Effect of *Sphaeranthus indicus* ethanol extract on tissue antioxidant activity in gentamicin induced nephrotoxic rats

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Summary

The purpose of the present study is to investigate whether the alcoholic extract of *Sphaeranthus indicus* could decrease the intensity of toxicity in albino rats. Gentamicin at a dose of 80mg/kg body weight was administered intraperitonially to albino male Wistar rats for 8 days. Then another set of animals were given the alcoholic extracts of *S. indicus* along with gentamicin treatment. The antioxidant levels, serum creatinine, serum urea etc were analyzed. The extracts could significantly decrease the gentamicin induced nephrotoxicity as inferred from the tissue antioxidant status in the drug administered animals. Remarkable change was observed in serum creatinine and urea levels. Lipid peroxidation in the kidney and liver tissues was also considerably reduced in *S. indicus* extract treated animals. The nephrotoxic rats showed lower activities of superoxide dismutase, catalase, glutathione transferase and reduced glutathione content in the liver and kidney which were restored to normal levels by treatment with *S. indicus* extract. The increased levels of lipid peroxidation in nephrotoxic rats were reverted back to normal levels after the treatment with *S. indicus* ethanol extract. These results suggest that it has protective effect against gentamicin induced nephrotoxicity which may be attributed to its antioxidant potential.

Key words: antioxidant, Asteraceae, free radical oxidative stress, gentamicin-induced renal damage, nephrotoxicity, *Sphaeranthus indicus*. 
INTRODUCTION

Gentamicin is a very effective antibiotic in treating Gram-negative infections. Unfortunately, in 10–20% therapeutic courses it causes renal failure. The exact mechanism of gentamicin-induced nephrotoxicity still remains unclear. It has been shown to enhance the generation of reactive oxygen species (ROS). ROS have been suggested as a causative agent of cell death in different pathological states including various models of renal diseases. The protective effects of some antioxidants such as manganese chloride and lycopene were investigated against lipid peroxidation induced by gentamicin and cisplatin. Free radicals are continuously produced by the body’s normal use of oxygen such as respiration and some cell mediated immune functions. Naturally, there is a dynamic balance between the amount of free radicals generated in the body and antioxidants to quench or scavenge them and protect the body against their deleterious effects [1]. In recent decades, a resurgent interest has been observed in traditional plant treatment for nephroprotective activity. As plants often contain substantial amounts of antioxidants, it has been suggested that an antioxidant action may be an important property of plant medicines associated with nephroprotective activity.

*Sphaeranthus indicus* Kurz (Asteraceae) is a medicinally important plant used in folk medicine and widely distributed in tropical Asia, Africa and Australia. All parts of the plant possess medicinal uses and have been reported to have beneficial effects on several ailments. This plant known as ‘Gorakmundi’ in Hindi is found abundantly in the plains all over India, ascending to a latitude of 1500 m in the hills, especially as a weed in the rice fields. The juice of the plant is styptic and diuretic and it is said to be useful against liver and gastric disorders [2]. Roots and seeds are used as stomachic and antihelmintic [3]. It is reported that flowers are highly alternative, depurative, cooling and tonic. They are also used as blood purifiers in skin diseases [4]. Though there is no scientific evidence to support the nephroprotective activity of *S. indicus*, tribal men of the chittoor district used it in the management of nephrotoxicity in combination with *Aerva lanata* [5]. The objective of this study was to ascertain the efficacy of this plant in the management of oxidative stress in gentamicin induced nephrotoxicity.

MATERIALS AND METHODS

Plant material

Plant was collected from Udupi district, Karnataka, India, in August 2006. It’s botanical identification was authenticated by Dr. Gopalkrishna Bhat, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher herbarium specimen PP 710 has been deposited in the Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal, India. The plants were shade dried and powdered.
Animals

Healthy adult albino male Wistar rats (100–200 g) aged 60–90 days were used for the study. The rats were housed in polypropylene cages and maintained under standard conditions (12:12 h light and dark cycle; 25±3°C; 35–60% humidity). The animals had free access to standard lab chow (Hindustan Lever Ltd. Mumbai, India) and tap water. The study was conducted after ethical committee clearance from the Institutional Animal Ethics Committee, KMC, Manipal (IAEC/KMC/07/2007–2008).

Preparation of ethanol extract

The shade-dried powdered plant was exhaustively extracted with 95% ethanol, using a Soxhlet apparatus. The total ethanol extract was then concentrated by distillation in vacuo and the residue was stored in dessicator and used for subsequent experiments. For animal studies, the dried extract was suspended in 2% gum acacia solution.

Preliminary phytochemical screening

The alcoholic extract was subjected to preliminary phytochemical analysis using standard methods [6].

High performance TLC

Qualitative densitometric high-performance thin-layer chromatography (TLC) analysis was performed to develop the characteristic fingerprint profile for the alcoholic extract of S. indicus. The extract and β-sitosterol, a biochemical marker, were dissolved in petroleum ether. Ten microlitres of the sample solutions were applied and the plates were developed in chloroform/ethyl acetate (85:15). Developed plates were sprayed with 10% methanolic sulfuric acid, heated at 105°C for 5 min and scanned densitometrically using a Camag TLC scanner 3 (CAMAG, Muttenz, Switzerland) at 600nm and documented.

Acute toxicity studies

The rats were fed with ethanol extract of S. indicus suspended in gum acacia (2% w/v) in increasing dose levels of 10, 30, 100, 300, 600, 1000 and 3000 mg/kg body weight [7]. The animals were observed continuously for 2 h for the gross behavioral changes and then intermittently every 2 h for a period of 24 h and finally at the end of 72 h to note for any signs of toxicity including death.
Gentamicin-induced renal injury

Five groups of six rats each were used in this model. Group I: Normal control rats were administered gum acacia daily for 18 days. Group II: normal rats were treated with ethanol extract of *S. indicus* 300 mg/kg for 18 days. Group III: Rats were treated with a single i.p. dose of gentamicin 80 mg/kg for 8 days was kept as nephrotoxic control. Group IV and Group V: Rats were treated with a single i.p. dose of gentamicin 80 mg/kg for 8 days followed by *S. indicus* ethanol extract 150 mg/kg and 300 mg/kg from 9th day to 19th day for 10 days.

At the end of the experimental period blood samples were collected for measuring serum urea and creatinine. Serum creatinine was determined by alkaline picric acid method using a diagnostic kit (Roche Diagnostics, Hitech, Mangalore, India). Serum urea was determined by diacetylmonoxime (DAM) reagent (modified Berthelot methodology) using a diagnostic kit (Roche Diagnostics, Hitech, Mangalore, India).

The rats were anaesthetized and sacrificed by cervical dislocation. The kidney sections were stained with hematoxyline and eosin and observed under light microscope for histopathological studies. The liver and kidney were excised, rinsed in ice cold saline and then homogenized with Tris-Hydrochloric buffer (pH 7.4). The tissue homogenates were used for the estimation of thiobarbituric acid reactive substance (TBARS) [8], reduced glutathione (GSH) [9], superoxide dismutase (SOD) [10], catalase (CAT) [11] and glutathione transferase (GST) [12].

Statistical analysis

Results were given as values of mean ±SE. Data were analyzed using one-way ANOVA followed by post hoc Scheffe’s test using SPSS computer software version 7.5. The statistical significance of difference was taken as p<0.05.

RESULTS

Co-chromatography of *S. indicus* extract along with β-sitosterol as a biochemical marker revealed the presence of β-sitosterol in the extract, with an retardation factor value of 0.55 (fig. 1). In acute toxicity studies ethanol extract was found to be safe in the doses used and there was no mortality up to a dose of 3000 mg/kg. Hence, the dose of 150 mg/kg and 300 mg/kg body weight were selected as test doses.
The effects of *S. indicus* ethanol extract on tissue antioxidant markers were studied. Table 1 shows the concentration of TBARS in the liver and kidney of normal and gentamicin induced nephrotoxic rats. Gentamicin caused an elevation of TBARS level which decreased upon administration of *S. indicus* ethanol extract as shown in table 1. GSH levels decreased in gentamicin treated rats (nephrotoxic control) in the liver and kidney were reverted back to normal level after treatment with the ethanol extract (nephrotoxic + *S. indicus*). Similar results were observed in glutathione transferase, catalase and SOD activity (values are shown in tab. 2 and 3). The gentamicin treated rats (nephrotoxic control) showed lower activities of superoxide dismutase, catalase and glutathione transferase in liver and kidney (tab. 2 and 3) which were restored to near normal levels by treatment with ethanol extract of *S. indicus*. Serum creatinine and urea level were significantly elevated in the gentamicin treated animals (nephrotoxic control) compared to the normal group (normal control). The increase of serum creatinine and urea levels were 8 and 10 fold, respectively. Treatment of animals with ethanol extract of *S. indicus* significantly reduced the elevated levels of serum creatinine and urea (tab. 4).

**Table 1.**

<table>
<thead>
<tr>
<th>group</th>
<th>kidney</th>
<th>liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal control</td>
<td>1.45 ± 0.33</td>
<td>1.41 ± 0.5</td>
</tr>
<tr>
<td>normal + <em>S. indicus</em> (300 mg/kg)</td>
<td>1.66 ± 0.32</td>
<td>1.6 ± 0.42</td>
</tr>
<tr>
<td>nephrotoxic control</td>
<td>4.21 ± 0.59</td>
<td>3.9 ± 1.02</td>
</tr>
<tr>
<td>nephrotoxic + <em>S. indicus</em> (150 mg/kg)</td>
<td>1.73 ± 0.35</td>
<td>1.83 ± 0.32</td>
</tr>
<tr>
<td>nephrotoxic + <em>S. indicus</em> (300 mg/kg)</td>
<td>1.9 ± 0.52</td>
<td>1.94 ± 1.2</td>
</tr>
</tbody>
</table>

Readings are values of mean ±SE, p<0.05 vs. nephrotoxic control.
Effect of ethanol extract of *S. indicus* in few selected biochemical variables indicative of oxidative stress in gentamicin induced renal damage (kidney)

<table>
<thead>
<tr>
<th>group</th>
<th>GSH [U/G/min]</th>
<th>GST</th>
<th>catalases [μg of H₂O₂/min mg protein]</th>
<th>SOD [U/G]</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal control</td>
<td>12.2±0.02</td>
<td>20.3±1.8</td>
<td>380.14±20.7</td>
<td>376.2±18.69</td>
</tr>
<tr>
<td>normal+ <em>S. indicus</em> (300 mg/kg)</td>
<td>12.6±1.2</td>
<td>22.4±2.2</td>
<td>361.2±18.6</td>
<td>360.4±16.71</td>
</tr>
<tr>
<td>nephrotoxic control</td>
<td>4.8±0.04</td>
<td>10.3±1.2</td>
<td>131.4±15.4</td>
<td>180.5±15.42</td>
</tr>
<tr>
<td>nephrotoxic + <em>S. indicus</em> (150 mg/kg)</td>
<td>11.8±1.3</td>
<td>19.7±1.4</td>
<td>362.5±20.3</td>
<td>368±12.18</td>
</tr>
<tr>
<td>nephrotoxic + <em>S. indicus</em> (300 mg/kg)</td>
<td>12.2±0.7</td>
<td>20.4±1.2</td>
<td>376.2±29.4</td>
<td>354±14.67</td>
</tr>
</tbody>
</table>

Readings are values of mean ±SE. U/G/min=units/gram/minute, p<0.05 vs. nephrotoxic control.

Effect of ethanol extract of *S. indicus* in few selected biochemical variables indicative of oxidative stress in gentamicin induced renal damage (liver)

<table>
<thead>
<tr>
<th>group</th>
<th>GSH [U/G/min]</th>
<th>SOD [μG]</th>
<th>GST [U/G/min]</th>
<th>Catalases [μg of H₂O₂/min/mg protein]</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal control</td>
<td>11.5±1.5</td>
<td>380.2±11.3</td>
<td>19.4±1.3</td>
<td>360.24±20.8</td>
</tr>
<tr>
<td>normal+ <em>S. indicus</em> (300 mg/kg)</td>
<td>10.8±1.3</td>
<td>382.3±11.5</td>
<td>20.2±1.4</td>
<td>371.32±28.4</td>
</tr>
<tr>
<td>nephrotoxic control</td>
<td>3.9±0.8</td>
<td>154.5±12.1</td>
<td>7.3±0.4</td>
<td>156.8±18.2</td>
</tr>
<tr>
<td>nephrotoxic + <em>S. indicus</em> (150 mg/kg)</td>
<td>12.65±1.8</td>
<td>379.3±12.9</td>
<td>17.5±2.8</td>
<td>364.4±22.1</td>
</tr>
<tr>
<td>nephrotoxic + <em>S. indicus</em> (300 mg/kg)</td>
<td>12.3±1.4</td>
<td>373.4±14.2</td>
<td>20.8±1.9</td>
<td>366.3±20.5</td>
</tr>
</tbody>
</table>

Readings are values of mean ±SE. U/G/min=units/gram/minute, p<0.05 vs. nephrotoxic control.

Effect of ethanol extract of *S. indicus* in gentamicin induced renal damage

<table>
<thead>
<tr>
<th>group</th>
<th>serum creatinine [mg/dl]</th>
<th>blood urea [mg/dl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal control</td>
<td>0.7±0.10</td>
<td>38.5±4.0</td>
</tr>
<tr>
<td>normal+ <em>S. indicus</em> (300 mg/kg)</td>
<td>0.9±0.10</td>
<td>39.5±0.05</td>
</tr>
<tr>
<td>nephrotoxic control</td>
<td>5.8±0.15</td>
<td>367.5±2.50</td>
</tr>
<tr>
<td>nephrotoxic + <em>S. indicus</em> (150 mg/kg)</td>
<td>1.2±0.09</td>
<td>88.5±10.21</td>
</tr>
<tr>
<td>nephrotoxic + <em>S. indicus</em> (300 mg/kg)</td>
<td>0.82±0.12</td>
<td>67.3±2.50</td>
</tr>
</tbody>
</table>

Readings are values of mean ±SE, p<0.05 vs. nephrotoxic control.

The presence of peritubular and glomerular congestion, tubular casts, epithelial degeneration, interstitial edema, blood vessel congestion and infiltration by inflammatory cells, which are features of acute tubular necrosis, were observed in the histopathological sections of the kidneys in the gentamicin treated nephrotoxic rats. The features of acute tubular necrosis in nephrotoxic rats were reverted back to normal after the treatment with ethanol extracts of *S. indicus* (tab. 5).
**Table 5.**

Effect of *S. indicus* ethanol extract on histopathological kidney damages induced by gentamicin

<table>
<thead>
<tr>
<th>Effect</th>
<th>normal control</th>
<th>normal+ <em>S. indicus</em> [300 mg/kg]</th>
<th>nephrotoxic control</th>
<th>nephrotoxic+ <em>S. indicus</em> [150 mg/kg]</th>
<th>nephrotoxic+ <em>S. indicus</em> [300 mg/kg]</th>
</tr>
</thead>
<tbody>
<tr>
<td>glomerular congestion</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>tubular casts</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>peritubular congestion</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>epithelial desquamation</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>blood vessel congestion</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>interstitial edema</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>inflammatory cells</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

(–) absent; (+) mild effect; (+++) severe effect

**DISCUSSION**

Gentamicin is a widely used aminoglycoside antibiotic inducing tubular necrosis [13-15]. Several reports have documented the pathogenesis of aminoglycoside-induced renal tubular cell injury such as derangement of lysosomal mitochondrial and plasma membrane structure [16-18]. Furthermore, results of many studies have shown that the altered concentrations of various biochemical indications of oxidative stress in kidney tissue are due to gentamicin [19-21]. Because of the obvious response of ROS in gentamicin-induced renal damage, several antioxidant agents have been used to block gentamicin-induced nephrotoxicity [22-24]. As it was mentioned above, oxidative stress is one of the causes of gentamicin-induced renal damage. Gentamicin causes rapid changes in membrane lipid composition. These changes may be induced by free radical initiated lipid peroxidation [25-26]. We have found the elevated lipid peroxidation in gentamicin-treated group.

The results also showed that catalase, GSH, GST and SOD levels were decreased significantly in gentamicin-treated rats. These biochemical indications of oxidative stress and antioxidant status were reversed by *S. indicus* ethanol treatment. We assume that these findings are in accordance with the antioxidant effect of *S. indicus*. Furthermore, these results are confirmed by the other studies, which have pointed to the inhibition of the development of renal failure by antioxidants in laboratory animals [27]. If intracellular free oxygen radical increase irreversible cellular injury process begins [28]. Scavenging of free oxygen radicals prevent irreversible cell injury and necrosis. A certain kind of enzymatic and non-enzymatic systems inactivates free radicals, these are antioxidants, iron, copper and a series of enzymes acting as free radical scavenging systems and breaking down hydrogen peroxide and superoxide anion. The free radical scavenging potential of the plant was studied by using different *in vitro* antioxidant models of screening and reported that the ethanol extract showed maximum scavenging of the radical [1].
TBARs are considered to be the index of endogenous lipid peroxidation, an indirect evidence of intensified free radical production. The TBARs levels in our study were found to be elevated in both liver and kidney of Gentamicin treated group and were significantly reduced upon administration of the extract. GSH known to protect the cellular system against the toxic effects of lipid peroxidation act as a free radical scavenger. The significant recovery of GSH and GST levels in both kidney and liver on treatment indicates the protective effect of the extract. SOD is an important defense system, which scavenges the superoxide radical by converting it into hydrogen peroxide and molecular oxygen, reduced content of SOD is observed in the liver and kidney of gentamicin treated may be due to increased production of reactive oxygen radicals. Upon treatment with the extract SOD levels were significantly increased.

The present study provides some useful tips into the antioxidant potency of *S. indicus* in gentamicin induced nephrotoxicity. However further work should be carried out to find out the absolute mechanism of action of the plant in experimental nephrotoxicity.

REFERENCES

2. Chadha YR. The wealth of India. The publications and Information Directorate. CSIR. New Delhi 1976;4:5.
Streszczenie

Celem niniejszej pracy było zbadanie, czy wyciąg alkoholowy ze *Sphaeranthus indicus* może zmniejszyć rozmiary uszkodzenia nerek u szczurów. Szczurom szczepu Wistar (samcom) podawano dootrzewnowo gentamycynę w dawce 80 mg/kg m.c. przez 8 dni. Druga grupa zwierząt równolegle z gentamycyną otrzymywała wyciąg alkoholowy ze *Sphaeranthus indicus*. Analizowano aktywność przeciwtleniającą, poziom kreatyniny i mocznika w surowicy. Podawanie tego wyciągu wyraźnie zmniejszało nefrotoksyczność indukowaną gentamycyną, co pozostawało w związku ze zmianami aktywności przeciwtleniającej u zwierząt. Istotną różnicę obserwowano w poziomie kreatyniny i mocznika w surowicy. U zwierząt, którym podawano wyciąg z *Sphaeranthus indicus*, także bardzo zmniejszyła się peroksydacja lipidów w tkankach nerek i wątroby. U szczurów ze uszkodzeniem nerek wystąpiła obniżona aktywność dysmutazy ponadtlenkowej, katalazy, transferazy glutatjonowej oraz obniżona zawartość glutatjionu w nerkach i wątrobie. Ich poziomy wróciły do normalnego poziomu po podaniu ekstraktu z *Sphaeranthus indicus*. Podwyższony poziom peroksydacji lipidów także wracał do normy po podaniu szczurom z objawami nefrotoksyczności etanolowego ekstraktu z *S. indicus*. Wyniki sugerują, że wyciąg ten może działać protekcyjnie, zapobiegając działaniu nefrotoksycznemu gentamycyny. Może to mieć związki z właściwościami przeciwtleniającymi wyciągu ze *Sphaeranthus indicus*.

Słowa kluczowe: przeciwtleniacz, *Astraceae*, stres oksydacyjny związany z wolnymi rodnikami, uszkodzenie nerek spowodowane gentamycyną, nefrotoksyczność, *Sphaeranthus indicus*