Althaea rosea L. ameliorates renal oxidative damage induced by gentamicin in rats

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Althaea rosea (AR) seeds are commonly used in renal disorders in Unani medicine. Hence, the present study was aimed to evaluate the nephroprotective activity of methanolic extract of AR seed (ME-AR) and its chloroform soluble (CHSF) and insoluble (CHIF) fractions in gentamicin-induced renal oxidative damage in Albino rats. The rats were divided into five groups of six animals each. Group I served as control. All five groups were received 1 % CMC (10 mL/kg), orally for 8 days. In addition to this, the groups III, IV and V were received ME-AR 103 mg/kg, CHSF 70 mg/kg, and CHIF 35 mg/kg respectively, orally for 8 days. Gentamicin was injected subcutaneously (100 mg/kg) in neck region in the volume of 1 mL/kg for the last 5 days for the group II (Toxicant), III, IV and V. Following treatment, the nephroprotective effects were evaluated with level of blood urea nitrogen (BUN), serum creatinine (SCr), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH) and lipid peroxidation (LPO) along with the histological observations among the experimental groups. Gentamicin intoxication induced changes of biochemical parameters were significantly prevented by test drug which was also evidenced by the histological observations.

Keywords: Nephroprotective, Althaea rosea, Gentamicin, Nephrotoxicity, Histopathology

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Althaea rosea (AR) belongs to the family Malvaceae. It is a stately ornamental plant, producing large single, semi-double, double or frilled flowers of many colours1. This is distributed from the East Mediterranean region to Central Asia and native to China and Greece. Althaea rosea is commonly found as an escape in waste places, along the roadsides2,3. The seeds of this plant are having anti-pyretic, diuretic, anti-inflammatory, demulcent and analgesic properties4,5,6. It has been used in traditional medicines for ailments like chest complaints, boils, abscesses, constipation, peptic ulceration, renal calculi, burning micturition, gastritis, cough, etc.2,3,7.

Phytochemical studies revealed that, Althaea rosea contains alkaloids, phenolic compounds (syringic, p-hydroxybenzoic and p-coumaric acids), flavonoids, steroids (β-sitosterol and stigmasterol), fatty acids (ricinoleic acid), etc.8,9.

Gentamicin is an aminoglycoside antibiotic, widely used in the treatment of Gram-negative infections. Though, gentamicin produces nephrotoxicity and ototoxicity, it is being continuously used due to its high bactericidal efficacy, limited bacterial resistance and low-cost. Aminoglycoside-induced nephrotoxicity is typically characterized by tubular necrosis without gross morphological changes in glomerular structures10 and increase in plasma creatinine and urea levels leading to severe renal failure11. Gentamicin induced renal oxidative damage due to generation of reactive oxygen species (ROS) in the kidney has been proven by large number of research studies10,12. Hence, the present study was aimed to evaluate the nephroprotective activity of methanolic extract of AR seed (ME-AR) and its chloroform soluble (CHSF) and insoluble (CHIF) fractions in gentamicin-induced renal oxidative damage in Albino rats.

Methodology

Plant material
Althaea rosea seeds were purchased from Khari Baoli, local market of Delhi and authenticated by Dr HB Singh, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi. Hundred gm crushed seeds of Althaea rosea were extracted by refluxing with
100 % methanol in distillation flask. The ME-AR was treated with boiling chloroform to get the CHSF of AR. The insoluble residue left after extraction with chloroform of ME-AR was dissolved in methanol to get the CHIF of AR.

Experimental animals

All the experiments were carried out in both sex of Albino rats of Wistar strain weighing between 150-180 g. All animals were housed in groups in polypropylene cage and maintained on a standard pellet diet and water ad libitum. The animals were kept under standard laboratory conditions at 25 ± 1°C temperature.

Experimental design

The rats were divided into five groups of six animals each. While, Group I (control) and group II (Toxicant) were received 1 % CMC (10 mL/kg), the groups III, IV and V were received 1 % CMC with higher dose (HD) of ME-AR 103 mg/kg, CHSF-AR 70 mg/kg, and CHIF-AR 35 mg/kg respectively, orally for 8 days. In addition to this, the animals in the groups II, III, IV and V were co-administrated with gentamicin subcutaneously (100 mg/kg) in neck region in the volume of 1 ml/kg for the last 5 days.

Estimation of parameters

Experimental animals were sacrificed on 9th day. The blood samples were collected from retro-orbital plexus and sera was estimated for blood urea nitrogen (BUN) and serum creatinine (SCr). Kidneys were removed for estimation of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), lipid peroxidation (LPO) and for histological examination.

Estimation of BUN was done by diacetyl monoxime method and SCr by alkaline picrate method. Post-mitochondrial supernatant (PMS) was obtained by the method of Fahamiya et al. The PMS was used as a source of enzymes for analysis. Estimation of LPO is carried out by the method of Fahamiya et al. and SOD activity was measured by Stevens et al. Assay for CAT activity was done by the method of Claiborne and GPx activity was assayed by the method of Mohandas et al. Estimation of GSH was determined by the method of Jollow et al.

Histological analysis

Histopathological examination was done under the light microscope (Olympus Cx21i) using 3µm thick sections cut stained with Hematoxylin and Eosin (H&E X 400) (Figs. 1-5).

Statistical analysis

Statistical evaluation was done by one way analysis of variance (ANOVA) followed by Dunnett’s post hoc t-test. The P-values of less than 0.05 have been considered as significant.

Results and discussion

Renal diseases are devastating, medical, social and economic problems which are affecting the world, including Sri Lanka. Gentamicin is an aminoglycosides widely used in the treatment of gram-negative infections. The average incidence of gentamicin nephrotoxicity is around 15 %. The toxicity of gentamicin is believed to be related with generation of reactive oxygen species (ROS) in kidney. It has been demonstrated that gentamicin-induced nephrotoxicity is characterized by direct tubular necrosis, which is localized mainly in the proximal tubules.

There are many herbs which have been tested and proven to improve kidney health and even reverse kidney damage. Gentamicin in a dose of 100 mg/kg induced nephrotoxicity has been documented in several studies. Previous studies demonstrate that the gentamicin reduces the glomerular filtration rate which leads impairment in glomerular function which is accompanied with increase level of blood urea and serum creatinine.

Antioxidants are any substance that delay or inhibits oxidative damage to a target molecule. At a time one antioxidant molecule can react with single free radicals and are capable to neutralize free radicals by donating one of their own electrons, ending the carbon-stealing reaction. Lipid peroxidation produces oxidative degradation of lipids. The products of lipid peroxidation damage the membranes, cells and even tissues. Malondialdehyde (MDA) is one of the products of lipid peroxidation which is used to assess oxidative stress. MDA reacts with thiobarbituric acid and produces red coloured products namely thiobarbituric acid reactive substance (TBARS) which can be measured colorometrically.

Human body has enzymatic and non enzymatic endogenous antioxidant defense systems. The enzymatic defense system includes different endogenous enzymes like SOD, CAT, GPx, glutathione reductase (GR) and non enzymatic defense system which include vitamin E, vitamin C, GSH, etc. One of the causes of renal injury
produce by gentamicin is by altering the concentrations of antioxidant systems. It has been proven by large numbers of research studies that co-administration of plant extract and phytoconstituents ameliorated the gentamicin-induced change in the antioxidant system.\textsuperscript{23,24,28,29}

In this study the effect of 8 days treatment of ME-AR (103 mg/kg) on renal function was investigated in normal rats (Table 1). No significant decrease or increase was found in biochemical parameters in the test drug group when compared with control group. Therefore, it is clear that there is no any adverse effect in kidney due to use of ME-AR.

The results of effect of higher dose of ME-AR and its CHSF and CHIF on BUN, Scr and LPO in

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose</th>
<th>BUN (mg/dl) Mean ± SE</th>
<th>Scr (mg/dl) Mean ± SE</th>
<th>LPO (nmol TBARS formed/h/gm tissue) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Vehicle</td>
<td>10 ml/kg</td>
<td>10.14 ± 0.90</td>
<td>0.739 ± 0.033</td>
<td>3.29 ± 0.27</td>
</tr>
<tr>
<td>Test drug</td>
<td>ME-AR</td>
<td>103 mg/kg</td>
<td>10.22 ± 0.65</td>
<td>0.709 ± 0.052</td>
<td>3.17 ± 0.07</td>
</tr>
<tr>
<td>Test drug</td>
<td>CHSF-AR</td>
<td>70 mg/kg</td>
<td>10.22 ± 0.65</td>
<td>0.709 ± 0.052</td>
<td>3.17 ± 0.07</td>
</tr>
<tr>
<td>Test drug</td>
<td>CHIF-AR</td>
<td>70 mg/kg</td>
<td>10.22 ± 0.65</td>
<td>0.709 ± 0.052</td>
<td>3.17 ± 0.07</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M from 6 animals in each group.

There is no statistically significant difference found in BUN and serum creatinine between two groups.
Table 2—Effect of higher dose of methanolic extract of *Althaea rosea* (ME-AR) and its fractions CHSF-AR and CHIF-AR on BUN, Scr and LPO in gentamicin induced nephrotoxicity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose</th>
<th>BUN (mg/dl)</th>
<th>Scr (mg/dl)</th>
<th>LPO (nmol TBARS formed/h/gm tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
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<td>Control</td>
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<td>0.739 ± 0.033</td>
<td>3.29 ± 0.27</td>
</tr>
<tr>
<td>Toxican</td>
<td>Gentamicin</td>
<td>100 mg/kg</td>
<td>28.35 ± 2.79###</td>
<td>1.562 ± 0.169###</td>
<td>9.2 ± 0.32 ##</td>
</tr>
<tr>
<td>Test drug</td>
<td>ME-AR + GM</td>
<td>103 mg/kg</td>
<td>11.87 ± 0.56**</td>
<td>(90.49 %)</td>
<td>0.758 ± 0.054**</td>
</tr>
<tr>
<td>Test drug</td>
<td>CHSF + GM</td>
<td>70 mg/kg</td>
<td>12.02 ± 1.35**</td>
<td>(89.67 %)</td>
<td>0.915 ± 0.021**</td>
</tr>
<tr>
<td>Test drug</td>
<td>CHIF + GM</td>
<td>35 mg/kg</td>
<td>13.58 ± 1.62**</td>
<td>(81.11 %)</td>
<td>0.957 ± 0.038**</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M from 6 animals in each group.

## p < 0.01, when compared with control group; **p < 0.01, when compared with toxicant group.

Values given in the parenthesis indicate inhibition.

Table 3—Effect of higher dose of methanolic extract of *Althaea rosea* (ME-AR) and its fractions CHSF-AR and CHIF-AR on CAT, SOD, GSH and GPX in gentamicin induced nephrotoxicity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose</th>
<th>Catalase [nmol H₂O₂ consumed/min/mg protein]</th>
<th>SOD (µM epinephrine oxidized/min/mg protein)</th>
<th>Glutathione peroxidase [nmol NADPH oxidized/min/mg protein]</th>
<th>Reduced glutathione [nmol GSH/gm tissue]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Vehicle</td>
<td>10 ml/kg</td>
<td>141.9 ± 6.98</td>
<td>146.3 ± 3.9</td>
<td>268.2 ± 16.2</td>
<td>0.65± 0.04</td>
</tr>
<tr>
<td>Toxican</td>
<td>Gentamicin</td>
<td>100 mg/kg</td>
<td>54.51 ± 4.2###</td>
<td>101.9 ± 2.4###</td>
<td>128.1 ± 9.6###</td>
<td>0.32± 0.04###</td>
</tr>
<tr>
<td>Test drug</td>
<td>ME-AR + GM</td>
<td>103 mg/kg</td>
<td>119.1 ± 4.1###</td>
<td>130.2 ± 3.0###</td>
<td>222.0 ± 18.0###</td>
<td>0.50 ± 0.02###</td>
</tr>
<tr>
<td>Test drug</td>
<td>CHSF + GM</td>
<td>70 mg/kg</td>
<td>96.12 ± 4.0**</td>
<td>128.6 ± 2.3*</td>
<td>210.8 ± 13.4###</td>
<td>0.47 ± 0.03*</td>
</tr>
<tr>
<td>Test drug</td>
<td>CHIF + GM</td>
<td>35 mg/kg</td>
<td>90.02 ± 3.1*</td>
<td>110.1 ± 3.1NS</td>
<td>166.7 ± 8.2*</td>
<td>0.40 ± 0.03NS</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M from 6 animals in each group.

## p < 0.001, when compared with control group; *p < 0.05, **p < 0.01, ***p < 0.001, when compared with toxicant group; NS- not significant.

Gentamicin induced nephrotoxicity are summarized in Table 2. The subcutaneous administration of gentamicin at 100 mg/kg/day caused renal dysfunction is evidenced by increase in BUN (279.58 %) and Scr (211.36 %) when compared with control group. Co-administration of higher dose of ME-AR (103 mg/kg), CHSF (70 mg/kg) and CHIF (35 mg/kg) (p.o.) with gentamicin significantly (p < 0.01) prevented the rise in BUN 90.49, 89.67, 81.11 % and Scr 97.69, 78.61, 73.51 %, respectively. Gentamicin treatment alone raised renal LPO level from 3.29 ± 0.27 (control group) to 9.2 ± 0.32 (gentamicin treated rats). The mean value of LPO in gentamicin (100 mg/kg/d) treated group was reduced by co-treatment with higher dose of ME-AR 103 mg/kg (4.12 ± 0.26), CHSF 70 mg/kg (4.36 ± 0.29) and CHIF 35 mg/kg (5.10 ± 0.33) (Table 2). Hence, gentamicin induced increased level of BUN, Scr and LPO were significantly inhibited by co-administration of ME-AR and its CHSF and CHIF-AR.

The results of effect of higher dose of ME-AR and its CHSF and CHIF on CAT, SOD, GSH and GPX in gentamicin induced nephrotoxicity are summarized in Table 3. The gentamicin (alone) treatment reduced the level of catalase to 54.51 ± 4.2 in the kidney when compared to control group (141.9 ± 6.98). The mean value of catalase significantly enhanced by co-administration of higher dose of ME-AR (119.1 ± 4.1##), and its CHSF (96.12 ± 4.0##) and CHIF 90.02 ± 3.1## with gentamicin. The gentamicin (alone) treatment reduced the level of SOD to 54.51 ± 4.2 in the kidney when compared to control group (141.9 ± 6.98). The mean value of SOD significantly increased by co-administration of higher dose of ME-AR (130.2 ± 3.0##), and its CHSF (128.6 ± 2.3##) and CHIF (125.9 ## ± 3.1##) with gentamicin (S.C.).

The level of Glutathione peroxidase (GPx) reduced to 128.1 ± 9.0 in gentamicin (alone) treated kidney when compared to control group (268.2 ± 16.2). The reduced level of GPx significantly enhanced by co-administration of higher dose of ME-AR (222.0 ± 18.0##), and its CHSF (210.8 ± 13.4**) and CHIF (166.7 ± 8.2**) with gentamicin (S.C.).

Gentamicin treatment alone reduced the renal GSH to 0.32 ± 0.04 when compared to control group (0.65 ± 0.04). However, the mean value of GSH significantly enhanced by co-administration of higher...
References


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