EFFECT OF METHANOLIC EXTRACT OF JASMINUM GRANDIFLORUM LINN LEAVES ON GENTAMICIN INDUCED NEPHROTOXICITY IN RATS

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ABSTRACT
In Indian traditional medicine, different parts of Jasminum grandiflorum Linn. (Oleaceae family) are valued highly for the treatment of Skin diseases, Ulcers, wounds, corns, ulcerative stomatitis, fixing loose teeth. In the present study, oral dose of 100 and 200 mg/kg/day of JGLE were studied for their protective effects in Gentamicin induced nephrotoxicity in wistar rats for 14 days. The adult wistar rats were divided evenly into five groups. Groups I, II & III served as untreated, toxic controls (Gentamicin 40mg/kg) & Standard group (Vitamin E 250mg/kg) respectively while groups III&V were treated with 100 and 200 mg/kg/day of JGLE hr before each dose of the nephrotoxicant (Gentamicin 40mg/kg) for 14 days. On the 15th day, blood samples were collected by retro orbital puncture and estimated renal biochemical parameters. In toxic control (Gentamicin 40mg/kg) group, attenuated elevations in the serum creatinine, blood urea, uric acid, blood urea nitrogen, reduced levels of total proteins and Increased levels of urine parameters (urine creatinine, urine urea, urine uric acid and albumin) in dose related fashion. In group IV & V groups (pretreated with JGLE) attenuated significantly decreased levels of blood parameters (serum creatinine, blood urea, uric acid, blood urea nitrogen) and increased levels of total proteins and also decreased levels of urine parameters (urine creatinine, urine urea, urine uric acid and albumin) when compared to toxic control group. In Conclusion the results suggest that the JGLE shown the significant nephroprotective activity could be due to inherent antioxidant property and free radical scavenging principle contained in the extract. In near the future, JGLE could constitute a lead to discovery of a novel compound for the treatment of drug induced nephrotoxicity.

Keywords
Gentamicin, Jasminum grandiflorum linn (JGLE), Nephrotoxicity, Nephroprotection.

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Introduction:

Kidneys are the major vital organs; their main function is to maintain total body fluid volume, its composition and acid base balance. Many environmental xenobiotics and drugs influence and interrupting these functions [1]. The incident of drug induced nephrotoxicity has been increasing, with the ever increasing number of drugs and easy availability over the counter medication, Non steroidal anti inflammatory drugs (NSAIDs). Antibiotics, Angiotensin converting enzyme inhibitors and contrast agents are the major culprit drugs contributory to kidney damage. Drug induced Acute renal failure (ARF) reported 20% of all ARF in an Indian study in that Amino glycosides accounted 40% of total cases. Amino glycosides are prototype drugs having nephrotoxicity as major side effect. Developing nephrotoxicity number of patient’s increases with duration of therapy reaching 50% with 14days or more of therapy [2].

Generation of free radicals in renal cortex plays important role in the pathogenesis of Gentamicin nephrotoxicity. Among them, antioxidants were shown to consistently protect and ameliorate the rats against this toxicity. In fact, extracts of several plants endowed with free radical scavenging activity have been shown to produce reliable reduction of Gentamicin induced nephrotoxicity [3].

The leaves of Jasminum grandiflorum Linn (Oleaceae) found extensively all over India and exhibit a wide ecological range. Traditionally the leaves are used in the treatment of odontalgia, fixing loose teeth, Ulcerative stomatitis, leprosy, skin diseases, Ottoorhexia, Otalgia, ulcers, wounds and corns, dysenorrhoa, Stangury. This species leaves have used in Indian folk medicine for treating ulcers, and also literature suggests that use of this plant as a diuretic and spasmylytic agent, which is given during the child birth [4], also having a variety of proven pharmacological activity [5-16]. And the leaf extract is having a valuable phytochemicals like galic acid, catechine, jasmine, 2-indoleoxygenase,hydroxyl cetophenone, oleanic acid, beta sitosterol, quercetin, isoquercetin, ursolic acidepinkamoxside are mostly supportive constituents for nephroprotective activity hence the present study was aimed to study the methanolic extract of Jasminum grandiflorum Linn leaves were selected to investigate the nephroprotective effect on Gentamicin induced nephrotoxicity in rats.

Material and Methods

Plant material:

Jasminum grandiflorum Linn leaves were collected from the surrounding gardens of Rajendranagar, Rangareddy district, Andrapradesh, India. The plant was identified and authenticated by the botanist Dr.A.Manohar Rao, Acharya N.G. Ranga Agricultural University, Rajendranagar, Hyderabad, Andrapradesh, India.

Preparation of extract:

Fresh leaves were collected, shade-dried and mechanically powdered. About 500g of the leaf powder were extracted 2000ml of Methanol by soxhlet apparatus for 24 hours. The Extract was dried at 40°C vacuum evaporator. The yield of extract was obtained 27% in our experimental condition.

Phytochemical screening:

Preliminary Phytochemical screening of the extract was subjected for the presence of flavonoids, alkaloids, phenolics, carotenoids, carbohydrates and glycosides [17].

Animals:

Albino rats of wistar strain of either sex weighing between 180-240g were used. At room temperature (25±2°C) they were housed in polyethylene cages in animal house, allowed to 4 days of acclimatization and maintained standard rat chow and standard laboratory conditions throughout the experiment. The study was conducted after obtaining approval by institutional ethical committee (IAEC) of Smt. Sarojini Ramulamma College of pharmacy bearing the number is 51/01/C/CPCSEA/2013/05.

Drugs and Chemicals:

Gentamicin used Ranbioric vial from Ranbaxy India, the other chemicals and analytic reagents grade obtained from Sicra (Prashanthi nagar, Hyd, A.P, India).

Toxicity Studies:

Rats were kept overnight fasting prior to drug administration. A total of five animals were used which received a single oral dose (2000 mg/kg, b.wt.) of JGLE. After the administration of JGLE, food was withheld for further 3-4 h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h and daily thereafter for a period of 14 days. Once daily cage side observations included changes in skin and fur, eyes and mucous membrane (nasal) and also respiratory rate, circulatory (heart rate and blood pressure), autonomic (salivation, lacrimation, urinary incontinence and defecation) and central nervous system (tremors and convulsion) changes and Mortality was determined over a period of 2 weeks [18]. We did not find any changes including morbidity and mortality in rats.

Selection of Dose of the Extract:

LD50 was done as per OECD guidelines for fixing the dose for biological evaluation. The LD50 of the extract as per OECD guidelines falls under class four values with no signs of acute toxicity at 2000 mg/kg. The biological evaluation was carried out at doses of 100 and 200 mg/kg body weight.
Methodology:
The present animal study experiment was conducted in Gentamicin drug induced nephrotoxicity. The rats were systematically randomized in to five groups of six rats per group. Twelve to fourteen hours before each of experiment began, the rats were fasted of feed but distilled water was made available ad libitum. In this Gentamicin model, group I and II rats, that served as untreated (0.9% normal saline 10ml/kg/day) and toxic controls (single dose of Gentamicin 40 mg /kg of for 14days, i.p) respectively, Group III named as standard group rats were pretreated with single oral dose of Vit E 250mg/kg b.wt, 1 hour before the intraperitoneal injection of Gentamicin 40 mg/kg b.wt of, for 14 days. And group IV&V rats were pretreated with single doses of 100 & 200mg/kg of JGLE 1 hour before the intraperitoneal injection of Gentamicin 40 mg/kg of, for 14days[19].

Evaluation of Biochemical Parameters:
Prior to termination of the experiment on day 14 after completion of dosing, animals were kept in metabolic cages for urine collection. On the 14th day, the rats were fasted overnight. On 15th day fasted rats were anesthetized with chloroform, blood samples were drawn periodically through retro orbital puncture. The serum creatinine, blood urea, blood uric acid and blood urea nitrates (BUN) and urine sample for (Urine Creatinine, Urine urea, and Urine uric acid) were analyzed using Robonik Diagnostic kits (Robonik (India) PVT. Ltd., Mahape, Navi Mumbai, India.)

Statistical analysis:
Results were presented as statistically by using Graphpad prism version 5.0. All results were expressed as Mean±SD, analyzed by one way analysis of variance followed by “Dennett’s test.

Results
Effect of JGLE on Body weight:
Table 1 shows that effect of single dose daily 100 and 200 mg/kg/day of oral JGLE on animal body weights of Gentamicin nephrotoxic rats treated for 14days. As shown in the table 1, daily Gentamicin i.p 40mg/kg for 14days induced significant progressive weight loss seen in7th day to 14th day in toxic control group rats. Other treated groups are compared to toxic group very less weight loss was observed.

Table 1. Effect of JGLE on animal bodyweights

<table>
<thead>
<tr>
<th>Group No</th>
<th>Treatment</th>
<th>Animal Body Weight (g)</th>
<th>0 Day</th>
<th>7th Day</th>
<th>14th Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control 0.9% Normal saline for 14 Days</td>
<td>208.2±5.193</td>
<td>212.8±5.037</td>
<td>216.7±5.922</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Toxic control Gentamicin40mg/kg for 14days</td>
<td>195.2±4.579</td>
<td>189.7±4.320</td>
<td>178.8±7.441</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>STD Vit-E (250mg/kg) +Gentamicin (40mg/kg) for 14days</td>
<td>200.5±12.68</td>
<td>206.0±32.29</td>
<td>201.2±25.23</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Test 1 JGLE (100mg/kg)+Gentamicin (40mg/kg) for 14days</td>
<td>202.2±15.80</td>
<td>197.5±15.71</td>
<td>194.2±15.89</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Test 2 JGLE (200mg/kg)+ Gentamicin (40mg/kg) for 14days</td>
<td>224.0±5.831</td>
<td>220.2±6.080</td>
<td>217.7±5.574</td>
<td></td>
</tr>
</tbody>
</table>

Values are Mean±SD; n=6. P value p<0.05, **p<0.01, ***p<0.001 vs. Toxic Control

Effect of Blood and urine Parameters:
Table 2 and 3 shows that effect of single dose daily 100 and 200 mg/kg/day of oral JGLE on biochemical compounds, As shown in the table 2, daily Gentamicin intraperitoneal injection (40mg/kg) for 14days induced progressively increases SerumCreatinine, blood urea, uric acid, and blood urea nitrates (BUN) concentration levels in group II (toxic control) rats. other treated groups including standard group are compared to toxic control group, significantly decreased levels of the Serum creatinine, blood urea, uric acid, and blood urea nitrates (BUN) were observed. In urine analysis, progressively increases Urine Creatinine, Urine urea, and Urine uric acid concentration levels in toxic control group (Gentamicin). Other treated groups are compared to toxic control group significantly decreased Urine Creatinine, Urine urea, and Urine uric acid concentration levels were observed.
Table 2. Effect of JGLE on blood parameters

<table>
<thead>
<tr>
<th>Group No</th>
<th>Treatment</th>
<th>Serum Creatinine (mg/dl)</th>
<th>Blood Urea (mg/dl)</th>
<th>Uric Acid (mg/dl)</th>
<th>BUN (mg/dl)</th>
<th>Total Proteins (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control</td>
<td>0.9% Normal saline for 14 Days</td>
<td>0.432±0.082</td>
<td>24.58±1.821</td>
<td>2.342±0.456</td>
<td>18.37±0.956</td>
</tr>
<tr>
<td>II</td>
<td>Toxic control</td>
<td>Gentamicin (40 mg/kg for 14 Days)</td>
<td>1.865±0.421</td>
<td>52.99±2.060</td>
<td>7.030±0.698</td>
<td>53.78±1.818</td>
</tr>
<tr>
<td>III</td>
<td>STD</td>
<td>Vit-E (250mg/kg) + Gentamicin (40 mg/kg for 14 Days)</td>
<td>0.507±0.090***</td>
<td>38.92±1.306***</td>
<td>2.768±0.8744***</td>
<td>22.83±2.512***</td>
</tr>
<tr>
<td>IV</td>
<td>Test 1</td>
<td>JGLE (100mg/kg) + Gentamicin (40 mg/kg for 14 Days)</td>
<td>1.042±0.323***</td>
<td>46.41±2.928***</td>
<td>5.145±1.400**</td>
<td>38.95±3.719***</td>
</tr>
<tr>
<td>V</td>
<td>Test 2</td>
<td>JGLE (200mg/kg) + Gentamicin (40 mg/kg for 14 Days)</td>
<td>0.715±0.115***</td>
<td>40.29±1.457***</td>
<td>3.493±0.953***</td>
<td>25.14±1.783***</td>
</tr>
</tbody>
</table>

Values are Mean±SD; n=6. P value p<0.05, **p<0.01, ***p<0.001 Vs. Toxic Control

Table 3. Effect of 40mg of JGLE on Urine Parameters

<table>
<thead>
<tr>
<th>Group No</th>
<th>Treatment</th>
<th>Urine Creatinine (mg/dl)</th>
<th>Urine Urea (mg/dl)</th>
<th>Urine Uric Acid (mg/dl)</th>
<th>Albumin (g/24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal Control</td>
<td>0.9% Normal saline for 14 Days</td>
<td>10.88±1.727</td>
<td>65.67±1.729</td>
<td>4.477±0.5934</td>
</tr>
<tr>
<td>II</td>
<td>Toxic control</td>
<td>Gentamicin (40 mg/kg for 14 Days)</td>
<td>36.60±2.725</td>
<td>86.26±2.764</td>
<td>14.46±0.9467</td>
</tr>
<tr>
<td>III</td>
<td>STD</td>
<td>Vit-E (250mg/kg) + Gentamicin (40 mg/kg for 14 Days)</td>
<td>13.22±0.9972***</td>
<td>71.80±1.977***</td>
<td>9.933±1.291***</td>
</tr>
<tr>
<td>IV</td>
<td>Test 1</td>
<td>JGLE (100mg/kg) + Gentamicin (40 mg/kg for 14 Days)</td>
<td>25.05±2.414***</td>
<td>80.79±1.933***</td>
<td>12.65±0.8678*</td>
</tr>
<tr>
<td>V</td>
<td>Test 2</td>
<td>JGLE (200mg/kg) + Gentamicin (40 mg/kg for 14 Days)</td>
<td>18.96±2.059***</td>
<td>73.50±1.603***</td>
<td>11.72±0.9111***</td>
</tr>
</tbody>
</table>

Values are Mean±SD; n=6. P value p<0.05, **p<0.01, ***p<0.001 Vs. Toxic Control

Effect of JGLE kidney weight changes:

Table 4 shows that effect of single dose daily 100 and 200 mg/kg/day of oral JGLE on the Gentamicin treated rats. As shown in the table 4, daily Gentamicin intraperitoneal injection 40mg/kg for 14days induced progressively increases kidney weight in toxic control group. Other treated groups are compared to toxic control group, significantly decreases the kidney weight.
Table 4. Effect of JGLE on kidney weight changes.

<table>
<thead>
<tr>
<th>Group No</th>
<th>Treatment</th>
<th>Animal kidney Weight (g)</th>
<th>Right Kidney</th>
<th>Left Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>Normal control</td>
<td>0.9% Normal saline for 14 Days</td>
<td>0.809±0.022</td>
<td>0.827±0.020</td>
</tr>
<tr>
<td>II</td>
<td>Toxic Control</td>
<td>Gentamicin (40 mg/kg) for 14 Days</td>
<td>1.649±0.6842</td>
<td>1.681±0.069</td>
</tr>
<tr>
<td>III</td>
<td>STD</td>
<td>Vit-E (250mg/kg) + Gentamicin (40 mg/kg) for 14 Days</td>
<td>0.866±0.0553***</td>
<td>0.884±0.056***</td>
</tr>
<tr>
<td>V</td>
<td>Test 1</td>
<td>JGLE (100mg/kg) + Gentamicin (40 mg/kg) for 14 Days</td>
<td>1.186±0.0305***</td>
<td>1.259±0.032***</td>
</tr>
<tr>
<td>V</td>
<td>Test 2</td>
<td>JGLE (200mg/kg) + Gentamicin (40 mg/kg) for 14 Days</td>
<td>1.057±0.0867***</td>
<td>1.116±0.091***</td>
</tr>
</tbody>
</table>

Values are Mean±SD; n=6. P value p<0.05, **p<0.01, ***p<0.001 Vs. Toxic Control

Discussion:

Large doses, continuous use and chronic uses of over the counter drugs, such as NSAIDs, amino glycosides is commonly associated with nephrotoxicity as well as hepatotoxicity in humans and animals. The use of Gentamicin, an amino glycoside antibiotic as a chemotherapeutic agent with a wide spectrum of activities against the Gram-negative as well as Gram-Positive bacterial infections but with high protection for the later, but is equally associated nephrotoxicity as its side effect. Gentamicin induces nephrotoxicity causing by renal phospholipidosis through inhibition of lysosomal hydrolases, such as phospholipases and sphingomyelinase in addition to causing oxidative stress [19].

Several studies now reported the importance of reactive oxygen metabolites in Gentamicin induced renal failure. Nephrotoxicity is usually associated with accumulation of drugs in renal cortex, dependent upon their affinity to kidneys and on kinetics of drug trapping process. Gentamicin at 40-80mg/kg bodyweight intraperitoneal injection, for six to 10 days in rats is known to cause significant nephrotoxicity. Gentamicin usually accumulates in the renal proximal tubules and enhances the hydrogen peroxide generation by the mitochondria, which is mostly derived from the dismutation of superoxide. Hydrogen peroxide generated during the Gentamicin induced oxidative stress in mitochondrial membranes releases iron from the mitochondria. The released iron makes a complex with Gentamicin and accelerates the oxidative stress. The use of antioxidants most consistent effects have been observed against the Gentamicin induced nephrotoxicity. The natural anti oxidants are safer than compare to the synthetic anti oxidants, which may cause unacceptable and serious side effects [20].

The investigated results reveals that the methanolic extract of Jasminum grandiflorum leaves having significant protective effect against the Gentamicin induced nephrotoxicity in rats, and the effect was found to be in a dose dependent manner. The present study demonstrate that Gentamicin induced renal failure as evident from elevated levels of blood parameters like serum creatinine, serum urea, uric acid, and blood urea nitrogen levels and decreased levels of total proteins (Table 2) and also elevated urine creatinine, urine urea, urine uric acid and albumin levels in renal failure/toxic control group (Table 3).

Animal body weights were significantly decreased in toxic control group increased in normal control group. Decreased body weights were also observed in treatment groups (JGLE 100 and 200 mg/kg body weight for 14days) but not significantly. The treatment with JGLE significantly restored the elevated serum creatinine, serum urea, uric acid, and blood urea nitrogen levels and decreased levels of total proteins and also significantly restored elevated urine creatinine, urine urea, urine uric acid and albumin levels indicating its nephroprotective effect. Significantly decreased levels of serum creatinine, blood urea, uric acid levels, blood urea nitrogen, increased levels of total proteins and also decreased levels of urine creatinine, urine urea, urine uric acid, albumin and significant reduction in kidney weights was observed when compared with toxic control group. The statistical significance of the nephroprotective activity of JGLE treated group and vitamin-E (standard group) treated group compared with toxic control group and were found that almost equal as both the groups gained same level of significance against the toxic control group in all parameters including serum creatinine, blood urea, uric acid levels, blood urea nitrogen, total proteins and all urine parameters. In vitro studies of Jasminum grandiflorum leaves elevated for its anti oxidant property revealed nitric oxide free radical scavenging effect [5].

In conclusion the effect of JGLE on Gentamicin induced rats (in-vivo), shown the significant nephroprotective effect in rats. The probable mechanism of nephroprotection by JGLE could be due to presence of variety of phytochemicals with its antioxidant property and free radical scavenging property and thus this plat could play promising role in the treatment of renal failure induced by nephrotoxicant like Gentamicin. Further study is needed for isolation and identification of the key phytochemical constituents which are responsible for the nephroprotective activity.

Acknowledgements:

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