ABSTRACT

Objective: To evaluate the potential nephroprotective and antioxidant activity of hydroalcoholic *Cissampelos pareira* (C. pareira) whole plant extract using gentamicin-induced rats. Methods: For studying acute toxicity study, single oral dose of 2g/kg hydroalcoholic extract of *C. pareira* was evaluated in rats by oral gavage. The nephroprotective activity was evaluated using gentamicin-induced model in rats. *In-vitro* antioxidant activity was evaluated by using DPPH assay and reducing power assay. *In-vivo* antioxidant activity was evaluated by using glutathione and lipid peroxidation estimations in gentamicin-induced rats. Hydroalcoholic *C. pareira* whole plant extract was given at a dose of 200 and 400 mg/kg p.o. Results: For acute toxicity testing rats administered with the extract at a dose 2 g/kg. the result showed no toxicity. Hydroalcoholic *C. Pareira* whole plant extract (200 and 400 mg/kg p.o) significantly decrease the elevated urinary glucose levels in the urine, decrease the elevated urea and creatinine levels in blood and increase the urinary creatinine levels in gentamicin-induced nephrotoxic rats. The extract had shown significant dose dependent increase in the DPPH and reducing power activity. There were a dose dependent decreasing and increasing of lipid peroxidation, glutathione levels in hydroalcoholic extract treated groups respectively. Conclusion: This study exhibits that hydroalcoholic *C. pareira* whole plant extract poses nephroprotective activity which may be due to its antioxidant activity.

Keywords Gentamicin, *Cissampelos pareira*, creatinine, urea, glucose, lipid peroxidation, glutathione, reducing power, DPPH.

INTRODUCTION

At present human beings are using different kinds of antibiotics to treat various infections. The aminoglycoside antibiotic like gentamicin is most frequently used drug for treating various infections caused by gram-negative organisms because of its rapid bactericidal activity and low resistance to those gram-negative organisms. But it is having major side effects like ototoxicity and nephrotoxicity. The nephrotoxicity is more frequent over ototoxicity[1]. Gentamicin has been confirmed to increase the generation of reactive oxygen species in the kidney which leads to renal anatomical and physiological damage. Furthermore, gentamicin treated rat kidneys are more prone to oxidative damage due to the generation of reactive oxygen species there by increased levels of lipid peroxidation and decreased levels of anti oxidant enzymes in gentamicin treated rat kidney[2]. Even though, gentamicin is having ototoxicity and nephrotoxicity still, it is using in clinical practices because of its potent bactericidal activity and less resistance, in those conditions a supportive therapy must be given to the patient to protect from the nephrotoxicity.

Therefore, the present work is to protect from the nephrotoxicity of the gentamicin by using naturally available antioxidants which are going to scavenge the reactive oxygen species. Antioxidants have been shown to improve signs of gentamicin induced nephrotoxicity[3]. A potential therapeutic approach to defend or reverse gentamicin induced oxidative damage and nephrotoxicity could have

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more importance for clinical consequences\(^6\). However, scientific reports on the exploration of \textit{C. pareira} for its effects on renal function are scarce. So the present study was designed to investigate the antioxidant and nephroprotective activity of \textit{C. pareira} hydroalcoholic extract in gentamicin induced rats.

**MATERIALS AND METHODS**

**Preparation of whole plant extract**

\textit{C. pareira} whole plant was collected from the Chittoor district of Andhra Pradesh and it is authenticated by K. Madava Chetty, Assistant professor, Sri Venkateswara University, Tirupathi. The whole plant was dried under shade and pulverized separately into coarse powder by a mechanical grinder. The resulting powder was used for the extraction. The powder was extracted directly with 70\% v/v ethanol, which was used for biological investigations and \textit{in-vitro} antioxidant studies, after subjecting it to preliminary qualitative phytochemical studies\(^7\). The extract was concentrated under reduced pressure and stored in vacuum desiccators. Dried extract was suspended in distilled water by using tween 80.

**Chemicals**

Assay kits for the estimation of urinary glucose, urinary creatinine, serum urea, serum creatinine and DPPH were purchased from transasia bio-medicals ltd, coral clinical systems, span diagnostics ltd, coral clinical systems and aldrich respectively. All the chemicals used were of analytical grade.

**Preliminary phytochemical investigation**

The preliminary phytochemical screening was carried out for qualitative identification of phytoconstituents\(^8\).

**Experimental animals**

Albino wistar rats weighing 150–250g was procured from Biogen, Bangalore. They were maintained in the animal house of Gautham College of Pharmacy. Animals were maintained under controlled condition of temperature at 27\(^\circ\) ± 2\(^\circ\) C and 12-h light-dark cycles. They were housed in polypropylene cages and a free access to standard pellets and ad libitum. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Gautham College of Pharmacy, Bangalore (REF-IAEC/021/12/2010) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (Reg No: 491/01/c/CPCSEA), Govt. of India.

**Determination of acute toxicity**

**Experimental animals and procedure**

Foe the determination of the dose LD\(_{50}\) albino rats of weighing 160–220g were used for the study. They were nulliparous and non-pregnant. These were acclimatized to laboratory conditions for one week prior to start of dosing. The \textit{C. pareira} extract was suspended in distilled water by using tween 80, to prepare a dose of 2g/kg. The doses were selected according to the OECD guideline no.425. The procedure was divided into two phases. Phase I (observation made on day one) and phase II (observed the animals for next 14 days of drug administration). Two sets of healthy rats (each set of 3 rats) were used for this experiment. First set of animals were divided into three groups, each of one in a group. Animal were divided into three groups, each of one in a group. Animals were fasted overnight with water ad libitum. Animals received a single dose of 2g/kg was selected for the test, as the test item was a source from herb. After administration of extract, food was withheld for 3–4 hrs. if the animal dies, conduct the main test to determine the LD\(_{50}\). If the animal survives, dose four additional animals sequentially so that a total of five animals are tested. However, if three animals die, the limit test is terminated and the main test is performed. The LD\(_{50}\) is greater than 2g/kg, if three or more animals survive. If an animal unexpectedly dies late in the study, and there are other survivors, it is appropriate to stop dosing and observe all animals to see if other animals will also die during a similar observation period. Late deaths should be counted the same as other deaths. The same procedure was repeated with another set of animals to nullify the errors.

**Evaluation of nephroprotective activity**\(^7\)\(^8\)

Rats were randomly assigned into 4 groups of each 6 animals each. Group-I was kept as normal control receiving saline (0.5 mL, i.p.) for 8 consecutive days and animals of groups II,III and IV were administered gentamicin 80 mg/kg/day, i.p. for 8 consecutive days. Group I and II received vehicle by oral gavage for 11 days and group III and IV were orally administered with hydroalcoholic extract of \textit{C. pareira} suspended in tween 80 at a dose of 200 mg/kg and 400 mg/kg which was started 3 days prior to the gentamicin treatment and continued along with 8 days gentamicin treatment.
Group-I: Kept as normal control received saline (0.5 mL, i.p.) for 8 days.

Group-II: Gentamicin treated group, (80 mg/kg/day, i.p.) for 8 consecutive days.

Group-III: Gentamicin (80 mg/kg/day, i.p) nearly for 8 days in addition to this they also received 200 mg/kg, p.o. of hydroalcoholic extract of *C. pareira* which was started 3 days prior to the gentamicin treatment and continued with eight days gentamicin treatment.

Group-IV: Gentamicin (80 mg/kg/day, i.p) nearly for 8 days in addition to this they also received 400 mg/kg, p.o. of hydroalcoholic extract of *C. pareira* which was started 3 days prior to the gentamicin treatment and continued with eight days gentamicin treatment.

At the end of the study, the animals were kept in individual metabolic cages for 24-hours urine collection. At the last day the animals were sacrificed under combination of ketamine (60 mg/kg) and xylazine (5 mg/kg) given intraperitoneally. Blood samples were collected via cardiac puncture in plain plastic tubes, kept aside for 1 hour at 4 ºC and centrifuged to separate serum. The serum was processed for the estimation of serum creatinine, serum urea. The urine samples were collected for the estimation of urinary glucose, urinary creatinine levels. The kidney samples were used for the *in-vivo* antioxidant studies.

**In-vitro antioxidant studies**

*In-vivo antioxidant studies*

**Glutathione estimation**

Tissue samples were homogenized in ice cold trichloroacetic acid (1 gm tissue plus 10 mL 10% TCA) in a tissue homogenizer. Glutathione measurements were performed using a modification of the Ellman procedure (Aykae, et.al.). Briefly, after centrifugation at 3000 rpm for 10 min. 0.5 mL supernatant was added to 2 mL of 0.3 M disodium hydrogen phosphate solution. A 0.2 mL solution of dithiobisnitrobenzoate (0.4 mg/mL in 1% sodium citrate) was added and the absorbance was measured at 412 nm. Ascorbic acid was used as the reference material. All the tests were performed in triplicate and the results averaged. Increased absorbance of the reaction mixture indicated the increased reducing power.

The % reducing power was calculated by using the formula

\[
\% \text{ increase in absorbance} = \left( \frac{A_{\text{test}} - A_{\text{control}}}{A_{\text{control}}} \right) \times 100 \%
\]

**Lipid peroxidation**

Stock solution of TCA-TBA-HCl reagent: 15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25 N hydrochloric acid. This solution may be mildly heated to assist in the dissolution of the thiobarbituric acid.

Combine 1.0 mL of biological sample (0.1–2.0 mg of membrane protein or 0.1–0.2 µmol of lipid phosphate) with 2.0 mL of TCA-TBA-HCl and mix thoroughly. The solution is heated for 15 min in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. the absorbance of
the sample is determined at 535 nm against a blank that contains all the reagents minus the lipid. % decrease in absorbance is directly proportional to the decrease in the levels of lipid peroxidation. Hence, % decrease in absorbance is calculated.

**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 ≤ 0.05.

**RESULTS**

**Phytochemical investigation**

Phytochemical investigation revealed that the plant extract contains alkaloids, flavanoids, carbohydrates, glycosides, proteins, tannins but saponins are absent.

**Acute toxicity study**

In both phase I and II procedures, none of the animals did not show any toxicity upon single administration of 2000 mg/kg, p.o. Thus, a low dose 200 mg/kg, p.o and a high dose 400 mg/kg, p.o were selected for the present study.

**Effect on physical and biochemical parameters**

Treatment of gentamicin, 80 mg/kg/day, i.p for 8 consecutive days, produced significant nephrotoxicity in rats. Change in body weight, there was significant decrease in body weight (p<0.001) (Table 1 and Figure 1). Urinary levels of glucose, creatinine and serum levels of urea, creatinine, there was significant increase in urinary glucose (p<0.001), serum urea (p<0.001), serum creatinine (p<0.001) and significant decrease in urinary creatinine (p<0.05) when compared with the normal control group (Table 2 and Figure 2, 3, 4 and 5). Whereas co-administration of C. pareira hydroalcoholic extract at a dose of 200 and 400 mg/kg/day was found to significantly improved in change in body weight, urinary creatinine and significantly decreased urinary glucose, serum urea, serum creatinine when compared to gentamicin treated group.

**In-vitro antioxidant**

The C. pareira hydroalcoholic extract shown significant dose dependent increase in the DPPH radical scavenging

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Change in body weight(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Normal saline</td>
<td>9.633 ± 1.327</td>
</tr>
<tr>
<td>Group-II</td>
<td>Gentamicin 80 mg/kg. I.P</td>
<td>-10.50 ± 2.872&quot;</td>
</tr>
<tr>
<td>Group-III</td>
<td>Gentamicin + HACP 200 mg/kg. P.O</td>
<td>1.500 ± 2.078&quot;</td>
</tr>
<tr>
<td>Group-IV</td>
<td>Gentamicin + HACP 400 mg/kg. P.O</td>
<td>4.00 ± 2.543&quot;</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n=6 in each group;*** significantly different at P<0.001,** significantly different at P<0.01.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Urinary glucose</th>
<th>Urinary creatinine</th>
<th>Serum urea</th>
<th>Serum creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Normal saline</td>
<td>1.987 ± 1.028</td>
<td>3.997 ± 0.487</td>
<td>26.35 ± 0.870</td>
<td>0.551 ± 0.204</td>
</tr>
<tr>
<td>Group-II</td>
<td>Gentamicin 80 mg/kg. I.P</td>
<td>95.55 ± 10.28&quot;</td>
<td>1.775 ± 0.410</td>
<td>66.94 ± 7.223&quot;</td>
<td>2.108 ± 0.317&quot;</td>
</tr>
<tr>
<td>Group-III</td>
<td>Gentamicin + HACP 200 mg/kg. P.O</td>
<td>27.81 ± 7.410&quot;</td>
<td>1.775 ± 0.410</td>
<td>35.56 ± 4.385&quot;</td>
<td>1.218 ± 0.205&quot;</td>
</tr>
<tr>
<td>Group-IV</td>
<td>Gentamicin + HACP 400 mg/kg. P.O</td>
<td>18.12 ± 4.450&quot;</td>
<td>4.442 ± 0.612&quot;</td>
<td>28.06 ± 3.632&quot;</td>
<td>0.773 ± 0.204&quot;</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n=6 in each group;*** significantly different at P<0.001,** significantly different at P<0.01,* significantly different at P<0.05.
Figure 1: Effect of HACP on Change in Body Weights in Gentamicin Induced Nephrotoxic Rats.

Figure 2: Effect of HACP on Urinary Glucose Levels in Gentamicin Induced Nephrotoxic Rats.

Figure 3: Effect of HACP on Urinary Creatinine Levels in Gentamicin Induced Nephrotoxic Rats.
Protective effect of cissampelos pareira linn. Extract on gentamicin-induced nephrotoxicity and oxidative damage in rats

activity, and also significant dose dependent increase in the reducing property (Table 3 and Figure 6 and 7).

In-vivo antioxidant

There was a marked increase in lipid peroxidation in gentamicin treated rat kidneys whereas C. pareira hydroalcoholic extract had shown dose dependent inhibition of lipid peroxidation levels in rat kidneys, 200 mg/kg/day p.o and 400 mg/kg/day p.o had shown 58.91%, 72.00% inhibition respectively. There was a marked depletion of glutathione levels in gentamicin treated rat kidneys whereas C. pareira hydroalcoholic extract showed a dose dependent increase in the levels of glutathione, 200 mg/kg/day p.o and 400 mg/kg/day p.o had shown 36.01%,

![Figure 4](image1.png)

**Figure 4:** Effect of HACP on Blood Urea Levels in Gentamicin Induced Nephrotoxic Rats.

![Figure 5](image2.png)

**Figure 5:** Effect of HACP on Blood Creatinine Levels in Gentamicin Induced Nephrotoxic Rats.

| Table 3: In-vitro antioxidant activity, DPPH and reducing power of C. pareira hydroalcoholic extract |
|---|---|---|---|---|
| Group | DPPH Absorbance | DPPH % Decrease | Reducing power Absorbance | Reducing power % Increase |
| Control | 1.253 ± 0.023 | – | 0.276 ± 0.008 | – |
| Ascorbic acid 50 µg | 0.107 ± 0.003*** | 91.46 | 0.533 ± 0.002*** | 93.11 |
| HACP 50 µg | 1.166 ± 0.003*** | 6.94 | 0.340 ± 0.011*** | 23.18 |
| HACP 100 µg | 1.034 ± 0.005*** | 17.47 | 0.385 ± 0.007*** | 39.49 |
| HACP 150 µg | 0.917 ± 0.001*** | 26.81 | 0.424 ± 0.002*** | 53.62 |
| HACP 200 µg | 0.739 ± 0.002*** | 41.02 | 0.452 ± 0.004*** | 63.76 |
| HACP 250 µg | 0.519 ± 0.003*** | 58.57 | 0.486 ± 0.002*** | 76.08 |

Values are mean ± SEM; n=6 in each group;*** significantly different at P<0.001.
Protective effect of *Cissampelos pareira* Linn. Extract on gentamicin-induced nephrotoxicity and oxidative damage in rats

**DISCUSSION**

Gentamicin is an important aminoglycoside antibiotic which is effective against gram negative microorganisms both in human and animals. Gentamicin is slightly bound to plasma proteins and is not metabolized in the body. It is excreted in unmodified form by the kidney\textsuperscript{12,13}. Gentamicin, an effective and widely used aminoglycoside antibiotic against severe infections is known to be potentially nephrotoxic despite close attention to its pharmacokinetics and dosing schedules that limits its long term clinical use\textsuperscript{14}. Several strategies, mechanisms and agents were utilized to prevent gentamicin nephropathy in animal model, however, their use to treat human subjects in clinical practice could not be achieved \textsuperscript{15,16}. The real mechanism

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>LP Absorbance</th>
<th>LP % Inhibition</th>
<th>GSH Absorbance</th>
<th>GSH % Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>Normal Saline</td>
<td>0.129 ± 0.006</td>
<td>–</td>
<td>1.885 ± 0.019</td>
<td>–</td>
</tr>
<tr>
<td>Group-II</td>
<td>Gentamicin 80 mg/kg. I.P</td>
<td>0.443 ± 0.015\textsuperscript{***}</td>
<td>–</td>
<td>1.03 ± 0.033\textsuperscript{***}</td>
<td>–</td>
</tr>
<tr>
<td>Group-III</td>
<td>Gentamicin + HACP 200 mg/kg. O.P</td>
<td>0.182 ± 0.009\textsuperscript{***}</td>
<td>58.91</td>
<td>1.401 ± 0.016\textsuperscript{***}</td>
<td>36.01</td>
</tr>
<tr>
<td>Group-IV</td>
<td>Gentamicin + HACP 400 mg/kg. O.P</td>
<td>0.124 ± 0.004\textsuperscript{***}</td>
<td>72.00</td>
<td>1.701 ± 0.006\textsuperscript{***}</td>
<td>65.72</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; n=6 in each group; \textsuperscript{***} significantly different at P<0.001.

Figure 6: Effect of HACP on DPPH Radical Scavenging Activity

![Figure 6](image_url)

Figure 7: Effect of HACP on Reducing Power Activity

![Figure 7](image_url)

65.72% increase in glutathione levels respectively in rat kidneys (Table 4 and Figure 8 and 9).
Protective effect of cissampelos pareira linn. Extract on gentamicin-induced nephrotoxicity and oxidative damage in rats

by which gentamicin has been shown both in-vitro and in-vivo studies to enhance the generation of reactive oxygen species (ROS). Abnormal production of ROS may damage some macromolecules, to induce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage [17,18]. Therefore, the diminution of nephrotoxicity would enhance its clinical value. Several agents that scavenge or interfere with the production of ROS have been used successfully to ameliorate gentamicin nephropathy [19]. In this present study, we identified that C. pareira hydroalcoholic extract had shown nephroprotective activity against gentamicin induced nephrotoxicity by its antioxidant activity. It was also found to be hepatoprotective [20], antinociceptive and antiarthritic [21], anti-inflammatory [22], and antifertility [23] activities besides other health benefits.

From the result of our study it was shown that administration of gentamicin elevated the levels of urinary glucose, serum urea, serum creatinine and lipid peroxidation levels in the kidneys whereas decreased urinary creatinine and glutathione levels in the kidneys. These results are in good agreement with those previously reported [24,3]. Our results also showed that treatment with different doses of C. pareira reduced the levels of urinary glucose, serum urea, serum creatinine and lipid peoxidation in kidneys whereas increases the urinary creatinine and glutathione levels in kidneys, dose dependently. In-vitro antioxidant study, DPPH radical scavenging activity and reducing power activity of the C. pareira hydroalcoholic extract had shown that the plan extract is having antioxidant activity. Hence natural and synthetic antioxidants and free radical scavengers are claimed to provide nephroprotective action against gentamicin induced nephrotoxicity. The probable mechanism of nephroprotection by C. pareira hydroalcoholic extract may be due to its antioxidant and free radical scavenging activity which increases renal mitochondrial antioxidant system [25], so thereby it can protect the kidneys from gentamicin toxicity. We are concluding that the C. pareira hydroalcoholic extract may have promising role in the treatment of gentamicin induced nephrotoxicity. However, further studies are needed to identify and isolate the active constituent which is responsible for the protective activity.

Figure 8: Effect of HACP on Tissue Lipid Peroxidation in Gentamicin Induced nephrotoxicity.

Figure 9: Effect of HACP on Tissue Glutathione in Gentamicin Induced Nephrotoxicity.
REFERENCES


