Therapeutic effect of ethanolic extract of *Hygrophila spinosa* T. Anders on gentamicin-induced nephrotoxicity in rats

K J Bibu*, A D Joy & K A Mercey

Department of Pharmacology and Toxicology, *Department of Statistics
Faculty of Veterinary and Animal Sciences, Mannuthy, Thrissur 680 651, India

Received 3 December 2009; revised 10 May 2010

Therapeutic effect of ethanolic extract of *Hygrophila spinosa* in gentamicin-induced nephrotoxic model of kidney injury in male Sprague-Dawley rats was studied. Rats were administered with gentamicin at a dose of 80 mg/kg intraperitoneally (ip) to induce nephrotoxicity. Kidney function was assessed by measuring serum creatinine and urea. Kidney superoxide dismutase, lipid peroxidation, catalase and reduced glutathione were also measured in control and treated rats. *H. spinosa* extract showed free radical scavenging activities at doses of 50 and 250 mg/kg with a predominant activity at 250 mg/kg. The ethanolic extract also caused a reduction in serum creatinine and urea levels. Histopathological studies were conducted to confirm the therapeutic action of the plant extract. The results demonstrated that the ethanolic extract of whole plant of *H. spinosa* evinced the therapeutic effect and inhibited gentamicin-induced proximal tubular necrosis.

**Keywords:** Free radical scavenging, Gentamicin, *Hygrophila spinosa*, Therapeutic

Nephrotoxicity is caused by several xenobiotic substances damaging renal proximal tubule, the portion of the nephron with greater sensitivity to nephrotoxic effects. Some of the chemicals which cause damage to the proximal tubule are antibacterial agents such as cephaloridine and aminoglycosides, anticancer drugs such as cisplatin and industrial chemicals such as cadmium, hexavalent chromium, mercury and palladium. Nephrotoxicity is the major side effect of aminoglycosides, especially gentamicin, accounting for 10 to 15% of all cases of acute renal failure. The defective drug excretion adversely affects the different systems of the body. Since kidneys are the major organs of drug excretion, the occurrence of nephrotoxicity is of great concern.

Herbal products have a special place in the world of pharmaceuticals. Side effects of conventional medicine, efficiency of plant-derived drugs and growing interest in natural products have increased scientific interest in medicinal plants. In the last few decades, there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and less side effects. India, being a treasure trove of medicinal plants, contributes much to these. Many regions in Kerala, especially the Western Ghats, still remain to be explored for unidentified medicinal herbs.

*Hygrophila spinosa*, T. Anders. (Kokilaksha in Sanskrit) Syn. *Asteracantha longifolia*, Nees., Syn. *Hygrophila auriculata* (K.Schum.) Heine., Syn. *Hygrophila schulli* (Ham.) is a well-known medicinal plant (family: Acanthaceae) found in paddy fields and marshy areas. The plant is widely distributed throughout India, Sri Lanka, Burma, Malaysia and Nepal. The whole plant has medicinal properties and it is being used in Ayurveda for various ailments like jaundice, hepatic obstruction, rheumatism, inflammation, pain, as a diuretic, aphrodisiac and in the treatment of dropsy, scanty urine and ascites. The plant is known to possess hypoglycemic activity in human subjects, antitumor, haematinic, anti-nociceptive, hepatoprotective, free radical scavenging and lipid peroxidation activities.

Hitherto no research work has been undertaken to explore the therapeutic effect of this plant or to investigate the effects of *Hygrophila spinosa* on the levels of antioxidant enzymes like superoxide dismutase, lipid peroxidation, catalase and reduced glutathione in kidney tissue. Hence, the present study is focused to evaluate the therapeutic effect and

*Correspondent author
Telephone: +91-487-2370665; Ext 249
Mobile: +91-9895297842
E-mail: bibujohn@rediffmail.com
antioxidant potentials of ethanolic extract of whole plant of *Hygrophila spinosa* (*H. spinosa*) against gentamicin-induced nephrotoxicity and validate the ethnobotanical and clinical claims of the plant.

**Materials and Methods**

*Drugs and chemicals*—Gentamicin sulphate (GM) was procured from TTK Pharma Limited, Raja Annamalaiapuram, Chennai, India. The other chemicals were of analytical grade. The enzyme kits used for analysis were obtained from Merck Specialities Private Limited, India.

*Experimental animals*—The study was conducted in 32 adult male Sprague Dawley rats weighing 200-250 g. The rats were purchased from Small Animal Breeding Station, Mannuthy, Thrissur, Kerala, India. The animals were housed in appropriate cages in a well ventilated room with a 12:12 h L:D cycle. They were maintained under identical feeding and management practices in the laboratory. An acclimatization period of four days was allowed before the commencement of the experiment. The animals were maintained as per the rules and regulations of Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA).

*Plant materials and preparation of extracts*—The whole plant of *Hygrophila spinosa* was collected from the marshy areas of Muringoor, Mukundapuram taluk, Thrissur district, Kerala, India. The plant was authenticated by Dr K T Prasanna Kumari, Professor, Department of Plant Breeding and Genetics, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala, India and a voucher specimen (No:-2068) has been kept at Department of Pharmacology and Toxicology, College of Veterinary and Animal Sciences, Mannuthy, Kerala, India. The whole plant of *H. spinosa* was air-dried at room temperature and coarsely powdered using an electrical pulverizer. The powders obtained were extracted using a soxhlet apparatus with 95% ethanol. The ethanolic extracts were then concentrated in a rotary vacuum evaporator under reduced pressure and temperature (55°C). The yield of the extract was 8.97% on dry matter basis.

*Acute toxicity study of the plant extracts*—An acute toxicity study of the ethanolic extract was conducted using acute toxic class method as per Organisation of Economic Co-operation and Development (OECD) guidelines 42513.

*Phytochemical analysis*—The ethanolic extract of whole plant of *H. spinosa* were tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins14.

*Experimental design*—The experiment was approved by the Institutional Ethics Committee. 46th Faculty Research Council meeting with order number R1/71783/2006 and a code number for the experiment as AD/182-00-00/05VC(12)KAU/PG and the animals (32) were randomly divided into following 4 groups of 8 animals each. The experiment was conducted for a period of 30 days.

- Group I (controls): administered with vehicle (0.125% Tween 80) po for 30 days.
- Group II (GM): received GM at a dose rate of 80 mg/kg, ip for 8 consecutive days. This dose was proved to be nephrotoxic15.
- Group III: received GM at a dose rate of 80 mg/kg, ip for 8 consecutive days + ethanolic extract of *H. spinosa* at a dose rate of 50 mg/kg, po from 9th day to day 30.
- Group IV: received GM at a dose rate of 80 mg/kg, ip for 8 consecutive days + ethanolic extract of *H. spinosa* administered at a dose rate of 250 mg/kg, po from 9th day to day 30.

The dose of 250 mg/kg, po was selected based on the earlier works12. The lower dose was selected as 1/5th of the higher dose.

*Blood and tissue sampling*—Blood was collected from the retro orbital plexus from all the rats on days 0, 9, 15 and 30 under mild ether anaesthesia for the assay of serum creatinine and urea. On 30th day, animals were sacrificed by cervical dislocation. Right and left kidneys were rapidly removed and processed for morphological and histological examination, superoxide dismutase16, lipid peroxidation17, catalase18 and reduced glutathione19 determinations.

*Serum biochemical assays*—Serum was separated by centrifuging at 3200 rpm for 10 min. Serum creatinine was determined based on Jaffe kinetic method without deproteinisation in Genesys spectrophotometer. Urea was estimated according to the Urease GLDH method (kinetic UV test) in semi automatic blood analyzer, Microlab 200.
Histopathological examinations—Representative samples of kidney obtained from the dissected animals were fixed in 10% formalin. They were then processed and paraffin embedded. The sections were stained with haematoxylin and eosin. The sections were examined in detail under light microscope.

Statistical analysis—Data are expressed as mean ± SE. The results of superoxide dismutase, lipid peroxidation, catalase, reduced glutathione and creatinine were analysed using analysis of variance (ANOVA) and the comparison of parameters between different groups were evaluated by Duncan’s multiple range test. Since significant difference in the urea levels was noted on day 0, analysis of covariance was done to nullify the effect of day 0 on other treatment days and the comparison of the parameter between different groups were made by Duncan’s multiple range test.

Results

Acute toxicity study—In acute toxicity studies, the high doses of the extracts (5 g/kg) did not produce any signs of toxicity and mortality. At the doses used, the plant extract did not significantly affect the body weights of the treated rats or the weights of the kidneys relative to the body weights. This suggests that the extract, at the doses used, caused no adverse effects on feed intake or metabolism. Therefore, the approximate LD50 should be above 5 g/kg.

Phytochemical analysis—Phytochemical analysis of the ethanolic extract of H. spinosa revealed the presence of steroids, phenolic compounds, flavonoids, diterpenes and saponins.

Effect of H. spinosa and gentamicin treatments on kidney superoxide dismutase, lipid peroxidation, catalase and reduced glutathione levels—The kidney superoxide level decreased in GM-treated rats as compared to control animals (P<0.05). The superoxide dismutase levels after administration with GM for eight days followed by treatment with ethanolic extract of H. spinosa at doses 50 and 250 mg/kg showed significant (P<0.05) increase in superoxide dismutase levels as compared to GM-treated rats (Table 1). Lipid peroxidation, measured by the formation of thio barbituric acid reactive substances, was significantly (P<0.05) increased in GM-treated rats as compared to controls. The results indicated that Group IV produced significant (P<0.05) reduction in lipid peroxidation when compared to GM-treated rats (Table 1). Group III also differ significantly from GM-treated group (P<0.05). In GM-treated rats, catalase levels significantly (P<0.05) reduced as compared to controls. All the treatment groups showed a significant increase in catalase levels (P<0.05) (Table 1). Similarly, reduced glutathione levels were significantly increased in all the treatment groups as compared to GM-treated animals which showed a reduction in reduced glutathione levels as compared to controls (Table 1).

Effect of H. spinosa and gentamicin treatments on serum creatinine and serum urea levels—Serum creatinine level showed a considerable increase in all the groups except in the control animals after GM administration for eight days. H. spinosa treatment groups showed a significant (P<0.05) decrease in creatinine level after 14 days of treatment when compared to GM-treated group. On 30th day, the creatinine levels were almost similar to healthy control rats except in Group II. Serum urea concentration was also markedly elevated with GM. On 9th day, significant increase in the urea levels was noted in Group IV. Groups II and III did not differ significantly (P>0.05). On 15th day, Groups II and III were examined in detail under light microscope.

Table 1 — Effect of ethanolic extract of H. spinosa on GM-induced changes in superoxide dismutase, lipid peroxidation, catalase and reduced glutathione levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>Superoxide dismutase (units/mg of protein)</th>
<th>Lipid peroxidation (mM/100 mg of tissue)</th>
<th>Catalase (units/assay mixture) (250 mg)</th>
<th>Reduced glutathione (mg/100g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2.494 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.367 ± 0.43&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.988 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>133.525 ± 3.23&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>1.612 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
<td>36.400 ± 0.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.722 ± 0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>81.337 ± 2.97&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>4.317 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.342 ± 0.96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.932 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>249.350 ± 1.90&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>9.676 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.716 ± 0.60&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.381 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>289.037 ± 2.89&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means bearing the same superscript within a column do not differ significantly at P<0.05

Group I – control; Group II – positive control (GM alone); Group III – H. spinosa treated group-50 mg/kg po; Group IV – H. spinosa treated group-250 mg/kg po.
did not differ significantly so as Groups I and IV. On 30th day, Groups I, III and IV did not differ significantly. *H. spinosa* treatment prevented GM-induced increase in blood urea. The time course of serum creatinine and urea levels is displayed in Table 2.

Effect of *H. spinosa* and gentamicin treatments on histopathology—In control group, the microscopic examination of the kidney revealed normal architecture. The tubular structures were largely intact without the presence of any mononuclear infiltrates in the interstitium. In GM-treated group (Group II), there were extensive proximal tubular necrosis and loss of the lining epithelium and these features were predominantly subcapsular. Besides, there were interstitial oedema, perivascular oedema and multiple focal collections of mononuclear cells in the interstitium. The glomerular changes were quite marked (Fig. 1B). In *H. spinosa* ethanolic 50 mg/kg treated group (Group III), there were areas of tubular degeneration and necrosis along with areas of perivascular oedema and tubulo-interstitial mononuclear cell infiltrates at different foci throughout the cortex. In *H. spinosa* ethanolic 250 mg/kg treated group (Group IV), the proximal tubular epithelial cells showed varying degrees of regeneration but slight degenerative changes. Besides this, there were scattered small foci of mononuclear cell infiltration confined to subcapsular area. The epithelial cells of the proximal convoluted tubules were more or less intact (Fig. 1D).

**Discussion**

Nephrotoxicity occurs as a disturbance in renal function due to various adverse drug interactions, inadequate elimination of radioactive contrast materials and chemicals. It is of great concern in patients with renal failure. Nephrotoxicity may limit the clinical usefulness of many diagnostic and therapeutic agents; recognition of factors associated with higher risk for renal injury is of great importance. However, the end point of nephrotoxicity is always cell death; therefore, it is important to identify the mechanism in addition to the site of action, in order to formulate a strategy for damage prevention. The strategies aimed at ameliorating the nephrotoxicity are of clinical interest.

The results of phytochemical screening of the ethanolic extract of *H. spinosa* which showed the presence of steroids, phenolic compounds, flavonoids, diterpenes and saponins are in accordance with the earlier works. Flavonoids are reported to possess antioxidant activity. The flavonoids present in the ethanolic extract of *H. spinosa* at the dose rate of 250 mg/kg contribute to the therapeutic efficacy possibly by its antioxidant activity.

In the present study, the ethanolic extract of *H. spinosa* exhibited significant antioxidant effect in a dose-dependent manner by increasing the levels of superoxide dismutase, reduced glutathione, catalase and by decreasing the levels of lipid peroxidation. Earlier works have reported that the ethanolic extracts of *Hygrophila auriculata* produced an increase in the activity of superoxide dismutase, catalase, glutathione, glutathione peroxidase and decrease in lipid peroxidation suggesting the antioxidant property at higher dose rate. Superoxide dismutase levels were significantly decreased in GM-treated groups which correlates with earlier findings. Lipid peroxidation, measured by the formation of thio barbituric acid reactive substances, was significantly increased in GM-treated groups. This was in agreement with earlier studies. In the present study, the ethanolic extract of *H. spinosa* at lower doses could alleviate GM-induced toxicity.

### Table 2 — Effect of ethanolic extract of *H. spinosa* on GM-induced changes in serum creatinine and urea levels

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
<td>9th day</td>
</tr>
<tr>
<td>I</td>
<td>0.54 ± 0.05a</td>
<td>0.67 ± 0.03a</td>
</tr>
<tr>
<td>II</td>
<td>0.45 ± 0.03a</td>
<td>2.81 ± 0.36c</td>
</tr>
<tr>
<td>III</td>
<td>0.47 ± 0.03a</td>
<td>2.84 ± 0.27b</td>
</tr>
<tr>
<td>IV</td>
<td>0.48 ± 0.03a</td>
<td>3.90 ± 0.39a</td>
</tr>
</tbody>
</table>

Means bearing the same superscript within a column do not differ significantly at P< 0.05
Group I – control; Group II – positive control (GM alone); Group III – *H. spinosa* treated group- 50 mg/kg po; Group IV – *H. spinosa* treated group-250 mg/kg po.
doses did not considerably reduce the increased lipid peroxidation level. But higher doses of the extract showed considerable reduction in lipid peroxidation level which correlates with the earlier works. The ethanolic extract of *Hygrophila auriculata* caused a reduction in lipid peroxidation at doses of 100 and 250 mg/kg. In the present study, all the plant extract treatment groups showed a significant increase in the catalase activity. Catalase activity was significantly reduced in GM-treated groups when compared with the control group. Gentamicin treatment caused a significant decrease in reduced glutathione level which agrees with the earlier findings. The treatment with the ethanolic extract of *H. spinosa* showed considerable increase in the reduced glutathione levels. Grape seed extract was found to increase the reduced glutathione level in GM-induced nephrotoxicity. The studies conducted revealed that reduced glutathione level was increased by dimethyl sulfoxide in gentamicin-induced nephrotoxicity. Similar results were shown by Ebselen, a selenoorganic drug, in gentamicin-induced renal toxicity. 

Fig. 1 — Photomicrographs of normal control, GM control and *H. spinosa* 50 and 250 mg/kg treated groups. (a) Normal group: A-intact glomerulus; B-intact proximal convoluted tubules. (b) Gentamicin group: A-marked glomerular changes; B-extensive tubular necrosis; C-mononuclear cell infiltration in the interstitium. (c) Ethanolic *H. spinosa* (50 mg/kg) treated group: A-tubular degeneration and necrosis; B-perivascular oedema; C-different foci of mononuclear infiltration. (d) Ethanolic *H. spinosa* (250 mg/kg) treated group: A-minimal glomerular changes; B-regenerative proximal tubules; C-foci of mononuclear cell infiltration. [Figs a-c: H & E × 100 and Fig. d: H & E × 400].
damage. Thus the present study proves the free radical scavenging activity of *H. spinosa* which is attributed to its therapeutic efficacy.

Serum creatinine and serum urea were significantly reduced in the treatment groups in a dose-dependent manner. Serum creatinine and serum urea (serum markers of kidney function) have been considered the most important manifestations of severe tubular necrosis of kidney. Gentamicin has been reported to produce nephrotoxicity even at the therapeutic dose level mainly because it is accumulated in the proximal tubular cells and cause local necrosis and aggravate the toxicity further at high dose level. The ethanolic extract of *H. spinosa* at higher dose showed a significant reduction in serum creatinine level on 15th day while their lower dose showed a gradual decrease in serum creatinine. This clearly indicates that the test drug protected the kidney from the toxic effect of GM preferably at high dose. Elevation of serum creatinine and serum urea which were marked on 9th, 15th and 30th day of the experiment in GM group indicated the severe tubular necrosis. The ethanolic extract of *H. spinosa* at low dose (Group III) does not reduce the serum urea level on 15th day and the serum urea level was comparable to GM group but the high dose of the extract showed significant reduction which was comparable to control group. By the end of the experiment, the serum urea levels were significantly reduced in the treatment groups. Having protected the kidney from the toxic effects of GM, the test drug has been shown to possess striking therapeutic efficacy in ameliorating nephrotoxicity at high dose.

Histologically, GM group showed severe proximal tubular necrosis and loss of lining epithelium along with mononuclear cell infiltrations. In the ethanolic extract of *H. spinosa* 250 mg/kg treated group, varying degrees of regeneration and only small foci of mononuclear infiltration could be seen. There were areas of degeneration and mononuclear cell infiltration at different foci in other the treatment group. The histopathological studies further confirm the therapeutic effect of *H. spinosa*. Ethanolic extract of *H. spinosa* (250 mg/kg) showed predominantly regenerative stages than that of the other treatment group. The epithelial cells of the proximal convoluted tubules of this group were intact. In this group, the glomerular changes were scanty. These histopathological findings supported by the results of antioxidant and serum biochemical parameters demonstrated the more therapeutic effect of ethanolic extract of *H. spinosa* at the dose rate of 250 mg/kg than the other treatment group. The antioxidant activity is mainly due to the presence of flavonoids. The therapeutic activity may be attributed to the excretion of the toxic substances along with increased diuresis due to high potassium content of the plant.

Thus, the findings of the present study validate the therapeutic effect of *H. spinosa* for the management of renal disorders possibly by inhibiting the free radical mediated process. Further investigation of these promising protective effects of *H. spinosa* against GM-induced renal injury may have a considerable impact on developing feasible strategies to treat patients with renal failure.

**Acknowledgement**
The authors thank Dr A J Mammen, Department of Pathology, Faculty of Veterinary and Animal Sciences, Mannuthy, Thrissur, for technical assistance and Dr K T Prasanna Kumari for identifying and authenticating the whole plant of *Hygrophila spinosa*. The financial support provided by the Kerala Agricultural University is also acknowledged.

**References**

10. Singh A & Handa S S, Hepatoprotective activity of graveolens and *Hygrophila auriculata* against paracetamol.


