Evaluation of Nephroprotective Activity of Fruits of *Ficus hispida* on Cisplatin-Induced Nephrotoxicity

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**ABSTRACT**

Traditional medicaments, chiefly obtained from plants have played a vital role in sustaining disease free human existence on this planet. In spite of overwhelming influence of modern science and tremendous advances made in the production of synthetic drugs, traditional medicaments designed as herbal drugs in different places in the literature have retained their place in the therapy. In this context the folk medicine *Ficus hispida* was studied for its nephroprotective activity. The nephroprotective effect of fruits of *Ficus hispida* (Moraceae) were investigated by acute toxicity studies, estimation of biochemical parameters and *in vitro* antioxidant studies. In our investigation methanolic extract showed significant nephroprotective activity than nephrocuration on cisplatin induced nephrotoxicity.

**Key words:** *Ficus fruits*, cisplatin, nephrotoxicity, free radical scavenging activity, nephroprotective effect.

**INTRODUCTION**

Nephrotoxicity comprises renal disorders produced by a wide range of drugs, diagnostic agents and chemicals. Nephrotoxicity is not an uncommon event, which can cause significant morbidity and can be easily overlooked. There are a growing number of hospitalized patients who develop drug-induced renal problems because increasing number of potent drugs has been added to the therapeutic arsenal in recent years.[1] Different classes of drugs[2,3,4] by virtue of immunological mechanisms or direct toxicity initiate certain stereotyped renal response. These drugs include antibiotics (aminoglycosides, tetracyclines, acyclovir, etc.), chemotherapeutic and immunosuppressants (cisplatin, methotrexate, mytomycin, cyclosporine, etc.,) radio contrast agents and NSAIDs.

Search for nephroprotective agents has made man turn to alternative medicine. It is a well documented fact that a number of medicinal plants show beneficial effects in renal disorders.[5-11] It is suggested that the nephroprotective activity of the plants are due to their antioxidant potential.[12] Currently much interest is paid to medicinal plants with nephroprotective potential. The genus *Ficus* comprising of 700 species belongs to the family Moraceae. The plant *Ficus hispida* (FH) is a shrub or small tree having rough leaved fig and all parts are more or less hispid-pubescent. FH is commonly known as Kathumber (English), Katgular (Hindi), Verse-atti pandhu (Telugu), Peyatti (Tamil) and Kakadumbura (Sanskrit) is widely distributed in India, Sri Lanka, Myanmar and southern regions of the Republic China, in damp localities and in shady places.[13] All parts of *Ficus hispida* are bitter, cooling acrid and astringent. Indian Medicinal Plants (IMPs) mentions its use in “Kapha’, dysentery, ulcers, biliousness, psoriasis, anaemia, piles, jaundice, hemorrhage of the nose and mouth and blood diseases.[14]

Literatures documents its wide range of biological activities[15-20] such as antidiarrhoeal, antitussive, anti-ulcer, antipyretic, anti-inflammatory, antioxidant, sedative, anticonvulsant, hepatoprotective and cardioprotective activities in leaves and hypoglycaemic activity in bark. Phytochemical studies revealed that the whole plant of *Ficus hispida* contains alkaloids (phenanthroindolizidine and biphenyl hexahydroindolizine) and flavanoids (β-amyrin, hispidine, bergapten and β-sitosterol) lupeol acetate, β-sitosterol and β-amyrin acetate in bark whereas leaves contain steroids and terpenoids.[21]

The tribal people in Tirumala hills (Andhrapradesh, India) use FH fruits for renal disorders. Lack of experimental data for its nephroprotective activity, prompted us to study the nephroprotective activity of FH fruits against cisplatin induced nephrotoxicity.
MATERIALS AND METHODS

Collection
Ficus hispida was collected from talakona forest of chittoor district, Andhra Pradesh, India, during september and was authenticated by Dr Madhavashetty, Dept of Botany, SV University, Tirupati, India.

Extraction
The fresh fruits were cut into small pieces shade dried and coarsely powdered. The coarse powder (100 g) was extracted with hot methanol in a Soxhlet extractor for 20 h. The methanol was removed by distillation under reduced pressure and controlled temperature using rotary vacuum evaporator. 25 g of methanolic extract of Ficus hispida fruits were subjected to fractionation and yield of 10 g is obtained.

Chemicals
Cisplastin was purchased from Sigma Aldrich Co, St Couis, USA. Trichloroacetic acid, thiobarbituric acid, o-phosphoric acid, diacetyl monoxime, thio semicarbazide, sodium tungstate, sodium nitroprusside are purchased from SD fine-Chem Ltd (India) and Merck (India). All the chemicals used are of analytical grade.

Preparation of test and standard solutions
The stock solutions of extracts of FH fruits and the standard antioxidants rutin, butylated hydroxyl anisole, α-tocopherol and anticancer drug cisplatin were dissolved in dimethyl sulfoxide (DMSO) separately and used for the in vitro antioxidant tests. The stock solutions were serially diluted with the DMSO to obtain lower dilutions.

Phytochemical screening
Preliminary phytochemical screening of different percentage of ethyl acetate fractions revealed the presence of fixed oil, fats, steroids, flavanoids, triterpenoids and alkaloids[22]. Lieberman-Burchard test, Shinoda’s test[23] and Hirschohin test given positive results and confirms the presence of steroids, flavanoids and triterpenoids. Presence of alkaloids was confirmed by the positive results obtained from Mayer’s, Dragendorff’s, Wagner’s and Hagner’s tests. Presence of tannins was confirmed by ferric chloride test and purple colour produced by the Modified Borntrager's test indicates the presence of glycosides.

Animal model
The animal toxicity study was conducted on male Wister albino rats weighing 150-200 g. The animals were deprived of food for 24 h but allowed free access to water in the same ambience. The animals were housed under standard conditions of temperature (23°C ± 1°C) and were acclimatized to 12 h light. The animals were fed with commercial rat feed pellets (Gold Mohur pellets, Bangalore) and were given water ad libitum. The ethical clearance was obtained from institutional animal ethic committee.

EXPERIMENTAL PROTOCOL

Acute toxicity and gross behavioural studies
Acute toxicity studies were carried out for methanolic extract using Acute Toxic Method as described in OECD (Organization of Economic Co-operation and Development) Guidelines No. 423. Animals were given increasing doses of 30, 100, 300, 600 and 1000 mg/kg, p. o. of the methanolic extract suspended in 2 % tween-80 solution. The animals were observed continuously for 2 h gross behavioural changes and intermittently once every 2 h and finally at the end of 24 and 72 h to note any toxic sign.

Treatment schedule
The Wister albino rats were divided into seven groups eight animals each.[24] Four groups served as control, cisplatin-treated, prophylactic control, curative control, one group of animals received prophylactic dose (500 mg/kg, p.o.) and other two group of animals received curative doses (250 and 500 mg/kg, p.o.).

Group 1 - Control: Rats received 2 % Tween 80 orally for 10 days.

Group 2 - Cisplatin treated: Rats were injected intraperitoneally with single dose of cisplatin (CP) 5 mg/kg, dissolved in 2 % Tween 80 on the first day of the experiment.

Group 3 - Prophylactic control: Rats were given 2 % Tween 80, p.o. for 10 days, on 11th day cisplatin 5 mg/kg, i.p.

Group 4 - Prophylactic activity: Rats were given extract of FH 500 mg/kg, p.o. for 10 days, on 11th day cisplatin 5 mg/kg, i. p.

Group 5 - Curative control: Rats were injected single dose of cisplatin 5 mg/kg, i. p. dissolved in saline on the 1st day of the experiment and normal saline is given on 6th day onwards.

Group 6 - Curative activity: Rats were injected single dose of cisplatin 5 mg/kg, i. p. dissolved in saline on the 1st day of the experiment and 6th day onwards crude extract of FH 250 mg/kg, p. o. for 10 days.

Group 7 - Curative activity: Rats were injected single dose of cisplatin 5 mg/kg, i. p. dissolved in saline on the 1st day of the experiment and 6th day onwards methanolic extract of FH 500 mg/kg, p. o. for 10 days.

At the end of the experimental period (i.e on 11th day of the experiment), the animals were anaesthetized with ether.
and blood was withdrawn through heart puncture. The kidneys were immediately removed and rinsed with ice-cold saline. Serum was separated from blood collected for biochemical parameters. Nephroprotective activity of FH was screened by percentage change in body weight of each rat before and after treatment and also by estimating the blood urea nitrogen (BUN; Diacetyl monoxime method), serum total protein (S_TP; Biuret method), serum creatinine and urinary creatinine (SC and UC; Alkaline picrate method), WBC count, creatinine clearance (Cr_cl) and urinary total proteins.\textsuperscript{[23]}

**IN VITRO ANTIOXIDANT ACTIVITY**

**Thiobarbituric acid test**

**Preparation of rat kidney homogenate**

Albino rat kidney was removed and washed with ice-cold saline. The brain was homogenized in ice-cold buffer (pH 7.4) using Teflon homogenizer. The supernatant was stored at -70°C.

**Assay**

Lipid peroxidation assay was evaluated as the malonyl dialdehyde (MDA) production according to the described method with some modifications.\textsuperscript{[26, 27]} Lipid peroxidation was initiated by adding ferric chloride (100 µM) to a mixture containing the rat kidney homogenate (0.25 ml) and different concentrations of extracts in a total volume of 0.75 ml. The reaction mixture was incubated for 20 min at 37°C and 20 % of the homogenate is taken in 1.5 % KCl. To 1 ml of homogenate, 20 % of 2.5 ml of trichloroacetic acid is added. The mixture is centrifuged at 3500 rpm for 10 min. The resulting pellet was dissolved in 2.5 ml of 0.05 M sulphuric acid and 3 ml of 0.38 % of thiobarbituric acid is added and incubated at 37°C for 30 min. The contents were then extracted into 4 ml of n-butanol and absorbance was measured spectrophotometrically at 530 nm.

**Nitric oxide scavenging activity**

Nitric oxide is a free radical and scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Nitric oxide was generated from sodium nitroprusside and measured by the modified Griess Ilosvog reaction.\textsuperscript{[24,28]} The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (1 ml), and extracts (0.4, 0.6, 0.8 and 1 mg/ml) or standard solution (1 ml) was incubated at 25°C for 2 h. After incubation, 0.5 ml of the reaction mixture containing nitrite was removed; 1 ml of sulphanallic acid reagent (0.33 % in 20 % glacial acetic acid) was mixed and allowed to stand for 5 min for complete diazotization. Then 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min. The absorbance of chromophore formed measured at 542 nm. The experiment was repeated in triplicate and percentage scavenging effect was calculated.

**Histopathological studies**

Animals from each group were sacrificed on the day of withdrawal of blood and kidneys were isolated. The kidney sections were stained with hematoxylin and eosin and observed under light microscope.\textsuperscript{[29]}

**Reducing power**

Different dose of methanolic extract FH were diluted to get 150, 250 and 400 µg/ml concentrations.\textsuperscript{[26,27]} Test concentrations are mixed with phosphate buffer (2.5 ml, 0.02 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion of trichloro acetic acid (2.5 ml, 10 %) added to the mixture which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.05 ml, 0.1%) was added and the absorbance was measured at 700 nm.

**Statistical analysis**

The data were expressed as mean ± SE, statistical analysis was performed by one-way ANOVA followed by post-hoc student-Neuman Keuls test. \(P\) values < 0.001 were considered as significant.

**RESULTS**

**Acute toxicity**

The acute toxicity showed no animal died even at 1000 mg/kg and hence the extract was treated as non-toxic. Therefore as per the CPCSEA guideline 420 it was thought that 1000 mg/kg is the LD_{50} cut off dose. Hence, 250 and 500 mg/kg were selected as test doses for further investigations.

**Nephroprotective activity screening**

Nephroprotective activity was assessed by measuring the following parameters

**Body weight**

Body weight was recorded in alternative days. The body weight of the animals receiving CP 5 mg/kg, i. p. decreased when compared to the control animals [table-1]. However, when the methanolic extract was given at prophylactic and curative doses the body weight increased significantly.

**Biochemical parameters**

Biochemical parameters BUN, SC, \(S_{TP}\) and \(Cr_{cl}\) levels were increased significantly and also reduction in the WBC count is observed. Methanolic extract of FH reversed these effects moderate to significantly by the administration of 250 and 500 mg/kg of methanolic extract. The reports were given in the table-1 and 2.
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**DISCUSSION**

The clinical use of cisplatin, a potent anticancer agent used in solid tumors of testes, ovary, breast, lungs, bladder, etc is limited by its renal toxicity.\(^{30,31}\) Studies suggest that the

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<th>Table 1: Effect of methanolic extract of fruits of Ficus hispida in cisplatin induced renal damage (values are mean ± SE of 6 replications)</th>
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BUN: Blood urea nitrogen; SC: serum creatinine; STP: serum total protein (Biuret method); *P < 0.01 (compared to control); **P < 0.001 (compared to cisplatin treated group). One way ANOVA followed by post hoc Student-Newman-Keuls Test.

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<th>Table 2: Effect of methanolic extract of fruits of Ficus hispida in cisplatin induced renal damage (values are mean ± SE of 6 replications)</th>
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*P < 0.01 (compared to control); **P < 0.001 (compared to cisplatin treated group). One way ANOVA followed by post hoc Student-Newman-Keuls Test.

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<th>Table 3: Effect of methanolic extract of fruits of Ficus hispida on cisplatin induced lipid peroxidation in rat kidney homogenate (values are mean ± SE of 6 replications)</th>
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*P < 0.01 (compared to control); **P < 0.001 (compared to cisplatin treated group). One way ANOVA followed by post hoc Student-Newman-Keuls Test.

**Lipid peroxidation**

CP increases MDA level where as methanolic extract of FH showed moderately decreased MDA levels at the doses of 250 and 500 mg/kg. However prophylactic dose (500 mg/kg) of methanolic extract showed significant reduction in MDA level. Increased MDA table-3 level by CP is reversed by methanolic extract of FH.

**Histopathology**

Intraperitoneal administration of CP (5 mg /kg) induced severe biochemical changes as well as oxidative damage in kidney. CP treated rat kidney exhibited marked congestion of the glomeruli with glomerular atrophy, desquamation of tubular epithelial cells and induced acute renal necrosis [Fig-1 (A-G)].

**Nitric oxide scavenging activity**

Methanolic extract of FH in 0.8 and 1.0 mg /ml showed significant (40 % and 49 % inhibition) nitric oxide scavenging activity [table-4].

**Reducing power**

Reducing power of the extract increased with the increase in concentrations (150, 250, 350 and 400 µg/ ml) of the methanolic extract of fruits of FH [table-5].
Figure 1: Histopathological study results of cisplatin induced nephrotoxicity in rat kidney.
A) Normal kidney showing normal organization of tubular epithelial cells and glomeruli, B) Cisplatin treated rat kidney showing infiltration of cells tubular congestion and glomerular atrophy, C) Rat kidney treated with cisplatin (Prophylactic control) showing glomerular congestion and congestion of inter-tubular blood vessels, D) Prophylactic group (500 mg/ kg, p.o) showing regenerative changes in glomeruli and tubules, E) Curative control showing congestion in glomeruli, F) Curative group (250 mg/ kg, p.o) showing normality of tubular epithelial cells and glomeruli, G) Curative group (500 mg/ kg, p.o) showing normality of tubular epithelial cells and glomeruli,
exhibited marked congestion of glomeruli with glomerular levels of MDA. But prophylactic group showed increased showed moderate protection against CP induced elevated lipid peroxidation studies, the extract in curative group significant at the higher dose (500 mg/kg, p.o.). In the functional impairment. However, the protection is more shown partial protection against CP induced renal and the curative regimen (250 and 500 mg/kg, p.o.). The extract The methanolic extract was administered in two doses in concentrations in the renal tissues before the damage occurs. the protective agent must be present in sufficient weight. The renal clearance data was markedly improved by the administration of FH. Therefore it is important that protection was observed in case of reduction of body dose of extract of FH (500 mg/kg, p.o) significantly decreases the elevation of BUN and SC in rats. Partial protection was confirmed in acute renal necrosis. In curative regimen atrophy and presence of casts in tubular epithelial cells indicating acute renal necrosis. In curative regimen (500 mg/kg), mild degenerative changes and congestion were observed. However, in 250 mg/kg features of tubular necrosis persisted. In preventive regimen, congestion of the glomeruli was reduced; degeneration of tubular cells was not observed indicating significant protection against the injury. One of the proposed mechanism for CP induced free radical damage is by increasing the activity of calcium-independent nitric oxide synthase. Antioxidants modulate this free radical damage due to their interaction with biomolecules. Methanolic extract of fruits of FH showed moderate nitric oxide scavenging activity and significant reducing power indicating its antioxidant potential.

Analysis of all the parameters i.e., biochemical parameters, histopathological changes in kidney clearly shows that the methanolic extract of fruits of FH shown protection in both prophylactic and curative regimen; the protection is more significant in prophylactic regimen. The exact mechanism of the protection need to be established and that helps in the further development in the related studies. Further studies are required for isolation of active constituents and to understand the mechanism of protection.

**REFERENCES**


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**Table 4: Nitric oxide scavenging activity of methanolic extract of fruits of Ficus hispida**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc (mg/ml)</th>
<th>Absorbance</th>
<th>% Inhibition</th>
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<tr>
<td>1</td>
<td>Control</td>
<td>0.984 ± 0.007</td>
<td>—</td>
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<tr>
<td>2</td>
<td>0.4</td>
<td>0.746 ± 1.74*</td>
<td>24</td>
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<tr>
<td>3</td>
<td>0.6</td>
<td>0.672 ± 0.006</td>
<td>32</td>
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<tr>
<td>4</td>
<td>0.8</td>
<td>0.586 ± 0.008</td>
<td>40</td>
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<tr>
<td>5</td>
<td>1.0</td>
<td>0.501 ± 0.007</td>
<td>49</td>
</tr>
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</table>

Sodium nitroprusside (5mM) was mixed with different concentrations of crude extract and incubated at 25ºC for 2 hrs. Griess reagent was added and absorbance of chromophore formed was read at 546 nm. Control experiment was also carried out in a similar manner.

**Table 5: Reducing power of methanolic extract of fruits of Ficus hispida**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Conc (mg/ml)</th>
<th>Absorbance</th>
<th>% Inhibition</th>
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<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.319 ± 0.005</td>
<td>—</td>
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<tr>
<td>2</td>
<td>150</td>
<td>0.242 ± 0.003</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>0.218 ± 0.008</td>
<td>32</td>
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<tr>
<td>4</td>
<td>350</td>
<td>0.192 ± 0.008</td>
<td>40</td>
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<tr>
<td>5</td>
<td>400</td>
<td>0.183 ± 0.006</td>
<td>43</td>
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Different doses of methanolic extract of Ficus hispida were mixed in 1 ml of distilled water so as to get 150, 250, 350 and 400 µg/ml concentration. Then mixed with phosphate buffer and potassium ferri cyanide and the mixture was incubated at 50ºC for 20 min. To this trichloroacetic acid was added and centrifuged at 3000 rpm for 10 min. The upper layer of the solution was mixed with distilled water and FeCl3 and the absorbance was measured at 700 nm.