RESEARCH PAPER

Nephroprotective activity of *Zanthoxylum armatum* bark extract against gentamicin-induced nephrotoxicity in experimental rats

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Abstract

Background/Aim: To evaluate the nephroprotective potential of *Zanthoxylum armatum* (*Z. armatum*) bark extract (MZA) against gentamicin (GM) induced nephrotoxicity in experimental rats.

Methodology: Wistar albino rats, weighing between 150 and 200 grams, were split into five groups: normal saline, oral gentamicin 40 mg/kg for 42 days, and oral methanolic extract of *Zanthoxylum armatum* bark at 100, 200, and 400 mg/kg for 42 days. Analyses of serum creatinine, blood urea, blood urea nitrogen, uric acid, renal enzymatic and non-enzymatic antioxidants, lipid peroxidation, and kidney histopathology were carried out.

Results: Electrolyte imbalance, elevated renal biomarkers, decreased antioxidant enzyme activity, and increased lipid peroxidation all showed that gentamicin significantly impaired the kidneys and produced oxidative stress ($p < 0.001$). Over the course of 42 days, treatment with the methanolic extract considerably and dose-dependently reduced these changes, restoring renal function indicators, electrolyte balance, and antioxidant status, extract demonstrating the extract's strong nephroprotective and antioxidant efficacy.

Conclusion: These results substantiate the ethnomedical usage of *Zanthoxylum armatum* bark extract by providing strong evidence that it functions in the kidney as a powerful scavenger of free radicals to counteract the harmful effects of GM in both biochemical and histological parameters.

Key words: *Zanthoxylum armatum,* nephrotoxicity, extract, antioxidant, blood, biochemical, oxidative stress.

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Introduction

The third most prevalent cause of acute kidney injury (AKI), a common kind of acute renal illness, is drug abuse that culminates in nephrotoxicity. The mechanisms via which AKI increases the likelihood of negative effects are based on uncertainty.[1] Therefore, using animal models to test new therapies and understand physiological processes is crucial. Animal models are essential for comprehending the pathophysiology of kidney diseases and creating reliable preclinical testing procedures for innovative therapeutics.[2] Although a number of alternative models have been created, rodents are the most widely used experimental model for AKI and chronic kidney disease (CKD).[3] One of the interesting features of pre-renal AKI is the reversible recovery. Oxidative stress and inflammatory response are important factors in the development of AKI, which is induced by a number of intricate pathways.[4-5] several medications, chemicals, and environmental pollutants, including antibiotics, significantly change the structure and function of different tissues and cause several negative consequences in the colon, liver, kidney, and heart.[6]

Severe infections of the abdomen and urinary system are often treated with aminoglycoside medicines.[7] Gentamicin (GM) is still regarded as a crucial aminoglycoside antibiotic for treating potentially fatal bacterial infections. Nephrotoxicity and ototoxicity, however, continue to be significant obstacles to its successful long-term clinical use. The buildup of GM in the renal proximal convoluted tubules, which results in tubular necrosis, appears to be linked to the specificity of GM nephrotoxicity.[8] GM is known to produce a variety of morphologic, metabolic, and functional changes in the kidney.[9] Gentamicin-induced renal toxicity is a complex phenomena characterized by elevated plasma creatinine and urea levels, severe proximal renal tubular necrosis, gradual deterioration, and renal failure.[10] Aminoglycoside-induced nephrotoxicity has been linked to the production of reactive oxygen species (ROS) in the kidney. The sensitivity to oxidative damage, which may change in response to oxidative stress, is significantly influenced by the cellular antioxidant level. Numerous investigations have asserted that the nephroprotective benefits of medications in gentamicin-induced kidney injury are mostly dependent on their antioxidant properties.[11-14]

Zanthoxylum armatum DC. (family: Rutaceae), also known as Timur, is a fragrant perennial shrub or small tree with thick, glabrous leaves with straight prickles that can reach a height of 6 m. The northeastern region of Bhutan, India, China, Pakistan, North and South Korea, Japan, Laos, North Vietnam, Taiwan, and Myanmar are all affected by it.[15] Alkaloids, sterols, terpenoids, phenolics, lignans, coumarins, benzenoids, flavonoids, fatty acids, amino acids, L-sesamin, geraniol, linalool, L-asarin, methyl cinnamate, alkenic acids, glycosides, limonene, linayl acetate, linalool, methyl cinnamate, armamide, L-planinin, citral, and sabines are among the plant's bioactive substances.[16] The fruits, stem, leaves, and bark of Z. armatum DC. have all been employed as appetizers, antipyretics, diuretics, and anti-inflammatory drugs in several traditional medicinal procedures. The stem bark aqueous extract of *Zanthoxylum armatum* DC. has been used to investigate the antioxidant and antidiabetic properties and has been proven to be non-toxic at various dosage rates.[17] Z. armatum DC. is highly sought after both domestically and abroad due to its pharmacological characteristics.[18]

Nephroprotective and antioxidant qualities are among the components of herbal treatments that help prevent kidney injury.[19] The goal of the current study was to assess Z. armatum DC. methanolic extract's nephroprotective effectiveness against gentamicin-induced nephrotoxicity in Wistar albino rats. The reduction of oxidative stress, hematological alterations, and kidney damage were deemed the primary outcome measures. To the best of our knowledge, its traditional claim of nephroprotective ability has not been supported by any scientific findings. Thus, the goal of the current investigation was to show how Z. armatum bark extract (MZA) protects experimental rats' kidneys from gentamicin-induced damage.

Methods

Chemicals

Gentamicin from the parenthetically specified source (Nicholas, Mumbai, India). All of the analytical-grade chemicals were purchased from Qualigens Fine Chemicals in Mumbai, India, and Sigma Chemicals Co. in the United States.

Preparation of plant extract

The entire *Z. armatum* plant was collected in February from the Uttarkashi, Uttarakhand. The plant specimens (923) were authenticated by BSI Dehradun, Uttarakhand. After being shade-dried and roughly ground, the bark was extracted. 500 g of powdered material was extracted using a Soxhlet apparatus and 100% methanol for 72 hours. Before being used, the extract was filtered, condensed under low pressure, and kept at 4 °C. 56.9 g of solid residue (yield 11.38% w/w) was the result. The extracted material underwent further pharmacological analysis.

Animals

For a week before to and during the studies, six to eight mature female and male wistar rats (weighing between 150 and 200 grams) were housed in a departmental animal house in a well-ventilated room at 22±1°C with 12-hour light and dark cycles. The animals were fed a regular mouse pellet diet (Amrut, India), but they were allowed unlimited access to water until 18 to 24 hours before to the experiment. The experiment was conducted in accordance with the guidelines established by the CPCSEA, India's Institutional Animal Care Committee.

Acute toxicity study

To find out if the aqueous extracts had any harmful consequences, a different experiment was conducted. After a 12-hour fast, the rats were split into seven groups of six rats per cage: drug-treated test groups and vehicle-treated control groups. The rats in each test group received different oral doses of the methanolic extract (100, 200, 400, 800, 1600, and 3200 mg/kg body weight). The vehicle alone (DMSO 0.5%; 1 mL/kg body weight) was administered to every rat in the control groups. Over the course of 24 hours to 21 days, the rats in the test and control groups were given access to food and water, and behavioural changes were monitored for indications of either acute or chronic poisoning. During this time, the number of deaths brought on by the extracts was noted. The median lethal dosage (LD50) of each extract was calculated using log dose-response graphs that were created for each extract.[20]

Experimental design

Six experimental groups of eight Wistar albino rats each were assigned at random. For 42 days, Group A, the normal control, was given 0.5% carboxymethyl cellulose (CMC) orally and norma saline intraperitoneally. Gentamicin was given orally once for 42 days at a dosage of 40 mg/kg to Group B, which served as the positive control. For 42 days, groups C, D, and E were given oral gentamicin (40 mg/kg) along with the methanolic extract at dosages of 100, 200, and 400 mg/kg, respectively. Every seven days, the animals' body weight, water consumption, urine volume, urine pH, and urine protein/albumin were measured (Days 7, 14, 21, 28, 35, and 42). At baseline (Day 0), Day 7, Day 28, and Day 42, blood samples (≈100–200 µL, micro-sampling) and urine were obtained to evaluate serum BUN, creatinine, uric acid, albumin, total protein, and calcium. On Day 43, the animals were slaughtered, and parts of their kidneys were removed, weighed, and utilized for histological examination and antioxidant enzyme measurements (SOD, CAT, GSH, and GPx).

Urine and serum biochemical study

Using commercially available kits and a Thermo Scientific Spectro 20D+ spectrophotometer, blood urea nitrogen (BUN), uric acid, and creatinine levels in serum as well as calcium, total protein, albumin, sodium, and potassium concentrations in urine were measured spectrophotometrically in accordance with the manufacturer's instructions (Tulip Diagnostics Private Limited, Goa, India).

Preparation of tissue homogenate

Following the experiment, the kidneys were removed, weighed, and homogenized using a Potter Elven Jem glass homogenizer at a concentration of 10% (w/v) in cold Tris buffer (10 mM, pH 7.4). After centrifuging homogenates at a high speed of 12,000 rpm for 20 minutes at 4 degrees Celsius, the sediment and supernatant were utilized for further biochemical measurements.

Measurement of renal antioxidants

Using the Kakkar et al. [21] approach, which is based on the suppression of nicotinamide adenine dinucleotide-phenazine methosulfate-nitro blue tetrazolium formazan production, superoxide dismutase (SOD) was measured in tissue supernatant. By calculating the rate at which hydrogen peroxide, the enzyme's substrate, degrades, the activity of catalase (CAT) in tissue supernatant was determined spectrophotometrically at 240 nm.[22] Using Ellman's reagent (dinitrothiobenzoic acid) as a colouring reagent, the reduced glutathione (GSH) level in tissue supernatant was determined spectrophotometrically using the technique outlined by Beutler et al.[23]

Measurement of renal lipid peroxidation

According to Buege and Aust, the thiobarbituric acid test was used to measure malonaldehyde as an indicator for lipid peroxidation (LPO).[24]

Histopathology Examination

After being cleaned with regular saline, the kidney was preserved in a 10% neutral buffered formalin solution, embedded in paraffin, and examined histopathologically.[25] Hematoxylin and eosin were used to stain the 2 µm thick, deparaffinized, and hydrated sections. Senior pathologists observed kidney tissue slices under a light microscope at a magnification of 400x.

Data Analysis

SPSS version 25.0 was used for the statistical analysis. Mean ± SD was used to express the quantitative data. Group comparisons were conducted using the Tukey post hoc tests and one-way ANOVA. Statistical significance is defined as $p < 0.05$. A renowned pathologist conducted the quality histopathology investigations.

Results

Effect of MZA on body weights and kidney weights against gentamicin-induced animals

The effects of MZA on body and kidney weights in different groups following gentamicin induction are displayed in Tables 1 and 2. The findings made it abundantly evident that the standard and test groups' body weights were much higher than those of the disease control group. On the other hand, gentamicin induction also caused alterations in kidney weights, whereas extract therapy caused healing. The results demonstrated that the body weight of the animals in Group B (those treated with gentamicin) was significantly ($p < 0.05$) lower than that of the normal control group (Group A). However, mice co-treated with methanolic extract at 100 mg/kg (Group C), 200 mg/kg (Group D), and 400 mg/kg (Group E) showed a significant increase in body weight ($p < 0.001$, $p < 0.01$, and $p < 0.01$, respectively) in comparison to the toxic control group (Group B).

Table 1: Effect of MZA on general parameters in gentamicin (GM)-induced nephrotoxicity in Wistar albino rats. Values are expressed as Mean ± SEM (n = 8).

Parameters Studied (Unit)	Day	Group A (Control)	Group B (GM 40 mg/kg)	Group C (GM + MZA 100 mg/kg)	Group D (GM + MZA 200 mg/kg)	Group E (GM + MZA 400 mg/kg)
Change in body wt. (g)	7th	3.02 ± 0.18	1.85 ± 0.15 **a	2.60 ± 0.14 *b	2.85 ± 0.13 **b	2.95 ± 0.14 **b
	14th	3.05 ± 0.19	1.62 ± 0.13 ***a	2.70 ± 0.15 **b	2.90 ± 0.13 **b	2.98 ± 0.14 ***b
	21st	3.04 ± 0.20	1.48 ± 0.12 ***a	2.78 ± 0.15 ***b	2.94 ± 0.14 ***b	3.01 ± 0.15 ***b
	28th	3.06 ± 0.23	1.52 ± 0.12 *a**	2.85 ± 0.16 *b**	2.98 ± 0.14 b	3.01 ± 0.15 *b**
	35th	3.07 ± 0.24	1.50 ± 0.12 ***a	2.88 ± 0.15 **b	3.00 ± 0.14 **b	3.05 ± 0.15 ***b
	42nd	3.10 ± 0.25	1.48 ± 0.11 ***a	2.90 ± 0.16 ***b	3.05 ± 0.15 ***b	3.08 ± 0.16 ***b

Where, 'a = Significant compared to normal control, b = Significant compared to GM control. * < 0.05, ** < 0.01, *** < 0.001, ns = not significant'.

GM-treated rats (Group B) had significantly ($p < 0.001$) greater kidney weight than the control group. Co-treatment with methanolic extract at 100 mg/kg ($p < 0.01$), and 400 mg/kg ($p < 0.001$)

significantly reduced kidney weight, indicating a protective effect against GM-induced renal hypertrophy.

Table 2. Effect of MZA on kidney weight in gentamicin (GM)-induced nephrotoxicity in Wistar albino rats. Values are expressed as Mean ± SEM (n = 8).

Parameters Studied (Unit)	Day	Group A (Control)	Group B (GM 40 mg/kg)	Group C (GM + MZA 100 mg/kg)	Group D (GM + MZA 200 mg/kg)	Group E (GM + MZA 400 mg/kg)
Kidney wt. (g)	43rd	0.65 ± 0.02	0.72 ± 0.03 *a	0.70 ± 0.03 b	0.68 ± 0.03 b	0.66 ± 0.03 b

Where, 'a = Significant compared to normal control, b = Significant compared to GM control. * < 0.05, ** < 0.01, *** < 0.001, ns = not significant'.

Effect of MZA on urinary creatinine and total protein levels against gentamicin-induced animals

Urinary albumin and total protein excretion were significantly higher in the gentamicin-treated rats (Group B) than in the control group ($p < 0.001$), suggesting substantial renal tubular and glomerular damage. Proteinuria was significantly ($p < 0.01$) attenuated when Z. armatum methanolic extract (Groups C–E) were administered together. In comparison to the hazardous group (Group B), the higher dose of MZA (Group E) showed the largest reduction in urine albumin levels ($p < 0.01$) among the treated groups, demonstrating improved nephroprotective effectiveness (Table 3).

Table 3. Effect of MZA on urinary albumin levels and total protein in experimental rats

Parameters Studied (Unit)	Day	Group A (Control)	Group B (GM 40 mg/kg)	Group C (GM + MZA 100 mg/kg)	Group D (GM + MZA 200 mg/kg)	Group E (GM + MZA 400 mg/kg)
Albumin (g/dL)	7th	0.76 ± 0.02	0.89 ± 0.05 a	0.85 ± 0.05 ns	0.81 ± 0.04 *b	0.78 ± 0.04 b
	14th	0.77 ± 0.02	0.92 ± 0.06 a	0.83 ± 0.05 ns	0.79 ± 0.04 *b	0.75 ± 0.04 b
	21st	0.77 ± 0.01	0.88 ± 0.06 a	0.82 ± 0.05 ns	0.77 ± 0.04 b	0.73 ± 0.04 *b
	28th	0.77 ± 0.01	0.84 ± 0.06 a	0.80 ± 0.05 ns	0.75 ± 0.04 *b	0.71 ± 0.04 b
	35th	0.77 ± 0.01	0.83 ± 0.05 a	0.79 ± 0.05 ns	0.74 ± 0.04 *b	0.70 ± 0.04 b
	42nd	0.77 ± 0.01	0.82 ± 0.05 a	0.78 ± 0.05 ns	0.73 ± 0.04 *b	0.69 ± 0.04 b
Total Protein (g/dL)	7th	3.64 ± 0.02	4.60 ± 0.03 *a	4.30 ± 0.04 b	4.10 ± 0.03 b	3.90 ± 0.05 *b
	14th	3.65 ± 0.02	4.70 ± 0.03 *a	4.20 ± 0.04 b	3.95 ± 0.03 b	3.72 ± 0.05 *b
	21st	3.65 ± 0.01	4.62 ± 0.03 *a	4.12 ± 0.04 b	3.85 ± 0.03 b	3.60 ± 0.05 *b
	28th	3.65 ± 0.01	4.52 ± 0.02 *a	4.05 ± 0.04 b	3.78 ± 0.03 b	3.53 ± 0.05 *b
	35th	3.65 ± 0.01	4.48 ± 0.02 *a	4.00 ± 0.04 b	3.74 ± 0.03 b	3.50 ± 0.05 *b
	42nd	3.65 ± 0.01	4.45 ± 0.02 *a	3.96 ± 0.04 b	3.70 ± 0.03 b	3.47 ± 0.05 *b

Where, 'a = Significant compared to normal control, b = Significant compared to GM control. * < 0.05, ** < 0.01, *** < 0.001, ns = not significant'.

Effect of MZA on urinary calcium, potassium, magnesium and sodium levels

When compared to the normal control group, gentamicin treatment significantly disrupted blood electrolyte levels, as seen by changes in potassium, sodium, and calcium concentrations ($p < 0.05$–0.001). Electrolyte levels returned to normal in a dose-dependent manner when the methanolic extract was administered concurrently. When compared to the gentamicin control group, the medium and high dosages of the extract (200 and 400 mg/kg) demonstrated statistically significant improvement ($p < 0.05$–0.001), with the highest dose showing values closest to the normal control. These results show that the methanolic extract significantly protects against gentamicin-induced electrolyte imbalance (Table 4).

Table 4. Effect of MZA on urinary calcium, potassium, and sodium levels

Parameters Studied (Unit)	Day	Group A Normal Control	Group B GM 40 mg/kg	Group C GM + MZA 100 mg/kg	Group D GM + MZA 200 mg/kg	Group E GM + MZA 400 mg/kg
Potassium (mmol/L)	7th	3.20 ± 0.02	3.30 ± 0.03 a*	3.25 ± 0.02 ns	3.22 ± 0.02 ns	3.20 ± 0.02 b*
	14th	3.20 ± 0.02	3.28 ± 0.03 a*	3.23 ± 0.02 ns	3.20 ± 0.02 b*	3.19 ± 0.02 b*
	21st	3.20 ± 0.02	3.26 ± 0.03 a*	3.22 ± 0.02 ns	3.19 ± 0.02 b*	3.18 ± 0.02 b*
	28th	3.20 ± 0.02	3.23 ± 0.02 a*	3.20 ± 0.02 ns	3.17 ± 0.02 b*	3.18 ± 0.02 b*
	35th	3.21 ± 0.02	3.20 ± 0.02 ns	3.19 ± 0.02 ns	3.16 ± 0.02 b*	3.18 ± 0.02 b*
	42nd	3.21 ± 0.02	3.18 ± 0.02 ns	3.19 ± 0.02 ns	3.15 ± 0.02 b**	3.17 ± 0.02 b**
Sodium (mmol/L)	7th	74.10 ± 1.80	76.50 ± 1.88 a**	72.10 ± 1.10 a*	74.50 ± 1.85 b*	74.40 ± 1.60 b*
	14th	74.12 ± 1.82	76.10 ± 1.87 a**	72.00 ± 1.05 a*	74.40 ± 1.82 b*	74.30 ± 1.58 b*
	21st	74.14 ± 1.84	75.80 ± 1.86 a**	71.80 ± 1.00 a**	74.35 ± 1.80 b*	74.25 ± 1.55 b*
	28th	74.16 ± 1.86	75.40 ± 1.87 a**	71.66 ± 0.95 a**	74.30 ± 1.78 b*	74.22 ± 1.53 b*
	35th	74.18 ± 1.87	75.10 ± 1.84 a*	71.55 ± 0.92 a**	74.28 ± 1.75 b*	74.20 ± 1.51 b*
	42nd	74.20 ± 1.88	74.90 ± 1.82 ns	71.48 ± 0.90 a**	74.25 ± 1.72 b*	74.18 ± 1.50 b*
Calcium (mg/dL)	7th	8.92 ± 0.02	10.95 ± 0.02 a***	6.80 ± 0.03 a***	8.40 ± 0.05 b**	8.15 ± 0.05 b**
	14th	8.93 ± 0.02	10.93 ± 0.02 a***	6.65 ± 0.03 a***	8.25 ± 0.05 b**	7.95 ± 0.05 b**
	21st	8.93 ± 0.01	10.92 ± 0.01 a***	6.55 ± 0.02 a***	8.15 ± 0.05 b**	7.88 ± 0.06 b**
	28th	8.93 ± 0.01	10.90 ± 0.01 a***	6.47 ± 0.02 a***	8.05 ± 0.05 b**	7.81 ± 0.06 b**
	35th	8.94 ± 0.01	10.88 ± 0.01 a***	6.40 ± 0.02 a***	7.95 ± 0.05 b**	7.75 ± 0.06 b**
	42nd	8.94 ± 0.01	10.86 ± 0.01 a***	6.35 ± 0.02 a***	7.88 ± 0.05 b***	7.70 ± 0.06 b***

Where, 'a = Significant compared to normal control, b = Significant compared to GM control. * < 0.05, ** < 0.01, *** < 0.001, ns = not significant'.

Effect of MZA on serum creatinine, BUN, and uric acid levels in experimental rats

Serum creatinine, blood urea nitrogen (BUN), and uric acid levels were significantly elevated as compared to the normal control group at all experimental time points ($p < 0.001$), indicating a severe and persistent decline in renal function induced by gentamicin treatment (40 mg/kg). Gentamicin-induced elevations in these renal indicators were considerably reduced by co-treatment with methanolic extract (MZA) at dosages of 100, 200, and 400 mg/kg in a dose-dependent manner ($p < 0.01$–0.001 against gentamicin control). By the conclusion of the trial period, the highest dose of MZA (400 mg/kg) provided the most noticeable nephroprotective effect, with significant reductions in creatinine, BUN, and uric acid levels that were almost normal. These results show that MZA protects renal functional integrity in a dose-responsive manner and successfully reduces gentamicin-induced nephrotoxicity (Table 5).

Table 5. Effect of MZA on serum creatinine, BUN, and uric acid levels in experimental rats

Parameter	Day	Group A Normal Control	Group B GM 40 mg/kg	Group C GM + MZA	Group D GM + MZA	Group E GM + MZA

				100 mg/kg	200 mg/kg	400 mg/kg
Creatinine	7th	0.70 ± 0.01	1.85 ± 0.03 ***a	1.70 ± 0.03 **b	1.48 ± 0.02 ***b	1.15 ± 0.02 ***b
	14th	0.69 ± 0.01	1.92 ± 0.02 ***a	1.62 ± 0.03 **b	1.36 ± 0.02 ***b	1.05 ± 0.02 ***b
	28th	0.68 ± 0.00	1.98 ± 0.02 ***a	1.54 ± 0.03 **b	1.21 ± 0.02 ***b	0.89 ± 0.01 ***b
	43rd	0.67 ± 0.01	1.94 ± 0.02 *a	**1.51 ± 0.03 b	1.17 ± 0.02 ***b	0.86 ± 0.01 ***b
BUN	7th	16.10 ± 0.18	26.70 ± 0.05 ***a	23.90 ± 0.07 **b	21.34 ± 0.06 **b	17.45 ± 0.06 ***b
	14th	15.94 ± 0.16	27.92 ± 0.04 ***a	22.72 ± 0.06 **b	19.95 ± 0.04 **b	16.35 ± 0.05 ***b
	28th	15.83 ± 0.19	28.89 ± 0.02 ***a	21.46 ± 0.06 **b	18.31 ± 0.04 **b	14.89 ± 0.05 ***b
	43rd	15.75 ± 0.18	28.40 ± 0.03 *a	20.90 ± 0.05 **b	17.50 ± 0.04 **b	14.10 ± 0.05 ***b
Uric Acid	7th	2.18 ± 0.07	3.10 ± 0.02 ***a	3.06 ± 0.05 **b	2.88 ± 0.04 ***b	2.65 ± 0.03 ***b
	14th	2.16 ± 0.07	3.18 ± 0.02 ***a	3.04 ± 0.05 **b	2.81 ± 0.03 ***b	2.57 ± 0.03 ***b
	28th	2.13 ± 0.08	3.22 ± 0.01 ***a	3.01 ± 0.06 **b	2.73 ± 0.04 ***b	2.49 ± 0.03 ***b
	43rd	2.10 ± 0.08	3.18 ± 0.02 ***a	2.98 ± 0.05 **b	2.68 ± 0.03 ***b	2.45 ± 0.03 ***b

Where, 'a = Significant compared to normal control, b = Significant compared to GM control. * < 0.05, ** < 0.01, *** < 0.001, ns = not significant'.

Effect of MZA on oxidative markers

Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione (GSH) activities were significantly reduced during all observation periods (days 7–43) following gentamicin administration (40 mg/kg). Malondialdehyde (MDA) levels were concurrently elevated when compared to the normal control group ($p < 0.001$). These changes show significant lipid peroxidation and oxidative stress brought on by gentamicin use. A strong treatment-dependent impact was confirmed by one-way ANOVA, which showed statistically significant differences between the experimental groups for all antioxidant measures at each time point ($p < 0.001$).

Co-administration of the methanolic extract (MZA) resulted in a dose- and time-dependent restoration of antioxidant status; SOD, CAT, and GSH levels were significantly improved at 200 and 400 mg/kg, and MDA concentrations were significantly lower than those of the gentamicin control group ($p < 0.001$). GPx activity showed modest recovery at lower extract dosages, but at the maximum dose, which showed near-normalization of all antioxidant parameters, there was a notable boost. The strong nephroprotective and antioxidant capacity of the methanolic extract was supported by post-hoc analysis (Tukey/Dunnett), which verified that the higher extract dosages and conventional medication successfully reduced gentamicin-induced oxidative damage (Table 6).

Table 6. Effect of MZA on oxidative markers in experimental rats

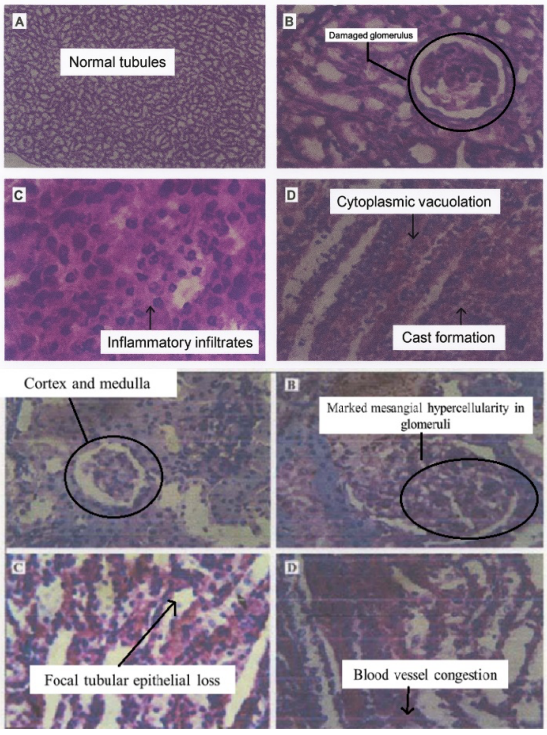
Parameter (Unit)	Day	Group A Normal Control	Group B GM 40 mg/kg	Group C GM + MZA 100 mg/kg	Group D GM + MZA 200 mg/kg	Group E GM + MZA 400 mg/kg
SOD (U/mg)	7th	12.44 ± 0.22	7.25 ± 0.20 ***a	9.40 ± 0.21 **b	10.15 ± 0.20 ***b	11.10 ± 0.22 ***b
	14th	12.45 ± 0.21	7.02 ± 0.19 ***a	9.82 ± 0.22 **b	10.60 ± 0.20 ***b	11.45 ± 0.23 ***b
	28th	12.45 ± 0.21	6.72 ± 0.18 ***a	10.35 ± 0.22 ***b	11.02 ± 0.20 ***b	11.86 ± 0.24 ***b
	43rd	12.45 ± 0.22	6.60 ± 0.18 ***a	10.50 ± 0.23 ***b	11.10 ± 0.21 ***b	11.90 ± 0.24 ***b
CAT (U/mg	7th	18.20 ±	10.20 ±	14.55 ±	15.25 ±	16.60 ±

)		0.33	0.23 ***a	0.26 **b	0.27 ***b	0.28 ***b
	14th	18.22 ± 0.32	9.95 ± 0.22 ***a	15.05 ± 0.25 **b	15.80 ± 0.27 ***b	16.95 ± 0.29 ***b
	28th	18.23 ± 0.32	9.45 ± 0.21 ***a	15.67 ± 0.25 **b	16.42 ± 0.27 ***b	17.34 ± 0.30 ***b
	43rd	18.23 ± 0.33	9.30 ± 0.21 ***a	15.80 ± 0.25 **b	16.60 ± 0.27 ***b	17.50 ± 0.30 ***b
GSH (µmol /mg)	7th	9.50 ± 0.18	4.75 ± 0.16 ***a	5.85 ± 0.18ns	6.42 ± 0.18ns	7.45 ± 0.20 ***b
	14th	9.51 ± 0.18	4.50 ± 0.16 ***a	6.10 ± 0.18ns	6.88 ± 0.19ns	7.85 ± 0.20 ***b
	28th	9.52 ± 0.18	4.21 ± 0.15 ***a	6.58 ± 0.18ns	7.35 ± 0.19ns	8.25 ± 0.21 ***b
	43rd	9.52 ± 0.18	4.10 ± 0.15 ***a	6.70 ± 0.18ns	7.50 ± 0.19ns	8.35 ± 0.21 ***b
GPx (U/mg)	7th	7.82 ± 0.17	3.45 ± 0.13 ***a	3.55 ± 0.14ns	3.75 ± 0.15ns	4.02 ± 0.16ns
	14th	7.84 ± 0.16	3.34 ± 0.13 ***a	3.65 ± 0.14ns	3.95 ± 0.15ns	4.20 ± 0.17ns
	28th	7.85 ± 0.16	3.22 ± 0.12 ***a	3.75 ± 0.14ns	4.10 ± 0.15ns	4.35 ± 0.17ns
	43rd	7.86 ± 0.16	3.05 ± 0.12 ***a	3.90 ± 0.14ns	4.30 ± 0.15ns	4.55 ± 0.17ns
MDA (nmol /mg)	7th	2.42 ± 0.10	5.55 ± 0.13 ***a	4.40 ± 0.12 **b	4.10 ± 0.12 **b	3.75 ± 0.11 **b
	14th	2.41 ± 0.10	5.65 ± 0.13 ***a	4.22 ± 0.12 **b	3.95 ± 0.11 **b	3.55 ± 0.11 **b
	28th	2.41 ± 0.10	5.75 ± 0.14 ***a	4.05 ± 0.12 **b	3.72 ± 0.11 **b	3.35 ± 0.10 **b
	43rd	2.41 ± 0.10	5.90 ± 0.14 ***a	3.80 ± 0.12 **b	3.40 ± 0.11 **b	3.05 ± 0.10 **b

Where, 'a = Significant compared to normal control, b = Significant compared to GM control. * < 0.05, ** < 0.01, *** < 0.001, ns = not significant'.

Histopathological studies

The kidney histology of Group I rats displays normal tubules and epithelial lining. Gentamicin-treated rats (Group B) showed signs of acute nephrotoxicity, including blood vessel congestion, tubular necrosis, tubular epithelial degeneration, and mesangial hypercellularity in the glomeruli. Mesangial hypercellularity in proximal tubular cells and mild to moderate cytoplasmic vacuolation were seen in the kidneys of rats co-treated with low-dose MZA (100 mg/kg, Group C). Animals in Group III are exhibiting comparably typical behaviour. Animals in Groups IV and V exhibit a decrease in the characteristics listed in Group II. Fig. 1 displayed the outcomes.



A)

B)

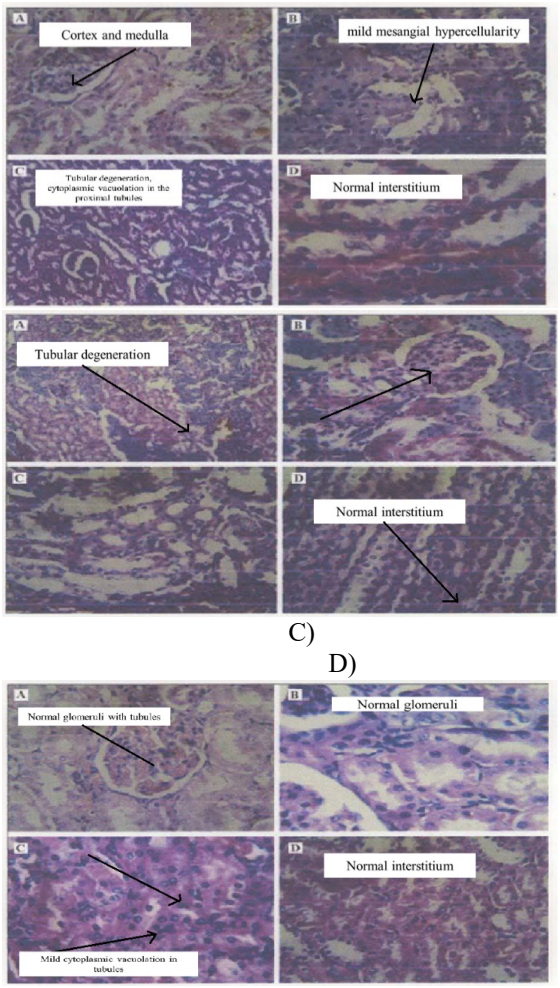


Figure 1. Histopathology results of kidney tissues of A) Normal rats (control); B) Group B (Gentamicin 40mg/kg) rats; C) Group C (GM + MZA 100 mg/kg) rats; D) Group D (GM + MZA 200 mg/kg) rats; E) Group E (GM + MZA 400 mg/kg) rats.

Discussion

In general, nephrotoxicity is an undesirable side effect of chemotherapy. The majority of chemotherapy medications target pathways necessary for cell division.[26] The significance of reactive oxygen metabolites in gentamicin-induced kidney injury has now been shown by a number of investigations.[27] Depending on the medications' affinity for the kidneys and the speed of the drug trapping process, nephrotoxicity is typically linked to the drugs' accumulation in the renal cortex.[28] There is ample evidence of the nephrotoxicity of aminoglycoside antibiotics, particularly gentamicin, the most widely used molecule.[29] Oxygen-free radicals are thought to be significant mediators of GM-induced acute renal failure, according to a number of studies.

The primary bactericidal action of gentamicin, which is a member of the aminoglycoside family of medications, is the suppression of protein synthesis. Ototoxicity and nephrotoxicity are two of gentamicin's main drawbacks.[30] In animal models, AKI is caused by nephron damage in the kidneys. Animal models of kidney injury are often developed with gentamicin antibiotics. The doses of antibiotics used to cause kidney damage vary greatly. Phospholipidosis and gentamicin toxicity are closely related.[31] Numerous therapies have been proposed to address this issue, including genetic modifiers, inflammatory mediators, and antioxidants.[32] Many antioxidants have been studied to protect the physiological traits and kidney function, but none have yet been proven to be clinically useful. In light of this concern, efforts have been made to reduce kidney injury by substituting natural antioxidant sources for synthetic antioxidants.[33]

The study's findings demonstrated that gentamicin at a dose of 40 mg/kg causes significant nephrotoxicity, as shown by the kidneys' histological alterations, which include tubular necrosis, tubule dilatation, tubular epithelial cell degeneration with casts in the tubular lumen, cell infiltration in the interstitium, glomeruli congestion, and extensive necrosis accompanied by changes in the corresponding biochemical parameters, such as elevated serum urea, creatinine, uric acid, and blood urea nitrogen. The current biochemical and histological results in the gentamicin-treated group are consistent with earlier findings.[34,35] By considerably lowering the scores of histological damages in comparison to the gentamicin-treated group, concurrent administration of Z. armatum appears to lessen the severity of the gentamicin-induced renal necrosis, preserving the tubular histology. At the greatest dose, tubule regeneration was clearly visible. A clear indication of the extract's nephroprotective properties is the restoration of the glomerulus and renal tubules in the Z. armatum-treated group.

Z. armatum DC. has been used extensively in the traditional indigenous medicinal practices of several ethnic groups in India and Nepal. Z. armatum DC. seeds and bark are used as an aromatic and carminative, as well as a tonic for fever and dyspepsia.[36] Berberine is a non-basic quaternary benzylisoquinoline plant alkaloid with a recognized medicinal history in Ayurvedic and Chinese medicine. The rhizome, stem bark, and roots of many important medicinal plants contain the active substance berberine.[37] Alkaloids are a type of basic nitrogen-containing chemical compounds that are mostly found in plants; however, they are also infrequently found in fungi and animals. Among the many physiological and pharmacological characteristics of alkaloids, which are secondary metabolites of plants, are antibacterial, anti-inflammatory, antioxidant, analgesic, and anti-tumor actions. There is an

abundance of resources for drug development because of these qualities.[38] When berberine reacts with its hydrogen donors, it reduces the radical to the matching hydrazine, demonstrating its antioxidant properties.[39] As evidenced by the radical scavenging activity, Zanthoxylum armatum DC. The extract contains active substances that can provide free radical hydrogen and neutralize the potentially hazardous entity. The Z. armatum DC. The extract's overall antioxidant activity indicated the presence of strong reducing agents. It has been shown that the ability of several medicinal plant extracts to lower phosphomolybdate is positively correlated with their polyphenol and flavonoid content.[40]

In contrast to previously published acute models that used an 8-day treatment period, the current investigation was prolonged to 42 days in order to test the methanolic extract's sustained nephroprotective capabilities after prolonged administration, as well as the sub-chronic nephrotoxic effects of gentamicin. The results of this study unequivocally show that *Zanthoxylum armatum* DC. has considerable protective potential against acute renal damage caused by gentamicin in the rat model. This study's renoprotective impact seems to be multifaceted, involving stability of antioxidant defense mechanisms, reduction of oxidative stress, suppression of inflammatory mediators, and preservation of renal functional indicators. Gentamicin-induced oxidative damage to renal tissues was well mitigated by treatment with the methanolic extract, which led to a dose-dependent restoration of endogenous antioxidants and a significant decrease in lipid peroxidation.

Conclusion

The current study's findings demonstrate that *Z. armatum* DC. can effectively treat gentamicin-induced nephrotoxicity in a rat model. Numerous traits, such as anti-inflammatory, antioxidant, organoprotective, and the ability to block biochemical parameters that lead to renal impairment, may be the cause of this *Z. armatum* DC.'s therapeutic importance is highlighted by its capacity to control oxidative stress and inflammation, two key pathways in the pathophysiology of drug-induced nephrotoxicity. The *Z. armatum* DC. extract decreased LPO levels while increasing antioxidant enzyme levels. The study adds to the increasing amount of data supporting the use of ethnomedicinal plants as supplemental or alternative nephroprotective medicines, especially in situations when traditional treatments are either ineffective or have negative side effects. However, additional research is needed to clarify the exact molecular mechanisms, identify active phytoconstituents, evaluate long-term safety, and validate efficacy through sophisticated preclinical and carefully monitored human clinical trials to strengthen the translational value of these findings.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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