NEPHROPROTECTIVE ACTIVITY OF THE ETHANOLIC EXTRACT OF BOUGAINVILLAE SPECTABILIS LEAVES AGAINST GENTAMYCIN INDUCED RENAL DYSFUNCTION IN WISTAR RATS

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<td><strong>Article history</strong></td>
<td>The current study aimed at evaluating the protective effects of <em>Bougainvillae spectabilis</em> (Nyctaginaceae family) on renal dysfunction of Gentamycin induced wistar albino rats using biochemical approaches. Pinitol, betacyanine, flavonoids, tannins and alkaloids are the constituents reported for <em>B. spectabilis</em>. Nephrotoxicity is considered a prominent side effect of Aminoglycoside antibiotic, Gentamicin. The drug was induced intra peritoneally at a dose of 80 mg/ kg body weight for 9 days. One of the main factors in for Gentamycin induced Nephrotoxicity is the Oxidative stress. Ethanolic extract of <em>B. spectabilis</em> decreased the increased levels of blood urea, serum creatinine, urinary protein, and extent of renal dysfunction at dose levels of 200 and 400 mg/kg body weight in wistar albino rats.</td>
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**Keywords**
- Nephroprotective Activity
- Gentamycin
- Bougainvillae Spectabilis
- Renal Dysfunction

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INTRODUCTION

Kidney is the major organ in urinary system that eliminates waste material from the blood and excretes out from body via urine. Kidney plays a major in maintaining electrolyte balance, fluid homeostasis and blood pressure. In case any kidney injury occurs, our body fails to clear body wastes, excess urine and blood electrolytes (potassium & magnesium).

Among kidney associated problems, Nephrotoxicity is a common disorder and is usually observed when body is exposed some drug or toxin [1]. Renal dysfunction is a term that represents the failure of kidney to excrete the waste. Nitrogenous waste products formed through the metabolic reactions are retained in the blood [2]. In addition to that, fluid and electrolyte balance gets disturbed with endocrine dysfunction. Generally, renal failure is of two types, Acute and chronic renal failure [3].

Gentamycin, a aminoglycoside antibiotic used mostly for gram-negative bacterial infections. Prolonged use of Gentamycin leads to either Nephrotoxicity or ototoxicity. Nephrotoxic grade for different aminoglycoside antibiotics is in the order, Neomycin > Gentamycin > Tobramycin [4]. Nearly 30% patients show nephrotoxic signs after being treated with high doses of aminoglycoside antibiotics [5].

Use of Herbal plant extracts is considered the best way in the traditional medicine for treating nephrotoxicity. Chemical agents are equally effective in controlling nephrotoxicity but they often produce side-effects. Because of this reason, researchers select different plants of medicinal value and extract phytochemicals from them that possess sufficient activity to cure kidney disorders without any side effects.

Bougainvillaea spectabilis is a medicinal plant belonging to the family Nyctaginaceae. It is a woody ornamental plant and inhabitant of warmer climates [6]. Generally, it is called as paper flower plant and native of Brazil [7]. B. spectabilis is a medicinal plant and various parts of the plant are traditionally used in the treatment of various disorders like diarrhoea, cough and sore throat, hepatitis and leucorrhoea. The main part is Leaves of B. spectabilis are used [8] it possess medicinal properties like Anti-diabetic [7,9,10,11], Anti-fertility [6], Anti-viral [12], Anti-microbial [13]. Anti-inflammatory [14], Hepatoprotective [15] Anti oxidant [16], Anti-carcinogenic, Anti allergic, Anti-mutagenic activities [17], Larvicidal [10] and is also a source of bioactive agents [18,19]. The leaves of B. spectabilis possess the following phytoconstituents: D-pinitol, Betacyanin, flavanoids, alkaloids and tannins [20,21].

The present study aimed at evaluating the nephroprotective activity of the ethanolic extract of leaves of B. spectabilis on gentamycin induced renal dysfunction in wistar albino rats.

MATERIAL AND METHODS

Animals:

Healthy adult male and female wistar albino rats weighing approximately 180-250g were used in this study. They were purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animals were housed in groups of six and maintained under standard conditions (Temperature 27 ± 2°C, relative humidity 44-56% and light/ dark cycles of 10-14 hours respectively) and fed with standard rat diet and purified drinking water ad libitum for 1 week before during the experiment. All experiments and protocols described in present study were approved by the Institutional Animal Ethical Committee (IAEC) of P.Rami Reddy Memorial College of Pharmacy (1423/PO/a/11/CPCSEA/104/2012).

All the experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals.

Chemicals:

Gentamycin, Ethanol, Sodium hydroxide and Trichloro Acetic acid (TCA) were purchased from SD fine chemicals Ltd Mumbai. All other reagents used in this study with high purity were obtained from Erba diagnostic Ltd. India.

Plant material

Collection of plant:

Bougainvillae spectabilis (Nyctaginaceae) leaves were collected from Tirupati, Chittoor district, Andhra Pradesh, India, in the month of December 2016. The plant was authenticated by Dr. Madhava Chetty, Asst.Professor. Department of Botany, S.V. University, Tirupathi, A.P.

Preparation of plant extract:

Collected Bougainvillae spectabilis leaves were cut into small pieces and shade dried at room temperature and makes a coarse powder using grinder mixture and then passed through 40 mesh sieve. About 1000gm of powdered drug was taken and extracted with aqueous ethanol by soxhlet apparatus. The extraction was carried out until the drug becomes exhausted. The solvent was recovered from their extract by distillation under reduced pressure. The dried extract thus obtained was kept in a dessicator and served as control group. Animals were then observed continuously for 2hrs, and intermittently for 6 hr and at the end of 24 hrs; number of deaths were noted to determine LD₅₀ of the extract.
Nephroprotective activity
Group I: Animals were administered with equivalent volumes of 0.1 ml i.p of normal saline (0.9% w/v NaCl) for 9 days.
Group II: Animals were received 80 mg/kg/day i.p of gentamycin for 9 days to induce nephrotoxicity.
Group III: Animals received 400 mg/kg/oral dose of ethanolic extract of Bougainvillae spectabilis throughout the study period.
Group IV-V: Animals were received 80 mg/kg/day i.p of gentamycin for 9 days to induce nephrotoxic and 200 mg/kg, 400 mg/kg Bougainvillae spectabilis extracts were given respectively to the animals from 10th to 21st day of study.

Biochemical parameters in serum
Nephroprotective activity was performed to determine significant effect of B. spectabilis on serum renal markers such as Uric acid, Bilirubin Urea Nitrogen, Total Protein, Urea, Albumin and Creatinine concentrations. Biochemical parameters such as Uric acid, Urea, Creatinine, Urine volume were estimated through urine samples. Additionally morphological parameters like Body weight and Kidney weight were estimated separately.

Histopathological studies
Administration of ethanolic extract of the Bougainvillae spectabilis demonstrated reduced renal dysfunction, induced by gentamycin.

Statistical analysis
The statistical analysis of results obtained after performing nephroprotective activity were carried out using one way analysis variation (ANOVA) followed by student ‘t’ test. p values < 0.05 were considered as significant.

RESULTS AND DISCUSSION:

Preliminary phytochemical screening
The ethanolic extract of B. spectabilis showed the presence of flavanoids, glycosides, phlobotannins, steroids, tannins and alkaloids. Saponins and terpenoids are absent.

Acute Oral Toxicity Study of Bougainvillae Spectabilis
Administration of ethanolic extract of Bougainvillae spectabilis orally produced no observable side effects, including death, upto 400 mg/kg body weight in rats even after 9 days of observation.

Nephroprotective Activity
Biochemical Parameters in Serum:

Blood Urea Nitrogen (BUN):
Blood urea nitrogen measures the amount of nitrogen in the blood. Urea nitrogen comes from breakdown product of protein. Increased BUN level suggests decreased kidney function. In this present study also observed the increased level of protein in gentamycin induced rats. Supplementation of Bougainvillae spectabilis decreased the BUN levels in gentamycin induced rats. Normal BUN levels are 35.80±0.5099, Gentamycin treated group increases the BUN levels 69.40±0.5099, plant treated group receiving low dose are 66.20±0.3742 showed decrease BUN level and high dose are 51.40±0.5099 showed significant decrease BUN levels as compared to gentamycin treated group.

Urea:
Urea is an end product of protein catabolism. Urea gets easily filtered through glomerulus, reabsorbed from both proximal and distal parts of nephron and gets excreted through urine in high concentrations. Urea excretion through urine and serum urine levels is considered an estimate of kidney function [22].

Drugs such as Allupuriniol, Gentamycin, Indometacin and some diuretics enhance the urine levels [23]. In this study, Gentamycin is used to increase urea levels in rats. Further administration of Bougainvillae spectabilis restored the enhanced urea levels in gentamicin-induced rats. Levels of Urea in normal group-39.40±0.5099, control group increases the serum urea level are 66.20±0.3742 showed decrease BUN level and high dose are 51.40±0.5099 showed significant decrease BUN levels as compared to gentamycin treated group.

Total Protein:
Proteinuria is considered as an early sign of kidney disease where the glomerulus seems to be impermeable for the filtration of plasma proteins. Further renal tubules fail to reabsorb water and electrolytes and do not maintain the normal volume and composition of body fluids within the required limits. Proteinuria is a feature of kidney disease and thus its detection is important for the identification of kidney dysfunction [24].

From the results of current study, it was found that proteins levels increased gradually in Gentamycin induced rats as compared to normal rats. Free radicals produced through Gentamycin induction causes toxicity due to which nephron gets damaged and thereby loss of proteins through urine. Administration of Bougainvillae spectabilis regularized the serum protein levels in gentamycin induced rats [25]. Group VI & V showed significant decrease in serum levels of Total protein are 4.360±0.05099 (low dose), 4.180±0.05831 (high dose). When compared with Group II -3.420±0.03742 (control). The normal group treated with high dose of extract (Group III-400mg/kg) showed similar effect as normal group without any treatment (Table no.1).
Creatinine:  
Similar to urine, creatinine is the end product of muscle catabolism. Creatinine is eliminated constantly by the kidneys. In clinical medicine, serum creatinine concentrations are considered as a measure of renal function. Serum creatinine concentration is somehow related to glomerular filtration rate [26], and thus believed to be an index of kidney function. If kidneys don’t function properly, creatinine levels in the blood rises significantly [22]. Gentamycins found to increase creatinine levels in the body [23]. Thus administration of our drug *Bougainvillae spectabilis* restores creatinine levels in gentamycin treated rats. Normal creatinine levels are 0.4400±0.05099, Gentamycin treated group as control are increases the serum creatinine level 0.7600±0.05099, plant treated group with low dose 0.3800±0.05831 and high dose 0.3000±0.07071 decreases the serum creatinine level as compared to control group.

Table no. 1 Effect of Ethanolic Extract of *Bougainvillae spectabilis* leaves on Biochemical parameters in serum.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>BUN</th>
<th>UREA</th>
<th>PROTEIN</th>
<th>CREATININE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>35.80±0.5831</td>
<td>39.40±0.5099</td>
<td>3.760±0.05099</td>
<td>0.4400±0.05099</td>
</tr>
<tr>
<td>II</td>
<td>69.40±0.5099</td>
<td>67.60±0.5099</td>
<td>8.420±0.03742</td>
<td>0.7600±0.05099</td>
</tr>
<tr>
<td>III</td>
<td>40.60±0.5099</td>
<td>42.80±0.3742</td>
<td>6.580±0.05831</td>
<td>0.3400±0.6782</td>
</tr>
<tr>
<td>IV</td>
<td>66.20±0.3742</td>
<td>8.420±0.03742</td>
<td>6.840±0.5099</td>
<td>0.3800±0.05831</td>
</tr>
<tr>
<td>V</td>
<td>51.40±0.5099</td>
<td>44.80±0.5831</td>
<td>6.380±0.03742</td>
<td>0.3000±0.7071</td>
</tr>
</tbody>
</table>

All values are shown in mean ±SEM and n=6. ***indicates p<0.001 when compared with normal group. *Indicates p<0.05, **indicates p<0.01, *** indicates p<0.001, when compared with control group.

Fig. no. 1: Effect of Ethanolic Extract of *Bougainvillae Spectabilis* (EEBS) on a) Bilirubin Urea Nitrogen (BUN) (mg/dL), b) Uric acid (mg/dL), c) Protein (g/dL), d) Creatinine (mg/dL).

Morphological Parameters:

a) Body Weight:

Gentamycin treated animals (Group II) exhibited a significant decrease in their body weight as compared to normal group (Group I). In contrast, significant increase in the body weight of animals was observed with animals treated with 200 mg/kg and 400 mg/kg of ethanolic extract of *Bougainvillae spectabilis* (p<0.001) respectively as compared to Group II shown in Table no.2.
b) Kidney Weight:
Group (G-II) rats induced with gentamycin exhibited a significant increase in kidney weight on 14th day when compared to normal group (G-I). The groups (G-IV& V) receiving EEBS (200 & 400 mg/kg) showed a significant decrease in kidney weight as compared to control group (G-II) shown in Table no.2.

Table:no. 2 Effect of Ethanolic Extract of *Bougainvillae Spectabilis* leaves on Morphological Parameters.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>BODY WEIGHT (gms)</th>
<th>KIDNEY WEIGHT (gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>158.6 ± 0.5099***</td>
<td>1.088 ± 0.008602***</td>
</tr>
<tr>
<td>II</td>
<td>134.4 ± 0.5099###</td>
<td>1.200 ± 0.007071###</td>
</tr>
<tr>
<td>III</td>
<td>168.6 ± 0.5099***</td>
<td>0.8900 ± 0.007071***</td>
</tr>
<tr>
<td>IV</td>
<td>143.2 ± 0.5831***</td>
<td>0.9640 ± 0.005099***</td>
</tr>
<tr>
<td>V</td>
<td>148.6 ± 0.5099***</td>
<td>0.9160 ± 0.005099***</td>
</tr>
</tbody>
</table>

All values are shown in mean ±SEM and n=6. ### indicates p<0.001 when compared with normal group. *Indicates p<0.05, **indicates p<0.01, *** indicates p<0.001, when compared with control group.

![Fig no.2: a) Effect of Effect of Ethanolic Extract of Bougainvillae spectabilis on Body Weight, b) Effect of Ethanolic Extract of Bougainvillae spectabilis on Kidney Weight in Wistar albino rats.](image)

Anti-oxidant parameters

Catalase (CAT):
CAT is an important constituent of antioxidant defense system and catalyses hydrogen peroxide into water. Inhibition of such protective system leads to increased sensitivity to free radical induced cellular damage. Normal CAT levels are 4.780±0.05831, Gentamycin induced group showed decreased CAT level are 3.000±0.07071, plant treated groups of low dose 4.320±0.05831and high dose 4.160±0.05099 showed significant increase in CAT levels shown in Table no.3.

Glutathione:
Glutathione plays a major role in cell biology as it is associated with cellular defence against xenobiotics and some naturally occurring harmful compounds, such as free radicals and hydro peroxides. It is usually a ubiquitous thiol containing tripeptide, present everywhere in the body. Glutathione is an indicator of cell viability and functionality. Depletion of GSH levels is an indicative of number of diseases such as kidney, cancer, cardiovascular and neurodegenerative diseases. Cytotoxic agents, before elimination through urine get exposed to kidneys and thus their concentration in kidney cells is critical [27].

In the present study, declined serum and GSH levels are observed with Gentamycin induced rats while it is still normal in control rats. Reduced GSH concentrations are a result of its increased use for neutralizing the free radicals generated from Gentamycin. Administration of *Bougainvillae Spectabilis* to gentamicin induced rats restores the glutathione levels. Levels of Glutathione in normal rats 4.520±0.05831, control group decreases the GSH levels are 3.200±0.07071, low dose of plant treated group 4.200±0.06633and high dose 4.260±0.09274 shows increased GSH levels as compared to control group shown in Table no.3.
Table no. 3 Effect of Ethanolic Extract of Bougainvillae Spectabilis leaves on Anti-oxidant parameters.

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>CAT (H₂O₂ consumed gram tissue)</th>
<th>GSH (µg of GSH/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4.950 ± 0.4958**</td>
<td>4.550 ± 0.4958**</td>
</tr>
<tr>
<td>II</td>
<td>3.117 ± 0.06009***</td>
<td>3.183 ± 0.06009***</td>
</tr>
<tr>
<td>III</td>
<td>4.683 ± 0.06009***</td>
<td>5.233 ± 0.1054***</td>
</tr>
<tr>
<td>IV</td>
<td>4.250 ± 0.09574***</td>
<td>4.250 ± 0.1478**</td>
</tr>
<tr>
<td>V</td>
<td>4.217 ± 0.1990***</td>
<td>4.283 ± 0.1493**</td>
</tr>
</tbody>
</table>

*a*CAT: Catalase, GSH: Reduced glutathione.
The above values are expressed in Mean ± SEM, **##,# indicates P<0.001 when compared to normal group. **indicates p<0.01, ***indicates P<0.001 when compared to control group.

**Histopathological Studies:**

Histopathological studies of the Kidney sections from normal animal (Fig.4) and normal animal treated with EEBS alone (Fig.5) showed essentially unremarkable histopathological changes, whereas those from gentamycin treated rats (Fig.6) exhibited severe vacolations, glomerular and tubular congestion with abnormal Bowmen's capsule, blood vessel congestion, necrosis, epithelial cell desquamation, and presence of tubular cast with few inflammatory cells. These structural changes were markedly improved when GM treatment was accompanied by *B. spectabilis* extract (Fig.7). Some small intracellular vacuoles could be seen in proximal tubular cells. However, no necrosis of these cells was identified (Fig.8). There were no definite changes in other parts of the nephron, including glomeruli and blood vessels, in all treatment groups.

![Fig no. 4. Normal group.](image1)

![Fig no. 5. Standard group.](image2)
CONCLUSION

The present work establishes the effect of Bouganvillea spectabilis on the decreasing the nephrotoxicity induced by Gentamycin on wistar rats. Gentamycin is a broad spectrum antibiotic, being used to treat many disorders. Nephrotoxicity is considered an adverse effect induced often with the use of some drugs. Induced drugs cause release of oxidants that inturn produces toxicity that destruct nephrons, the functional units of kidney. Thus, current study is towards reducing the toxic effects to kidney by using medicinal plant along with antibiotics like gentamycin. The detailed scientific investigation of plant, B.spectabilis provides detailed information that will be helpful in treating drug induced toxicity.

Abbreviations

EEBS - Ethanolic Extract Bougainvillae spectabilis
BUN - Bilirubin Urea Nitrogen
CAT - Catalase
GSH - Reduced Glutathione

REFERENCES


