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RESEARCH ARTICLE

Attenuation of Gentamicin-Induced Acute Kidney Injury by Methanolic Leaf Extract of *Bauhinia variegata* L. in Wistar Rats

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Abstract: Aminoglycoside antimicrobial therapy, particularly involving gentamicin, remains a primary intervention against severe Gram-negative bacteremia; however, its clinical administration is frequently compromised by dose-dependent nephrotoxicity. Gentamicin-induced acute kidney injury is characterized by proximal tubular necrosis, generation of reactive oxygen species, and impaired renal clearance leading to retention of circulatory nitrogenous waste products. Bioactive botanical constituents capable of counteracting intrarenal oxidative degradation provide potential protective strategies against drug-induced renal insult. *Bauhinia variegata* L. synthesizes a broad spectrum of secondary metabolites, including polyphenols, flavonoids, and tannins with significant antioxidant activity. Intraperitoneal injection of gentamicin at 100 mg/kg/day for eight consecutive days in Wistar albino rats precipitated acute renal dysfunction, marked by substantial increases in serum creatinine (1.25 ± 0.05 mg/dL vs. normal control 0.70 ± 0.02 mg/dL) and blood urea nitrogen (76.11 ± 0.92 mg/dL vs. normal control 24.40 ± 0.97 mg/dL), along with systemic catabolic loss of body weight (-9.03 ± 1.54%). Simultaneous oral treatment with methanolic leaf extract of *Bauhinia variegata* (MEBV) at 600 mg/kg/day significantly blunted these pathophysiological elevations, maintaining serum creatinine at 0.86 ± 0.02 mg/dL and blood urea nitrogen at 28.03 ± 0.20 mg/dL, while normalizing urinary solute excretion profiles and minimizing weight loss (-3.24 ± 0.23%). These results indicate that MEBV possesses notable nephroprotective capacity against gentamicin-induced renal damage, driven primarily by radical-scavenging actions and maintenance of structural tubular integrity.

Keywords: *Bauhinia variegata*; Gentamicin; Nephrotoxicity; Acute kidney injury; Nitrogenous waste.

1. Introduction

The mammalian kidney maintains systemic metabolic homeostasis, osmoregulation, fluid volume, and electrolyte balance through highly coordinated filtration, reabsorption, and secretion mechanisms [1]. Because the renal vasculature receives roughly 20% to 25% of total cardiac output, renal tissue is exposed to substantial circulating volumes of xenobiotics, therapeutic drugs, heavy metals, and environmental toxins [2]. Active tubular transport systems concentrated within the proximal convoluted tubule, specifically the S1 and S2 segments, facilitate high intrarenal bioaccumulation of chemical agents, rendering these anatomical structures vulnerable to toxic damage [3, 4].

Toxicant-induced nephrotoxicity manifests clinically across a spectrum ranging from subclinical tubular transport alterations to severe acute tubular necrosis and acute kidney injury [5]. Cellular pathways governing chemical insult include mitochondrial membrane depolarization, microvascular vasoconstriction leading to localized ischemia, intracellular overload of toxic metabolites, and accelerated production of reactive oxygen species [6, 7]. When cellular endogenous antioxidant defenses comprising superoxide dismutase, catalase, and reduced glutathione are overwhelmed, excess free radicals trigger lipid peroxidation of polyunsaturated fatty acids within cellular membranes [8, 9]. This oxidative cascade leads to membrane fluid disruption, loss of selective permeability, cell death via apoptotic or necrotic pathways, and decline in overall glomerular filtration rate [10]. Gentamicin is a clinically vital aminoglycoside antibiotic prescribed for severe Gram-negative bacterial infections, including septicemia, endocarditis, and pyelonephritis [11]. However, therapeutic utility is restricted by dose-limiting nephrotoxicity, which occurs in approximately 10% to 25% of treatment regimens [12].

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The pathogenesis of gentamicin nephrotoxicity begins with electrostatically driven binding of the polycationic drug to acidic phospholipids located on the brush border membrane of proximal tubular epithelial cells, followed by endocytosis via the megalin-cubilin receptor complex [13, 14]. Following endocytosis, gentamicin concentrates inside endosomes and lysosomes, where it inhibits lysosomal phospholipases and sphingomyelinase [15]. Lysosomal accumulation induces phospholipidosis, vesicle rupture, and cytosolic release of both gentamicin and aggressive lysosomal hydrolases [16]. Within the cytosol, gentamicin targets mitochondria, inhibiting respiratory complex I and complex III activity [17]. This inhibition causes electron leakage and triggers rapid accumulation of superoxide anion radicals ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$) [18]. Free radical proliferation damages cellular proteins, disrupts nuclear DNA, and induces lipid peroxidation [19]. Simultaneously, gentamicin triggers nuclear factor kappa-B (NF-κB) signaling, inducing local synthesis of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) [19, 20]. The combination of cellular oxidative stress, inflammation, and proximal tubular cell desquamation obstructs tubular lumens, causes intrarenal vasoconstriction, reduces glomerular filtration, and leads to nitrogenous waste accumulation in circulation [18-20].

Bauhinia variegata L. (Family: Caesalpiniaceae), commonly known as Kanchanara or Mountain Ebony, is an arboreal species distributed across tropical and subtropical regions of Asia [1, 2]. Traditional ethnomedical systems utilize various plant parts including leaves, bark, roots, and flowers for managing inflammatory states, glandular enlargements, metabolic imbalances, and gastrointestinal disorders [3-7]. Phytochemical characterizations of *Bauhinia variegata* leaves confirm an abundance of polyphenolic compounds, including flavonoids (quercetin, rutin, kaempferol, and luteolin), gallic acid derivatives, condensed tannins, saponins, triterpenoids, and phytosterols [8-13]. Polyphenols act as powerful biological antioxidants because their phenolic hydroxyl groups donate hydrogen atoms or electrons to neutralize radical species such as $O_2^{\bullet}$ and $OH^{\bullet}$ [10, 12]. Additionally, plant flavonoids possess metal-chelating capacity, binding redox-active ferrous iron (Fe^{2+}) to prevent Fenton reaction cascades [11, 13].

Figure 1. Leaves of *Bauhinia variegata*

Given the importance of oxidative damage and inflammatory signaling in aminoglycoside-induced renal injury, therapeutic administration of phytochemicals from *Bauhinia variegata* could be a viable nephroprotective option [10-13, 17]. This study evaluates the nephroprotective capacity of the methanolic leaf extract of *Bauhinia variegata* (MEBV) against gentamicin-induced acute kidney injury in a rodent model [14-17].

2. Materials and Methods

2.1. Extraction and Phytochemical Screening

Fresh leaves of *Bauhinia variegata* L. were collected from verified botanical sources and authenticated by taxonomic authorities at Kakatiya University, Warangal, Telangana, India [10-12]. Voucher specimens were deposited in the institutional herbarium. Collected leaf material was cleaned with distilled water, shade-dried at controlled ambient temperature (25 ± 2°C) for 14 days, and milled into a coarse powder using an industrial mechanical grinder [8, 11]. The powder was extracted with 99.5% methanol using a Soxhlet apparatus at 60-65°C over a 72-hour period [9, 13]. The resulting filtrate was processed under reduced pressure at 40°C using a rotary vacuum evaporator. The residual extract was dried completely in a vacuum desiccator to yield the final dry methanolic leaf extract of *Bauhinia variegata* (MEBV). Qualitative phytochemical screening was carried out using established analytical methods to verify the presence of flavonoids, tannins, saponins, alkaloids, phenolics, triterpenoids, and glycosides [8, 9, 11].

2.2. Experimental Animals

Adult Wistar albino rats of either sex (150 - 200 g) were used for the experiment [15, 16]. Animals were housed in polypropylene cages with sterile bedding under standard environmental conditions (23 ± 2°C temperature, 55 ± 5% relative humidity, and 12-hour

light/dark cycles) [15]. Animals had access to commercial rodent pellet diet and water *ad libitum*. All experimental procedures complied with institutional guidelines for animal care and experimental use [15, 16].

2.3. Experimental Protocol

Following a 7-day laboratory acclimatization period, animals were randomly divided into three distinct groups (n = 6 per group) [15]:

1. Group I (Normal Control): Received daily oral administration of vehicle (distilled water, 5 mL/kg/day) for 8 consecutive days.
2. Group II (Toxic Control): Received intraperitoneal (i.p.) injections of gentamicin sulfate at 100 mg/kg/day for 8 consecutive days to induce acute nephrotoxicity [17-19].
3. Group III (Test Group - MEBV + Gentamicin): Received oral administration of MEBV at 600 mg/kg/day once daily for 8 consecutive days, simultaneously with intraperitoneal injection of gentamicin sulfate (100 mg/kg/day) [15-17].

The experimental dose of MEBV (600 mg/kg) was selected based on acute oral toxicity testing and previous pharmacological evaluations showing safety and therapeutic efficacy [15-17]. The experimental methodology is shown in Figure 1.

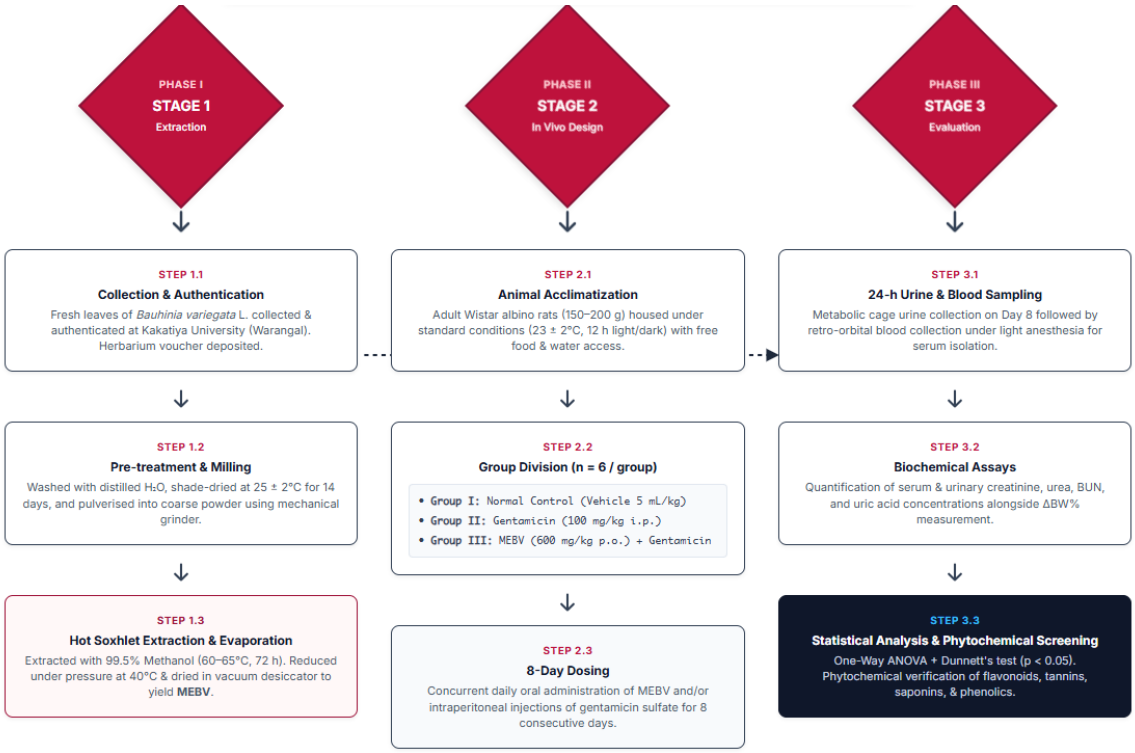

Figure 2. Experimental methodology process flow. Stage 1: Collection, extraction of *Bauhinia variegata* L. leaves, and MEBV yield preparation. Stage 2: *In vivo* experimental grouping and 8-day treatment administration in Wistar albino rats. Stage 3: 24-hour urine collection, serum bioassays, nitrogenous waste parameter evaluation, and statistical analysis.

2.4. Biochemical Assays

Body weights were recorded on Day 1 and Day 8 prior to animal sacrifice [15]. Percentage change in body weight (ΔBW%) was calculated as:

$$\Delta BW\% = \left(\frac{\text{Body Weight}_{\text{Day 8}} - \text{Body Weight}_{\text{Day 1}}}{\text{Body Weight}_{\text{Day 1}}} \right) \times 100$$

On Day 8, following final dosing, rats were placed in individual stainless-steel metabolic cages for 24-hour urine collection [15, 17]. Urine samples were collected in tubes containing 0.02% sodium azide, centrifuged at 3000 rpm for 10 minutes to remove sediment, and stored at -20°C for quantification of urinary creatinine, urea, and uric acid concentrations [15, 17].

Blood samples were subsequently obtained under light anesthesia from the retro-orbital plexus using micro-capillary tubes [15, 16]. Blood was allowed to clot for 30 minutes at room temperature and centrifuged at 4000 rpm for 15 minutes at 4°C to isolate serum. Serum samples were assayed for serum creatinine, blood urea nitrogen (BUN), and serum uric acid using commercial diagnostic kits according to manufacturer instructions [18-20].

2.5. Statistical Analysis

Experimental results are expressed as Mean ± Standard Error of the Mean (SEM). Group comparisons were performed using One-Way Analysis of Variance (ANOVA), followed by Dunnett's *post-hoc* multiple comparison test. Differences were considered statistically significant at $p < 0.05$ [15, 16].

3. Results and Discussion

3.1. Impact on Serum Nitrogenous Waste Products

Intraperitoneal gentamicin administration (100 mg/kg/day) for 8 days produced significant renal functional impairment in Group II animals compared to normal controls.

Serum creatinine in Group II increased to 1.25 ± 0.05 mg/dL relative to 0.70 ± 0.02 mg/dL in Group I ($p < 0.05$). Blood urea nitrogen (BUN) levels increased sharply from 24.40 ± 0.97 mg/dL in normal controls to 76.11 ± 0.92 mg/dL in toxic controls ($p < 0.05$). Serum uric acid elevated from 0.30 ± 0.03 mg/dL to 0.40 ± 0.02 mg/dL ($p < 0.05$). Co-treatment with MEBV at 600 mg/kg/day in Group III significantly attenuated these serum biochemical alterations. Serum creatinine was reduced to 0.86 ± 0.02 mg/dL, BUN decreased to 28.03 ± 0.20 mg/dL, and serum uric acid was reduced to 0.36 ± 0.02 mg/dL. The results are shown in Table 1.

Table 1. Effect of methanolic leaf extract of *Bauhinia variegata* on serum biochemical parameters in gentamicin-induced nephrotoxicity.

Experimental Group	Serum Creatinine (mg/dL)	Serum Uric Acid (mg/dL)	Blood Urea Nitrogen (mg/dL)
Group I (Normal Control)	0.70 ± 0.02	0.30 ± 0.03	24.40 ± 0.97
Group II (Gentamicin Control)	1.25 ± 0.05*	0.40 ± 0.02*	76.11 ± 0.92*
Group III (MEBV 600 mg/kg + Gentamicin)	0.86 ± 0.02#	0.36 ± 0.02#	28.03 ± 0.20#

*Mean ± SEM (n = 6). *p < 0.05 vs. Group I; #p < 0.05 vs. Group II (One-Way ANOVA followed by Dunnett's t-test)*

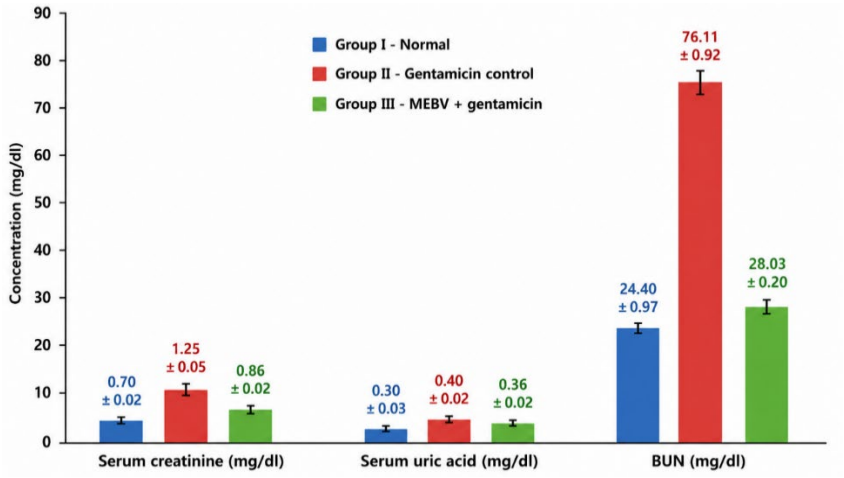

Figure 3. Effect of MEBV on Serum Creatinine, Uric acid and BUN

3.2 Effect on Renal Function and Excretion

In 24-hour urine collection analyses, gentamicin intoxication (Group II) produced marked increases in urinary excretion of creatinine, urea, and uric acid. Urinary creatinine increased from 69.00 ± 0.86 mg/dL (Group I) to 194.50 ± 1.77 mg/dL (Group II) ($p < 0.05$). Urinary urea increased from 17.73 ± 0.10 mg/dL to 32.67 ± 0.73 mg/dL ($p < 0.05$). Urinary uric acid rose from 3.91 ± 0.14 mg/dL to 18.76 ± 0.17 mg/dL ($p < 0.05$). Simultaneous administration of MEBV (600 mg/kg/day) in Group III significantly reduced these urinary elevations, bringing urinary creatinine down to 137.67 ± 2.08 mg/dL, urinary urea to 19.12 ± 0.18 mg/dL, and urinary uric acid to 8.28 ± 0.15 mg/dL.

Table 2. Effect of methanolic leaf extract of *Bauhinia variegata* on Kidney and Body Weight Percentage Change.

Experimental Group	Urinary Creatinine (mg/dL)	Urinary Urea (mg/dL)	Urinary Uric Acid (mg/dL)	Body Weight Change (%)
Group I (Normal Control)	69.00 ± 0.86	17.73 ± 0.10	3.91 ± 0.14	+2.77 ± 0.54
Group II (Gentamicin Control)	194.50 ± 1.77*	32.67 ± 0.73*	18.76 ± 0.17*	-9.03 ± 1.54*
Group III (MEBV 600 mg/kg + Gentamicin)	137.67 ± 2.08#	19.12 ± 0.18#	8.28 ± 0.15#	-3.24 ± 0.23#

*Mean ± SEM (n = 6). *p < 0.05 vs. Group I; #p < 0.05 vs. Group II (One-Way ANOVA followed by Dunnett's t-test).*

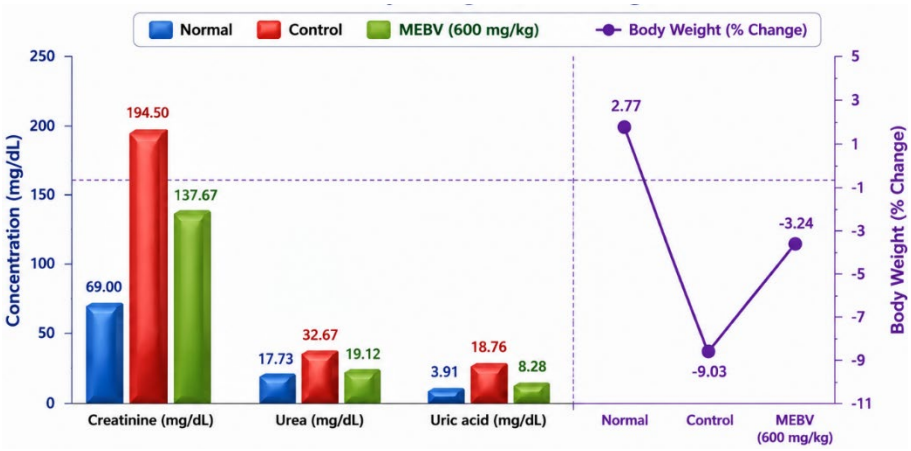

Figure 4. Effect of MEBV *Bauhinia variegata* on Kidney and Body Weight Percentage Change

3.2. Effect on Body Weight

Normal control animals maintained positive weight gain (+2.77 ± 0.54%) over 8 days. Gentamicin administration induced notable weight loss (-9.03 ± 1.54%, $p < 0.05$), reflecting acute toxicity. Co-treatment with MEBV (600 mg/kg/day) mitigated weight loss (-3.24 ± 0.23%), indicating reduced systemic toxicity.

4. Discussion

Gentamicin-induced acute kidney injury provides an established experimental model for evaluating candidate nephroprotective agents [17, 18]. The primary site of gentamicin toxicity is the renal cortex, specifically proximal convoluted tubules [19, 20]. Gentamicin accumulation inside epithelial cells initiates lysosomal swelling, membrane rupture, mitochondrial respiratory disruption, and radical generation [16-18].

A primary outcome of proximal tubular necrosis is reduced glomerular clearance capability [18, 19]. Serum creatinine and blood urea nitrogen serve as primary clinical indicators of functional filtration [18-20]. Under physiological conditions, creatinine is filtered at the glomerulus and excreted without major tubular reabsorption [19]. When tubular necrosis, lumen obstruction, or renal vasoconstriction occurs, glomerular filtration falls, causing creatinine and urea nitrogen accumulation in blood [18-20]. Gentamicin treatment in Group II produced a >78% increase in serum creatinine and a >3-fold rise in BUN, confirming acute renal injury.

Urinary parameters further reflected tubular injury. Gentamicin-induced damage alters tubular reabsorption, causing loss of solutes [18, 20]. Elevated urinary excretion of creatinine, urea, and uric acid in toxic control animals reflects tubular cell desquamation,

impaired concentrating ability, and membrane injury [18, 19]. Accompanying body weight loss in gentamicin-treated rats further illustrates systemic toxicity and metabolic disturbance secondary to acute renal failure [17-20].

Concurrent administration of MEBV at 600 mg/kg/day protected renal function against gentamicin damage. MEBV restored serum creatinine and BUN levels, improved urinary parameter profiles, and reduced weight loss.

This nephroprotection correlates directly with the phytoconstituent composition of *Bauhinia variegata* leaves [8-13]. Qualitative screening indicates that *Bauhinia variegata* leaves are rich in flavonoids (quercetin, kaempferol, rutin), polyphenols, and tannins [10-13]. Flavonoids function as effective radical scavengers [8, 12], donating hydrogen atoms to neutralize superoxide anions ($O_2^{\bullet}$), hydroxyl radicals ($OH^{\bullet}$), and peroxynitrite (ONOO-) [10, 13].

Active flavonoids such as quercetin and rutin upregulate endogenous antioxidant defenses through activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway [12, 17]. Nrf2 activation increases transcription of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [10]. By enhancing cellular enzymatic defenses, MEBV reduces lipid peroxidation, maintains mitochondrial integrity, and attenuates apoptotic signaling [11, 13].

Polyphenols and tannins in MEBV also exhibit anti-inflammatory effects by suppressing NF-ϰB signaling [10, 12, 19]. Suppression of NF-ϰB decreases pro-inflammatory cytokine expression (TNF-α, IL-1β), thereby lessening renal tissue inflammation and edema [17, 19].

Comparing these results with published nephroprotection literature supports the therapeutic potential of *Bauhinia variegata*. Studies evaluating natural antioxidants against aminoglycoside toxicity show that polyphenols protect renal architecture by preserving vascular endothelial function and maintaining renal perfusion pressure [10, 14, 17]. The reduction in serum creatinine (0.86 mg/dL) and BUN (28.03 mg/dL) achieved by MEBV treatment indicates functional recovery of the filtration barrier, comparable to reference antioxidant compounds in rodent toxicity models [15-18].

Although this study provides a clear evidence of nephroprotection, several aspects require further research:

1. Histopathological evaluation using Hematoxylin & Eosin (H&E) and Periodic Acid-Schiff (PAS) staining to assess tubular necrosis, cast formation, and structural preservation.
2. Measurement of tissue-specific oxidative stress markers, including renal malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activities.
3. Isolation, characterization, and bioassay-guided fractionation of specific bioactive compounds within MEBV.
4. Extended dose-response evaluations across a wider range (100 - 800 mg/kg) to establish precise ED_{50 values and safety profiles.

5. Conclusion

Methanolic leaf extract of *Bauhinia variegata* L. shows significant nephroprotective activity against gentamicin-induced acute kidney injury in Wistar albino rats. Oral administration of the extract effectively attenuates elevated serum creatinine, blood urea nitrogen, and serum uric acid levels, while normalizing urinary metabolite excretion patterns and minimizing systemic body weight loss. These protective outcomes are mediated by the radical-scavenging, antioxidant, and anti-inflammatory properties of its rich phytoconstituent composition, particularly flavonoids and phenolic compounds. *Bauhinia variegata* leaf extract represents a viable natural candidate for development as an adjunctive therapeutic agent to prevent aminoglycoside-induced nephrotoxicity, subject to further molecular and clinical validation.

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