Evaluation of the Renal Protective Efficacy of Ethanolic Extract of *Zinnia Angustifolia* Leaves in Gentamicin-Induced Nephrotoxicity Model in Wistar Rats

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Abstract

Nephrotoxicity, a major cause of acute kidney injury, is commonly associated with aminoglycoside antibiotics such as gentamicin. The present study evaluated the nephroprotective potential of Zinnia angustifolia ethanolic leaf extract in gentamicin-induced nephrotoxic rats. Nephrotoxicity was induced by intraperitoneal administration of gentamicin (100 mg/kg) for 7 days, followed by 28 days of treatment with Z. angustifolia extract (200 and 400 mg/kg, orally) or the standard drug Cystone (500 mg/kg). Biochemical assessments included serum creatinine, urea, blood urea nitrogen (BUN), uric acid, albumin, and total protein, along with physical parameters such as body weight, urine volume, and kidney weight. Histopathological evaluation of kidney tissues was performed to confirm structural alterations. Results showed that gentamicin significantly elevated serum urea, BUN, creatinine, and uric acid, and caused marked renal histological damage. Treatment with Z. angustifolia extract produced dose-dependent improvements in biochemical markers and histoarchitecture, with the 400 mg/kg dose showing effects comparable to Cystone. These findings suggest that Z. angustifolia exerts strong nephroprotective activity, likely mediated through its antioxidant and anti-inflammatory properties, and may serve as a promising natural therapeutic option against drug-induced nephrotoxicity.

Keywords: *Nephrotoxicity, Gentamicin, Zinnia angustifolia, Antioxidant, Nephroprotection.*

1. INTRODUCTION

Nephrotoxicity refers to the toxic injury of the kidneys caused by exposure to chemical substances, drugs, or environmental agents that impair renal function and structure. The kidneys, being highly vascularized and metabolically active organs, play a crucial role in maintaining homeostasis through filtration, excretion, and regulation of electrolytes and fluid balance. [1] Due to their role in eliminating xenobiotics, the kidneys are particularly vulnerable to toxic insults, making nephrotoxicity a significant concern in clinical pharmacology and toxicology.[2-3] A wide range of therapeutic agents have been implicated in nephrotoxicity, including aminoglycoside antibiotics[4] (e.g., gentamicin, tobramycin), non-steroidal anti-inflammatory drugs[5] (NSAIDs), chemotherapeutic agents[6] like cisplatin, and immunosuppressants such as cyclosporine and tacrolimus. These drugs can induce renal damage through mechanisms such as oxidative stress, mitochondrial dysfunction, and direct tubular toxicity.[7] In addition to pharmaceuticals, various industrial chemicals and environmental pollutants—such as ethylene glycol, carbon tetrachloride, and halogenated hydrocarbons—also pose nephrotoxic risks.[8] Heavy metals like lead, mercury, cadmium, and arsenic are particularly notorious for their cumulative nephrotoxic effects. These metals can disrupt renal tubular function, impair glomerular filtration, and provoke inflammatory responses, often leading to chronic kidney disease upon prolonged exposure. The nephrotoxicity induced by these agents is often dose-dependent and may be exacerbated by factors such as age, pre-existing renal conditions, and concurrent drug use.[9-10]

Drug-induced nephrotoxicity is one of the leading causes of acute kidney injury (AKI), especially in hospitalized patients. Among the various nephrotoxic agents, aminoglycoside antibiotics such as gentamicin are well-documented for their renal toxicity. Gentamicin accumulates in the renal proximal tubule cells, where it induces oxidative stress, disrupts mitochondrial function, and triggers inflammatory and apoptotic pathways.[11-12] These events lead to tubular necrosis, glomerular damage, and a marked decline in renal function, often reflected by elevated serum creatinine and blood urea nitrogen (BUN) levels.[13] The pathogenesis of nephrotoxicity involves multiple mechanisms, including the generation of reactive oxygen species (ROS), lipid peroxidation, and activation of pro-inflammatory cytokines.[14] These molecular events compromise the integrity of renal tissues and contribute to progressive renal failure if left unchecked. While several pharmacological agents are available to manage nephrotoxicity, their efficacy is often limited, and adverse effects may further complicate treatment.[15-16]

Zinnia angustifolia, commonly known as creeping zinnia, is a flowering herbaceous plant belonging to the family Asteraceae. Native to Mexico and the southwestern United States, it thrives in dry, sandy soils and is widely cultivated for its ornamental value due to its vibrant blooms and hardy nature. Beyond its aesthetic appeal, *Z. angustifolia* has gained recognition in traditional medicine for its therapeutic potential, particularly in the treatment of skin disorders, urinary tract ailments, and inflammatory conditions.[17-19] Phytochemical investigations have revealed that *Z. angustifolia* contains a diverse array of bioactive compounds, including flavonoids, saponins, alkaloids, and phenolic acids, which contribute to its pharmacological profile.[20-22] These constituents exhibit antioxidant, anti-inflammatory, analgesic, hepatoprotective, and antibacterial activities, making the plant a promising candidate

for drug development and integrative therapeutic applications. Recent studies have highlighted its diuretic and anti-inflammatory effects, which are particularly relevant in the context of nephrotoxicity, where oxidative stress and inflammation play central roles in renal injury. The presence of flavonoids and saponins in *Z. angustifolia* may help mitigate renal damage by scavenging reactive oxygen species (ROS), modulating cytokine expression, and stabilizing renal cellular architecture.[23-24]

2. METHODOLOGY

2.1 Collection and Authentication of Plant Material

Fresh leaves of Zinnia angustifolia were procured from the local market in Charbagh, Lucknow, Uttar Pradesh, India, during August, 2024. The plant specimen was authenticated by the Department of Botany at Banaras Hindu University (BHU), Varanasi.

2.2 Preparation of Ethanolic Extract

The collected leaves were washed thoroughly under running tap water to eliminate dust and surface contaminants. Cleaned leaves were shade-dried in a well-ventilated area to preserve thermolabile phytoconstituents. Once dried, the leaves were ground into coarse powder using a mechanical grinder. For extraction, the powdered material was subjected to cold maceration using 95% ethanol in a 1:5 (w/v) ratio. The mixture was kept in a sterile, airtight glass container at room temperature for 48 hours, with intermittent stirring every 6–12 hours to enhance solvent penetration and phytochemical release. After maceration, the extract was filtered using muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator and stored in a refrigerator at 4°C until further use.

2.3 Experimental Animals

Healthy adult Albino Wistar rats (150–200 g) of either sex were obtained from a CPCSEA-registered supplier. The animals were housed in polypropylene cages (six rats per cage) under controlled environmental conditions: temperature 25 ± 2°C, relative humidity 45–55%, and a 12-hour light/dark cycle. Rats were acclimatized for 7 days prior to experimentation and provided with standard pellet diet and water ad libitum. All experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Sardar Patel University, in accordance with CPCSEA guidelines.

2.4 Nephroprotective activity

Nephrotoxicity was induced in Groups II–V by intraperitoneal administration of Gentamicin (100 mg/kg/day) for 7 consecutive days. This model is well-established for mimicking acute renal injury via oxidative stress and tubular necrosis. A total of 24 rats were randomly divided into five groups (n = 4 per group) and treated for 35 days as follows

Table.1: Experimental Grouping and Treatment Protocols in Gentamicin-Induced Nephrotoxicity Model

Group	Treatment Type	Treatment Protocol	Dose
Group I	Normal Control	Received 0.9% NaCl orally once daily throughout the study period.	–
Group II	Disease Control	Gentamicin administered i.p. for 7 days to induce nephrotoxicity. No further treatment for the remaining 28 days.	100 mg/kg
Group III	Low-Dose Extract	After gentamicin induction, received *Z. angustifolia* extract orally for 28 days.	200 mg/kg
Group IV	High-Dose Extract	After gentamicin induction, received *Z. angustifolia* extract orally for 28 days.	400 mg/kg
Group V	Standard Treatment	After gentamicin induction, received Cystone orally for 28 days as a reference nephroprotective agent.	500 mg/kg

2.5 Biochemical Evaluation

Physical parameters included the regular monitoring of body weight of experimental animals throughout the study period, as variations in body weight reflect both general health status and drug-related toxicities. At the end of the experimental protocol, the animals were sacrificed, and kidneys were excised for the measurement of absolute and relative organ weight. In addition, the morphology of the kidneys, including their size, shape, and color, was observed to identify any gross pathological changes.

The biochemical assessment focused on key indicators of renal function and protein metabolism. Blood samples were analyzed for serum creatinine, urea, blood urea nitrogen (BUN), uric acid, albumin, and total protein. These parameters are widely recognized as reliable markers of kidney function, providing insight into filtration efficiency, protein turnover, and metabolic balance. Elevated levels of creatinine, urea, BUN, and uric acid are indicative of impaired renal clearance, whereas changes in albumin and total protein reflect alterations in protein metabolism and possible kidney damage. Together, these biochemical measurements served to evaluate the severity of nephrotoxicity and the protective effect of the treatments administered.

Table.2. Biochemical Parameters Used in Renal Function Analysis

Parameter	Method Used	Principle
Serum Uric Acid	Uricase–Peroxidase Method	Uric acid is oxidized by uricase to allantoin; the generated H_2O_2 is quantified via peroxidase reaction.

Serum Creatinine	Jaffe's Alkaline Picrate Method	Creatinine reacts with picrate in alkaline medium, forming a colored complex measured spectrophotometrically.
Serum Albumin	Bromocresol Green (BCG) Method	Albumin binds with BCG dye to form a green-colored complex, measured at 630 nm.
Serum Urea	Diacetyl Monoxime Method	Urea reacts with diacetyl monoxime under acidic conditions to produce a chromogen.
Blood Urea Nitrogen (BUN)	Calculated from Serum Urea	BUN value is derived from urea concentration using the conversion factor (BUN = Urea ÷ 2.14).
Total Protein	Biuret Method	Proteins react with copper ions in alkaline solution, forming a violet-colored complex.

2.6 Histopathological Evaluation

Kidney tissues were excised and fixed in 10% neutral buffered formalin. The samples were dehydrated through a graded alcohol series, embedded in paraffin, and sectioned at 5 μm thickness using a rotary microtome. The sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope at 10x and 40x magnifications to evaluate structural and pathological changes.

3. RESULT AND DISCUSSION

3.1 Effect of Treatments on Body Weight, Urine Output, and Kidney Weight

The data demonstrate the impact of different treatments on body weight, urine output, and kidney weight in gentamicin-induced nephrotoxic rats. The disease control group (G-II) showed a marked reduction in body weight and urine volume compared to the normal control (G-I), indicating renal impairment and systemic toxicity. Treatment with Zinnia angustifolia extract (G-III and G-IV) produced dose-dependent improvements, reflected by increases in body weight and urine output, with the high dose (400 mg/kg) showing effects comparable to the standard drug, Cystone (G-V). Kidney weights remained relatively stable across groups, though slight normalization was observed in treated animals.

Table.3: Impact of Treatments on Growth and Renal Indices in Gentamicin-Treated Rats

Group	Treatment	Dose (mg/kg)	Body Weight (g)	Urine (ml)	Kidney Weight (g)
G-I	Normal Saline (Control)	–	208.35 ± 14.92	8.60 ± 1.15	0.64 ± 0.09
G-II	Gentamicin	100 (i.p.)	137.10 ± 17.50	3.00 ± 0.58	0.46 ± 0.07

G-III	GN + *Zinnia angustifolia*	200 (oral)	145.67 ± 16.89	5.70 ± 0.80	0.58 ± 0.07
G-IV	GN + *Zinnia angustifolia*	400 (oral)	178.22 ± 15.35	8.35 ± 1.20	0.53 ± 0.08
G-V	GN + Cystone	500 (oral)	192.40 ± 15.65	7.20 ± 0.90	0.56 ± 0.07

The results are presented as Mean ± SEM for each group (n=4). Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test. Differences were considered statistically significant at $P<0.05$ compared with the normal control, while a threshold of $P<0.001$ indicated a highly significant change relative to the disease control group.

3.2 Effect of Treatments on Uric Acid, Creatinine, and Albumin Levels

The biochemical findings indicate that gentamicin (G-II) markedly elevated serum uric acid and creatinine while increasing albumin levels compared to the normal control (G-I), reflecting renal dysfunction and impaired filtration. Treatment with Zinnia angustifolia extracts (G-III and G-IV) significantly reduced uric acid and creatinine levels in a dose-dependent manner, suggesting restoration of renal clearance capacity. Albumin values also approached normal, indicating reduced protein leakage. The high-dose extract (400 mg/kg) exhibited effects closely matching those of the standard nephroprotective drug Cystone (G-V). These results support the nephroprotective potential of Z. angustifolia against gentamicin-induced toxicity.

Table.4: Impact of Zinnia angustifolia Extract and Standard Drug on Renal Biomarkers

Group	Treatment	Dose (mg/kg)	Uric Acid (mg/dl)	Creatinine (mg/dl)	Albumin (mg/dl)
G-I	Normal Saline (Control)	–	1.89 ± 0.22	0.40 ± 0.08	1.20 ± 0.15
G-II	Gentamicin	100 (i.p.)	2.85 ± 0.20#	2.60 ± 0.20#	1.50 ± 0.07#
G-III	GN + *Zinnia angustifolia*	200 (oral)	1.95 ± 0.19***	0.58 ± 0.15***	1.28 ± 0.10**
G-IV	GN + *Zinnia angustifolia*	400 (oral)	1.78 ± 0.23***	0.38 ± 0.14***	1.18 ± 0.09**
G-V	GN + Cystone	500 (oral)	1.75 ± 0.25***	0.54 ± 0.10***	1.15 ± 0.08***

Values are expressed as Mean ± SEM (n=4). Statistical analysis was carried out using one-way ANOVA followed by Dunnett's multiple comparison test. A probability value of $P<0.05$ was considered significant compared to the normal control, whereas **$P<0.001$ indicated a highly significant difference in relation to the disease control group.

3.3 Effect of Treatments on Urea, BUN, and Total Protein Levels

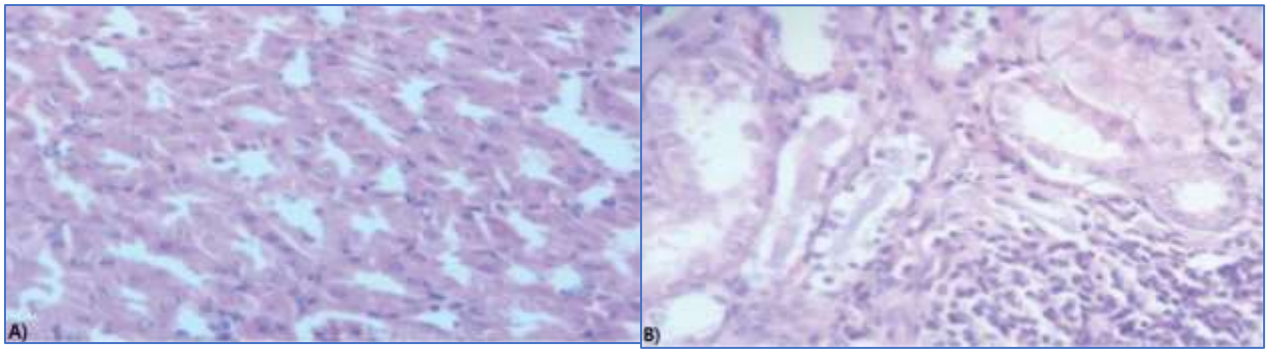
The results indicate that gentamicin administration (G-II) caused a significant elevation in serum urea and BUN levels compared to the normal control, confirming renal impairment, while total protein levels also showed a slight increase. Treatment with *Zinnia angustifolia* extract (G-III and G-IV) produced a dose-dependent improvement, as reflected by the reduction in urea and BUN values along with normalization of protein levels. The higher dose (400 mg/kg) demonstrated more pronounced nephroprotective effects, comparable to the standard reference drug Cystone (G-V). These findings suggest that Zinnia angustifolia exhibits substantial protective potential against gentamicin-induced renal dysfunction.

Table.5: Effect of *Zinnia angustifolia* Extract on Serum Urea, BUN, and Total Protein Levels in Gentamicin-Induced Nephrotoxicity in Rats

Group	Treatment	Dose (mg/kg)	Urea (mg/dl)	BUN (mg/dl)	Total Protein (g/dl)
G-I	Normal Saline (Control)	–	27.95 ± 1.80	12.45 ± 1.10	6.80 ± 0.25
G-II	Gentamicin	100 (i.p.)	42.15 ± 2.10#	21.67 ± 1.82#	8.10 ± 0.30#
G-III	GN + *Zinnia angustifolia*	200 (oral)	32.89 ± 3.15***	17.89 ± 1.92**	7.20 ± 0.28**
G-IV	GN + *Zinnia angustifolia*	400 (oral)	30.75 ± 2.35***	16.23 ± 1.50***	6.90 ± 0.22***
G-V	GN + Cystone	500 (oral)	31.78 ± 2.60**	16.98 ± 1.58***	7.00 ± 0.24***

All values are expressed as Mean ± SEM (n = 4). Data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. Significance was considered at $P < 0.05$. # denotes a significant difference versus the normal control, while *, **, and *** indicate $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively, when compared with the disease control.

3.4 Histopathological Studies



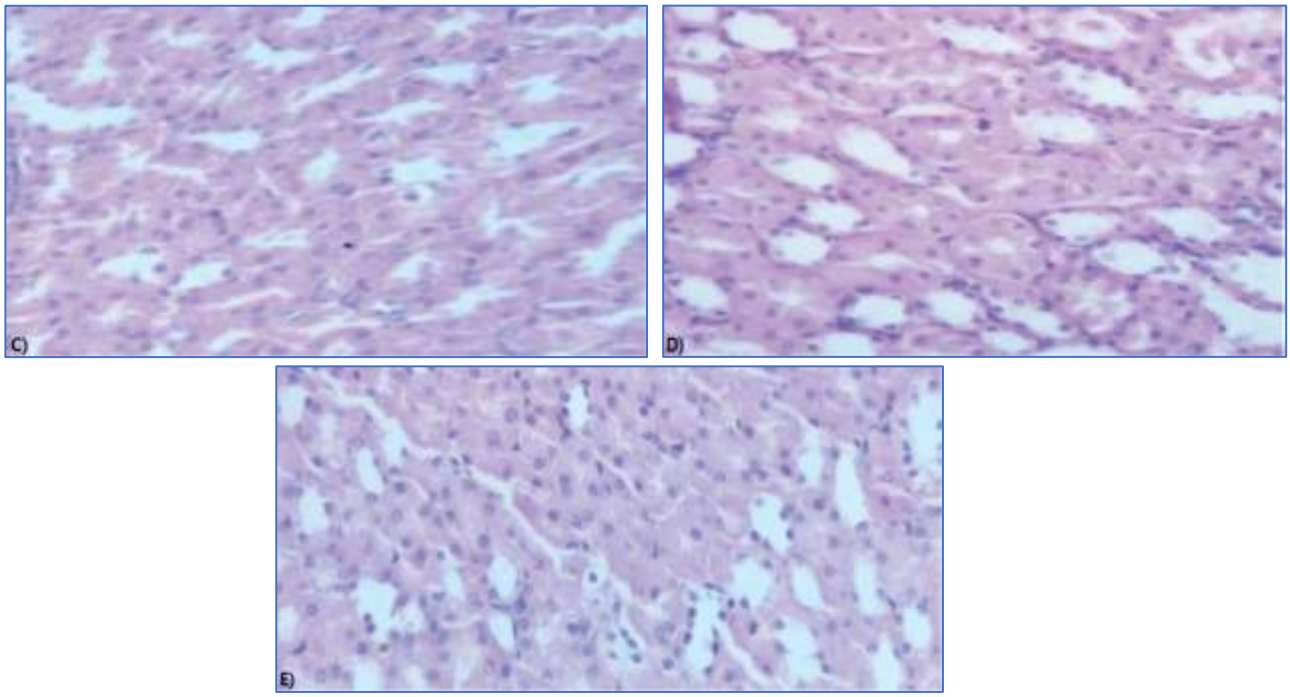


Fig.1: Histopathological Evaluation (A) Normal Control (Saline), (B) Disease Control (Gentamicin), (C) Standard Drug (Cystone) (D) Test Group (Zinnia 200 mg/Kg) (E) Test Group (Zinnia 400 mg/Kg)

Histological analysis of kidney tissues in the control group (G-I) showed normal renal architecture with intact glomeruli, Bowman's capsules, and tubules, without any pathological changes. In contrast, the toxicant group (G-II) exhibited marked glomerular distortion, capillary congestion, hemorrhage, and tubular damage, confirming acute nephrotoxicity. Groups treated with the protective agents (G-III, G-IV, and G-V) displayed notable improvements, with reduced congestion and tubular injury compared to G-II. Among them, the higher dose group (G-IV, 400 mg/kg) showed near-normal renal structure with minimal abnormalities, supported by biochemical values close to normal, indicating superior nephroprotection over the lower dose.

CONCLUSION

The present study clearly establishes that gentamicin administration produces significant nephrotoxic effects, reflected in altered biochemical markers such as elevated serum urea, BUN, creatinine, and uric acid, along with reduced protein balance, as well as pronounced histopathological damage including glomerular distortion, tubular injury, and hemorrhagic lesions. These findings confirm the reliability of the gentamicin-induced nephrotoxicity model. Treatment with *Zinnia angustifolia* extract demonstrated marked nephroprotective potential, restoring both biochemical and histological parameters toward near-normal values. The extract at 200 mg/kg offered moderate protection, while the higher dose (400 mg/kg) produced more pronounced improvements, as evidenced by preservation of renal architecture, reduced tubular damage, and normalization of biochemical indices. The efficacy of the higher dose was

comparable to that of the standard nephroprotective drug, Cystone, indicating the therapeutic relevance of the plant extract.

Overall, the results suggest that *Z. angustifolia* exerts dose-dependent protective effects against gentamicin-induced renal injury, likely through its antioxidant, anti-inflammatory, and tissue-preserving properties. These findings provide strong experimental support for its potential application as a natural nephroprotective agent and justify further exploration into its phytoconstituents and underlying mechanisms of action.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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