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Nephroprotective Activity of *Cissampelos Pareira* Linn. Extract Against Cispltin Induced Nephrotoxic Rats

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ABSTRACT

To appraise the nephroprotective and antioxidant activity of hydroalcoholic*Cissampelospareira* (*C.pareira*) whole plant extract using cisplatin induced nephrotoxic rats. *C.pareira*is a well know medicinal plant of Menispermaceae family which is widely used for ayurvedic medicine to treat various kidney complications. In the present study *C.pareira*was observedfor its acute toxicity studies by using OECD guidelines no.425. The nephroprotective activity was evaluated by using cisplatin induced nephrotoxic rats. *In-vivo* antioxidant activity was evaluated by using glutathione and lipid peroxidation estimation in cisplatin induced rats. One way ANOVA followed by Dunnett's test using Graph pad prism software. Statistical significance was set at $P \leq 0.05$. From the acute toxicity study two doses were selected as 200 and 400 mg/kg *p.o.* body weight for the present study. *C.pareira* extract significantly increases the body weights, decrease the elevated urinary glucose levels in the urine, decrease the urea and creatinine levels in blood and increase the urinary creatinine levels in cisplatin induced nephrotoxic rats. In the *in-vivo* antioxidant study there was a dose dependent decreasing and increasing of lipid peroxidation, glutathione levels in hydroalcoholic extract treated groups respectively. The histopathological investigation was also supported nephroprotective activity of *C.pareira*. All the results were shown that the plants *C.pareira*hydroalcoholic extract has significant nephroprotective activity.

Keywords: Nephrotoxicity, Cisplatin, Antioxidant.

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INTRODUCTION

Cisplatin is a potent antitumor agent and widely used for diverse spectrum of malignancies[1]. Even though it's tremendous use in the treatment of malignancies, the usage of cisplatin is contraindicated because of its nephrotoxicity and ototoxicity[2,3] along with various side effects including vomiting, nausea and diarrhea[4,5]. Cisplatin induces cellular damage and necrosis in the rat kidney especially in proximal convoluted tubular epithelial cells[6]. The main targets for the cisplatin are mitochondria, lysosomes, microsomes and it can alter the physiological functions of the kidney like change in urine volume, increase in blood urea nitrogen, urinary glucose levels and serum creatinine[7,8,9].Cisplatin induces nephrotoxicity by generating oxygen free radicals like superoxide anion and hydroxyl radicals and stimulates lipid peroxidation in renal tissue[10,11]. Superoxide dismutase (SOD) and *o-(*beta-hydroxyethyl)-rutoside are acting as a free radical scavenger and reported to be a partial protective agent against cisplatin induced nephrotoxicity[12,13]. Therefore, the generation of free radicals may play a crucial role in cisplatin induced nephrotoxicity. So, there is a continuous search for the agents which can reduce or ameliorate the nephrotoxicity induced by the cisplatin for which there is no remedy in allopathy. Therefore, the present work mainly focuses on naturopathy by using naturally available antioxidants which are going to scavenge the reactive oxygen species to ameliorate the nephrotoxicity. The plant *Cissampelospareira* (*C.pareira*) is used to treat Rakta and Medadhaatus according to Ayurveda. In folk medicine *C.pareira* is using for various disease like diarrhea, dropsy, cough and urinary difficulties[14,15]. Hence, in the present study we are intended to evaluate the nephroprotective activity of *C.pareira*hydroalcoholicextract.

MATERIALS AND METHOD

Preparation of Whole Plant Extract

The plant *C.pareira* was collected from Thirupathi and it was authenticated by Dr. K. Madavachetty, Department of Botany, Sri Venkateswara University, Tirupathi. The whole plant was washed and shade dried then the dried plant was pulverized into coarse powder. The resulting powder was used for hydroalcoholic extraction by using 70% ethanol. The extract was used for the investigation of nephroprotective and its *in-vivo* anti-oxidant activity. The extract was concentrated under reduced pressure and stored in vacuum desiccators. The dried extract was suspending in distilled water by using tween 80.

Chemicals

Kits for the estimation of urinary creatinine,urinary glucose,serum urea and serum creatinine were purchased from transasia bio-medical ltd, coral clinical systems, span diagnostics ltd. All the chemicals were of analytical grade.

Preliminary Phytochemical Investigation

For the qualitative identification of phytoconstituents we have done preliminary phytochemical screening[16].

Experimental Animals

Adult albino wistar rats weighing 180-250g was procured from Biogen, Bangalore. They were maintained in the animal house of Gautham College of Pharmacy. The animals were maintained at optimum conditions 27° ± 2° C and 12-h light-dark cycles. These animals were free access to standard pellets and water ad libitum. The study was approved by Institutional Animal Ethical Committee formed (REF-IAEC/021/12/2010) according to Committee for the purpose of Control and Supervision of Experiments on Animals (Reg No: 491/01/c/CPCSEA), Govt. of India.

Acute Toxicity Testing

As per the Organization for Economic Cooperation and Development (OECD) guidelines 425, the acute oral toxicity was carried out in wistar rats and the dose 2g/kg, which was used, did not show any kind of toxic effects on animals. So, maximum tolerated dose was determined as 2g/ kg, as the test item was a source from herb. From the maximum tolerated dose we have selected 200 mg/kg and 400 mg/kg as test doses.

Evaluation of Nephroprotective Activity

In the dose response experiment, 24 wister rats were randomly divided into 4 groups of 6 animals each. 1st and 2nd group of animals kept for negative and positive control respectively. 1st group was given with normal saline throughout the experiment and 2nd group was given with cisplatin (7.5 mg/kg, *i.p.*) on 7th day. 3rd and 4th groups of animal were given withcisplatin (7.5 mg/kg,*i.p.*) on 7thday and these two groups of animals were given with *C.pareira*extract 200 mg/kg*p.o*. 400 mg/kg*p.o.* respectively for 9 days, 6 days prior to the treatment of cisplatin, on 7th day along with cisplatin and 2 days after the treatment of cisplatin. At the end of the study all the animals were kept in metabolic cages for 24 hrto collect the urine samples, under mild anesthesia (ketamine 60 mg/kg, xyazine 5 mg/kg *i.p.*) all animals sacrificed to collect blood samples and kidney samples. Blood samples were collected via cardiac puncture, kept aside for 1 hr at 4ºC and centrifuged to separate serum. The serum samples were used for the estimation of creatinine, urea. The urine samples were collected to estimate urinary glucose, creatinine levels. The kidney samples were used for *in-vivo* antioxidant studies and histopathological studies.

***IN-VIVO* ANTIOXIDANT STUDIES**

Glutathione Estimation[17]

Kidney samples were homogenized in ice cold trichloroacetic acid (10ml 10%TCA plus 1 gm tissue). Modified Ellamn procedure (Aykae, et.all.) was used to estimate the glutathione estimation. Briefly, after centrifugation aat 3000rpm for 10 min. supernatant was collected from the supernatant 0.5 ml was added to 2 ml of 0.3 M disodium hydrogen phosphate solution. Add 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml in 1% sodium citrate) and absorbance was measure at 412nm immediately after mixing the solutions. Percentage increase in absorbance was calculated.

Lipid Peroxidation[17]

Trichloroaceticacis 15% w/v, thiobarbituric acid 0.375% w/v and hydrochloric acid 0.25 N were mixed together on mild heating to make sure of thiobarbituricacis dissolution, to form a stock solution of TCA-TBA-HCl stock solution. Take 2.0 ml of stock solution of TCA-TBA-HCl, mix with 1.0 ml of biological sample (0.1-2.0 mg of membrane protein or 0.1-0.2 µmol of lipid phosphate). The mixture was heated for 15 min in boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. the absorbance of the sample was determined at 535 nm against a blank. Percentage decrease in absorbance values was calculated.

Histopathological Examination

After scarification from each group kidneys were isolated from two animals and kept in 10% formalin solution for histopathologicalzz studies. Samples were embedded in paraffin, sectioned and stained with haematoxylin and eosin.

Statistical Analysis

The values were expressed as Mean ± SEM. The data analysed by using one way ANOVA followed by Dunnett's test using Graph pad prism software. Statistical significance was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

The present study was aimed to evaluate the nephroprotective activity of *C.pareira*hydroalcoholic extract against cisplatin induces nephrotoxic rats. Cisplatin induces nephrotoxicity by generating free radicals which will increases the lipid peroxidation and decreases the naturally available antioxidants in the body like glutathione, superoxide dismutase, catalase, and peroxidases in tissues. Some reports suggesting that cisplatin increases the calcium independent nitric oxide

synthesis alter the L-arginine metabolism leads to the generation of nitric oxide[18]. By taking all literature into consideration, the nephrotoxicity is due to the generation of free radicals. So that, those agents having antioxidant property might have amelioration effects on cisplatin induced nephrotoxicity. The phytochemical investigation revealed that the plant extract has alkaloids, flavanoids, carbohydrates, glycosides, proteins, tannins but saponins are absent. In the acute toxicity study, none of the animal has shown any toxicity upon administration of *C.pareira*hydroalcoholic extract(2 g/kg *p.o.*). So, a low dose of 200 mg/kg, *p.o.* and a high dose of 400 mg/kg, *p.o.*were selected for the present study. Treatment with cisplatin 7.5 mg/kg *i.p.* on 7th day produces significant nephrotoxicity. Physical parameter, the nephrotoxic rats were shown significant decrease in body weights ($p<0.001$), when compared with normal control (Table 1). Urinary levels of glucosecreatinine, and serum levels of urea, creatinine, there was a significant increase in urinary glucose ($p<0.001$), serum creatinine ($p<0.01$), serum urea ($p<0.001$) and significant decrease in urinary creatinine ($p<0.05$) when compared with normal control group. Whereas administration of *C.pareira*at a dose of 200mg/kg, 400 mg/kg were significantlyimproves the body weights ($p<0.01$), $p<0.001$) respectively. But there was no significant increase in urinary creatinine with the co-administration of *C.pareira*at a dose of 200 mg/kg. Whereas co-administration of extract at a dose of 400 mg /kg was significantly increase the urinary creatinine ($p<0.05$). The co-administration of *C.pareira*extract at a dose of 200 mg/kg, 400 mg/kg were shown a significant decrease in urinary glucose when compared with normal control ($p<0.05$), ($p<0.001$) respectively. Administration of extract at a dose of 200 mg/kg and 400 mg/kg were shown significant decrease in blood urea levels ($p<0.001$), but 200 mg/kg dose was not shown any significant decrease in urinary creatinine levels in plasma whereas 400 mg/kg was shown significant decrease in blood creatinine ($p<0.05$) (Table 2). In the present study the results were shown that administration of cisplatin elevated the levels of urinary glucose, blood creatinine and blood urea significantly and decreases the body weights, urinary creatinine levels significantly when compared with normal control. By the administration of *C.pareira*extract has shown the significant improvement in body weights, urinary creatinine levels and significantly decreased the urinary glucose, blood creatine and blood urea levels when compared with normal control. Cisplatin has shown significant increase in lipid peroxidation ($p<0.001$) and significant decrease in glutathione ($p<0.001$), whereas the co-administration of *C.pareira*extract was shown significant decrease in the lipid peroxidation ($p<0.001$) and also significant improvement in the glutathione levels ($p<0.001$) in the kidney tissues (Table 3). The animals treated with cisplatin has shown significant increase in lipid peroxidation and decreases the glutathione levels in the kidney tissues

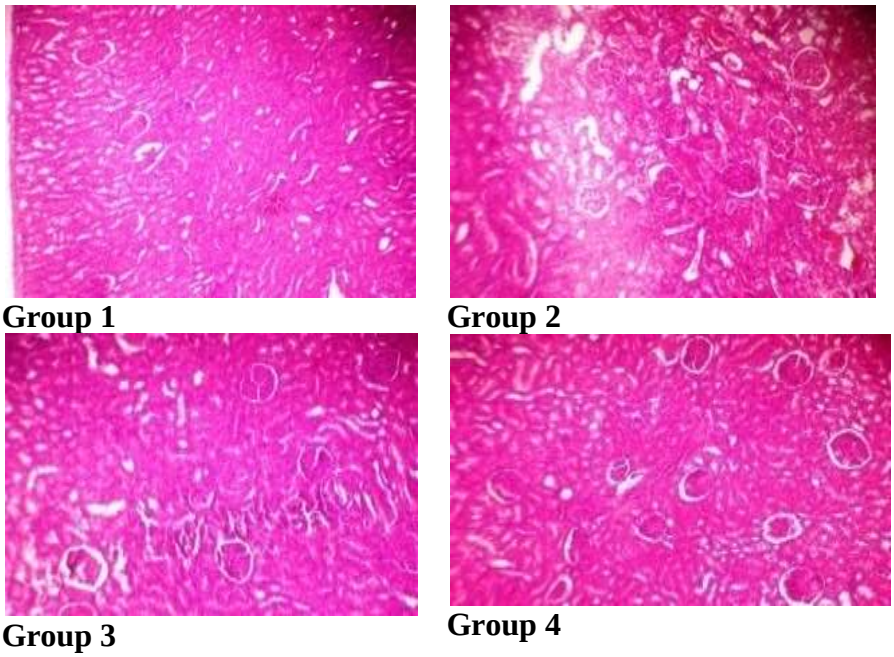
because of the generation of free radicals, whereas the co-administration of *C.pareira*extract has shown significant decrease in lipid peroxidation and increases the glutathione levels in the kidney tissues. The cisplatin treated group has shown tubular congestion, epithelial desquamation, glomerular congestion when compared with normal control. Whereas co-administration of extract showed minimal cellular damage and decrease the tubular congestion and glomerular congestion dose dependently (Table 4 and Figure 1). The histopathological study also proved that the *C.pareira* extract has nephroprotective activity by having antioxidant property.

Table 1: Effect of *C.pareira*Hydroalcoholic Extract on Change in Body Weight

Group	Treatment	Change in body weight (g) Mean ± SEM
Group-I	Normal saline	4.66 ± 1.56
Group-II	Cisplatin 7.5 mg/kg I.P.	-9.66 ± 2.108***
Group-III	Cisplatin 7.5 mg/kg + HACP 200 mg/kg P.O.	0.166 + 1.887**
Group-IV	Cisplatin 7.5 mg/kg + HACP 400 mg/kg P.O.	3.33 ± 1.97***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, ***P<0.001 and ** P<0.01. All values were compared with toxicant control;Toxic control was compared with normal control.

HACP: Hydro alcoholic extract of *Cissampelospareira* Linn.



Group 1 **Group 2**

Group 3 **Group 4**

Figure 1: Effect of *C.pareira* Hydroalcoholic Extract on Hystopathological Parameters

Table 2: Effect of *C.pareira*Hydroalcoholic Extract on Biochemical Parameters (mg/dL)

Group	Treatment	Urinary glucose	Urinary creatinine	Serum urea	Serum creatinine
Group-I	Normal saline	7.33 ± 5.81	1.72 ± 0.193	17.09 ± 1.62	1.12 ± 0.14
Group-II	Cisplatin 7.5 mg/kg I.P.	296.1 ± 18.15***	0.95 ± 0.06*	82.35 ± 5.63***	1.97 ± 0.12**
Groupp-III	Cisplatin 7.5 mg/kg + HACP 200 mg/kg P.O.	178.4 ± 40.25*	1.516 ± 0.23ns	36.60 ± 3.38***	1.77 ± 0.18ns
Group-IV	Cisplatin 7.5 mg/kg + HACP 400 mg/kg P.O.	100.6 ± 35.71***	1.64 ± 0.16*	25.80 ± 3.16***	1.40 ± 0.17*

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, *P<0.05 and ns, not significant. All values were compared with toxicant control; Toxic control was compared with normal control.

HACP: Hydro alcoholic extract of *Cissampelospareira* Linn.

Table 3: Effects of *C.pareira*Hydroalcoholic Extract on Tissue Lipid Peroxidation (LP) and Glutathione (GSH).

Group	Treatment	LP Absorbance	LP % Inhibition	GSH Absorbance	GSH % Increase
Group-I	Normal saline	0.179 ± 0.003	-	1.673 ± 0.044	-
Group-II	Cisplatin 7.5 mg/kg I.P.	0.711 ± 0.002***	-	0.924 ± 0.043***	-
Group-III	Cisplatin 7.5 mg/kg + HACP 200 mg/kg P.O.	0.195 ± 0.002***	72.57	1.352 ± 0.042***	46.32
Group-IV	Cisplatin 7.5 mg/kg + HACP 400 mg/kg P.O.	0.117 ± 0.002***	83.54	1.606 ± 0.072***	73.80

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where,***P<0.001.All values are compared with Toxicant control. HACP: Hydro alcoholic extract of *Cissampelospareira* Linn.

Table 4: Effect of *C.pareira*Hydroalcoholic Extract on Hystopathological Parameters

Group	Treatment	Tubular Congestion,	Epithelial Desquamation,	Glomerular Congestion
Group-I	Normal saline	-	-	-
Group-II	Cisplatin 7.5 mg/kg I.P	+++	++	+++
Group-III	Cisplatin 7.5 mg/kg + HACP 200 mg/kg P.O	++	++	++
Group-IV	Cisplatin 7.5 mg/kg + HACP 400 mg/kg	++	+	+

+ + + Presence of histopathological abnormalities; - Absence of histopathological abnormalities. HACP: Hydro alcoholic extract of *Cissampelospareira* Linn.

CONCLUSION

In the present study *C.pareira* extract has shown significant improvement in the physical and biochemical parameters of the extract treated groups when compared with the toxic control group. The extract has shown increased levels of glutathione and decreased the lipid peroxidation in the kidney homogenate. So, all these results indicating that the extract of *C.pareira* can protect the kidney from chemical toxicity.

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