15. Sakamoto S, Putalun W, Vimolmangkang S, Phoolcharoen W, Shoyama Y, Tanaka H, *et al*. Enzyme-linked immunosorbent assay for the quantitative/qualitative analysis of plant secondary metabolites. J Nat Med. 2018;72(1):32-42. DOI: 10.1007/s11418-017-1144-z.
16. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984;21(2):130–132. PMID: 6490072.
17. Zeb A, Ullah F. A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods. J Anal Methods Chem. 2016;2016:9412767,1-5. DOI: 10.1155/2016/9412767.
18. Spirlandeli AL, Deminice R, Jordao AA. Plasma Malondialdehyde as Biomarker of Lipid Peroxidation : Effects of Acute Exercise. Int J Sports Med. 2014;35(1):14-18. DOI: 10.1055/s-0033-1345132.
19. Bancroft, J.D, Stevens, A. Theory and practice of histological techniques.8ed London: Churchill Livingstone;2019. p.63-84.
20. Husain K, Whitworth C, Rybak LP. Time response of carboplatin-induced nephrotoxicity in rats. Pharmacol Res. 2004;50(3):291–300. DOI: 10.1016/j.phrs.2004.04.001.
21. Akhtar F, Azhar M, Aslam M, Javed K. Nephroprotective effect of Khar-E-Khasak Khurd (*Tribulus terrestris* Linn) on gentamicin-induced experimental nephrotoxicity in rats. Asain J Res Neph 2020; 3(3): 6-13.
22. Yadav HN, Sharma US, Singh S, Gupta YK. Effect of *Tribulus terrestris* in mercuric chloride-induced renal accumulation of mercury and nephrotoxicity in rat. J Adv Pharm Technol and Res. 2019;10(3):132–137. DOI: 10.4103/japtr.JAPTR_386_18.
23. Badr A, Fouad D. Anti-apoptotic and anti-inflammatory effects of olive leaf extract against cisplatin-induced nephrotoxicity in male rats. Int J Pharmacol. 2016;12(7):675–88. DOI: 10.3923/ijp.2016.675.688.
24. Raoofi A, Khazaei M, Ghanbari A. Protective effect of hydroalcoholic extract of *Tribulus terrestris* on cisplatin induced renal tissue damage in male mice. Int J Prev Med. 2015;6(11):1-7. DOI: 10.4103/2008-7802.151817.
25. Erisgin Z, Atasever M, Cetinkaya K, Dizakar SQA, Omeroglu S, Sahin H. Protective effects of *Nigella sativa* oil against carboplatin-induced liver damage in rats. Biomed Pharmacother. 2019;110:742–747. DOI: 10.1016/j.biopha.2018.12.037.
26. LiverTox. Clinical and research information on drug-induced liver injury. bethesda (md): national institute of diabetes and digestive and kidney diseases. 2012 Carboplatin. [Updated 2020]. Available from:

https://www.ncbi.nlm.nih.gov/books/NBK548565. Bookshelf ID: NBK547852. PMID:.31643176.

27. Kilany OE, El-Beltagy MA, El-Sherbeeny NA. *Tribulus terrestris* ameliorates carbon tetrachloride-induced hepatotoxicity in male rats through suppression of oxidative stress and inflammation. Environ Sci Pollut Res Int. 2020;27(20):24967–24981. DOI: 10.1007/s11356-020-08826-w.
28. Zhu W, Du Y, Meng H, Dong Y, Li L. A review of traditional pharmacological uses, phytochemistry, and pharmacological activities of *Tribulus terrestris*. Chem Cent J. 2017;11(1):60,1-16. DOI: 10.1186/s13065-017-0289-x.
29. Almasi F, Khazaei M, Chehrei S, Ghanbari A. Hepatoprotective effects of Tribulus terrestris hydro-alcholic extract on non-alcoholic fatty liver- induced rats. Int. J. Morphol. 2017;35(1):345-350. DOI: 10.4067/S0717-95022017000100054.
30. Su H, Lei CT, Zhang C. Interleukin-6 signaling pathway and its role in kidney disease: an update. Front Immunol. 2017; 8:405,1-10. DOI: 10.3389/fimmu.2017.00405.