Protective action of *Cuminum cyminum* against gentamicin induced nephrotoxicity

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**Abstract**

**Objective:** To evaluate the effect of aqueous extract of *Cuminum cyminum* (AEC) seeds on gentamicin induced nephrotoxicity in rats.

**Methods:** The animals are divided into five groups of six animals each; normal saline, AEC 200mg/kg p.o for 11 days, gentamicin 80 mg/kg i.p for 8 days, AEC 100 and 200 mg/kg for 3 days and concurrently with gentamicin for 8 days. End of the study urine sodium, potassium, glucose, creatinine, urea, serum urea, serum creatinine, urea and creatinine clearance. Lipid peroxidation through thiobarbituric acid reacting substances (TBARS) method and histopathological examination was done.

**Results:** Gentamicin treated group caused nephrotoxicity as evidenced by marked elevation of serum urea, creatinine and urine glucose. Decreased clearance of urea, creatinine and rise in lipid peroxidation level. The AEC 200 mg/kg showed marked decrease in elevated levels of serum urea, creatinine, lipid peroxidation and increased clearance compare to the AEC 100 mg/kg.

**Conclusion:** The *Cuminum cyminum* showed the protective action against gentamicin induced nephrotoxicity.

**Keywords:** Gentamicin, Nephrotoxicity, Malondialdehyde, Creatinine, *Cuminum cyminum*

**Introduction**

One of the serious side effects that limit the usage of aminoglycosides is nephrotoxicity, used for the treatment and management of Gram negative bacterial infections. There have been many studies in recent years suggesting a significant role for reactive oxygen species (ROS) in gentamicin treatment. Lipid peroxidation may occur in the course giving rise to free radical which is highly toxic to tissue. This caused by accumulation of aminoglycosides in the proximal convoluted tubules attributed to its toxicity.

Till date even though there is tremendous advance in the field of medicine there is no satisfactory treatment or precaution for this toxicity. It’s documented that 80% of the world’s population has faith in traditional medicine particularly herbal drugs for their primary health care. The *Cuminum cyminum* (species: Apiaceae synonyms Cumin, Jira, Jirake, Jirakam, Jiraka, Jila karra) is commonly used species in the daily which is reported for its antioxidant, astringent, digestive, carminative, anthelmintic, diuretic, antimicrobial, anticarcinogenic, anticonvulsant, hypolipidemic, antidiabetic and hepatoprotective etc. It consists of carvone, cuminol, cumin aldehyde, mixture of hydrocarbons, cymene or cymol, terpene, small quantities of a-pinene, ß-pinene, phellandrene, cuminic alcohol, hydrated cumin aldehyde hydrocumin and glucosides. In the present study the protective action of *Cuminum cyminum* on gentamicin induced nephrotoxicity is studied by the protective action on the oxidative stress with the light change in the Na+ and K+ excretion.

**Material and Methods**

**Plant material:**

A dried seeds of *Cuminum cyminum* was collected and it was identified and authenticated by Dr. Siddappa Botanist. A voucher specimen has been deposited at the department of pharmacology, Sree Siddaganga College of Pharmacy- Tumkur.

**Extraction and preparation of test drug:**

The seeds are chopped finely using a grinder. 500g of seeds powder was used for extraction using Soxhlet apparatus for 72 hours. The extract was evaporated under vacuum. The extract was stored in refrigerator. The required quantity of extract was dissolved in distilled water and used for study.

**Animals:**

Male albino rats weighing 200-250 g were used for the experiment. The animals were housed in group (six animals in each cage) and fed with standard diet and water ad libitum. Animal were kept in central animal house of Sree Siddaganga College of Pharmacy- Tumkur, with maintenance of room temperature (25 ± 2 °C) and light: dark exposure of 12:12 h. The experiments were carried out after obtaining prior approval from Institutional Animal Ethical Committee (IAEC) approval no. (SSCPT/IAEC/61/2008-09).

**Selection of dose:**

A dose is selected based on previous study.
Grouping of animal

The animals are divided into five groups of six animals each.

Group I: Control was given normal saline throughout the course.

Group II: Animals were given 200 mg/kg aqueous extract of cumin seeds orally for eleven days.

Group III: Animals were given intraperitoneal injections of gentamicin (80 mg/kg/bdy wt) for eight days.

Group IV: Animals were given 80 mg/kg of gentamicin intraperitoneal for eight days in addition to this they also received 100 mg/kg aqueous extract of cumin seeds, which is started prior to gentamicin injections and continued with the eight day gentamicin treatment.

Group V: Animals were given 80 mg/kg of gentamicin intraperitoneal for eight days in addition to this they also received 200 mg/kg aqueous extract of cumin seeds, which is started prior to gentamicin injections and continued with the eight day gentamicin treatment.

Biochemical assay:

At the end of the study, the animals were kept in individual metabolic cage for 24-hour urine collection. Before sacrificing the animal, blood was collected by orbital sinus under ether anesthesia. Estimation of urinary sodium and potassium was done using flame photometer. Blood urea concentration was determined by GLDH-kinetic method, using autoanalyser. Creatinine clearance was calculated after estimating the serum and urinary creatinine by alkaline picrate method.

Assessment of oxidative stress

Post mitochondrial supernatant preparation (PMS):

Kidneys were, perfused with ice cold saline (0.9% w/v NaCl) and homogenized in chilled KCl (1.17%) using a homogenizer. The homogenates were centrifuged at 800 g for 5 minutes at 4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 g for 20 minutes at 4°C to get the post mitochondrial supernatant which was used to assay.

Estimation of lipid peroxidation:

The malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed by TBARS method. The reaction mixture consisted of 0.2 ml of 8.1% w/v sodium lauryl sulphate, 1.5 ml of 20% v/v acetic acid solution adjusted to pH 3.5 with NaOH and 1.5 ml of 0.8% w/v aqueous solution of thiobarbituric acid was added to 0.2 ml of 10% w/v of PMS. The mixture was brought up to 4.0 ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0 ml distilled water and 5.0 ml of the mixture of n-butanol & pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm. TBARS were quantified using an extinction coefficient of 1.56 × 105 M⁻¹/cm⁻¹ and expressed as nmol of TBARS per mg protein. Tissue protein was estimated using Biuret method of protein assay and the renal MDA content expressed as nmol of malondialdehyde per milligram of protein.

Histopathological examination:

Kidneys from all the five groups was fixed in 10% neutral buffered formalin and processed to paraffin wax. Sections (5 microns) are stained with haematoxylin and eosin and were examined under light microscope. They were evaluated and assigned score as follows: score 0 = normal; 1 = areas of focal granulovascular epithelial cell degeneration and granular debris in tubular lumens with or without evidence of tubular epithelial cell desquamation of small foci (< 1% of total tubule population); 2 = tubular epithelial necrosis and desquamation easily seen but involving less than half of cortical tubules; 3 = more than half of proximal tubules showing desquamation of necrosis but involved tubules easily found; 4 = complete or almost complete tubular necrosis.

Statistical analysis:

The significance of differences among the groups was assessed using one way analysis of variance (ANOVA) followed by multiple comparison test. P values less than 0.05 were considered significant.

RESULTS

The data present in the table 1 revels that change in body weight was significantly decreased in gentamicin treated group compare to control and AEC treated showed significantly increase in body weight there was no much difference in AEC plus gentamicin, urine glucose was significantly increased in gentamicin treated group, co administration of AEC plus gentamicin showed decrease in glucose level in urine. The table 2 says about urine sodium, potassium was significantly decreased in gentamicin treated compared to control, were as in AEC and AEC plus gentamicin treated group there was significantly increased. Urine creatinine, urea was significantly lower and serum creatinine, urea was significantly higher than control; coadmisitation of AEC with gentamicin group showed significantly increased urine creatinine, urea and significantly decreased serum urea.

The Figures 1 shows about creatinine, urea clearance and lipiddperoxidation. The creatinine, urea clearance was significantly less in gentamicin treated group compare to control, coadmisitation of AEC with gentamicin showed increase in the clearance. The lipiddperoxidation level was more in gentamicin treated compare to control, coadmisitation AEC with gentamicin showed marked decrease in lipiddperoxidation.

Table 1: Effect of gentamicin and AEC on body weight and urine glucose.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in body weight (gm)</td>
<td>3.33 ±1.66</td>
<td>20a2.58***</td>
<td>-15a2.23***</td>
<td>-10.83±2.00</td>
<td>-2.50±1.70***</td>
</tr>
<tr>
<td>Urinary Glucose (mg/day)</td>
<td>Nil</td>
<td>Nil</td>
<td>19.850±2.856***</td>
<td>13.950±0.953</td>
<td>5.70±0.976***</td>
</tr>
</tbody>
</table>

Group II and III were compared with Group I, Group IV and V with Group III. Values are mean ± SEM of 6 animals in each group. Data analyzed by One way ANOVA followed by Tukey. Significant relative to *P < 0.05, **P < 0.025 and ***P < 0.01.
Table 2: Effect of gentamicin and AEC on urine sodium, potassium, creatinine, urea, serum creatinine and urea

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary Sodium (meq/day)</td>
<td>0.547±0.021</td>
<td>0.851±0.079**</td>
<td>0.311±0.029*</td>
<td>0.403±0.030</td>
<td>0.531±0.056*</td>
</tr>
<tr>
<td>Urinary Potassium (meq/day)</td>
<td>0.739±0.024</td>
<td>0.992±0.059**</td>
<td>0.520±0.056**</td>
<td>0.703±0.02*</td>
<td>0.775±0.044*</td>
</tr>
<tr>
<td>Urinary Creatinine (mg/dl)</td>
<td>1.607±0.085</td>
<td>1.912±0.121</td>
<td>0.532±0.103***</td>
<td>0.773±0.148</td>
<td>1.355±0.098***</td>
</tr>
<tr>
<td>Urinary Urea (mg/dl)</td>
<td>58.85±4.117</td>
<td>68.92±5.142</td>
<td>6.867±0.670***</td>
<td>14.32±2.997</td>
<td>57.67±3.787***</td>
</tr>
<tr>
<td>Serum Creatinine (mg/dl)</td>
<td>0.937±0.009</td>
<td>0.899±0.029</td>
<td>1.248±0.075</td>
<td>1.239±0.886</td>
<td>1.016±0.063</td>
</tr>
<tr>
<td>Serum Urea (mg/dl)</td>
<td>35.77±1.916</td>
<td>59.97±4.410*</td>
<td>128.7±8.899***</td>
<td>112.6±5.252</td>
<td>61.32±3.906***</td>
</tr>
</tbody>
</table>

Group II and III were compared with Group I, Group IV and V with Group III. Values are mean ± SEM of 6 animals in each group. Data analyzed by One way ANOVA followed by Tukey. Significant relative to *P < 0.05, **P < 0.025 and ***P < 0.01.

Figure 1: Effect of gentamicin and AEC on creatinine, urea clearance and lipid peroxidation.

Table 3: Scoring of Histopathology studies.

<table>
<thead>
<tr>
<th>Group</th>
<th>Scoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>0</td>
</tr>
<tr>
<td>Group II</td>
<td>0</td>
</tr>
<tr>
<td>Group III</td>
<td>4</td>
</tr>
<tr>
<td>Group IV</td>
<td>3</td>
</tr>
<tr>
<td>Group V</td>
<td>2</td>
</tr>
</tbody>
</table>

The histopathological results show that necrosis seen in the gentamicin treatment compared to the control. The histological changes in kidney of all the groups were graded and the results are expressed.
DISCUSSION

In recent years many workers have suggested that administration of aminoglycosides including gentamicin produces nephrotoxicity in human20,21. Proximal tubular cells are the major site of damage in patient treated with the gentamicin22. Gentamicin binds with cell wall phospholipids, blocking the chain reactions of phosphotidyl inositol which impairs cell integrity. It results by generation of ROS23. Gentamicin administration to rats enhances the production of H2O2 in renal cortical mitochondria as a result of increase in the production of superoxide anions24, 25. Superoxide anions and H2O2 may interact to form a reactive and unstable radical namely a hydroxyl radical. This radical is formed by the reaction between H2O2 and Fe2+ 26. Thus Fe2+ appears to play an important role in production of reactive oxygen radicals in gentamicin nephrotoxicity 27. When oxygen radical begins to accumulate, renal cells exhibit a defensive mechanism by various antioxidant enzymes. Reduced activity of one or more antioxidant enzymes by the toxic effect of gentamicin or decreased level caused by accumulation of H2O2 and hydroxyl radical are the triggering factors in gentamicin nephrotoxicity28.

The cationic form of aminoglycosides attaches to the acidic phospholipids in brush border enzymes this results in the leakage of intracellular ions (K+, Mg2+ and Ca2+) proteins and enzymes this results in the decreased glomerular filtration rate29. Thus increase in blood urea and creatinine decreased creatinine and urea clearance. There is glucosuria in gentamicin treated rats. This is due to the proximal tubular cell damage or necrosis30.

In this we investigated the antioxidant property of Cuminum cyminum showed marked changes in kidney function and histopathological protection of acute failure in rats treated with gentamicin. Increased in blood urea, serum creatinine, glucosuria and decreased creatinine and urea clearance was prevented by the Cuminum cyminum. It also showed the increased excretion of K+ and Na+ concentration compared to the gentamicin treated group which there by increase in the glomerular filtration rate.

Oxidative stress causes necrosis is well demonstrated in many experimental models. S-allylmercaptoctystine31, Kallikrein/kinin32, L-carnitine33, Vitamin E and Vitamin C34 attenuate gentamicin induced nephrotoxicity. N-acetylcyctisteine35 and arginine attenuates cyclosporine induced nephrotoxicity. In gentamicin treated rats there was significant increase in lipid peroxidation shows oxidative stress cases necrosis caused by production of hydrogen peroxide in isolated mitochondrial tissue this was supported by histopathological examination. In histological studies the gentamicin group showed necrosis but prior drug (aqueous extract of Cuminum cyminum) treated with gentamicin showed the protection against the cell damage.

Pretreatment with Cuminum cyminum was effective in preventing the rise of MDA induced by gentamicin. It has been reported that Cuminum cyminum noted for its invitro antioxidant property, hepatoprotective activity. Extract possesses potent free radical scavenging property. The present study demonstrates the potent antioxidant properties of the extract. Hence, it may be concluded that the mechanism of nephroprotection by Cuminum cyminum extract in gentamicin treated rats could be due to the antioxidant and free radical scavenging activity. Future study is to be needed to identify the phytoconstituents responsible for these pharmacological actions of Cuminum cyminum.

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REFERENCES


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