Hydroalcoholic extract of *Tribulus terrestris* protects against accumulation of platinum in rat kidney

Uma Shankar Sharma, Harlokesh Narayan Yadav*, Surender Singh, Yogendra Kumar Gupta

Introduction

Over the past few decades, a dramatic rise in the incidence of acute kidney injury (AKI) has been reported.1,2 Drug-induced nephrotoxicity contributes to the high prevalence and incidence of AKI in both hospitalized and non-hospitalized patients.3 The incidence of medical institution obtained AKI is 5–10 instances greater than that of community-acquired AKI and has an annual incidence of 0.15–7.2%.4 Cisplatin is more effective chemotherapeutic agents in cancers of the testis, bladder, lung, and ovary.5 One of the major limitations associated with cisplatin is nephrotoxicity.6 Cisplatin causes dose-dependent and cumulative nephrotoxic effect so that it might be possible to decrease the dose or withdrawal of the drug.

ABSTRACT

The present study has been designed to investigate the potential effects of the hydroalcoholic extract of *Tribulus terrestris* (TT) in cisplatin-induced nephrotoxicity and accumulation of platinum in kidney of Wistar rats. Pre-treatment of TT was started at a dose of 100/200/300 mg/kg oral once daily from day 1 to day 10. Nephrotoxicity was induced by administration of a single dose of cisplatin (8 mg/kg, i.p.) on the 7th day. Blood urea nitrogen (BUN), serum creatinine, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx) were measured. Renal histopathology, liver fatty acid binding protein (L-FAB), kidney injury molecule-1 (KIM-1), and platinum accumulation in kidney tissue were also determined by ICPMS. Single dose of cisplatin caused significant elevation of BUN, serum creatinine, MDA, KIM-1, L-FAB, and kidney platinum level, and fall in GSH, SOD, GPx, and histopathological changes in disease control as compared to normal control group (*P* < 0.001). Dose of TT 200 and 300 mg/kg significantly (*P* < 0.001) prevented abnormalities caused by cisplatin in the above parameters. The lower dose of TT 100 mg/kg did not show significant prevention as compared to the disease control group. TT 200 treated groups; platinum accumulation in kidney tissues was significantly different as compared to the control group. TT exerted significant protection against nephrotoxicity induced by cisplatin and to a certain extent it decreases the level of accumulated platinum in kidney tissues; hence, TT could be beneficial in the prevention of cisplatin-induced nephrotoxicity followed by reactive oxygen species, inflammation, and oxidative stress.

Keywords: Antioxidant, cisplatin, kidney injury molecule-1, liver fatty acid binding protein, nephrotoxicity, platinum, *Tribulus terrestris*
furthermore established that insurance against cisplatin-induced renal tissue harm in male mice.[13]

Therefore, the present study has been designed to explore the nephroprotective potential of hydroalcoholic extract of TT and its effect on the accumulation of platinum in the kidney of Wistar rats.

Materials and Methods

Experimental animals

Adult Wistar albino rats, weighing 170–230 g, were used for this study. Rats were procured from Central Animal Facility, All India Institute of Medical Sciences (AIIMS), New Delhi. The animals were acclimatized before conducting the experiments. The animals were housed in cages of 40 cm × 20 cm × 15 cm made up of polypropylene. They were kept in standard room conditions under the dark cycles and natural light and were fed rat diet and provide water *ad libitum*. The performance of experimental work was started after getting approval from the Institutional Animal Ethics Committee (944/IAEC/16). All experimental works were conducted in the Department of Pharmacology, AIIMS, New Delhi, 110029.

Drugs and chemicals

The hydroalcoholic extract of TT was purchased from Sunpure Pvt. Ltd. New Delhi. Cisplatin was obtained from Fresenius Kabi Oncology Limited, HP (India). The kidney function tests were done by kits purchased from Erba Mannheim for blood urea nitrogen (BUN) and serum creatinine. The glutathione peroxidase (GPx) activity was determined by BioVision Colorimetric assay kit. The rat kidney injury molecule-1 (KIM-1) and liver fatty acid binding (L-FABP) ELISA kits were purchased from Sincere Biotech Company China. All other chemicals were purchased locally and of highest purity grade.

Induction of nephrotoxicity and treatment protocol

Nephrotoxicity was induced Wistar in rats by single dose administration of cisplatin (8 mg/kg i.p.).[13] The rats were divided into six groups with six animals in each group [Table 1]. TT was dissolved in distilled water and administered orally once daily at a dose of 100, 200, and 300 mg/kg for 10 days.[13,14] The injection of cisplatin was administered at a dose of 8 mg/kg, (i.p.) once on the 7th day of treatment of TT. This nephrotoxic dose in rat had been standardized in our department.[13]

Assessment of renal function test

The BUN and serum creatinine levels had been measured using commercially accessible kits as per manufacturer’s instructions. Absorbance was measured by UV spectrophotometer at 510 nm for creatinine and 340 nm for BUN.

Assessment of oxidative stress

The kidney was isolated from the body, weighed, and 0.1 M sodium phosphate buffer (at pH 7.4) was added 10 times (W/V) followed by homogenization using homogenizer (details of homogenizer) at 800–900 rpm. The homogenate was used to estimate various biological parameters. Kidney tissue homogenate (10%) was prepared as explained above, and the parameters such as malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and GPx were estimated as per methods described in literature. Each experiment was done in duplicate. MDA, an indicator of lipid peroxidation, was investigated as described by Ohkawa et al., 1979.[15] Reduced GSH evaluated by the method performed by Ellman’s method 1959.[16] SOD activity was determined according to the method of Marklund and Marklund, 1974,[17] and GPx was done according to Olorunnisola et al., 2012.[18] Each experiment was done in duplicate. GPx activity was calculated by the following formula:

\[
\text{GPx activity} = \frac{B \times \text{Sample dilution}}{(T2 - T1)} \text{ mU/g}
\]

Where, B is the concentration of NADPH. Standard curve was prepared by plotting the concentration-absorbance of NADPH standard. The concentration of NADPH was determined by the linear standard curve.

AKI markers

KIM-1 and L-FABP were evaluated by ELISA kit’s according to the manufacturer’s instructions were followed. The absorbance values were plotted against the concentration of KIM-1, and the standard curve was generated. The concentration of KIM-1 was determined by the linear standard curve.[19] The readings of absorbance were plotted against the concentration of L-FABP and the standard curve was generated. The concentration of L-FABP was determined by the linear standard curve.[20] Each experiment was done in duplicate.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control group</td>
<td>No treatment</td>
</tr>
<tr>
<td>Cisplatin control group</td>
<td>Single dose of cisplatin 8 mg/kg was given</td>
</tr>
<tr>
<td>TT per se group</td>
<td>Normal rats were treated with TT at a dose of 300 mg/kg for 10 days</td>
</tr>
<tr>
<td>Cisplatin+TT (100mg/kg) treated group</td>
<td>Rats were treated with TT 100 mg/kg for 10 days and on the 7th day administered single dose of cisplatin 8 mg/kg</td>
</tr>
<tr>
<td>Cisplatin+TT (200mg/kg) treated group</td>
<td>Rats were treated with TT 200 mg/kg for 10 days and on 7th day administered a single dose of cisplatin 8 mg/kg</td>
</tr>
<tr>
<td>Cisplatin+TT (300mg/kg) treated group</td>
<td>Rats were treated with TT at a dose of 300 mg/kg for 10 days and on the 7th day administered single dose of cisplatin 8 mg/kg</td>
</tr>
</tbody>
</table>

TT: Tribulus terrestris
Histopathology

Para-formaldehyde fixed tissues were kept under the running tap water for 6–8 h to remove the fixative. After washing with increasing concentrations of alcohol and acetone, they were immersed in cedar oil for 2–3 days. Tissues were then embedded in paraffin wax and poured in a plastic mold, allowed to solidify at room temperature to make paraffin blocks. Paraffin blocks were sectioned (5 µm thick) with the help of microtome (Shandon AS 325), and thin tissue sections were allowed to float in warm water at 44°C. In warm water, tissues expand properly and prevent from undesired folding. Flattened floating tissue sections were taken on egg albumin and thymol pre-coated glass slides. Pre-coating of a glass slide with egg-albumin helps in tissue adhesion over the glass surface, while thymol prevents fungal growth and contamination. Slides were air dried and kept overnight at room temperature. All the steps were followed with extreme care to minimize tissue damage. Paraffin sections were then stained with Hematoxylin and eosin stain as standard protocol. Images were analyzed and captured. The histological features were examined microscopically by an expert pathologist.

Estimation of platinum in kidney tissue by inductive coupled plasma atomic emission spectroscopy (ICP-AES)

Weighed 500 mg tissue placed in digestion vessel unit and added 3 ml nitric acid and 1 ml hydrogen peroxide in the vessel. The same procedure was repeated in all vessels (11 vessels) following tight closure of vessel screw and was put in the digestion unit. The samples were digested in the digester; scheme 2 was selected in which temperature can rise up to 160°C. Further, the digested samples were run by ICP-AES.

Statistical analysis

The data were represented as the mean ± standard error of the mean. The data were analyzed using Wilcoxon Signed-Rank test with a direct comparison of respective groups. The prior $P < 0.05$. The data were analyzed using the standard statistical software for GraphPad prism version 5.03 (San Diego, CA, USA).

Results

Effect on BUN levels

As shown in Figure 1, the BUN levels in the cisplatin group were increased significantly ($P < 0.001$) when compared to the normal control group indicating nephrotoxicity. Administration of TT alone at doses of 100 and 200 mg/kg was unable to significantly reduce the cisplatin-induced increase in BUN level. Administration of TT at 300 mg/kg dose significantly reduced the cisplatin-induced BUN levels ($P < 0.001$) when compared to cisplatin control.

Effect on serum creatinine levels

Serum creatinine was estimated to assess the kidney function test. As illustrated in Figure 2, serum creatinine levels were increased significantly in the cisplatin control group when compared to normal control ($P < 0.001$). Administration of TT alone at doses of 100 and 200 mg/kg was unable to significantly reduce the serum creatinine level as compared to the cisplatin group. Moreover, the administration of TT at 300 mg/kg significantly ($P < 0.001$) reduced the level of serum creatinine as compared to the cisplatin control group.
Effect on SOD activity

The SOD activity was estimated to evaluate the oxidative stress level; the data are shown in Figure 3. Cisplatin treatment was found to significantly reduce the SOD activity as compared to normal control. However, the treatment of TT alone at all doses showed a significant increase in the SOD activity as compared to cisplatin control.

Effect on GPx levels

According to results represented in Figure 4, the GPx levels were significantly diminished in cisplatin-treated rats as compared to normal control (P < 0.001) which shows elevated oxidative stress. The treatment of TT at doses of 100, 200, and 300 mg/kg increased the GPx levels as compared to cisplatin-treated rats, but not to significant levels.

Effect on MDA levels

Figure 5 represents the data of the MDA experiment. It can be illustrated from the data that the treatment of cisplatin significantly
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Effect on reduced GSH levels

Figure 6 shows level of estimated GSH in different treatment groups. Results of the present study illustrated that GSH level significantly decreased in the cisplatin control group \( (P < 0.001) \) as compared to normal control which indicates oxidative stress. The treatment of TT at a dose of 100, 200, and 300 mg/kg increased the GSH levels but not at levels of significance.

Effect on KIM-1 levels

KIM-1 was measured to estimate the AKI in the proximal tubule of kidney; Figure 7 represents the KIM-1 data. The KIM-1 level was significantly \( (P < 0.001) \) increased in cisplatin-treated group as compared to normal control group. The administration of TT at a dose of 100/200 mg/kg was unable to reduce significantly the levels of KIM-1 as compared to the cisplatin control group. However, the administration of TT alone at a dose of 300 mg/kg significantly reduced the levels of KIM-1.

Effect on L-FABP protein levels

L-FABP is also explained about acute renal damage. Figure 8 represents the L-FABP data, which shows that the treatment of a single dose of cisplatin significantly \( (P < 0.001) \) increased the serum L-FABP levels as compared to the normal group representing nephrotoxicity. However, the administration of TT at a dose of 100/200/300 mg/kg was unable to significantly decrease the levels of L-FABP as compared to the cisplatin control group.

Effect on histopathological structural changes

All histopathology images were taken at the magnification of \( \times 20 \) and the structural alterations were expressed in grades [Figure 9]: Grade-1: Indicates <25% tubular alterations; Grade-2: Indicates 26–50% tubular alterations; Grade-3: Indicates 51–75% tubular alterations; and Grade-4: Indicates >75% tubular alterations. Figure 9a: Normal control, the structure of the glomerulus was normal and surrounded by tubules with intact epithelium lining. Figure 9b: In cisplatin control glomeruli were relatively unremarkable; tubules showed extensive necrosis of tubular epithelium with detachment from the basement membrane and no nuclei were seen in the cell, representing Grade-4 tubular alteration. Figure 9c: Few of the tubules illustrated intact epithelium. 10–15% tubules showed viable epithelium and rest of the tubules showed necrosis of tubular epithelium indicating Grade-3 alteration. Figure 9d: Structural changes were the same as Figure 9c, indicating Grade-3 alteration. Figure 9e: Structural alterations were the same as Figure 9c and d, indicating Grade-3 alteration. Figure 9f: Represents the per se group. There was no change as compared to normal control.

Effect on platinum accumulation

Table 2 shows the data for platinum accumulation in rat kidney tissue. Platinum was found to be significantly accumulated in cisplatin control rats as compared to normal control. However, TT 200 mg/kg significantly decreased the platinum concentration in kidney tissue in cisplatin control group.

Effect of Tribulus terrestris (TT) on BUN levels in cisplatin (8 mg/kg, i.p., once) induced nephrotoxicity. Data are presented as mean ± SD, \( (n=6) \). Cisplatin (CP) Control: CP at 8 mg/kg. TT 100/200/300 + CP: CP at 8 mg/kg in combination with Tribulus terrestris at 100, 200, 300 mg/kg, respectively. TT per se at 300 mg/kg: \( (a* P < 0.05) \) versus Normal control.

Effect of Tribulus terrestris (TT) on serum levels of Kim-1 in cisplatin (8 mg/kg, i.p., once) induced nephrotoxicity. Data are presented as mean ± SD, \( (n=6) \). Cisplatin (CP) Control: CP at 8 mg/kg. TT 100/200/300 + CP: CP at 8 mg/kg in combination with Tribulus terrestris at 100, 200, 300 mg/kg, respectively. TT per se at 300 mg/kg: \( (a* P < 0.05) \) versus Normal control.

Effect of Tribulus terrestris (TT) on serum levels of L-FABP in cisplatin (8 mg/kg, i.p., once) induced nephrotoxicity. Data are presented as mean ± SD, \( (n=5) \). Cisplatin (CP) Control: CP at 8 mg/kg TT 100/200/300 + CP: CP at 8 mg/kg in combination with TT at 100, 200, 300 mg/kg, respectively. TT per se at 300 mg/kg: \( (a* P < 0.05) \) versus Normal control.
It has been reported that cisplatin causes nephrotoxicity by the production of oxidative stress, mitochondrial dysfunction, and inflammation.\cite{18} Cisplatin converts into highly reactive form, and it interacts with thiol-containing molecules like GSH. Depletion or inactivation of endogenous antioxidants such as GSH and antioxidant enzymes by cisplatin leads to overproduction of reactive oxygen species (ROS), outoming in oxidative stress which leads to mitochondrial dysfunction.\cite{21}

The diagnosis of AKI on the basis of BUN and serum creatinine has its limitations. The BUN and serum creatinine are not specific as well as a sensitive method for detection of AKI. The factors which may affect its value are such as age, muscle mass, nutritional status, and infection. However, early detection of AKI is not possible by traditional markers. The KIM-1 is an important AKI marker because it is validated and quantified.\cite{13} The KIM-1 and L-FABP are the early, more sensitive and excellent markers for detection of tubular damage in AKI and therefore, both have been incorporated in this study.

TT a natural herb having various medicinal properties, i.e., diuretic, antioxidant, anti-inflammatory, aphrodisiac, antiurolithic, and antihypertensive.\cite{24} It is a flowering plant of the family Zygophyllaceae, commonly known as puncturevine. In the present study, hydroalcoholic extract of TT was used in cisplatin-induced nephrotoxic model and renal function tests, AKI markers, histopathological features, and biochemical profiles of the rat kidneys were estimated. The overall inference drawn from our study was that the hydroalcoholic extract of TT showed significant nephroprotective activity which can be attributed to antioxidant and anti-inflammatory properties of this herbal drug.

It has been reported that cisplatin is injurious to renal vasculature and causes reduce in renal blood flow chiefly to ischemic injury of the kidneys, performing as a minimize in the glomerular filtration rate which is mirrored as expanded BUN and serum creatinine levels.\cite{14} In our research, we also observed the increased levels of BUN and serum creatinine which are indicative of renal damage by cisplatin. The serum creatinine is one of the waste metabolic products which is produced by the breakdown of creatinine phosphate of muscle. Normally, serum creatinine is excreted through the kidneys, in particular by way of by glomerular filtration and in trace amount through proximal tubular secretion with a minute or no reabsorption. Thus, during the renal dysfunction, serum level of creatinine rises\cite{17} as well as, simultaneous decline in urinary creatinine clearance is also observed. In our study, drug treatment was started 7 days earlier to cisplatin administration. It was observed that the administration of a hydroalcoholic extract of TT at a dose of 300 mg/kg significantly decreased BUN and serum creatinine levels. It was found that TT alone improves the renal functions in cisplatin-induced nephrotomic rats.

It has been reported that increased level of oxidative stress leads to lipid peroxidation and depletion of antioxidants enzymes such as GSH, SOD, and GPx. Tissue MDA and GSH levels and total GPx and SOD activities are important indicators of oxidative stress since it is difficult to measure ROS directly. MDA level is related to the degree of membrane damage while GSH, GPx, and SOD play a crucial role in the oxidative/antioxidant balance. These enzymes neutralize ROS and protect cells from oxidative damage.\cite{21} In this study, treatment of nephrotomic rats with hydroalcoholic extract of TT (300 mg/kg) significantly attenuated the renal oxidative stress which was noted in terms of decreased levels of antioxidants enzymes such as GSH, SOD, and GPx and increased levels of MDA. Cisplatin also causes mitochondrial dysfunction and upregulate the ROS production through disruption of the respiratory chain. ROS causes lipid peroxidation, which is defined by an increased level of MDA as seen in the cisplatin-control group in this study also [Figure 5]. Due to high instability of ROS molecules, they react with cellular proteins membrane lipids and DNA resulting in cellular stress.

**Discussion**

Nephrotoxicity is one of the major limitations associated with the use of several pharmacological agents. It is proven that a major dose of cisplatin shows a better therapeutic effect by attenuating the growth of tumors. However, chronic exposure of higher doses of cisplatin tends to the renal accumulation of platinum which produces deleterious effects including irreversible renal damage, and now till date, there is no any effective treatment strategy available to overcome the cisplatin-induced nephrotoxicity.\cite{11} It has been reported that cisplatin causes nephrotoxicity by the production of oxidative stress, mitochondrial dysfunction, and inflammation.\cite{17} Cisplatin converts into highly reactive form, and it interacts with thiol-containing molecules like GSH. Depletion or inactivation of endogenous antioxidants such as GSH and antioxidant enzymes by cisplatin leads to overproduction of reactive oxygen species (ROS), outoming in oxidative stress which leads to mitochondrial dysfunction.\cite{21}

The diagnosis of AKI on the basis of BUN and serum creatinine has its limitations. The BUN and serum creatinine are not specific as well as a sensitive method for detection of AKI. The factors which may affect its value are such as age, muscle mass, nutritional status, and infection.

### Table 2: Effect on platinum accumulation

<table>
<thead>
<tr>
<th>Name of the group</th>
<th>Platinum concentration (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>BLQ (&lt;0.3)</td>
</tr>
<tr>
<td>CP control</td>
<td>8.61±1.05***</td>
</tr>
<tr>
<td>TT 200+CP</td>
<td>5.96±0.07***</td>
</tr>
<tr>
<td>TT 300 ppm</td>
<td>BLQ (&lt;0.3)</td>
</tr>
</tbody>
</table>

CP: Cisplatin, TT: Tribulus terrestris

SD. (n = 6). Cisplatin (CP) Control: CP at 8 mg/kg, TT 200 + CP: CP at 8 mg/kg in combination with TT at 200 mg/kg, TT per se at 300 mg/kg: (a*** P < 0.05) versus Normal control, (b* P < 0.05) versus Cisplatin control.
The depletion of cellular antioxidant has also been mentioned previously in cisplatin nephrotoxicity models. In this present study, it is also observed that the decline in GSH and other endogenous antioxidants such as SOD and GPx in the cisplatin control group (Figures 3 and 4). However, the pre-treatment with TT alone maintained GSH and other antioxidants at close normal levels in renal tissue. Our results are, therefore, in support of the earlier studies that demonstrated the antioxidant activity of TT.

According to the results of present study, the expression of KIM-1 and L-FABP was found to increase in the cisplatin control group as compared to the normal control [Figure 8] which confirms the cisplatin-induced nephrotoxicity. Treatment of hydroalcoholic extract of TT (300 mg/kg) produces significant renal protection noted in terms of decreases the KIM-1 levels in drug-treated group as compared to the cisplatin control group. Moreover, all three doses of TT, i.e., 100/200/300 mg/kg decreased (not significant) the levels of L-FABP and KIM-1 in drug-treated groups as compared to cisplatin control group.

A single injection of cisplatin in rats causes renal morphological changes including tubular necrosis, desquamation, and degeneration in the proximal and distal tubules, which appeared histologically as tubular atrophy. Administration of TT alone at a dose of 300 mg/kg significantly preserved the histology of renal tubular cells in cisplatin-treated rats. The results of the present study are found to be in support/accordance to the previous studies with TT.

Renal accumulation of metals in the form of crystals is common renal problems in which crystals in the urine produces stones and is known as urolithiasis. Long term of urolithiasis leads to irritation, renal inflammation, and urinary tract infection. It has been reported that TT having the anti-urolithiatic actions in an experimental model in rats. In present study, platinum was found to be significantly accumulated after a single administration of cisplatin (8 mg/kg) in the cisplatin control group as compared to normal control. Treatment of TT 200 mg/kg significantly decreased the platinum concentration in kidney tissue as compared to the cisplatin control group which may be by its anti-urolithiatic activity.

The results of the current study showed that administration of TT reduced cisplatin-induced inflammation and oxidative stress which were incriminated in the pathogenesis of renal dysfunction. The nephroprotective efficacy of these compounds could be attributed due to its antioxidant and anti-inflammatory properties. The present study has, additionally, mounted the renoprotective impact of TT in cisplatin-induced acute renal toxicity in rats. The treatment of TT ameliorated most of the harmful indication of cisplatin. This renoprotection of TT was clear from enhanced functional as well as morphological renal profiles, blunting of inflammation and oxidative stress and decreased platinum level in kidney. Our outcomes, in addition, supplied proof that this improvement used to be mediated by inhibition of the oxidative stress and AKI marker.

**Conclusion**

It can be concluded based on the results of the present study that hydroalcoholic extract of TT has nephroprotective activity. The anti-inflammatory and antioxidant activities of TT contributed to its nephroprotective activity; it also decreased the platinum concentration in kidney tissue at some extent. Thus, TT extract can be further corroborated for its employees as a valuable potential substitute in the treatment of the cisplatin-induced nephrotoxicity.

**Acknowledgment**

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**References**


