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Protective effect of the bark of *Thespesia Populnea* on Gentamicine induced Nephrotoxic rats

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Abstract

In this study, gentamicin (100 mg/kg BW/day i.p.) produces nephrotoxicity as evidenced by decrease in body weight, urine volume, elevate in kidney weight, blood urea, uric acid, serum creatinine, decrease creatinine clearance and renal tubular necrosis. The ethanolic and ethyl acetate extracts of *Thespesia populnea* at a dose of 400 mg/kg BW/day induced significant increase in urine volume, as compared to gentamicin treated group. Ethanolic extract (400 mg/kg BW/day p.o.) of *Thespesia populnea* (L) bark showed significant ($p < 0.001$) decrease serum urea (UR), uric acid (UA) and creatinine (CR) level. The findings suggest that the extract of *Thespesia populnea* possesses marked Nephroprotective activity with minimal toxicity.

Keywords: *Thespesia populnea*, nephroprotective, gentamicin, nephrotoxicity

Introduction

Thespesia populnea (L.) Soland ex. correa is ever green tree belongs to family Malvaceae widely distributed tropical and subtropical regions in coastal areas of the india and pacific oceans. It is commonly known as Milo or Portia tree [1]. It is used for treatment of various diseases in Indian system of medicine including antibacterial [2], antidiabetic [3], antiimplantation [4], antioxidant [5], Alzheimer [6], Antinociceptive and anti-inflammatory [7], diuretic [8], psoriasis [9], hepatoprotective [10] and wound healing [11]. It was traditionally used in urinary disease [12]. In present study, we investigated protective effect of the ethanolic and ethyl acetate extracts of *Thespesia populnea* bark in gentamicin induced nephrotoxic rats.

Table 1: Effect of 100 mg/kg BW/day i.p. gentamicin and graded oral *Thespesia populnea* bark extracts on body weight, kidney weight, urine volume, serum creatinine; blood urea and serum uric acid in treated rats for 8 days.

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
% Body Weight Change (gm)	7.060±0.8004	-9.370±0.4545 ^c	-7.060±0.1263**	-1.434±0.2925***	-8.750±0.3856 ^{ns}	-6.752±0.2991**
Kidney Weight (gm)	0.4760±0.0067	0.5820±0.0115 ^c	0.5260±0.0107**	0.4960±0.0107***	0.5760±0.0132 ^{ns}	0.5300±0.5300*
Urine Volume (ml)	3.800±0.1673	2.480±0.1200 ^c	3.320±0.1497***	3.560±0.1939***	2.800±0.1414 ^{ns}	3.160±0.1720***
Creatinine (mg/dl)	0.9000±0.04658	1.984±0.0455 ^c	1.556±0.1304**	1.080±0.0408***	1.866±0.0520 ^{ns}	1.638±0.09836*
Urea (mg/dl)	28.07±1.171	77.49±2.283 ^c	58.70±3.319***	39.85±2.843***	65.78±2.668*	63.21±1.968**
Uric Acid (mg/dl)	2.448±0.3374	10.25±0.8278 ^c	6.636±0.4838**	4.960±0.6021***	7.428±0.3265*	6.398±0.8288***

N=6 animals in a group; Values are expressed as Mean±SEM;

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ Vs Toxicant Control.

c-Indicate Gentamicin treated Gephrotoxicity.

ns-Indicate no significant

2. Experimental

2.1. Plant material

The fresh bark of *Thespesia populnea* (L.) was collected during the month of Feb. 2010 from the local areas & surroundings of Hubli, Dharwad in Karnataka, India.

The plant material was taxonomically identified and authenticated by Dr. B.D. Huddar, Professor and Head, Department of Botany, H.S.K. Kotambari Science College Hubli, Karnataka, India. The voucher specimen (KLESCOP/HBL/AUTH2010-11) has been deposited in the herbarium section of the Pharmacognosy Division, K.L.E.S College of Pharmacy, Hubli for future and further reference.

2.2. Preparation of the extracts

The bark of *Thespesia populnea* was shade dried at room temperature, pulverized and 100 g of coarse powder was defatted with petroleum ether (40-60°C) in a Soxhlet extractor. The above defatted material was extracted by continuous hot percolation (soxhlation) with Chloroform, Ethyl acetate Ethanol and Water in increasing order of their polarity. The solvent was removed under reduced pressure using rotary flash evaporator then finally dried in desiccators over sodium sulfite.

2.3. Animals

Male Wistar rats (150-200 g) were housed in clean polypropylene cages and maintained at 24±2 °C under 12 h (L:D) cycle and fed with standard laboratory diet and water *ad libitum*. It was procured from Sri Venkateswara Enterprises, Bangalore, India. The experimental protocol was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. The study was conducted after obtaining Institutional animal ethical committee clearance (Ref. KLESCOPH/IAEC Clear/2009-2010/06).

2.4. Nephroprotective activity

Thirty six male wistar rats weighing 150-200 g were used for study. Animals were divided into 6 groups of 6 animals each.

- **Group I:** Control rats (CON) that received normal saline (5 ml/Kg i.p.) and 0.5% carboxymethyl cellulose (CMC) (p.o.) for 8 days.
- **Group II:** Gentamicin-treated rats (GM) that received 100mg/kg BW/day GM (i.p.) and 0.5% CMC (p.o.) for 8 days.
- **Group III:** Rats received ethyl alcohol extract of *Thespesia populnea* bark (200mg/kg BW/day p.o.) + GM (100mg/kg BW/day i.p.) for 8 days.
- **Group IV:** Rats received ethyl alcohol extract of *Thespesia populnea* bark (400mg/kg BW/day p.o.) + GM (100mg/kg BW/day i.p.) for 8 days.
- **Group V:** Animals received Ethyl acetate extract of *Thespesia populnea* bark (200mg/kg BW/day p.o.) + GM (100mg/kg BW/day i.p.) for 8 days.
- **Group VI:** Animals received Ethyl acetate extract of *Thespesia populnea* bark (400mg/kg BW/day p.o.) + GM (100mg/kg BW/day i.p.) for 8 days.

After collection of blood animals were sacrificed by cervical dislocation under mild ether anaesthesia and kidneys were harvested, rinsed in saline and fixed in 10% formalin for histopathological studies^[13, 14].

2.5. Statistical analysis

The values were expressed as Mean ± SEM. Statistical analysis was performed by one way analysis of variance

(ANOVA) followed by Tukey multiple comparison test P values < 0.05 were considered as significant.

3. Results

The gentamicin 100 mg/kg BW/day for 8 days is reduced the body weight, urine volume, elevated to kidney weight, serum urea, serum creatinine, uric acid and histopathological observations reveals that there is a glomerular congestion, infiltration, inflammatory cells, tubular necrosis, peritubular necrosis in rats. The observations are studied to assess the gentamicin induced nephrotoxicity. Fig. no.1, 2.

Treating (group III & IV) with ethanolic extract (200 & 400 mg/kg BW/day) of *Thespesia populnea* (L.) bark showed significant decrease ($p < 0.001$) in concentration of serum urea, uric acid and creatinine compared to gentamicin treated group II.

Treatment with ethyl acetate extract (200 & 400 mg/kg BW/day) of *Thespesia populnea* (L.) bark significantly ($p < 0.05$) & ($p < 0.01$) decreases the serum urea, uric acid and creatinine levels in group V & VI compared to gentamicin treated group II. Ethanol and ethyl acetate extracts of *T. populnea* at a dose of 400 mg/kg BW/day induced significant increase in urine volume, as compared to control group II.

Oral administration of ethanolic extract of the *Thespesia populnea* bark at the dose of 400 mg/kg BW/day showed the more significant decrease in serum urea (UR), uric acid (UA) and creatinine (CR) level. However the higher dose of ethyl alcohol extract (400 mg/kg BW/day) animals has shown almost complete recovery from nephrotoxicity induced by gentamicin. The results compelled in Table no. 1 and graphically depicted Fig. no.1, 2.

4. Discussion

Gentamicin has been reported to produce nephrotoxicity even at normal therapeutic dose level (Smith *et al.*, 1980). Elevation of serum creatinine, uric acid and serum urea has been considered as the most important manifestation of severe tubular necrosis of kidney (Gilman *et al.*, 1992; Bennit *et al.*, 1982; Ali *et al.*, 2001). Gentamicin-induced nephrotoxicity is characterized by tubular necrosis, basal membrane disruption, mesangial cell contraction, proliferation and apoptosis, indicated by decreases in glomerular filtration and alteration in intraglomerular dynamics (Martínez-Salgado *et al.*, 2007). Histopathological findings showed that administration of gentamicin caused apoptosis, intracellular edema, basal membrane interruption, glomerulus narrowing of the Bowman's capsule and acute tubule necrosis (Souza *et al.*, 2008)^[15, 16]. Results of this study confirmed that gentamicin at a dose of 100 mg/kg produces significant nephrotoxicity as evidenced by decrease in body weight, urine volume, elevate in kidney weight, blood urea, uric acid, serum creatinine, decrease creatinine clearance and renal tubular necrosis.

The present study results showed that the Ethanolic extract of *Thespesia populnea* bark possesses potent nephroprotective activity. This plant contains different classes of phytochemicals such as flavonol glycosides like quercetin-7-O-rhamnoglucoside^[10], kaempferol-7-glucoside, kaempferol-3-monoglucoside, kaempferol-3-rutinoside, kaempferol-3-glucoside, kaempferol-5-glucoside, quercetin-3-glucoside, rutin, calyopterin isolated from heartwood^[17]. Two new sesquiterpenoid quinones -

thespesone, thespone and mansonones C, D, E and F isolated from heartwood.^[18]

The literature survey showed that quercetin flavonoid present in the *Thespesia populnea* (L.) plant and an earlier report suggests that quercetin has a marked protective effect on cadmium-induced nephrotoxicity that results from an increase in Metallothionein, a small cysteine-rich protein and eNOS (endothelial nitric oxide synthase) expression and the inhibition of COX-2 (cyclooxygenase-2) and iNOS (inducible nitric oxide synthase) expression^[19].

The alcoholic extract (400 mg/kg BW/day) of *Thespesia populnea* showed promising nephrocurative activity, where as ethyl acetate extract of *Thespesia populnea* possessed significant nephroprotective activity in the rat model of gentamicin-induced renal toxicity. These results suggest the

therapeutic utility of herbal *Thespesia populnea* bark extracts in renal injury

Conclusion

In Conclusion the gentamicin treated group there will be rise in serum marker such as urea, uric acid creatinine and decrease body weight, urine volume. The same was observed in kidney diseases in clinical practice and hence are having diagnostic importance in the assessment of kidney function.

Based on improvement in serum marker levels, histopathological studies and presence of phytoconstituents, it was concluded that the ethanolic extract of *Thespesia populnea* bark possesses nephroprotective activity.

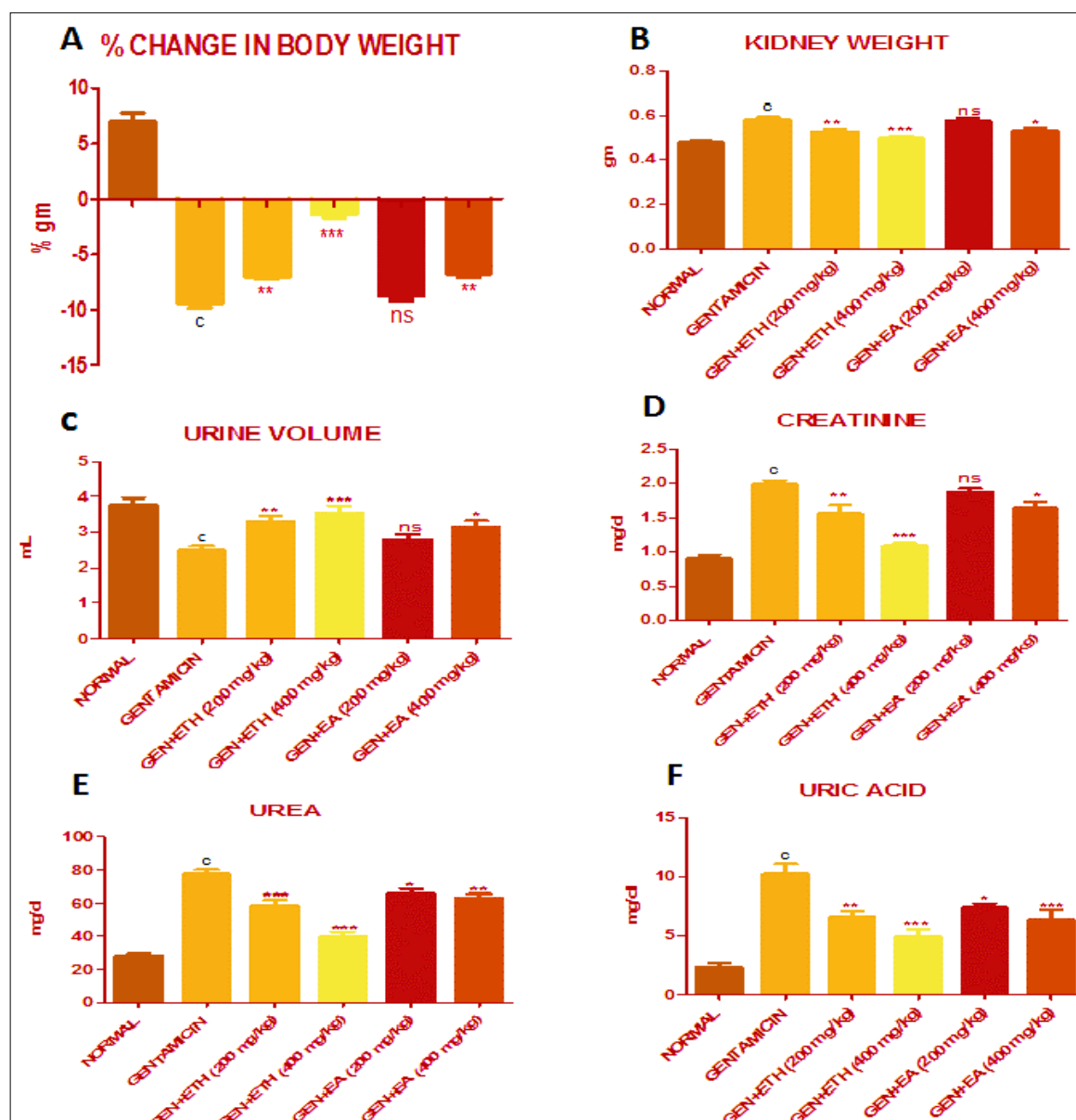


Fig. 1: A. Effect of Ethanolic & Ethyl acetate extract (200 and 400 mg/kg BW/day) of *Thespesia populnea* bark on gentamicin induced reduction in body weight.

B. Effect of Ethanolic & Ethyl acetate extract (200 and 400 mg/kg BW/day) of *Thespesia populnea* bark on gentamicin induced elevated in kidney weight.

C. Effect of Ethanolic & Ethyl acetate extract (200 and 400 mg/kg BW/day) of *Thespesia populnea* bark on gentamicin induced reduction in urine volume.

D. Effect of Ethanolic & Ethyl acetate extract (200 and 400 mg/kg BW/day) of *Thespesia populnea* bark on gentamicin induced elevated in serum creatinine levels.

E. Effect of Ethanolic & Ethyl acetate extract (200 and 400 mg/kg BW/day) of *Thespesia populnea* bark on gentamicin induced elevated in blood urea levels.

F. Effect of Ethanolic & Ethyl acetate extract of *Thespesia populnea* bark on gentamicin induced elevated in Serum uric acid levels.

Histopathological evaluation

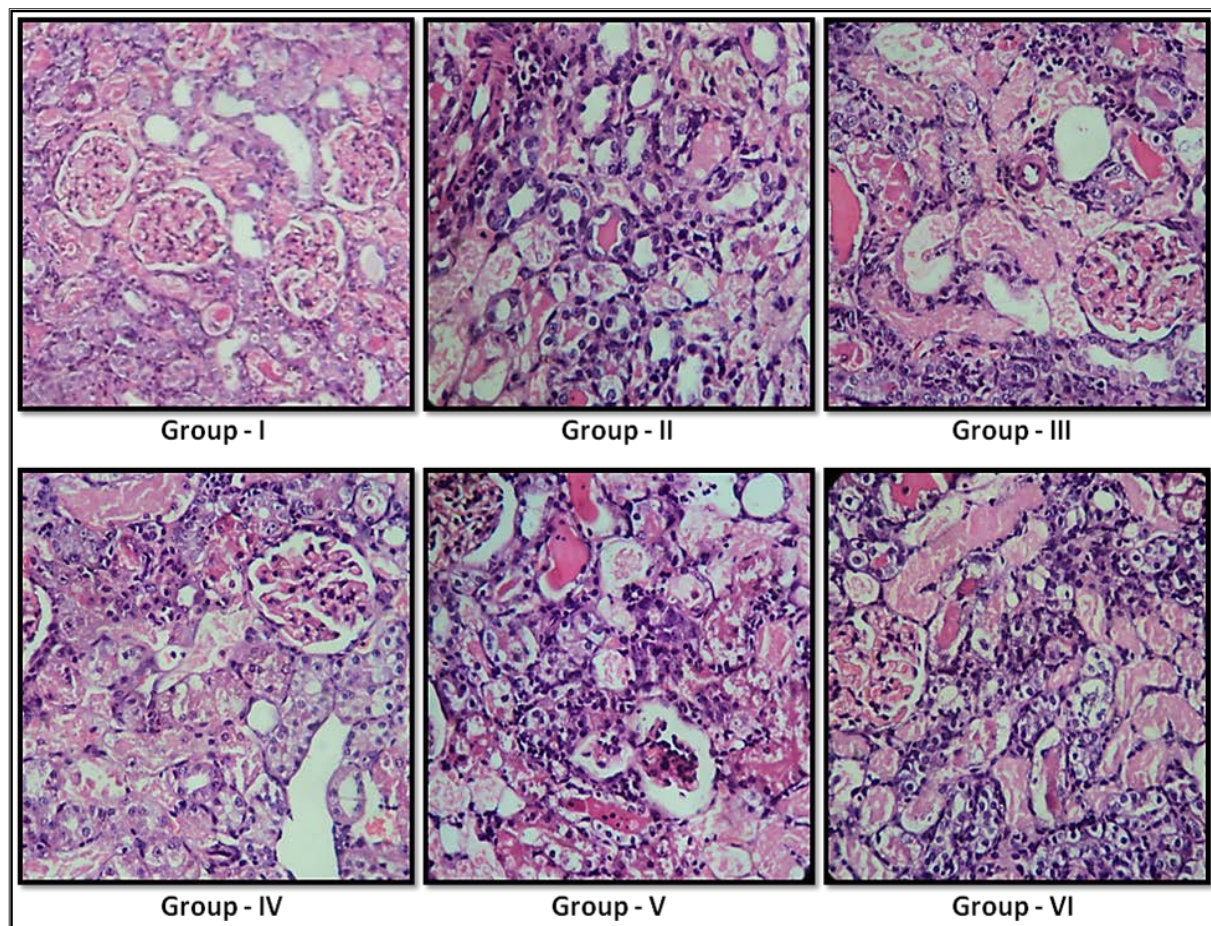


Fig 2: Microscopy of rat Kidneys sections from different treatment groups stained with haematoxylin and eosin magnified using a 45x objective.

Group I - Microscopy of Normal rat kidney,

Group II - Microscopy of Gentamicin treated rat kidney.

Group III-Microscopy of Gentamicin + Ethyl alcohol extract 200 mg/kg treated rat kidney.

Group IV-Microscopy of Gentamicin + Ethyl alcohol extract 400 mg/kg treated rat kidney.

Group V- Microscopy of Gentamicin + Ethyl acetate extract 200 mg/kg treated rat kidney.

Group VI-Microscopy of Gentamicin + Ethyl acetate extract 400 mg/kg treated rat kidney.

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