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Research Article
Combination of *Tribulus terrestris*, *Boerhaavia diffusa* and *Terminalia chebula* Reverses Cisplatin-induced Nephrotoxicity in Wistar Rats

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Abstract

Background and Objective: Cisplatin is a potent anti-cancer agent, however, its usage is limited due to its nephrotoxicity. To prevent cisplatin induced kidney toxicity we used a combination of dietary plant extracts, particularly, hydro-alcoholic extract of *Tribulus terrestris* (TT), *Boerhaavia diffusa* (BD) and *Terminalia chebula* (TC) in rats. **Materials and Methods:** Animals (n = 6 per group) were randomly assigned into six groups: A normal control, cisplatin control, the drug-combination armat doses of 198, 300 and 600 mg kg^{-1} of equal ratio of each of TT, BD and TC and the combination of 300 mg kg^{-1} per se group. All treatments were given orally once a day for 10 days. Nephrotoxicity was induced by single dose of cisplatin 8 mg kg^{-1}, i.p on the 7th day. Blood urea nitrogen (BUN), serum creatinine, malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GPx), renal histopathology, liver fatty acid binding protein (L-FAB), kidney injury molecule-1 (Kim-1) and platinum accumulation in kidney tissue were determined. **Results:** The pre-treatment of the combination of equal ratio of TT, BD and TC at 198, 300 and 600 mg kg^{-1} significantly ($p<0.05$) attenuated the cisplatin-induced nephrotoxicity, as indicated by decrease in BUN, serum creatinine, MDA and an increase in the concentration of GSH, SOD and GPx whereas combination100 and 200 mg kg^{-1} significantly decreases Kim and L-FABP and combination of 600 mg kg^{-1} significantly decreases the platinum accumulation in treated groups as compare to cisplatin control group. **Conclusion:** Our results suggest that the combination of TT, BD and TC may be efficacious in attenuating cisplatin-induced nephrotoxicity by decreasing the renal level of platinum, reactive oxygen species (ROS) and oxidative stress.

Key words: Nephrotoxicity, plant extracts, platinum, antioxidant, L-FAB, KIM-1

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Over the past 2 decades, there has been a significant increase in the occurrence of acute kidney injury (AKI)[1,2]. Drug induced nephrotoxicity has been reported as a major cause of the high incidence and prevalence of AKI[3]. The frequency of hospital acquired AKI is 5-10 times greater than that of community-acquired AKI, which has an estimated yearly occurrence[4] of 0.15-7.2%. Nonetheless, this estimate may be low due to the under reporting of AKI. Cisplatin is used in the treatment of cancers of the testis, bladder, lung and ovary[5]. However, the use of cisplatin is restricted due to its potential of nephrotoxicity[6]. Cisplatin produces renal dysfunction and toxicity at a dose[7] of 5-40 mg kg^{-1}. Indeed, to minimize the magnitude of nephrotoxicity, the dosage of cisplatin is decreased or treatment withdrawn[8]. Approximately 30% of patients treated with cisplatin have been reported to develop a significant decrease in renal function after the first course of treatment[9]. Cisplatin is transported into c renal cells by the organic cation transporters 2 (OCT2) and can induce cell death by increasing the levels of reactive oxygen species (ROS), in addition to releasing TNF-α, which produces a significant inflammatory response[10]. Cisplatin also induces injury in the renal vasculature, eliciting ischemic tubular cell death and a decrease in the glomerular filtration rate (GFR)[11]. It has already been reported in our lab that cisplatin use causes increase in BUN and creatinine levels[12].

Tribulus terrestris (TT), known as Gokshur, Gokharu or Puncture vine is a member of the family Zygophyllaceae has been extensively used in Indian and Chinese traditional medicine[13]. The TT has been reported to have anti-inflammatory, anti-oxidant, diuretic, anti-hypertensive and anti-urolithiatic efficacy[14-16]. It is considered to be an important component in Ayurvedic and Unani systems for the treatment of renal diseases[13]. The TT has been reported to have a prophylactic effect against ROS-induced damage in the renal endothelium and play a key role in attenuating vascular endothelial dysfunction and impaired enzyme activity[17].

The aerial part of *Boerhaavia diffusa* (BD), its root as well as the entire plant has restorative properties and it is a member of the family Nyctaginaceae and known as 'Punarnava'[18]. It is utilized by tribes in India and Arabic nations as an anti-oxidant[19]. It produces anti-inflammatory efficacy by reducing ROS levels[20]. The BD has been reported to have efficacy in treating ascites[21]. The BD can decrease kidney cell damage and oxidative trauma produced by urinary stones[22].

Terminalia chebula (TC) a member of the family Combretaceae has been deemed "a king of medicines" in Tibetas it has efficacy in promoting wound healing[23]. TC has been reported to have antioxidant, anti-inflammatory, anti-bacterial, anti-fungal, antiviral, as well as anti-mutagenic, anti-ulcerogenic, hepatoprotective, cardioprotective, anti-diabetic and retinoprotective efficacy[24-26].

The TT has been utilized for the treatment of kidney disorders and it removes calculi from the urine and bladder[27-28]. The TC has been reported to have efficacy in treating cadmium and gentamicin-induced nephrotoxicity and BD has also been used to treat drug-induced nephrotoxicity[26].

Kidney injury molecule-1 (Kim-1)[29,30] and liver fatty acid binding protein (L-FAB)[31,32] are tubular proteins that appear during tissue injury and in response to various proximal tubular toxins, such as aminoglycosides, amphotericin B, cis-platinum, etc. Renal tissue injury results in inflammation which may also result in decrease in GFR and tubular obstruction. The growing concern over the increasing use of metal additives and enhance accumulation of metal has made it necessary to adequately characterize the potential harmful effects and determine the safety of these agents. Given the reports suggesting that TT, TC and BD may attenuate renal dysfunction due to certain drugs, we designed experiments to assess the nephroprotective efficacy of the combination of TT, BD and TC in cisplatin-induced nephrotoxicity in adult male wistar rats.

MATERIALS AND METHODS

Experimental animals: The study was conducted at the Department of Pharmacology, All India Institute of Medical sciences, New Delhi, India in August, 2016. Wistar albino rats, weighing 200-250 g were used in this study. The animals were obtained from the Central Animal Facility, All India Institute of Medical Sciences (AIIMS), New Delhi, India. The animals were acclimatized for one week before conducting the experiments. The animals were housed in polypropylene cages (40×20×15 cm). No more than 4 animals were kept in one cage. The animals were kept in standard laboratory conditions under natural light and dark cycles and had *ad libitum* access to rat chow and water. Each experimental animal was allotted a cumulative animal number for the purpose of keeping track of the animals and for maintaining an experimental data register. The experimental work was started after obtaining approval from the Institutional Animal Ethics Committee (document number 944/IAEC/16). All experimental work was conducted in the Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, India, 110029.

Drugs and chemicals: The hydro-alcoholic extract of TT, BD and TC were purchased from Sunpure Pvt., Ltd., New Delhi, India. Cisplatin was obtained from Fresenius Kabi Oncology Ltd., Solan, HP. The determination of BUN and serum creatinine was done based on the instructions for the kits (Erba, Mannheim, Germany) Glutathione peroxidase activity was determined using a colorimetric assay kit (Biovisions, Milpitas, California). The rat Kim-1 and L-FABP ELISA kits were purchased from Sincere Biotec company, Beijing, China. All other chemicals were purchased locally and were of the highest purity grade.

Experimental protocol: Wistar rats (n = 6/group) were randomly divided into six groups as indicated below:

- **Group 1 (normal control):** Normal saline (1 mL kg^{-1} day^{-1}; p.o) for 10 days
- **Group 2 (cisplatin control):** Normal saline (1 mL kg^{-1} day^{-1}; p.o) was given for 10 days and on the 7th day of treatment, a single injection of 8 mg kg^{-1} i.p. of cisplatin was given
- **Group 3-5 (combination of TT with BD and TC 66, 100, 200 mg kg^{-1} day^{-1} each extract+cisplatin):** Combination of *Tribulus terrestris* (TT), *Terminalia chebula* (TC), *Boerhaavia diffusa* (BD) 66, 100 and 200 mg kg^{-1} day^{-1} each; p.o. were administered to wistar rats for a period of 10 days and on 7th day, a single injection of cisplatin at the dose of 8 mg kg^{-1} i.p. was given
- **Group 6 (TT, BD and TC):** A combination of *Tribulus terrestris* (TT) *Terminalia chebula* (TC) and *Boerhaavia diffusa* (BD) at a dose of 100 mg kg^{-1} day^{-1}, p.o for each drug was administered to rats for 10 days

Measurement of kidney function: Serum creatinine was determined using the Erba unit, according to manufacturer's instructions (Erba, Mannheim, Germany). The working reagent was set up by mixing reagent 1 (picric acid) and reagent 2 (an antacid reagent) in parallel volumes. The working reagent (1000 µL) was mixed with the test sample (100 µL) the absorbance at 510 nm was measured using a UV spectrophotometer (Specord 200, Analytic Jena AG, Germany). Each sample was analyzed in duplicate. The determination of blood urea nitrogen (BUN) in the serum samples was done using an Erba Mannheim kit, according to the manufacturer's instructions (GLDH-Urease technique). In brief, the working reagent (1000 µL) was mixed with the test (20 µL) and the absorbance at 340 nm was measured using a UV spectrophotometer.

Determination of oxidative stress: The rat kidneys were surgically removed, weighed and placed in 10 times (w/v) of 0.1 M sodium phosphate buffer (pH 7.4), followed by homogenization in a homogenizing tube at 800-900 rpm for 10 min. The homogenate was used to determine malondialdehyde (MDA) levels, glutathione levels (GSH), superoxide dismutase (SOD) and glutathione peroxidase (GPx) Each sample was done in duplicate. The MDA was measured as previously described[33]. The GSH levels were determined using the technique of Ellman[34]. The SOD activity was determined using the method of Marklund and Marklund[35]. The activity of GPx was determined using an assay kit (Biovision, Milpitas, California).

Measurement of markers of acute kidney injury: The kidney injury molecule 1 (kim-1) and liver-fatty acid binding proteins (L-FABP) were measured using ELISA kits based on the manufacturer's instructions.

Sample volumes of 10 µL tests were obtained, diluted with 40 µL of the diluent, mixed gently and covered with clouser plate film and incubated for 30 min at 37°C. The ELISA plate was washed using a washing buffer 5 times for 30 sec. About 50 µL of the HRP conjugate was added and the sample was incubated and washed as above. About 50 µL of chromogen solution Awas added to each well, followed by the addition of 50 µL of chromogen solution B. Each well was carefully mixed, protected from light by covering with an aluminum foil and incubated for 15 min at 37°C. The reaction was stopped by adding 50 µL of stop solution to each well (see an immediate change in color from blue yellow). Subsequently, the absorbance was immediately measured at 450 nm using a UV spectrophotometer. Each sample was done in duplicate.

Histopathological assessment: Para-formaldehyde fixed tissues were kept under running tap water for 6-8 h to evacuate the fixative. After washing with gradually increasing concentrations of alcohol and acetone, the samples were inundated with cedar oil for 2-3 days. The samples were then placed in paraffin wax and poured in a plastic mold and permitted to harden at room temperature to make a paraffin block. The paraffin blocks cut into 5 µm thick sections using a microtome (Shandon AS 325, Hampton, US) and the tissue sections were permitted to drift in warm water (44°C). The flattened gliding tissue sections were placed up against egg albumin and thymol pre-covered glass slides. The slides were air dried and kept overnight at room temperature.

The paraffin sections were then stained with using hematoxylin and eosin as previously described[36]. Pictures were

taken of the slides and the histology of each slide was viewed by an expert pathologist for observing the pathological change in the renal tissue.

Determination of platinum in kidney tissue using inductive coupled plasma atomic emission spectroscopy (ICP-AES): About 500 mg of tissue were placed in a digestion vessel unit and 3 mL of nitric acid and 1 mL of hydrogen peroxide were added. The samples were placed in a digester (Sineo Company) and scheme 2 was selected where the temperature was increased incrementally up to 160°C. Finally, the digested samples were assessed using inductively coupled plasma atomic emission spectroscopy (ICP-AES).

Statistical analysis: The data were represented as mean±SEM. The data were analyzed using Wilcoxon-Rankson test with direct comparison of respective groups. The prior p-value was less than 0.05. The data were analyzed using the standard statistical software for Graph pad prism version 5.03 (San Diego, CA, USA).

RESULTS

Effect of TT, BD and TC on renal function in animals administered cisplatin: A single i.p. injection of 8 mg kg^{-1} of cisplatin significantly ($p<0.05$) increased serum creatinine and BUN levels compared to the control group (Fig. 1). The pretreatment of animals with TT, BD and TC at doses of either 198, 300 or 600 mg kg^{-1} significantly ($p<0.05$) reduced the level of serum creatinine and BUN level as compare to cisplatin control group (Fig. 1a and b).

Effect of TT, BD and TC on oxidative stress in animals administered cisplatin: The animals in the cisplatin control group had a significant increase in tissue MDA levels (Fig. 2a), a significant decrease in SOD enzyme activity (Fig. 2b), GSH (Fig. 2c) and GPx content (Fig. 2d). The pretreatment of combination of TT, BD and TC (198, 300 and 600 mg kg^{-1}) significantly decreased the renal MDA levels ($p<0.05$) and significantly attenuated the decrease in of SOD activity, GSH levels and GPx activity as comparison to cisplatin control group ($p<0.05$).

Effect of TT, BD and TC on the levels of kidney injury molecule-1 (kim-1) in animals administered cisplatin: The levels of Kim-1 level were significantly ($p<0.05$) increased in animals treated with cisplatin compared to the normal control group. The administration of 198 mg kg^{-1} of combination of TT BD and TC did not significantly decrease the levels of Kin-1 moreover treatment of combination of 100

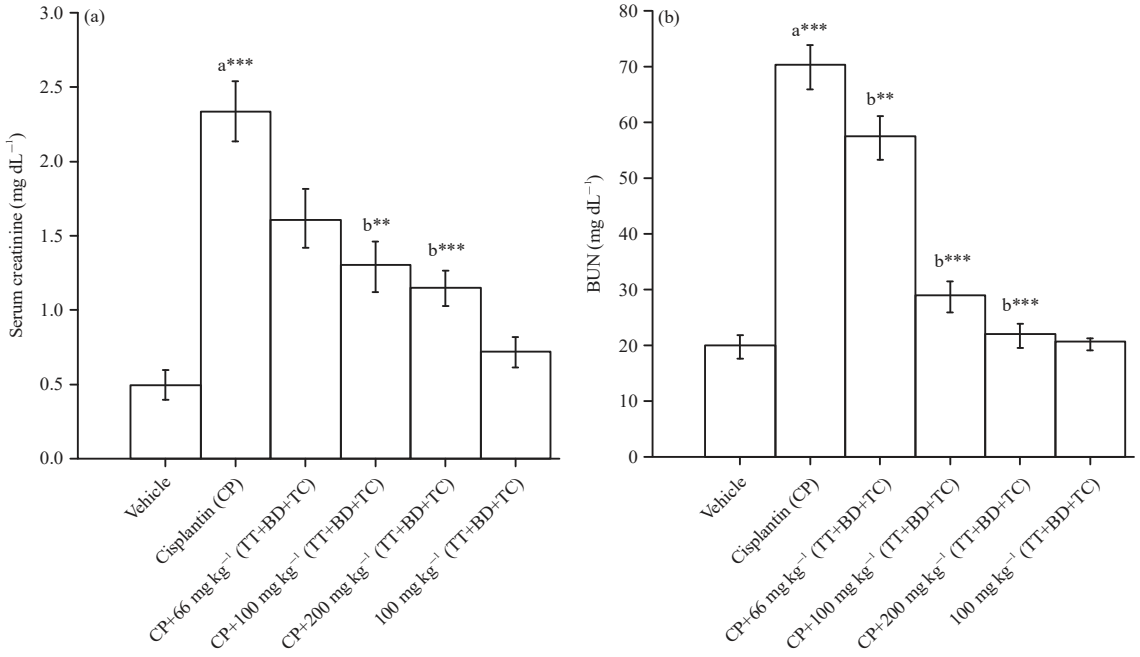

Fig. 1(a-b): Effect of combination of *Tribulus terrestris (TT)*, *Boerhaavia diffusa* (BD) and *Terminalia chebula* (TC) on (a) Serum creatinine and (b) Blood urea nitrogen (BUN) level in cisplatin induced nephrotoxicity

Each bar represents as mean±SD. Control: Cisplatin at 8 mg kg^{-1}, Comb: Cisplatin at 8 mg kg^{-1} in combination of TT with BD and TC at 66/100/200 mg kg^{-1} of equal ratio of each drug. Combination of TT with BD and TC at 100 mg kg^{-1} of equal ratio of each drug as per se group. a: $p<0.05$ vs. Normal control and b: $p<0.05$, vs. Cisplatin control

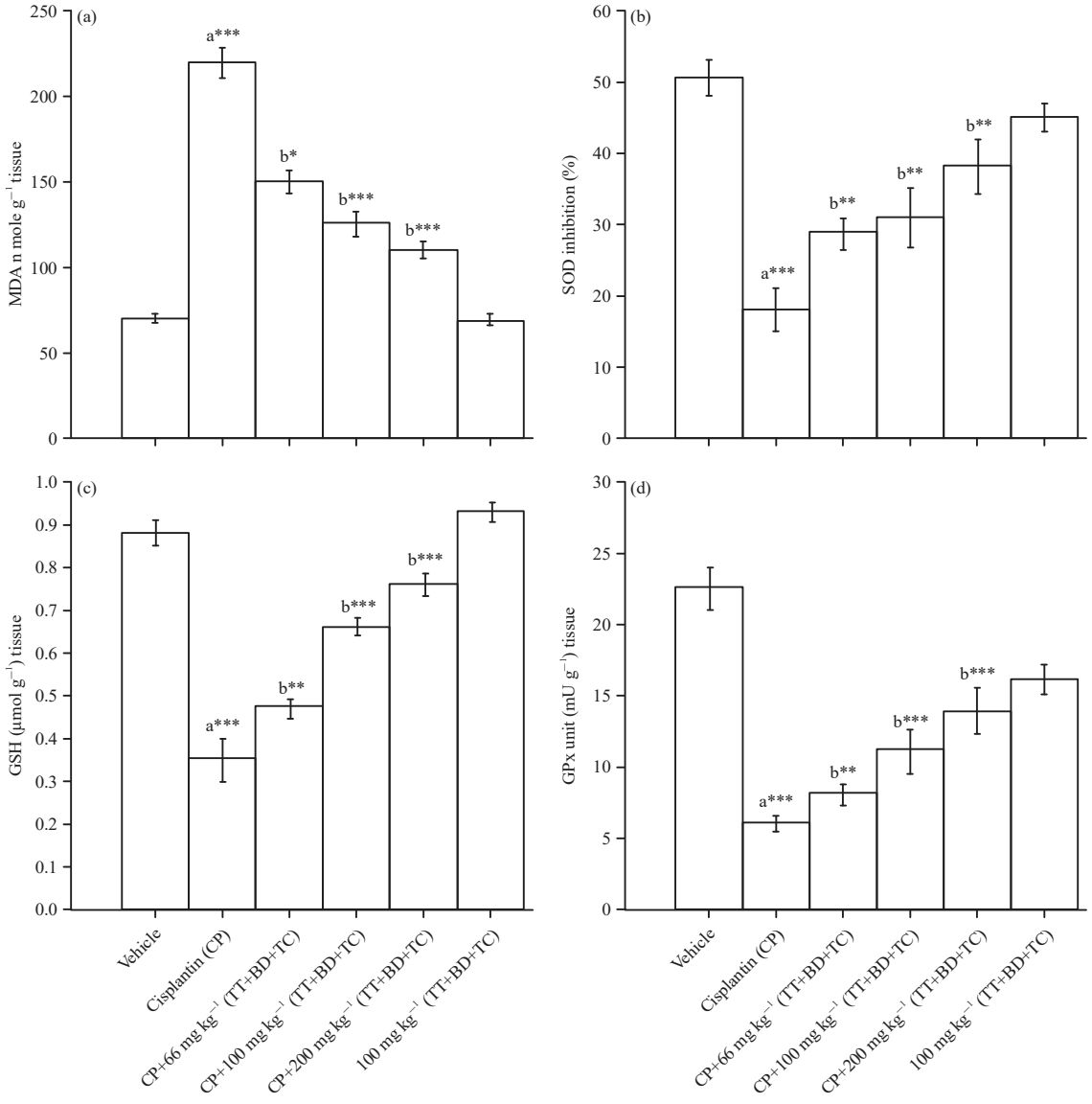

Fig. 2(a-d): Effect of combination of *Tribulus terrestris* (TT), *Boerhaavia diffusa* (BD) and *Terminalia chebula* (TC) on (a) MDA level, (b) SOD activity, (c) GSH content and (d) GPx level in cisplatin induced nephrotoxicity

Each bar represents as mean±SEM, Control: Cisplatin at 8 mg kg^{-1}, Comb: Cisplatin at 8 mg kg^{-1} in combination of TT with BD and TC at 66/100/200 mg kg^{-1} of equal ratio of each drug. Combination of TT with BD and TC at 300 mg kg^{-1} of equal ratio of each drug as per se group. a: p<0.05, vs. normal control and b: p<0.05, vs. cisplatin control

and 200 mg kg^{-1} of TT, BD and TC significantly (p<0.05) decreased Kim-1 levels compared to the cisplatin control group (Fig. 3).

Effect of TT, BD and TC on the levels of liver-fatty acid binding protein (L-FABP) in animals administered cisplatin: The administration of 8 mg kg^{-1} i.p. of cisplatin significantly increased (p<0.05) the serum levels of L-FAPB compared to the normal control group (Fig. 4). The administration of 198 mg kg^{-1} i.p. of TT BD and TC did not significantly decrease the levels of L-FABP, moreover administration of combination of 100 or 200 mg kg^{-1} i.p. each of TT BD and TC significantly (p<0.05) decreased the serum levels of L-FABP compared to the normal control group.

Effect on histopathological structural changes: The combination of TT, BD and TC at doses of 198, 300 and 600 mg kg^{-1}, respectively showed 50-75% intact/viable tubular epithelium and remaining were necrotized representing grade 1 protection (Fig. 5).

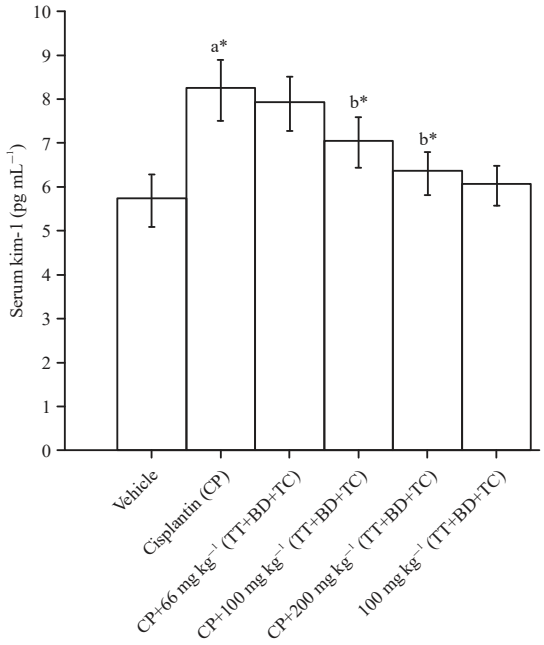

Fig. 3: Effect of combination of *Tribulus terrestris* (TT), *Boerhaavia diffusa* (BD) and *Terminalia chebula* (TC) on Kim-1 levels in cisplatin induced nephrotoxicity
Each bar represents as Mean±SEM, Control: Cisplatin at 8 mg kg^{-1}, Comb: Cisplatin at 8 mg kg^{-1} in combination of TT with BD and TC at 66/100/200 mg kg^{-1} of equal ratio of each drug. Combination of TT with BD and TC at 100 mg kg^{-1} of equal ratio of each drug as per se group. a: p<0.05, vs. normal control and b: p<0.05, vs. cisplatin control

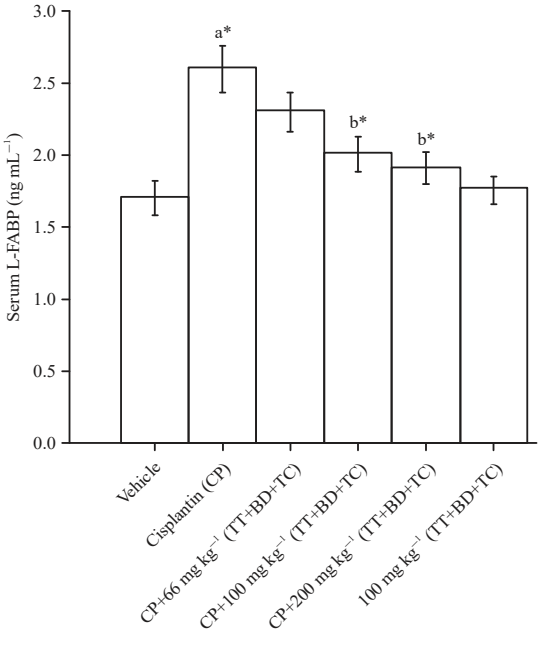

Fig. 4: Effect of combination of *Tribulus terrestris* (TT), *Boerhaavia diffusa* (BD) and *Terminalia chebula* (TC) on L-FABP levels in cisplatin induced nephrotoxicity
Each bar represents as Mean±SEM, Control: Cisplatin at 8 mg kg^{-1}, Comb: Cisplatin at 8 mg kg^{-1} in combination of TT with BD and TC at 66/100/200 mg kg^{-1} of equal ratio of each drug. Combination of TT with BD and TC at 100 mg kg^{-1} of equal ratio of each drug as per se group. a: p<0.05, vs. normal control and b: p<0.05, vs. cisplatin control

Table 1: Effect of *Tribulus terrestris* (TT), *Boerhaavia diffusa* (BD) and *Terminalia chebula* (TC) on platinum accumulation in the kidneys of male wistar rats

Treatment groups	Platinum concentration (μg g^{-1})
Normal control	BLQ (<0.3)
Cisplatin-control	8.68±0.23*^{a}
TT, BD and TC 600 mg kg^{-1} day^{-1}+cisplatin	6.10±0.22*^{b}
TT, BD and TC 300 mg kg^{-1} day^{-1}	BLQ (<0.3)

Animals in groups 2 and 3 were given 8 mg kg^{-1} i.p. of cisplatin on the seventh day. The animals in group 1 received vehicle (normal saline, 1 mL kg^{-1} day^{-1} p.o) on the 7th day of treatment. The animals in groups 3 and 4 were given combination of TT, BD and TC once a day for 7 days. The data are expressed as the Mean±SEM and n = 6 for each treatment group.*^{a}p<0.05 vs normal control, *^{b}p<0.05 vs cisplatin control

Effect on platinum accumulation: The administration of 8 mg kg^{-1} i.p. of cisplatin significantly (p<0.05) increased the accumulation of platinum in the kidneys compared to the normal control group (Table 1). It did not perform the 100 mg kg^{-1} due to some limitations. The combination 600 mg kg^{-1} (TT 200 mg kg^{-1}, BD 200 mg kg^{-1} and TC 200 mg kg^{-1} each) significantly (p<0.05) decreases the renal levels of platinum compared to cisplatin control group (Table 1).

DISCUSSION

Previously, it has been reported that one of the limiting adverse effects in the use of cisplatin is renal dysfunction that can progress to nephrotoxicity[37]. A number of studies suggest that cisplatin-induced nephrotoxicity in humans may result from oxidative stress, mitochondrial dysfunction and inflammation[38,39]. The depletion of endogenous antioxidants such as glutathione and decrease in the levels or activity of antioxidant enzymes (e.g., SOD) increases ROS levels, thereby exposing cells to oxidative stress[40,41]. Therefore, treatments that can decrease the likelihood of cisplatin-induced nephrotoxicity could be useful in patients treated with cisplatin. In the present study, it determined the effect of combination of TT, BD and TC on cisplatin-induced nephrotoxicity in Wistar rats by measuring renal function, acute kidney damage markers, histopathological features and the levels of certain antioxidants and activity of enzymes involved in the biosynthesis of anti-oxidants. Overall, these results suggested that the oral administration of TT, BD and

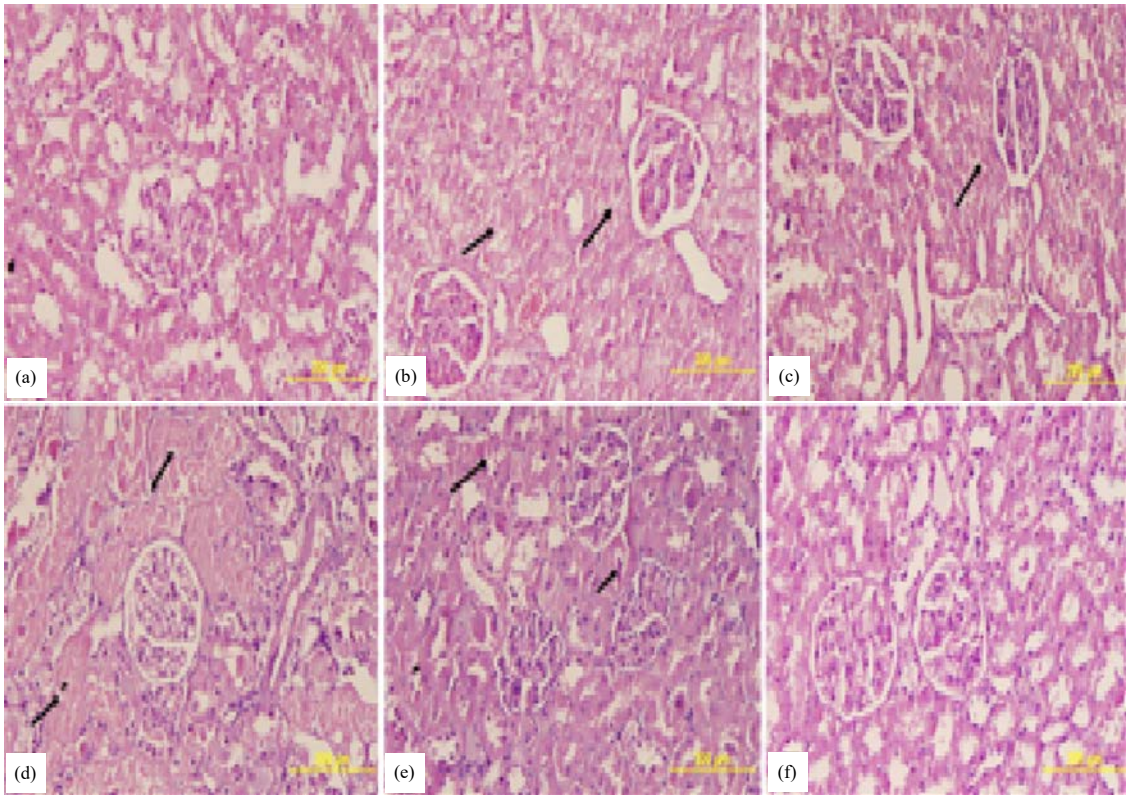

Fig. 5(a-f): Effect of combination of *Tribulus terrestris* (TT), *Boerhaavia diffusa* (BD) and *Terminalia chebula* (TC) on histopathological change in proximal and glomerulus tubular cell (a-f) in different experimental group in (8 mg kg^{-1}, i.p. once) induced nephrotoxicity

(a) Normal control treated kidney show normal morphology of tubules and glomeruli epithelial cell, (b) Cisplatin-induced kidney exhibit diffuse acute proximal tubular necrosis, glomerular shrinkage without inflammation and fibrosis, (c) kidney section from combination of TT with BD and TC+cisplatin at 200 mg kg^{-1} shows similar finding with tubular necrosis and glomerular atrophy, (d) kidney section from combination (100 mg kg^{-1}) of TT with BD and TC+8 mg kg^{-1} of cisplatin shows patchy tumor necrosis and preserved normal glomeruli, (e) Kidney section from combination (200 mg kg^{-1}) of TT with BD and TC+8 mg kg^{-1} of cisplatin shows preserved tubular epithelium and normal glomeruli and (f) Combination of TT with BD and TC per se group exhibit normal structure of proximal tubular cells and cortex

TC had nephroprotective effects in animals administered 8 mg kg^{-1} i.p. of cisplatin after 7 days of treatment with TT, BD and TC.

Clinically, it is well-established that serum creatinine and BUN levels are indicators of renal function[42]. The normal reference values for BUN and creatinine earlier reported by Karwasra *et al.*[12] ranged from 20-30 and 0.4-0.6 mg dL^{-1}, respectively. The values of BUN and creatinine indicative of severe kidney dysfunction ranged from 80-100 and 1.4-2.0 mg dL^{-1}, respectively[12].

These results indicate that the administration of combination of TT, BD and TC at 198 or 300 and 600 mg kg^{-1} given 7 days before cisplatin significantly decreased the cisplatin-induced increase BUN and serum creatinine levels. It hypothesized that the nephroprotective efficacy of TT, BD and TC could be due their attenuation of renal oxidative stress. Indeed as mentioned earlier, cisplatin nephrotoxicity may be in part due to an increase in ROS levels[12]. It is well established that increased oxidative stress induces lipid peroxidation and depletion of anti-oxidants like GSH and decreases the levels and activity of the enzymes SOD and GPx, which play a role in oxidative defense[43]. Tissue MDA and GSH levels and the activity of GPx and SOD have been reported to be indicators of oxidative stress[44]. The MDA levels have been reported to be positively correlated with the magnitude of membrane damage[45]. In addition, GSH, GPx and SOD play a critical role in maintaining the oxidative/anti-oxidant balance in cells[46]. For example, the enzymes SOD and GPx superoxide and peroxide radicals, respectively, thereby protecting cells from oxidative damage[40,43].

In this study, the administration of a single dose of 8 mg kg^{-1} i.p. of cisplatin significantly decreased the levels of

GSH, GPx, SOD and increased the levels of MDA. These findings are consistent with previous studies indicating the ROS are involved in the pathogenesis of cisplatin-induced nephrotoxicity[37]. Results indicated that the administration of combination of either of 198, 300 or 600 mg kg^{-1} of TT, BD and TC significantly attenuated the renal oxidative stress induced by cisplatin as evidenced by the decrease in the levels of creatinine and BUN and increase in the level of GSH, SOD and GPx. Consistent with these results, it has been reported that TT significantly decreases cisplatin-induced apoptosis in mouse kidney[47]. The TT has been reported to decrease ROS and increase the removal of gravel from the urine and calculi from the bladder of mice administered cisplatin[48]. The BD has been shown to significantly attenuate gentamicin-induced nephrotoxicity in mice[49]. The TT BD significantly enhances mitochondrial function and inhibits the release of cytochrome c[50]. Based on the results of this study and others[37,48], it hypothesized that the nephroprotective effects of the combination of TT, BD and TC may be due to antioxidant and anti-inflammatory efficacy[47,49].

In this study, a single administration of 8 mg kg^{-1} i.p. of cisplatin on the seventh day of treatment significantly increased the accumulation of platinum in animals as compare to control group. The findings of this study were consistent with literature showing that kidney accumulates and retains platinum to a greater degree than other organs[51,52]. The administration of combination of 600 mg kg^{-1} of TT, BD and TC significantly alter the renal accumulation of platinum as compared to animals given only vehicle. The combination of TT, BD and TC causes excretion of platinum from kidneys. As stated earlier given that platinum accumulation can produce renal toxicity, similarly in this study combination of TT, BD and TC was attenuating cisplatin-induced toxicity by significantly decreasing the levels of platinum in the kidneys.

A number of studies indicated that increase in the level of the proteins Kim-1 and L-FAB represent a biomarker for renal damage[53]. Furthermore, Kim-1 and L-FAB have predictive value for AKI in patients undergoing cardiac surgery[54,55]. In this study, cisplatin, as previously reported by Abdel-Kader *et al.*[56], significantly increased the expression of Kim-1 and L-FAB in the glomeruli and renal tubules of Wistar rats. The combination treatment at dose of 300 and 600 mg kg^{-1} significantly decreased the expression of Kim-1 and L-FABP. As previously reported by Vaidya *et al.*[3] and Abdel-Kader *et al.*[56], a single injection of 8 mg kg^{-1} i.p. of cisplatin produced a number of renal morphological changes, including tubular necrosis, desquamation and degeneration in the proximal and distal tubules, which appeared histologically as tubular atrophy[3]. The findings are consistent with previous lab findings stating that BD decreases creatinine and BUN levels and also increase the levels of antioxidant such as GSH, SOD and GPx. In these result maximum renal protection was observed at 600 mg kg^{-1} day^{-1}. In the human relevance dose it reflects near about 48 g day^{-1} for an 80 kg human that seems to be very high. Combination of 300 mg kg^{-1} also produced significant renal protection noted in terms of BUN, serum creatinine, acute kidney injury markers i.e., L-FAB and Kim-1 and also other oxidative stress markers. Moreover, it is possible to increase the length of treatment by using the low dose of the combination of TT, BD and TC which may be effective for the patient taking cisplatin.

CONCLUSION

This combination of herbal drug decrease the platinum accumulation in rat kidney is a novel finding of this research. Provided these results can be extended to humans, they suggest that the combination of TT, BD and TC could be used in the pharmacotherapy of nephrotoxicity due to cisplatin and possibly other drug.

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SIGNIFICANCE STATEMENT

This study discovered the this combination of herbal drug decrease the platinum accumulation in rat kidney is a novel finding of this research. That can be beneficial for patients with nephrotoxic effects of platinum, frequently use herbal or nutrient supplements and/or concomitant medications with their treatment. This study helps the researchers to uncover the critical areas of dose monitoring in metal accumulation patients that many researchers were not able to explore. Thus a new theory on combination herb-drug interaction may be arrived at.

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