



ANTIOXIDANT AND NEPHROPROTECTIVE EFFECTS OF METHANOLIC FLOWER EXTRACT OF DELONIX REGIA IN GENTAMICIN-INDUCED NEPHROTOXICITY IN WISTAR RATS

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Abstract:

Background: Gentamicin is an effective aminoglycoside antibiotic whose clinical use is limited by dose-related renal tubular injury, oxidative stress and loss of renal function. Delonix regia is rich in phenolic and flavonoid constituents, but the nephroprotective potential of its flowers has not been adequately characterized.

Objective: To evaluate the in vitro antioxidant activity and in vivo nephroprotective effect of a methanolic flower extract of D. regia in rats with gentamicin-induced nephrotoxicity.

Methods: Flowers were defatted with petroleum ether and extracted with methanol by Soxhlet extraction. Preliminary phytochemical tests, total phenolic content, total flavonoid content and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity were determined. Wistar rats were allocated to five groups (n=6): normal control, gentamicin control (40 mg/kg, intraperitoneally), silymarin (200 mg/kg, orally) plus gentamicin, and D. regia extract (100 or 200 mg/kg, orally) plus gentamicin for 14 days. Urine volume, body weight, serum creatinine, blood urea nitrogen (BUN) and renal histology were assessed.

Results: The methanolic extraction yield was 2.04%. Total phenolic and flavonoid contents were 53.0 mg gallic acid equivalents/g and 17.5 mg rutin equivalents/g, respectively. The extract produced concentration-dependent DPPH scavenging with an IC₅₀ of 49.48 µg/mL, compared with 21.60 µg/mL for ascorbic acid. Gentamicin increased serum creatinine (4.90±0.13 mg/dL) and BUN (61.0±0.79 mg/dL) and reduced urine volume (5.99±0.77 mL/24 h) and body weight (149.22±2.66 g). The 200 mg/kg extract group showed the greatest recovery among extract-treated groups: creatinine 1.00±0.06 mg/dL, BUN 29.91±0.54 mg/dL, urine volume 9.81±0.26 mL/24 h and body weight 212.0±3.74 g. Histology showed dose-related preservation of renal architecture.

Conclusion: Methanolic D. regia flower extract exhibited in vitro antioxidant activity and attenuated functional and histological manifestations of gentamicin nephrotoxicity, with the 200 mg/kg dose producing the stronger effect. Chemical standardization and mechanistic renal oxidative-stress measurements are required before therapeutic translation.

Key Words: Delonix Regia; Gentamicin; Nephrotoxicity; Antioxidant; Phenolics; Flavonoids; Wistar Rats

1. Introduction:

An abrupt decline in renal function characterizes acute kidney injury and is frequently identified by changes in serum creatinine and urine output. Drug-induced injury is an important preventable contributor to acute kidney injury, particularly when nephrotoxic drugs accumulate in renal tubular cells [1]. Aminoglycosides remain useful for severe Gram-negative infections, but gentamicin can cause proximal tubular injury after intracellular accumulation. The resulting lysosomal dysfunction, mitochondrial impairment, reactive oxygen species generation, inflammation and cell death collectively reduce glomerular filtration and disturb tubular handling of water and solutes [2-5].

Oxidative stress is a central, although not exclusive, component of gentamicin nephrotoxicity. Experimental studies have shown increased production of superoxide, hydrogen peroxide and hydroxyl radicals, depletion of endogenous antioxidant defenses, lipid peroxidation and structural tubular damage [5-7]. This mechanistic profile provides a rationale for investigating plant-derived phenolics and flavonoids, which can scavenge radicals, chelate redox-active metals and modulate cellular antioxidant pathways. Nevertheless, in vitro radical scavenging alone does not establish renal protection; therefore, functional biochemical outcomes and tissue morphology should be evaluated in an appropriate animal model.

Delonix regia (Bojer ex Hook.) Raf., commonly known as gulmohar or royal poinciana, is a leguminous ornamental tree used in several traditional systems of medicine. Different parts of the plant contain flavonoids, phenolic acids, tannins, sterols and other secondary metabolites and have demonstrated antioxidant, anti-inflammatory, antimicrobial and metabolic effects in preclinical studies [8-10]. Methanolic and hydroalcoholic extracts of D. regia leaves and flowers have shown free-radical scavenging activity, supporting further investigation of their pharmacological potential [9, 10].

Evidence directly linking D. regia flower extract with protection from aminoglycoside-induced renal injury remains limited. The present study therefore evaluated (i) extraction yield and preliminary phytochemical composition, (ii) total phenolic and flavonoid contents, (iii) DPPH radical scavenging activity and (iv) the effect of two oral doses of methanolic D. regia flower extract on urine volume, body weight, serum creatinine, BUN and renal histology in gentamicin-treated Wistar rats. We hypothesized that the extract would improve renal outcomes in a dose-related manner.

2. Materials and Methods:

2.1 Chemicals and Reagents:

Methanol, petroleum ether, gallic acid, rutin, ascorbic acid, DPPH, Folin-Ciocalteu reagent, sodium carbonate, aluminium chloride and reagents for phytochemical screening were of analytical grade. Gentamicin and silymarin were used for induction and reference treatment, respectively.

2.2 Plant Material, Authentication and Preparation:

Fresh flowers of *D. regia* were collected during. The material was authenticated by, and a voucher specimen was deposited in. Approximately 300 g of flowers were cleaned, shade-dried for 3 days, oven-dried at 45°C for a further 3 days, pulverized and stored in a closed container until extraction.

2.3 Extraction:

Powdered flower material was first defatted with petroleum ether using a Soxhlet apparatus. The marc was then extracted continuously with methanol at approximately 60°C. The methanolic extract was concentrated under reduced pressure using a rotary evaporator at approximately 40°C and stored in an airtight container. Percentage yield was calculated as: extract yield (%) = [mass of dried extract (g)/mass of plant material extracted (g)] × 100.

2.4 Preliminary Phytochemical Screening:

Petroleum ether and methanolic extracts were screened qualitatively for alkaloids, carbohydrates, glycosides, flavonoids, tannins/phenolic compounds, proteins/amino acids, saponins and steroids/triterpenoids using conventional color and precipitation reactions. Results were recorded as present (+) or absent (-).

2.5 Total Phenolic Content:

Total phenolic content was determined using the Folin-Ciocalteu colorimetric method with gallic acid as the calibration standard [11]. Absorbance was measured against a reagent blank at the wavelength specified in the laboratory protocol. Results were calculated from the gallic acid calibration curve and expressed as milligrams of gallic acid equivalents per gram of dried extract (mg GAE/g).

2.6 Total Flavonoid Content:

Total flavonoid content was measured by an aluminium chloride colorimetric method using rutin as the reference standard [12]. Results were obtained from the rutin calibration curve and expressed as milligrams of rutin equivalents per gram of dried extract (mg RE/g).

2.7 DPPH Radical Scavenging Assay:

The free-radical scavenging activity of the methanolic extract was determined by the DPPH method adapted from Brand-Williams et al. [13]. Extract or ascorbic acid solutions at 20, 40, 60, 80 and 100 µg/mL were reacted with DPPH solution. Absorbance was measured against an appropriate blank, and percentage inhibition was calculated as [(Acontrol - Asample) / Acontrol] × 100. IC50 values were estimated from the concentration-response relationship.

2.8 Experimental Animals and Ethical Approval:

Thirty adult Wistar rats weighing approximately 200±50 g were used. Animals were housed under controlled laboratory conditions (22±2°C) with a standard pellet diet and water ad libitum and were acclimatized before experimentation. The study protocol was reviewed and approved by CPCSEA registration no. Animal reporting should comply with ARRIVE 2.0 [14].

2.9 Acute Oral Toxicity:

Acute oral toxicity of the methanolic extract was assessed in accordance with OECD Test Guideline 423 [15]. Animals were observed after oral administration up to 2,000 mg/kg for mortality, behavioral changes and clinical signs. No toxicity was reported at the limit dose. Doses of 100 and 200 mg/kg were selected for the nephroprotective experiment.

2.10 Experimental Design and Treatment:

Animals were allocated into five groups (n=6 per group) and treated for 14 consecutive days. The intended design, reconstructed from the laboratory record, is shown below.

2.11 Urine Collection, Blood Sampling and Tissue Collection:

At the end of treatment, rats were placed individually in metabolic cages, and 24-h urine volume was measured. On day 15, blood was collected under light ether anesthesia by retro-orbital puncture, allowed to clot and centrifuged to obtain serum. Animals were then euthanized, and kidneys were removed for histological examination.

2.12 Biochemical Analysis:

Serum creatinine was estimated by a Jaffé-based colorimetric procedure described by Slot [16]. BUN was measured using the diacetyl monoxime/Berthelot-type method, as described by Fawcett and Scott [17]. Concentrations are reported in mg/dL.

2.13 Histopathology:

Kidney samples were fixed, processed, embedded, sectioned and stained for light microscopy. Representative images were used to compare normal architecture, gentamicin-induced injury and treatment-associated preservation.

2.14 Statistical Analysis:

Data are presented as mean ± SEM (n = 6). The source record states that groups were compared by one-way analysis of variance and that p<0.05 was considered statistically significant.

3. Results:

3.1 Extraction Yield and Phytochemical Profile:

Petroleum ether defatting yielded 1.71 g from 284 g of material (0.60%), while methanolic extraction yielded 6.12 g from 299.82 g of material (2.04%). The methanolic extract tested positive for alkaloids, carbohydrates, flavonoids, tannins/phenolic compounds and saponins. Glycosides, proteins/amino acids and steroids/triterpenoids were not detected in the methanolic extract (Table 1).

Table 1: Extraction yield of *D. regia* flowers

Group	Description	Treatment
I	Normal control	Normal saline, 1 mL/kg/day

II	Gentamicin control	Gentamicin 40 mg/kg/day, intraperitoneally
III	Reference treatment	Silymarin 200 mg/kg/day, orally + gentamicin 40 mg/
IV	Low-dose extract	D. regia methanolic extract 100 mg/kg/day, orally +
V	High-dose extract	D. regia methanolic extract 200 mg/kg/day, orally +

Table 2: Preliminary phytochemical screening

Extract	Material extracted (g)	Dried extract (g)	Yield (%)
Petroleum ether extract	284	1.71	0.6
Methanolic extract	299.82	6.12	2.04

Note: + - detected

3.2 Phenolic, Flavonoid and DPPH Antioxidant Results:

The methanolic extract contained 53.0 mg GAE/g total phenolics and 17.5 mg RE/g total flavonoids. DPPH inhibition increased with concentration from 44.11% at 20 µg/mL to 60.54% at 100 µg/mL. The extract IC₅₀ was 49.48 µg/mL, whereas the ascorbic acid IC₅₀ was 21.60 µg/mL, indicating lower radical-scavenging potency than that of the reference antioxidant (Table 3 and Figure 1).

Table 3: Antioxidant-related measurements of the methanolic extract

Phytochemical class	Petroleum ether extract	Methanolic extract
Alkaloids	+	+
Carbohydrates	-	+
Glycosides	+	-
Flavonoids	-	+
Tannins/phenolics	-	+
Proteins/amino acids	-	-
Saponins	+	+
Steroids/triterpenoids	-	-

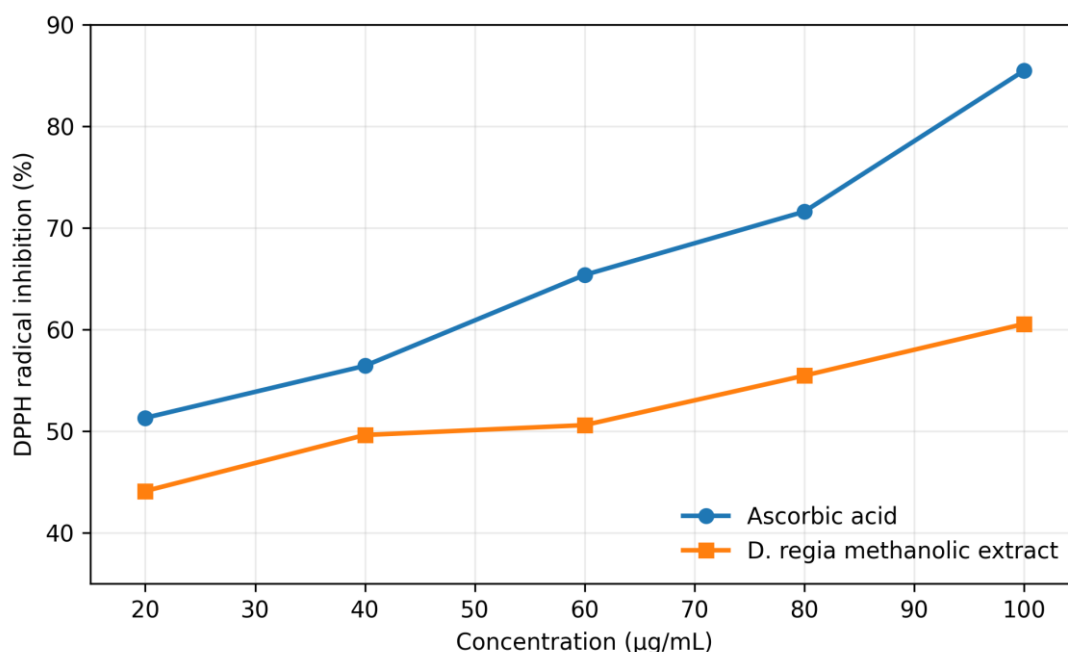


Figure 1: Concentration-dependent DPPH radical scavenging by methanolic D. regia flower extract and ascorbic acid. Values were reconstructed from the source dataset; replicate-level variation was not available

3.3 Effects on Urine Volume, Body Weight and Renal Biochemical Markers:

Gentamicin administration was associated with a marked deterioration in all measured general and renal-function variables. Relative to normal controls, gentamicin-treated rats had lower urine volume and body weight and substantially higher serum creatinine and BUN. Co-treatment with the extract shifted each outcome toward the normal-control value in adose-related pattern. The 200mg/kg dose reduced creatinine by approximately 79.6% and BUN by 51.0% relative to the gentamicin-control mean, while increasing urine volume by approximately 63.8%. Silymarin produced values closest to those of the normal control (Table 4). These percentages are descriptive statistics based on group means and should not be interpreted as inferential effect estimates.

Table 4: General and renal-function outcomes after 14 days of treatment

Measurement	Result
Total phenolic content	53.0 mg GAE/g extract
Total flavonoid content	17.5 mg RE/g extract
DPPH IC ₅₀ , methanolic extract	49.48 µg/mL
DPPH IC ₅₀ , ascorbic acid	21.60 µg/mL

Values are mean±SEM (n=6). GM, gentamicin; BUN, blood urea nitrogen. The original record states one-way ANOVA with p<0.05 but does not document the post-hoc test or exact group-wise p values; these must be verified from raw data.

3.4 Renal Histopathology:

Representative renal sections from normal controls showed relatively preserved glomerular and tubular architecture. The gentamicin-control section showed pronounced structural disruption, including tubular dilatation/vacuolation and loss of normal tissue organization. Silymarin-treated kidneys showed substantial preservation. The 100 mg/kg extract group displayed partial improvement, whereas the 200 mg/kg group showed more evident preservation of glomerular and tubular morphology (Figure 2). Because the original record did not include a validated lesion score, magnification or blinded quantitative assessment, the histological findings are interpreted qualitatively.

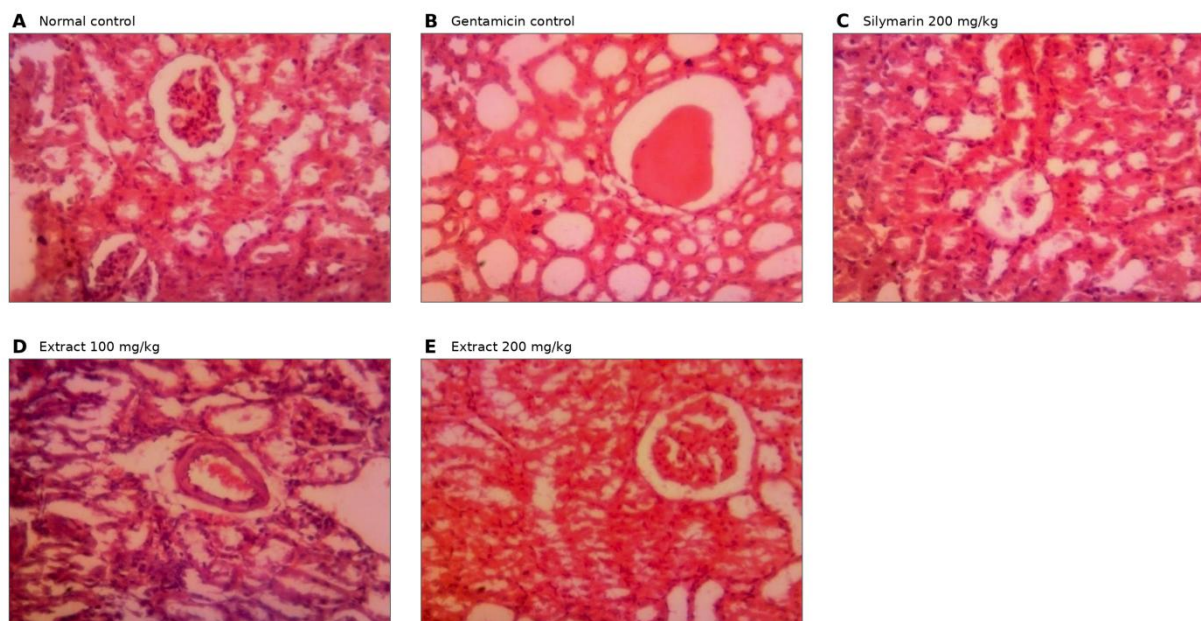


Figure 2: Representative kidney histology: (A) normal control; (B) gentamicin control; (C) silymarin 200 mg/kg plus gentamicin; (D) D. regia extract 100 mg/kg plus gentamicin; and (E) D. regia extract 200 mg/kg plus gentamicin. Stain and original magnification were not documented in the source file and must be confirmed before publication.

4. Discussion:

The present study combined phytochemical and antioxidant characterization with an *in vivo* gentamicin-nephrotoxicity model. The central finding was that oral methanolic *D. regia* flower extract improved urine output, body weight, serum creatinine, BUN and qualitative renal histology in a dose-related pattern. The higher extract dose produced values substantially closer to normal control than the lower dose and approached the response observed with silymarin. These findings support the working hypothesis that the flower extract contains constituents capable of attenuating gentamicin-associated renal injury. Gentamicin is filtered at the glomerulus and taken up by proximal tubular epithelial cells through megalin/cubilin-mediated pathways. Intracellular accumulation disrupts lysosomes, phospholipid metabolism and the homeostasis of mitochondria and the endoplasmic reticulum, promotes oxidant formation and activates inflammatory and cell-death pathways [2-7]. The high creatinine and BUN values and reduced urine volume in the gentamicin group are consistent with loss of filtration and tubular function. Qualitative tubular disorganization in the corresponding histological section further supports successful induction of nephrotoxicity.

The methanolic extract contained several classes of secondary metabolites, notably flavonoids and tannin/phenolic compounds. Its total phenolic content was 53 mg GAE/g and total flavonoid content was 17.5 mg RE/g. These classes can donate hydrogen atoms or electrons to reactive species, chelate transition metals and influence endogenous antioxidant signaling. Previous studies of *D. regia* have reported appreciable phenolic content and radical-scavenging activity in leaves, flowers and other tissues [8-10]. The DPPH IC₅₀ of 49.48 µg/mL confirms moderate *in vitro* radical-scavenging capacity. However, the greater potency of ascorbic acid indicates that the crude extract is a complex, less concentrated antioxidant mixture. A biologically plausible interpretation is that phenolic and flavonoid constituents reduced one or more oxidant-dependent components of tubular injury, thereby preserving renal architecture and function. However, this mechanism was not directly tested in kidney tissue. Renal malondialdehyde, reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase, inflammatory cytokines and tubular injury biomarkers such as kidney injury molecule-1 were not measured. Accordingly, the study demonstrates an association between an antioxidant-rich extract and nephroprotection, but it cannot establish that *in vivo* antioxidant action mediated the renal effect.

The dose-response pattern strengthens the biological interpretation. Compared with the gentamicin control mean, the 200 mg/kg extract reduced serum creatinine from 4.90 to 1.00 mg/dL and BUN from 61.0 to 29.91 mg/dL, while urine volume increased from 5.99 to 9.81 mL/24 h. The low dose produced intermediate values. Similar patterns have been reported with other phenolic-rich plant preparations in gentamicin models, where biochemical improvement accompanies reduced tubular necrosis and vacuolation [18, 19]. Nevertheless, cross-study comparison should be cautious because gentamicin dose, route, duration, species, extract preparation and reference treatment vary substantially.

Several methodological limitations affect publication strength. First, plant authentication details and a herbarium voucher were not reported, limiting reproducibility. Second, the extract was not chemically standardized by chromatographic fingerprinting or quantification of marker compounds. Third, the source record did not provide full randomization, allocation concealment or blinding details. Fourth, "either sex" was stated without reporting sex distribution or sex-stratified outcomes. Fifth, the statistical

record lacks the post-hoc procedure, exact p values, assumption checks and raw data. Sixth, histology was presented without stain, magnification, lesion-scoring criteria or blinded evaluation. Finally, the reported final body weights are unusually different between groups; baseline and longitudinal weight measurements should be checked to exclude transcription or allocation errors.

Before submission, the raw dataset should be reanalyzed using a prespecified model and an appropriate multiple-comparison procedure. Individual data points should be retained and, where possible, displayed. Future work should include extraction fingerprinting by HPLC/UPLC-MS, quantification of marker compounds, renal oxidative stress and inflammatory endpoints, creatinine clearance or cystatin C, urinary protein and injury biomarkers, and blinded semi-quantitative histopathology. Such improvements would distinguish a descriptive screening study from a mechanistically persuasive nephroprotection study.

5. Conclusion:

Methanolic flower extract of *D. regia* showed moderate DPPH radical-scavenging activity and contained measurable phenolic and flavonoid constituents. In gentamicin-treated Wistar rats, oral administration at 100 and 200 mg/kg shifted urine volume, body weight, serum creatinine, BUN and renal morphology toward normal values, with the 200 mg/kg dose producing the stronger response. The findings support nephroprotective potential, but they should be interpreted as preclinical and exploratory until plant authentication, extract standardization, complete statistical analysis and quantitative histopathology are documented. Direct measurement of renal oxidative stress and inflammatory mechanisms is also needed.

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