

# Effect of the Ethanolic Extract of *Scoparia dulcis* in Cisplatin induced Nephrotoxicity in wistar rats

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## ABSTRACT

**Objective:** To evaluate the nephroprotective potential of ethanolic extract of aerial parts of plant *Scoparia dulcis* against cisplatin induced nephrotoxicity in Wistar rats. **Materials and Methods:** Nephrotoxicity was induced by a single intra-peritoneal administration of Cisplatin (6 mg/kg) in wistar rats. Nephroprotective potential of plant was tested at two different doses in curative regimen and for a single dose of 400 mg/kg in prophylactic control. Nephrotoxicity was characterised by induced marked renal failure, significant increase in blood urea nitrogen, serum creatinine level, lipid peroxidation levels and significant decrease in urinary creatinine clearance. Histopathological studies confirmed tubular necrosis. Supplementation of ethanolic extract of *Scoparia dulcis* reduced the elevated serum creatinine, blood urea nitrogen levels, lipid peroxidation levels and improved the creatinine clearance. **Results and Discussion:** Our experimental results suggest that supplementation of *Scoparia dulcis* during cisplatin therapy reduces the risk of cisplatin induced nephrotoxicity in a dose dependent manner in curative regimen. The prophylactic regimen also possessed significant nephroprotection against cisplatin toxicity. The protective effect of *Scoparia dulcis* in curative and prophylactic regimen may be due to the antioxidant property of *Scoparia dulcis*. These findings suggest the significant nephroprotection of *Scoparia dulcis* against cisplatin nephrotoxicity.

**Key words:** Nephrotoxicity, *Scoparia dulcis*, Cisplatin, lipid Peroxidation, anti-oxidant.

## INTRODUCTION

*Scoparia dulcis* is a perennial herb, belongs to the family Scophulariaceae is distributed throughout the tropical and subtropical region of the world and is known as Vas-sourinha in India.<sup>1</sup> Traditionally the fresh or dried plant has been used as a remedy for treating diseases such as; stomach ailments, hypertension, diabetes, inflammation bronchitis, haemorrhoids, analgesic, antipyretic and urinary disorders. Plant is also used for upper respiratory bacterial and viral infections, to relieve from all types of pain, to tone balance, strengthen heart function, for venereal diseases and urinary tract infections.<sup>2</sup> The leaf of *Scoparia dulcis* is used for diabetes in India. Recently studies have been carried on the extract of aerial parts of *Scoparia dulcis* for potential uses including pain relieving antispasmodic, anti inflammatory activities.<sup>3</sup>

Plant is also reported to possess cytotoxic, anti cancerous,<sup>4</sup> antimicrobial, anti malarial,<sup>5</sup> anti ulcer, antacid,<sup>6</sup> anti diabetic, anti cholesterol<sup>7</sup> and antioxidant actions.<sup>8</sup>

The cisplatin is a water soluble anticancer drug, organic complex formed by an atom of platinum surrounded by chloride and ammonium atoms. The mechanism of action include after entering the cell, the chlorine ions dissociates leaving a reactive complex. This complex reacts with water and then interacts with DNA resolving in denaturation of DNA chain. Cisplatin also damages cell mitochondria arrests cell cycle in G<sub>2</sub> phase inhibits ATPs activity, alters the cellular transport system and eventually causing apoptosis,<sup>9</sup> inflammation,<sup>10</sup> necrosis and death in cells. Although it is a potent

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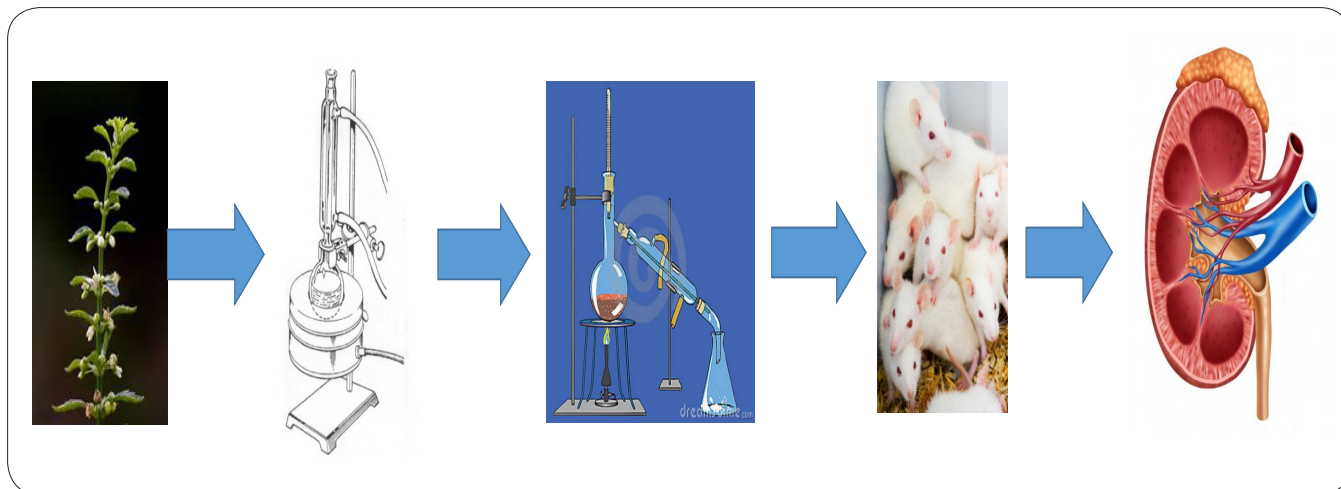
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### Graphical Abstract

anticancer, the major drawback associated with cisplatin is kidney damage. Previous reports showed evidences of cisplatin predominantly localized in the S<sub>3</sub> segments of proximal tubules in the corticomedullary region.<sup>11</sup>

In allopathy till date there is no effective drug reported to treat the nephrotoxicity of cisplatin. The effectiveness of herbal extracts for the urinary diseases is as old as humankind itself. Eventhough *Scoparia dulcis* used as an ethnomedicine against urinary disorders, there is no pharmacological validation for its traditional use. Hence the present study was designed to establish the nephroprotective property of arial parts of *Scoparia dulcis*. The ethanolic extract was used to evaluate the cisplatin induced nephrotoxicity and dysfunction in wistar rats.

## MATERIALS AND METHODS

### Materials

#### Plant material collection and authentication

The aerial parts of plant *Scoparia dulcis* was collected from Idukki, Kerala, India and authenticated by the department of botany, Nagarjuna Ayurveda Centre, Alakode, Thodupuzha, India. A specimen voucher was deposited in college (Visveswarapura Institute of Pharmaceutical Sciences) herbarium for future references. The drug cisplatin was a gifted sample from Caplin Point Laboratories, Pondicherry, India. All other chemicals used were analytical grade.

#### Preparation of extract

The plant (1000 gm) was shade dried and coarsely powdered for extraction with alcohol at 60-70°C for 3 hour and the filtrate was concentrated under vaccum. The residue collected (20 gm) was thick, green in colour and gumaceous in nature and stored at below 20°C. The required extract was dissolved in suitable vehicle and used for investigation.

## Pharmacological Studies

### Acute toxicity studies

Acute toxicity studies were performed on albino mice of either sex weighed (25-30 gm). The acute oral toxicity study<sup>12</sup> was carried out as per guide lines set by organisation for economic corporation and development (OECD) received from committee for the purpose of control and supervision of experiments on animals. One tenth of median lethal dose (LD<sub>50</sub>) was taken as effective dose.<sup>13</sup>

### Animal selection

Wistar rats weighing between 200 and 250 g were selected for nephroprotector activity. The animals were acclimatised to standard laboratory conditions at (25 ± 0.2°C and maintained for 12 hour day and night cycle). They were provided with regular rat chow and drinking water. The animals care and experimental protocols were in accordance with Institutional Animal Ethical Committe (IAEC, Reg.No.152/1999/CPCSE).

### Experimental model for Nephroprotector activity

Wistar rats were weighed (200-250 g) and divided into 6 groups of six animals each. Group-I, received (1% tween 80) for 15 days and Group-II, (curative control) received cisplatin (6 mg/Kg, i.p; single dose) on the first day and vehicle (1% tween 80) from day 6- day 15<sup>th</sup>. Group-III, received cisplatin on first day and plant extract (200 mg/Kg) from day6 to day 15<sup>th</sup>. Group-IV, treated with cisplatin on first day and plant extract (400 mg, higher dose) from day 6 to day 15<sup>th</sup>. Group-V, acts as a prophylactic control in which the animals are given with vehicle from day 1 to day 10<sup>th</sup> and cisplatin on 11<sup>th</sup> day. Group-VI, is treated with plant extract (*Scoparia dulcis*) from day 1 to day 10<sup>th</sup> and cisplatin on 11<sup>th</sup> day. On 16<sup>th</sup> day blood and urine are collected from all six groups for biochemical estimation. The estimation

**Table 1: Effect of extracts on Serum Parameters on 15<sup>th</sup> day**

| Groups n=6 | BUN (mg/dl)              | Serum creatinine (mg/dl) | Lipid Peroxidation (percentage inhibition) |
|------------|--------------------------|--------------------------|--|
| Group I    | 25.3 ± 1.4               | 0.5 ± 0.6                | -  |
| Group II   | 49.4 ± 1.6               | 2.7 ± 0.7                | -  |
| Group III  | 35.4 ± 0. <sup>a</sup>   | 2.0 ± 0.5 <sup>a</sup>   | 18 <sup>a</sup>                            |
| Group IV   | 26.7 ± 1.3 <sup>ab</sup> | 0.9 ± 0.4 <sup>ab</sup>  | 40 <sup>ab</sup>                           |
| Group V    | 51.9 ± 1.7               | 2.3 ± 0.5                | -  |
| Group VI   | 30.8 ± 0.9 <sup>c</sup>  | 1.1 ± 0.8 <sup>c</sup>   | 19 <sup>c</sup>                            |

Mean±SD, n=6, \*significant pharmacological activity, \*\*Potent pharmacological activity.

<sup>a</sup>P<0.05 compared with curative control ; <sup>ab</sup>P<0.01.compared with curative control ; <sup>c</sup>P<0.01.compared with prophylactic control.

of biochemical parameters include Blood urea nitrogen (BUN, Diacetyl monooxime method), serum creatinine (Alkaline picrate method), Serum total proteins (Biuret method), Urinary protein (Sulphosalicylic acid method) using UV-Visible spectrophotometer by following standard methods. For histopathological studies and lipid peroxidation studies in kidney, the rats from each group have to be anaesthetised using sodium phenobarbitone (60 mg/Kg) and kidneys were isolated.

### Serum Analysis

The blood was collected from the retro-orbital on 15<sup>th</sup> day from animals and they were anaesthetized using sodium phenobarbitone (60 mg/Kg). The serum was separated by centrifugation at 10000 rpm for 10 min and analysed for biochemical parameters such as serum creatinine, (Alkaline picrate method), blood urea nitrogen (Diacetyl monooxime method)<sup>14</sup> and uric acid.<sup>15</sup>

### Urine Analysis

All animals were kept in individual metabolic cages and urine samples were collected. The animals had free access to drinking water during the urine collection period. The

urine was collected to analyse creatinine clearance and urinary protein. (Sulphosalicylic acid method).<sup>16</sup>

### In vivo lipid peroxidation in cisplatin induced nephrotoxicity

The degree of lipid peroxide formation was assayed by monitoring thiobarbituric reactive substance formation. Stock solution of 15% w/v trichloroacetic acid (TCA), 0.375 % w/v of thiobarbituric acid (TBA) and 0.25 N Hydrochloric acid (HCl). The solution was mildly heated to assist the dissolution of TCA. Further combine 1 mL of biological sample (1-2 mg of membrane protein or 0.1-0.2 μ mole of lipid phosphate) with 2 mL of TCA-TBA HCL and mixed thoroughly. Then the solution was heated for one hour in a boiling water bath and allowed to cool. After cooling, the flocculent precipitate was removed by centrifugation at 2500 rpm for 2 min and at 535 nm against the blank. The concentration of malondialdehyde of the sample was calculated by following formula using an extinction coefficient 1.56x10<sup>5</sup> M<sup>-1</sup>cm<sup>-1</sup>% inhibition.

$$\text{Inhibition (\%)} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

### Histopathological studies

The extraneous tissues of isolated kidneys were cleaned off and preserved in neutral formalin (10%) solution. Formalin preserved samples of kidneys from various groups were studied for histopathological changes during experimental section of kidneys stained with haematoxylin and eosin were observed under standard microscope. The centrifuged homogenate was used for lipid peroxidation studies.

### Statistical Analysis

All data are subjected to one way ANOVA followed by Dunnetts test. The P value of <0.05 was considered statistically significant. The statistical analysis was performed using Prism Software for windows.

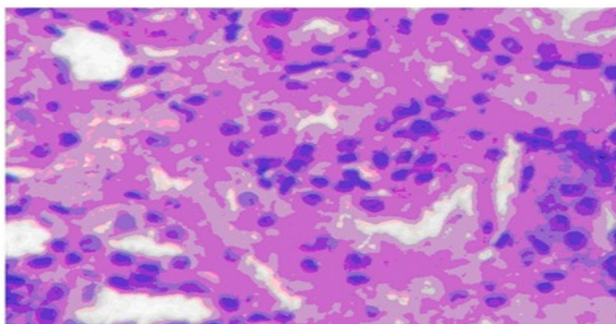
**Table 2: Effect of ethanolic extract on urine parameters**

| Groups | Creatinine clearance (ml/h/100 g Bd.Wt) | Urine totalprotein (mg/day) | Urine volume (ml/day)   |
|--------|---|-----------------------------|-------------------------|
| I      | 17.8 ± 1.3                              | 5.7 ± 0.8                   | 12.13 ± 0.7             |
| II     | 8.4 ± 0.2                               | 16.8 ± 2.5                  | 4.2 ± 0.2               |
| III    | 11.5 ± 0.3 <sup>a</sup>                 | 12.5 ± 0.3 <sup>a</sup>     | 6.4 ± 0.3 <sup>a</sup>  |
| IV     | 14.7 ± 0.2 <sup>ab</sup>                | 7.1 ± 0.5 <sup>ab</sup>     | 10.6 ± 0.2 <sup>b</sup> |
| V      | 7.2 ± 0.2                               | 14.9 ± 1.2                  | 5.9 ± 0.2               |
| VI     | 12.8 ± 0.2 <sup>c</sup>                 | 9.2 ± 0.3 <sup>c</sup>      | 9.8 ± 0.2 <sup>c</sup>  |

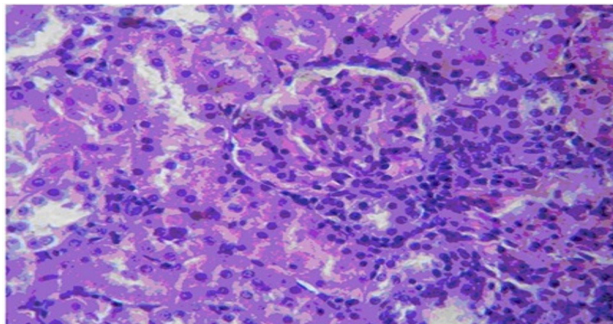
Mean±SD, n= 6, \*Significant pharmacological activity, \*\*Potent pharmacological activity.

<sup>a</sup>P<0.05, <sup>ab</sup>P<0.01 ; <sup>a</sup>P<0.05 compared with curative control ; <sup>ab</sup>P<0.01.compared with curative control ; <sup>c</sup>P<0.01.compared with prophylactic control.

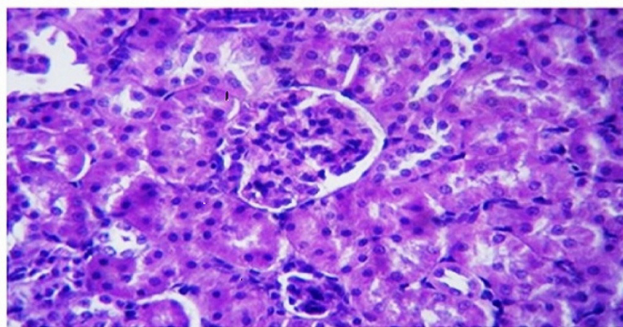




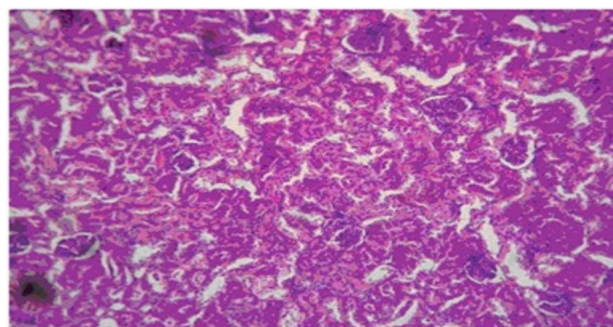
**Figure 1:** The normal control showed (Figure 1) the structure of kidney with normal glomerulal, proximal and distal tubules and with normal interstitium and blood vessels



**Figure 2:** A positive control ie, cisplatin treated group showed (Figure 2) glomeruline unremarkable, most of the tubules showed desquamation along with epithelial casts and showed massive total necrosis



**Figure 3:** Curative regimen treatment done with 200 mg/Kg ethanolic extract showed (Figure 3) structure of the kidney with maintained glomerulal, proximal and distal tubules, there were less interstitial congestion and showed recovery of interstitial congestion



**Figure 4:** Curative regimen treatment done with 400 mg/Kg ethanolic extract showed (Figure 4), structure of kidney with maintained glomerulal, proximal and distal tubules, there was no congestion and indicated interstitial congestion like normal

## RESULTS

### Effect of *Scoparia dulcis* on serum parameters

The effect of *Scoparia dulcis* on serum parameters is represented in Table 1. Animals which received only cisplatin induced nephrotoxicity characterised by increased levels of serum creatinine, and Blood Urea Nitrogen (BUN) when compared with the normal control. Upon supplementation of *Scoparia dulcis* to the cisplatin treated animals with (Grp III & IV), showed a significant reduction in BUN and serum creatinine was observed. The group IV with higher dose of 400 mg/kg body weight of *Scoparia dulcis* reduced the values to the extent of  $p < 0.01$  when compared to positive control. This shows a dose dependent effect of extract in curative regimen. In prophylactic regimen the treatment with extract (Grp VI, 400 mg/kg) for ten days showed a significant reduction in serum marker levels in group VI when compared to normal control.

### Effect of *Scoparia dulcis* on urine parameters

The effect of cisplatin and extract on the renal functions was represented in Table 2. The administration of cisplatin caused significant reduction ( $P < 0.05$ ) in creatinine clearance and increased the excretion of urinary

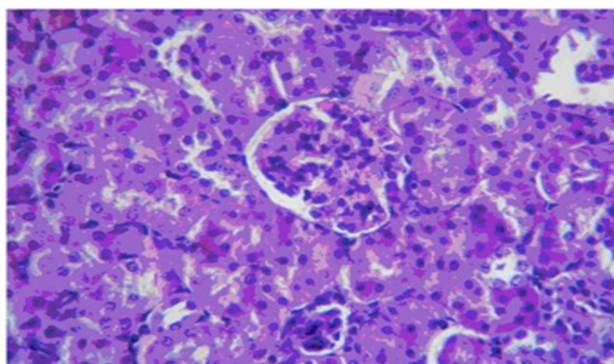
protein in curative control and preventive control when compared to the normal control groups. In curative regimen, animals which were treated with extracts in (Groups III and IV), increased creatinine clearance and reduced the elevated levels of total protein excretion. Animals of the preventive regimen (Group-VI) showed significant protection against cisplatin induced effects.

### Lipid Peroxidation

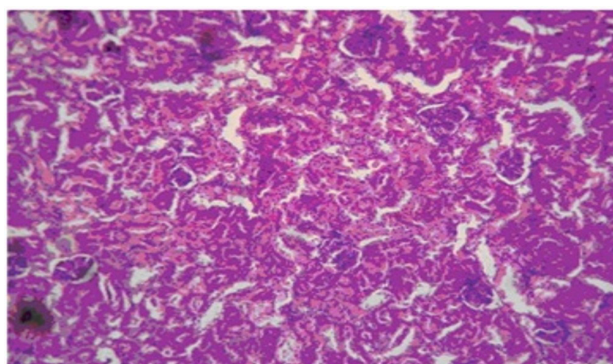
Administration of cisplatin caused significant increase in the levels of Malon Dialdehyde (MDA) in curative control (Group-II) and preventive control (Group-V) when compared to normal control group ( $p < 0.05$ ). This indicated that increased lipid peroxidation in Group-II and V animals. A significant reduction in MDA level was observed in animals treated with plant extracts (Groups -III animals (200 mg/Kg) and Group - IV animals (400 mg/Kg) compared to curative control group. Animals treated with extract for ten days prior to the cisplatin administration decreased MDA levels.

### Histopathological Studies

The above findings were supported by histopathological studies. Group II showed marked renal toxicity, characterized by congestion of glomeruli, presence of inflam-



**Figure 5: Prophylactic control showed (Figure 5), glomeruli unremarkable, tubules desquamation is seen along with epithelial casts, interstitium mild lymphocytic infiltration, and vessels unremarkable and showed residual interstitial nephritis**



**Figure 6: Prophylactic treatment done with 400 mg/Kg showed (Figure 6) structure of kidney with maintained glomeruli, proximal and distal tubules and showed less congestion**

matory cells, desquamation of tubular epithelial cells etc. Both prophylactic and curative regimen showed good protection against cisplatin induced nephrotoxicity compared to control groups. (Figure 1-6)

## DISCUSSION

The alcoholic extract of *Scoparia dulcis* was evaluated at two curative doses (200 mg/kg and 400 mg/kg) and one prophylactic dose (400 mg/kg). Our experimental results suggested that supplementation of *Scoparia dulcis* during cisplatin therapy reduces the risk of cisplatin induced nephrotoxicity in a dose dependent manner. The curative regimen showed significant amount of activity to prevent the effects of cisplatin, especially the higher dose of 400 mg/kg possessed significant protection of  $p < 0.01$ . The extract also showed significant preventive effect due to presence of sufficient amount of extract in animals to prevent the effects of cisplatin. Histopathological studies and lipid peroxidation studies support these findings.

Kidney is a complex and dynamic organ. Although excretion of wastes is the primary function, it also plays a significant role in regulation of total homeostasis. Regulation of extracellular volume and control of electrolyte and acid base balance are also important functions of kidney. A toxicological insult to the kidney could affect any one or all of these functions. The toxic effects of affected kidney will be reflected as decreased elimination of wastes, an increase in blood urea nitrogen, and an increase in plasma creatinine. These parameters are clinical indices of nephrotoxicity. We are vulnerable to nephrotoxicity due to some drugs and chemicals. Cisplatin is an effective anticancer drug which induces nephrotoxicity and there is no known drug available which effectively treat the nephrotoxicity of cisplatin. Reports suggested a single dose of intraperitoneal administration of cisplatin

(6 mg/Kg) induced nephrotoxicity.<sup>17</sup> Even though the mechanisms are clearly not known, several mechanisms have been suggested for cisplatin induced nephrotoxicity i.e., apoptosis, inflammatory mechanism and generation of reactive oxygen species.<sup>18</sup> The nephrotoxicity due to cisplatin is a rapid process due to the reaction with the proteins in renal tubules. The renal damage is produced within one hour after administration. Hence the presence of protective agent in the renal tissues may reduce the toxic effects of cisplatin. This is the rationale behind the prophylactic treatment of plant extract.

Previous evidences have shown that cisplatin induced nephrotoxicity is by initiation of lipid per oxidation.<sup>19</sup> There were also reports suggesting cisplatin exerts its nephrotoxicity by generation of free radicals.<sup>20</sup> The general toxicity mechanism involves oxidative stress by the generation of reactive oxygen species such as hydrogen peroxide, superoxide anion, and hydroxyl radicals which are generated under normal cellular conditions and they are immediately detoxified by endogenous antioxidant enzymes superoxide dismutase (SOD), Catalase (CAT), and non enzymatic compounds as reduced glutathione (GSH). But the cisplatin accumulation in kidneys causes an imbalance in antioxidants due to loss of copper and zinc resulting in depletion of SOD and peroxidation of lipids.<sup>21</sup> Hence the antioxidants and free radical scavengers of natural or semi synthetic and synthetic origin provide nephroprotection.

Earlier reports proved that plants possessing antioxidant principles such as *pongamia pinnata*,<sup>22</sup> *crataeva nurv-ala*<sup>23</sup> exhibited nephroprotector activity against cisplatin induced renal damage. Tannins and flavanoids are well known for their antioxidant properties and free radical scavenging abilities.<sup>24</sup> A relationship between oxidative stress and renal toxicity has been documented in several animal experimental models.<sup>25</sup> *Scoparia dulcis* is one such plant rich in antioxidant constituents and was used



by tribals of Kerala for kidney diseases.<sup>26</sup> The leaves of *Scoparia dulcis* were reported to contain flavanoids scutellariin, and 7-o-methyl scutellariin. A flavone cirsiatkaoside was also extracted from *Scoparia dulcis*.<sup>27</sup> The nephroprotective effect of *Scoparia dulcis* may be due to the presence of well known antioxidants that can scavenge free radicals.

The measurement of lipid peroxidation is an efficient method of monitoring oxidative damage in tissues, which is determined by measurement of thiobarbituric acid substance. The concentration of lipid peroxidation products reflects the degree of oxidative stress and the malondialdehyde concentration (MDA) concentration is a measure of lipid peroxidation. Reactive oxygen species causes peroxidation of membrane lipids with devastating effects on functional and structural states. The preservation and restoration of cellular membrane integrity depends on protective and repair mechanism against renal tissue oxidative damage. Our data showed that cisplatin induced lipid peroxidation and was significantly reduced by the administration of plant extract in both curative and preventive regimen. The percentage of inhibition of lipid peroxidation was very significant

in higher dose of curative regimen and also in the preventive ie, prophylactic regimen. These results indicate that the extract is involved in the protection of normal cell structure and function of homeostasis.

As cisplatin is believed to induce oxidative stress which leads to a chain of complex biochemical reactions resulting in cellular damage by free radicals, here our data showed that the administration of *Scoparia dulcis* extract protects and also repairs the structure and functional changes due to their antioxidant potential.

## CONCLUSION

Since our present study reveals the potential of *Scoparia dulcis* as a nephroprotective agent, it is suggested for further validation, quantitative and qualitative studies which may help in designing a novel drug in the area of nephrotoxicity and related problems.

## ACKNOWLEDGEMENTS

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## SUMMARY

- Cisplatin induces nephrotoxicity in wistar rats.
- Nephrotoxicity was characterized by marked renal failure, increased serum parameters and lipid peroxidation levels.
- Supplementation of *scoparia dulcis* during cisplatin therapy reduces nephrotoxicity in a dose-dependent manner.
- *Scoparia dulcis* also exhibited significant protection against cisplatin nephrotoxicity and can be used as prophylactic for nephrotoxicity.

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