Effect of different leaf extracts of *Bombax ceiba* on gentamicin induced nephrotoxicity in albino rats

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ABSTRACT: The ethylacetate, n-butanol, aqueous extract of *Bombax ceiba* was investigated for its protective effects on gentamicin-induced renal toxicity in rats. Nephrotoxicity was induced by intraperitoneal injection of gentamicin (80 mg/kg) for 8 days. The effect of different extracts (200mg/kg) given orally either with gentamicin administration was assessed by biochemical and histopathological changes of kidneys. n-butanolic and aqueous extracts protected the rats from alteration in serum levels of urea, creatinine, MDA((Malondialdehyde) better when co-administered with gentamicin through reversing the mild tubular necrosis than severe tubular necrosis.

KEYWORDS: Nephrotoxicity, gentamicin, *Bombax ceiba*.

INTRODUCTION

Bombax ceiba is commonly known as silk cotton tree and semal which belongs to family Bombacaceae. It is one of the important medicinal plants in tropical and subtropical India and also occurs in Sri Lanka, Pakistan, Bangladesh, Myanmar, Malaysia, Java, Sumatra and Northern Australia. It has number of traditional uses and its medicinal usage has been reported in the Indian traditional systems of medicine such as Ayurveda, Siddha and Unani. The various parts of B. ceiba have been reported for hypotensive and antiangiogenic, analgesic, antiulcer, antioxidant, hepatoprotective and antimicrobial activities. Also it was used for the treatment of sexual debility bleeding wounds and vaginal infections¹. Since there is no scientific evidence found for Nephroprotective potential of B. ceiba leaves extract where as leaves are frequently used in tribals of Aravali region, so in the present study, an attempt has been made for evaluation of Nephroprotective potential of different extracts of B. ceiba leaves.

Nephrotoxicity is a major adverse effect of amino glycoside antibiotics; reversible renal impairment being reported in 10 – 15 % of all courses of therapy with aminoglycosides². Gentamicin induced renal damage is widely used model for inducing nephrotoxicity in experimental animals. Though the exact mechanism of gentamicin induced nephrotoxicity remains unknown, oxidative stress has been suggested to play a major role in renal damage. Increased generation of hydrogen peroxide and other oxygen free radicals leading to peroxidation of phospholipids membranes has been attributed to aminoglycoside exposure in vitro. Increase in renal cortical lipid peroxidation in gentamicin treated rats has also been reported.

Various compounds with antioxidant properties have been reported to prevent nephrotoxicity induced by gentamicin in animals. Antioxidant property of *Bombax ceiba* is well demonstrated.³ Thus *Bombax ceiba* aqueous leaf extract has been found to be protective against radiation injury by modulating glutathione and antioxidant enzymes.

MATERIALS AND METHODS

1. PROCUREMENT OF RAW MATERIAL AND ITS AUTHENTIFICATION

Plant Material: The plant Bombax ceiba was collected from upper ki Oden, Aravali mountain region in Nathdwara during the month of April to June 2013. The leaves were identified by Dr. K.P. Dahariwal, Botanist at S.M.B govt.PG. College Nathdwara. The leaves were taken and dried in shade. Then the shade-dried leaves were made into coarse granules and were used for different investigation.

2. PREPARATION OF DIFFERENT EXTRACTS
The shade dried leaves of Bombax ceiba were powdered. About 1 kg of the dried powder was extracted first with petroleum ether at 60° to 65 °C by continuous hot percolation, using Soxhlet apparatus. This process was continued by using solvents of increasing polarity (n.butanol, ethyl acetate, water). The extraction was continued for 72 hours in each solvent. The crude extracts obtained after carrying out the above procedure were redissolved in respective solvents and the experiments were carried out to establish the presence of compounds.

3. Toxicological studies

Albino rats of Wistar strain (150-200 gm body weight) of either sex and healthy young adult female Swiss albino mice, nulliparous, non-pregnant and weighing 25-32 gm procured from in house animal facility of Institute. All animals were housed under standard conditions of temperature 25±2 °C, 65±10% relative humidity, 12:1 2 hr Light: Dark cycle and fed with standard pellet diet (Trimurti foods, Udaipur, India) and water ad libitum. Experimental protocols were approved by Institutional animal ethical committee (Registration No. 1202/08/CPCSEA dt. 02.04.2008) of Geetanjali Medical College & Hospital "Academic Research Wing" Udaipur, India.

Determination of Acute toxicity (ALD<sub>50</sub>)

The acute toxicity was determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline No. 420) method of CPCSEA was adopted for toxicity studies. 3 mice in each group were randomly selected. They were treated with aqueous suspension of Extracts i.e. Ethyl acetate extract (ETE), Butanolic Extract (BTE) and Aqueous Extract (AE) at 2,000 mg/kg body weight as a single oral dose. The animals were observed for morbidity and mortality at 1, 2, 4, and 6 hr on the day of dosing and once a day thereafter up to 14 days. The study was performed in accordance with Organization for Economic Co-operation and Development (OECD) test guideline No.420.

4. Nephrotoxicity study

- Gentamycin induced model:

Nephrotoxicity study was done by below procedures and results are decipated in the table.no

4.1 Procedure:

Healthy adult albino rats of either sex of wister strain weighing 150-250 g. were used after approval of Institutional Animal Ethical Committee. They were kept on standard pellet diet. The animals were divided into five groups of three each.

Group-I received received only distilled water orally and by IP rout throughout experiment period,

Group-II received gentamicin (80 mg/kg/d) i.p. The dose 80 mg/kg/d have been shown to produce nephrotoxicity.

Group-III received gentamicin (IP) at 80 mg/kg concurrently with n-butanolic leaf extract of Bombax ceiba (200 mg/kg)given by oral gavage once daily for eight days

Group–IV received gentamicin (IP) at 80 mg/kg concurrently with ethylacetate leaf extract of Bombax ceiba (200 mg/kg)given by oral gavage once daily for eight days.

Group–V received gentamicin (IP) at 80 mg/kg concurrently with aqueous leaf extract of Bombax ceiba (200 mg/kg)given by oral gavage once daily for eight days.

Twelve hour after the last treatment, all rats were anesthetized by IP injection of ketamine (35mg/kg) and blood samples were immediately obtained by intracardiac aspiration. The serum was rapidly separated and processed for determination of urea, creatinine,MDA. After the animals were sacrificed, postmortem examination was performed. The rat kidneys were identified and carefully dissected out en bloc for histopathological examination. Pieces of kidney from each group were fixed immediately in 10% neutral formalin for a period of at least 24 h, dehydrated in graded (70–100%) alcohol, embedded in paraffin, cut into 4–5 μm thick sections, stained with Hematoxylin–eosin and finally observed under a light microscope. The sections were evaluated for the pathological changes of nephrotoxicity mainly in proximal tubules such as: swelling, loss of brush borders, desquamation and intraluminal cast formation.

Assessment of renal damage:

Renal damage was assessed by biochemical investigations and histopathological examination.

Biochemical assay:

a) Estimation of blood urea was done colorimetrically by DAM method<sup>14</sup>.

b) Estimation of serum creatinine was done by Picric Acid method<sup>15</sup>.

c) Assessment of oxidative stress: Lipid peroxides were estimated in plasma by using thiobarbituric acid (TBA) method<sup>16</sup>. this method measures MDA.
MALONDIALDEHYDE (Malondialdehyde) reactive products. The results obtained were expressed in nmols/ml.

RESULTS

TOXICOLOGICAL STUDIES:
The Acute toxicity all extracts (ETE, BTE and AE) was determined in albino mice, showed no mortality on 2000mg/kg. Therefore 2000mg/kg dose was considered as a safe dose, so 1/10th and 1/5th (200mg and 400mg) of that were we can select for all in vivo experiments as sub maximal and maximal dose.

NEPHROPROTECTIVE STUDY:

Table.1 Toxicological studies of different extracts of *Bombax ceiba*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dose (mg/kg)</th>
<th>No. of animals used</th>
<th>Mortality</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate extract of Leaves</td>
<td>2000</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Butanolic extract of Leaves</td>
<td>2000</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aqueous extract of Leaves</td>
<td>2000</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Statistical Analysis:
The results were given as the mean ± SEM. One-way analysis of variance (ANOVA) were used to compare data between groups for changes in serum levels of creatinine, BUN, potassium, sodium, and chloride using SPSS, version 11. The level of significance was set at 0.05.

Table2: The effect of n-butanol extract of *Bombax ceiba* on serum levels of urea, creatinine and MDA in different groups. G1: control, G2: gentamicin only, G3: ethyl acetate extract and gentamicin (G+EEBC), G4: n-butanol extract and gentamicin (G+BEBC), G5: aqueous extract and gentamicin (G+AEBC)

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Experimental group</th>
<th>Mean (±SD)</th>
<th>P * value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>G1</td>
<td>10.11(±2.13)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>47.44(±12.96)</td>
<td></td>
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<tr>
<td></td>
<td>G3</td>
<td>28.29(±5.05)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G4</td>
<td>12.73(±1.18)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G5</td>
<td>20.76(±2.54)</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>G1</td>
<td>0.78(±0.12)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>3.66(±0.93)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>2.03(±0.59)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G4</td>
<td>1.29(±0.19)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G5</td>
<td>1.36(±0.25)</td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>G1</td>
<td>1.1(±0.05)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>G2</td>
<td>2.8(±0.12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G3</td>
<td>2.32(±0.46)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G4</td>
<td>1.09(±0.06)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>G5</td>
<td>1.6(±0.06)</td>
<td></td>
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</tbody>
</table>

Histopathological evaluation.
Figure 1  Nephroprotective effect of Bombax cieba extract on gentamicin induced kidney damage. Histological finding of (A) normal kidney (Control Group), (B) Group 2 (G) received gentamicin showing severe tubular necrosis and intraluminal cast (C) in Group 3 (G-EEBC) showing tubular necrosis. in (D) Group 4 (G+BEBC) showing normal renal tubules with mild swelling and in (E) Group 5 (G+AEBC) showing normal renal tubules with some tubular necrosis. (original magnification x100).
Figure 2: Nephroprotective effect of bombax ceiba n-butanolic extract on gentamicin induced kidney damage. (A) Histological finding of Group 2 (G) received gentamicin showing sever tubular necrosis and intraluminal cast. (B) Histological finding of Group 4 (G+BEBC) showing mild tubular swelling. (original magnification x400).

Discussion:

Urea, creatinine, MDA and electrolytes are markers of kidney function. The alterations in the levels of these markers in gentamicin treated group shown in this study have also been reported by others when toxic doses of gentamicin are administered. Gentamicin induces oxidative stress through induction of reactive oxygen species such as free radical, superoxide, hydroxyl radical anion and hydrogen peroxide. When ROS are generated as a consequence to tissue injury induced by gentamicin and is not eliminated, it will attack different cell components as DNA, RNA, proteins, lipids and enzymes leading to many degenerative processes in the renal cells manifested as glomerular disease, renal ischemia, perfusion injury and eventually acute renal failure. This side effect was evident in the present study by the significant alteration in urea, creatinine, MDA and electrolytes serum levels of this group. Such effects have also been documented previously.

Furthermore the histopathological changes seen in the kidneys section in gentamicin treated group supports the concept of gentamicin-induce renal toxicity and explains the significant alteration in kidney function tests found in this study. As the proximal tubule accumulates this antibiotic highly because of its physio-chemical properties therefore it is the main part of kidney affected by gentamicin toxicity which was obvious in the sections of kidneys of gentamicin treated groups. The histo-pathological changes were similar to others findings.

The co-treatment of Bombax ceiba extract(n-butanolic and aqueous) attenuated gentamicin induced renal oxidative damage in rats. These effects were evident from the significant decrease in serum levels of urea, creatinine, uric acid compared to gentamicin and they were close to those in the control untreated group. These effects are assumed to be related to the antioxidant property of mangiferin through scavenger of free oxygen radical released as a consequence of oxidative damage as reported in numerous studies.

Conclusion:

Herbal medicine is defined as "the art and science of restoring a sufferer to health by the use of plant remedies." Herbal remedies consist of portions of a plant (e.g. leaves, roots, stem) as opposed to specific chemicals isolated and extracted in the laboratory. Herbal remedies are unpurified plant extracts containing several substituents, which often work together synergistically.

Research for herbal drugs are done because of the effectiveness, easy availability, low cost and comparatively being devoid of serious toxic effects (time tested) popularized, herbal remedies. Acute renal failure (ARF), characterized by sudden loss of the ability of the kidneys to excrete wastes, concentrate urine, conserve electrolytes, and maintain fluid balance, is a frequent clinical problem, particularly in the intensive care unit, where it is associated with a mortality of between 50% and 80%. The use of herbal products accounts for nearly 35% of all cases of acute renal failure. Nephropathy following intake of Chinese herbs is well documented. Many herbal drugs due to presence of antioxidant potential in them can be used for renal tragedies.

Oxidative stress and lipid peroxidation are early events related to radicals generated during the renal impairment. Also the generation of reactive oxygen species has been proposed as a mechanism by which many chemicals can induce nephrotoxicity increases the lipid peroxidation and suppresses the antioxidant defense mechanisms in renal tissue Bombax ceiba has proved that it has antioxidant activity so it work to protect acute renal failure also.

Role of Bombax ceiba in protecting the acute renal failure is that it contains flavanoids and phenolic compound which have detected in phytochemical investigation in this study. The main flavanoid mangiferin is present in the bombax ceiba has antioxidant potential. Mangiferin was mainly present in the n-butanolic extract which was detected in HPTLC work. Hence n-butanolic extract was given better antioxidant activity in comparison to other extracts. The
n-butanolic extract in nephrotoxicity study where it gave better nephroprotective action when it was given to the rats at a dose of 200mg/kg this dose was considered as safe dose in toxicological activity. n-butanolic extract when given to rats with gentamycin it attenuated the induced renal oxidative damage doing by gentamycin. Hence these effects were evident for significant decrease serum level of urea, creatinine potassium etc. These effects were happens due to mangiferin presence in extract which scavage the free oxygen radical that released due to oxidative damage done by gentamycin. Hence bombax can be used in acute renal failure.

REFERENCES
7) Schrier Robert & etal, Acute renal failure definitions, diagnosis, pathogenesis, and therapy, The Journal of Clinical Investigation, 2013,