

Nephroprotective Effect of Hydroalcoholic Leaf Extract of *Tagetes erecta* L. Against Gentamicin-Induced Nephrotoxicity in Wistar Rats

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Abstract—Gentamicin is a widely used aminoglycoside antibiotic whose clinical value is limited by proximal tubular toxicity. This study evaluated the nephroprotective effect of the hydroalcoholic (ethanol:water, 70:30 v/v) leaf extract of *Tagetes erecta* L. (TE) against gentamicin-induced nephrotoxicity in Wistar rats. Leaves were defatted with petroleum ether, extracted by the Soxhlet method and screened for phytoconstituents. Thirty rats were divided into five groups (n = 6): normal control; toxicant control (gentamicin 10 mg/kg/day intraperitoneally (i.p.) for 5 days); standard (silymarin 100 mg/kg orally (p.o.) + gentamicin); and TE 50 or 100 mg/kg p.o. + gentamicin. Serum alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, albumin and uric acid were estimated, and kidneys were examined histologically. The extract contained flavonoids, tannins, saponins, glycosides, alkaloids and triterpenoids. Gentamicin significantly increased ALP (286.58 ± 0.33 U/L), BUN (41.48 ± 0.64 mg/dL) and uric acid (12.54 ± 0.36 mg/dL) and decreased albumin (2.80 ± 0.04 g/dL) compared with normal rats ($p < 0.001$), whereas the rise in creatinine was not significant. TE 100 mg/kg significantly lowered ALP (211.92 ± 3.84 U/L) and uric acid (8.29 ± 0.15 mg/dL) and restored albumin (4.45 ± 0.18 g/dL) ($p < 0.001$). Both doses reduced BUN, with a greater reduction at 50 mg/kg. Tubular necrosis, casts and inflammatory infiltration seen after gentamicin were attenuated by the extract, most clearly at 100 mg/kg. *T. erecta* leaf extract, therefore, offers partial protection against gentamicin-induced renal injury.

Index Terms—Gentamicin, Hydroalcoholic extract, Nephroprotective activity, Silymarin, *Tagetes erecta*, Wistar rats.

I. INTRODUCTION

The kidneys maintain fluid, electrolyte and acid–base balance, eliminate metabolic waste products and perform important endocrine functions. The proximal convoluted tubules, which reabsorb a large fraction of the glomerular filtrate, are particularly exposed to circulating toxic substances and are therefore highly susceptible to injury [1], [2]. Drug-induced nephrotoxicity is an important and frequently preventable cause of acute kidney injury [3], [4]. Aminoglycoside antibiotics, and gentamicin in particular, are still used for serious Gram-negative infections because of their rapid, concentration-dependent bactericidal action and low cost, but renal toxicity develops in a considerable proportion of treated patients, limiting both the dose and the duration of therapy [5], [6]. Gentamicin is freely filtered at the glomerulus, and part of the filtered drug is taken up by proximal tubular epithelial cells through the megalin–cubilin endocytic receptor complex, after which it accumulates in lysosomes, the Golgi apparatus and the endoplasmic reticulum [7], [8].

Intracellular accumulation of gentamicin leads to phospholipidosis, mitochondrial dysfunction, and excessive generation of reactive oxygen species, including hydroxyl radicals. These changes lead to lipid peroxidation, inflammation and apoptotic or necrotic death of tubular cells [9]–[11]. The resulting tubular necrosis, epithelial desquamation, and cast formation reduce glomerular filtration and cause retention of urea and other nitrogenous waste products, as reflected in serum markers such as blood

urea nitrogen, creatinine, and uric acid [5], [12], [13]. Because oxidative stress is central to this injury, antioxidant agents, including plant flavonoids and standardised extracts such as silymarin, have been widely investigated as nephroprotectants in experimental aminoglycoside models [10], [14]–[16]. *Tagetes erecta* L. (Asteraceae), commonly known as African or Aztec marigold, is native to Mexico and is now cultivated widely, including in India, as an ornamental and medicinal plant [17]. Plants of the genus are rich in flavonoids, thiophenes, carotenoids such as lutein, terpenoids and phenolic acids [18], [19]. Traditional uses of *T. erecta* include the treatment of cold-related ailments and the promotion of urination [17], and a hydroethanolic flower extract produced diuretic, natriuretic and calcium-sparing effects in normotensive and hypertensive rats [20]. Other reported activities include antioxidant and cytotoxic [19], anxiolytic [21] and antidepressant-like [22] effects, and crude extracts produced no signs of acute toxicity in mice at the doses tested [23].

Despite these reports and the plant's traditional use for renal complaints, the effect of *T. erecta* leaves on drug-induced kidney injury has not been adequately studied. The present study, therefore, evaluated the nephroprotective effect of the hydroalcoholic leaf extract of *T. erecta* against gentamicin-induced nephrotoxicity in Wistar rats. Serum biochemical markers of renal function and histopathological examination of kidney tissue were used as end points, with silymarin as the reference standard.

II. MATERIALS AND METHODS

A. Drugs and Chemicals

Gentamicin was used as the nephrotoxicant and silymarin as the standard nephroprotective drug. Serum alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, albumin and uric acid were estimated using standard analytical procedures. All other chemicals and solvents used were of analytical grade.

B. Collection and Authentication of Plant Material

Leaves of *Tagetes erecta* L. were collected from the hillside area of Tirumala, Andhra Pradesh, India, in April 2025. The plant material was identified and authenticated by Dr. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara

University, Tirupati, Andhra Pradesh, and an authentication certificate was issued.

C. Preparation of the Hydroalcoholic Extract

The collected leaves were reduced to a fine powder and extracted by the hot continuous (Soxhlet) method [24]. The powder was packed in a thimble and first extracted with petroleum ether to remove fatty material. The defatted marc was then extracted with ethanol and water at a 70:30 (v/v) ratio. The extract was filtered through Whatman filter paper and concentrated by drying at room temperature. The dried extract was stored in an airtight container for phytochemical and pharmacological studies. The percentage yield of each extract was calculated relative to the weight of the powdered leaf material.

D. Preliminary Phytochemical Screening

The extracts were qualitatively screened for alkaloids, carbohydrates, flavonoids, steroids, triterpenoids, and proteins using standard procedures [24]–[26].

1) *Alkaloids: Dragendorff's test*: a few milligrams of extract in 5 mL of distilled water were acidified with 2 M hydrochloric acid, and 1 mL of freshly prepared Dragendorff's reagent was added; an orange or orange-red precipitate indicated alkaloids. *Hager's test*: a few drops of Hager's reagent were added to 1 mL of the extract; a yellow precipitate indicated the presence of alkaloids. *Wagner's test*: 1 mL of dilute hydrochloric acid and a few drops of Wagner's reagent were added to 1 mL of extract; a yellow or brown precipitate indicated alkaloids. *Mayer's test*: a few drops of Mayer's reagent were added to 1 mL of the extract; a pale yellow precipitate indicated the presence of alkaloids.

2) *Carbohydrates: Molisch's test*: 2 mL of extract was mixed with 20% alcoholic α -naphthol, and 2 mL of concentrated sulphuric acid was poured along the side of the tube; a red-violet ring at the junction indicated carbohydrates. *Benedict's test*: 0.5 mL of extract was heated with 5 mL of Benedict's solution; a brick-red colour indicated the presence of reducing sugars. *Fehling's test*: 2 mL of extract was heated with 1 mL of a mixture of equal parts of Fehling's solutions A and B; a red precipitate indicated the presence of reducing sugars. *Anthrone test*: 0.5 mL of extract was mixed with 2 mL of anthrone reagent; a blue-green colour indicated carbohydrates.

3) *Flavonoids: Shinoda test*: a few magnesium turnings and 10 drops of dilute hydrochloric acid were added to 0.5 mL of extract and warmed; a pink or reddish-brown colour indicated the presence of flavonoids.

4) *Steroids and triterpenoids: Liebermann–Burchard test*: a few milligrams of extract were dissolved in acetic anhydride, heated and cooled, and 1 mL of concentrated sulphuric acid was added along the side of the tube; a green colour indicated the presence of steroids/triterpenoids. *Salkowski test*: 1 mL of concentrated sulphuric acid was added to a chloroform solution of the extract; a red colour indicated steroids.

5) *Proteins: Biuret test*: 5–8 drops of 10% w/v copper sulphate solution were added to 1 mL of extract made alkaline with sodium hydroxide; a violet-red colour indicated proteins. *Millon's test*: 5–6 drops of Millon's reagent were added to 1 mL of extract; a white precipitate that turns red on heating indicated the presence of proteins.

6) *Saponins, tannins, glycosides and fixed oils*: These constituents were tested using the standard qualitative procedures described in the literature [25], [26].

E. Experimental Animals and Ethical Approval

Thirty healthy Wistar albino rats weighing 100–150 g were procured from a CPCSEA-approved animal supplier. The animals were housed six per cage in

sanitised cages under hygienic conditions at 22–26 °C and 60–70% relative humidity with a natural light–dark cycle, and received a standard pellet diet and clean water *ad libitum*. They were acclimatised to laboratory conditions before the start of the study. The study protocol was prepared in accordance with CPCSEA guidelines [27] and approved by the Institutional Animal Ethics Committee (IAEC) of Malla Reddy College of Pharmacy (Approval No. MRCP/CCSEA/IAEC/2024-25/MOCOL/06, dated 09/04/2025). The study is reported in line with the ARRIVE guidelines [28].

F. Dose Selection and Experimental Design

Gentamicin was given at 10 mg/kg/day intraperitoneally (i.p.) to induce nephrotoxicity. Silymarin (100 mg/kg) was given orally (p.o.) as the standard drug. The extract doses (50 and 100 mg/kg, p.o.) were selected based on the available literature and previous pharmacological reports. Thirty rats were randomly divided into five groups of six animals each (Table I). Group I (normal control) received normal water. Group II (toxicant control) received gentamicin 10 mg/kg i.p. Group III (standard) received silymarin 100 mg/kg p.o. along with gentamicin 10 mg/kg i.p. Groups IV and V received the hydroalcoholic leaf extract of *T. erecta* (TE) at 50 mg/kg and 100 mg/kg p.o., respectively, along with gentamicin 10 mg/kg i.p. All treatments were given daily for five consecutive days.

TABLE I EXPERIMENTAL DESIGN AND TREATMENT SCHEDULE

Group	Animals	Group name	Treatment	Dose (mg/kg)	Route	Duration
I	1–6	Normal control	Normal water	—	p.o.	—
II	7–12	Toxicant control	Gentamicin	10	i.p.	5 days
III	13–18	Standard	Silymarin + gentamicin	100 + 10	p.o. + i.p.	5 days
IV	19–24	Treatment (low dose)	TE extract + gentamicin	50 + 10	p.o. + i.p.	5 days
V	25–30	Treatment (high dose)	TE extract + gentamicin	100 + 10	p.o. + i.p.	5 days

TE, *Tagetes erecta* hydroalcoholic leaf extract; p.o., oral; i.p., intraperitoneal.

G. Collection of Blood and Tissue

On the sixth day, the animals were euthanised using carbon dioxide. Blood samples were collected via the retro-orbital route and centrifuged at 4000 rpm for 15 min to separate the serum, which was used to estimate

biochemical parameters. The kidneys were then removed for histopathological examination.

H. Estimation of Serum Biochemical Parameters

Serum ALP, BUN, creatinine, albumin and uric acid were estimated using the respective standard analytical procedures [13], [29].

I. Histopathological Examination

The kidneys were preserved in 10% formalin, processed, sectioned with a microtome and stained with haematoxylin and eosin (H&E). The sections were examined under a light microscope at 100× magnification for glomerular and tubular changes, casts and inflammatory infiltration.

J. Statistical Analysis

Data are expressed as mean $\pm$ standard error of the mean (SEM) for six animals per group. Differences among groups were analysed using one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparisons test, with the toxicant control group as the reference. A p-value < 0.05 was considered statistically significant. Statistical analysis was performed using GraphPad Prism version 5.0.

III. RESULTS

A. Extractive Yield

Soxhlet extraction of the powdered leaves yielded 17.23% with petroleum ether and 11.38% with the hydroalcoholic solvent (Table II).

TABLE II PERCENTAGE YIELD OF *Tagetes erecta* LEAF EXTRACTS

S. No.	Plant name	Part used	Petroleum ether extract (% w/w)	Hydroalcoholic extract (% w/w)
1	<i>Tagetes erecta</i>	Leaves	17.23	11.38

B. Preliminary Phytochemical Screening

The hydroalcoholic extract contained alkaloids, carbohydrates, steroids, triterpenoids, saponins, tannins, flavonoids, amino acids, and glycosides, whereas fixed oils and fats were absent. The petroleum ether extract contained only steroids/triterpenoids, fixed oils, and fats (Table III).

TABLE III PRELIMINARY PHYTOCHEMICAL SCREENING OF *Tagetes erecta* LEAF EXTRACTS

S. No.	Phytoconstituent	Petroleum ether extract	Hydroalcoholic extract
1	Alkaloids	—	+
2	Carbohydrates	—	+
3	Steroids and triterpenoids	+	+
4	Saponins	—	+
5	Tannins	—	+
6	Flavonoids	—	+
7	Amino acids	—	+
8	Glycosides	—	+
9	Fixed oils and fats	+	—

+, present; —, absent.

C. Effect on Serum Alkaline Phosphatase

Gentamicin increased serum ALP more than three-fold, from 85.21 ± 1.61 U/L in the normal control group to 286.58 ± 0.33 U/L in the toxicant control group ($p < 0.001$; Table IV). Both doses of the extract lowered ALP significantly compared with the toxicant group ($p < 0.001$). The reductions were 7.5% at 50 mg/kg (265.02 ± 1.06 U/L) and 26.1% at 100 mg/kg (211.92 ± 3.84 U/L), indicating a dose-related effect, although ALP in both extract groups remained well above the normal value. Silymarin reduced ALP to 63.28 ± 1.37 U/L, which was below the normal control value.

TABLE IV EFFECT OF HYDROALCOHOLIC EXTRACT OF *Tagetes erecta* ON SERUM ALKALINE PHOSPHATASE (U/L)

Animal	Normal control	Toxicant control	Standard	TE low dose	TE high dose
Treatment	Normal water	Gentamicin	Silymarin + GM	TE 50 + GM	TE 100 + GM
Dose (mg/kg)	—	10	100 + 10	50 + 10	100 + 10
R1	90.20	286.40	65.30	265.00	220.20

Animal	Normal control	Toxicant control	Standard	TE low dose	TE high dose
Treatment	Normal water	Gentamicin	Silymarin + GM	TE 50 + GM	TE 100 + GM
Dose (mg/kg)	—	10	100 + 10	50 + 10	100 + 10
R2	80.05	286.00	60.20	268.20	202.20
R3	88.58	288.20	62.00	265.00	220.00
R4	82.88	286.20	68.80	260.50	208.50
R5	86.88	286.40	63.40	264.60	220.20
R6	82.68	286.30	60.00	266.80	200.40
Mean ± SEM	85.21 ± 1.61	286.58 ± 0.33####	63.28 ± 1.37***	265.02 ± 1.06***	211.92 ± 3.84***

Values are individual animals (R1–R6; marking codes H, B, T, HB, BT, HT) with mean ± SEM (n = 6). One-way ANOVA followed by Dunnett's test vs toxicant control: ####p < 0.001 toxicant vs normal control; *p < 0.05, **p < 0.01, ***p < 0.001 vs toxicant control; ns, not significant. GM, gentamicin 10 mg/kg i.p.; TE, *Tagetes erecta* hydroalcoholic leaf extract.

D. Effect on Blood Urea Nitrogen

BUN rose from 11.37 ± 0.01 mg/dL in normal rats to 41.48 ± 0.64 mg/dL after gentamicin (p < 0.001; Table V). BUN was significantly lower than in the toxicant group after treatment with silymarin (35.71 ± 0.87 mg/dL; p < 0.001), TE 50 mg/kg (33.57 ± 0.61 mg/dL;

p < 0.001) and TE 100 mg/kg (37.50 ± 1.03 mg/dL; p < 0.01). The reduction was greater with the lower dose (19.1%) than with the higher dose (9.6%), and BUN in all treated groups remained about three times the normal value.

TABLE V EFFECT OF HYDROALCOHOLIC EXTRACT OF *Tagetes erecta* ON BLOOD UREA NITROGEN (MG/DL)

Animal	Normal control	Toxicant control	Standard	TE low dose	TE high dose
Treatment	Normal water	Gentamicin	Silymarin + GM	TE 50 + GM	TE 100 + GM
Dose (mg/kg)	—	10	100 + 10	50 + 10	100 + 10
R1	11.39	43.10	34.65	33.10	33.14
R2	11.33	43.13	39.69	35.79	39.65
R3	11.40	39.99	35.65	33.99	39.10
R4	11.36	41.31	34.15	31.17	39.50
R5	11.33	41.99	33.96	33.90	36.30
R6	11.39	39.33	36.13	33.45	37.30
Mean ± SEM	11.37 ± 0.01	41.48 ± 0.64####	35.71 ± 0.87***	33.57 ± 0.61***	37.50 ± 1.03**

Values are individual animals (R1–R6; marking codes H, B, T, HB, BT, HT) with mean ± SEM (n = 6). One-way ANOVA followed by Dunnett's test vs toxicant control: ####p < 0.001 toxicant vs normal control; *p < 0.05, **p < 0.01, ***p < 0.001 vs toxicant control; ns, not significant. GM, gentamicin 10 mg/kg i.p.; TE, *Tagetes erecta* hydroalcoholic leaf extract.

E. Effect on Serum Creatinine

Mean serum creatinine was 0.88 ± 0.04 mg/dL in normal rats and 1.09 ± 0.14 mg/dL in gentamicin-treated rats (Table VI). This increase was not statistically significant (one-way ANOVA, p = 0.29)

and was largely due to a single animal with a value of 1.78 mg/dL. Creatinine in the silymarin (1.02 ± 0.09 mg/dL), TE 50 mg/kg (1.11 ± 0.02 mg/dL), and TE 100 mg/kg (0.98 ± 0.04 mg/dL) groups did not differ significantly from that in the toxicant group.

TABLE VI EFFECT OF HYDROALCOHOLIC EXTRACT OF *Tagetes erecta* ON SERUM CREATININE (MG/DL)

Animal	Normal control	Toxicant control	Standard	TE low dose	TE high dose
Treatment	Normal water	Gentamicin	Silymarin + GM	TE 50 + GM	TE 100 + GM
Dose (mg/kg)	—	10	100 + 10	50 + 10	100 + 10
R1	0.78	1.78	1.23	1.10	0.85
R2	1.05	1.07	0.88	1.01	0.88
R3	0.88	1.08	1.02	1.12	1.02
R4	0.85	0.88	1.32	1.20	1.05
R5	0.88	0.85	0.83	1.12	1.00
R6	0.85	0.87	0.82	1.13	1.08
Mean ± SEM	0.88 ± 0.04	1.09 ± 0.14 (ns)	1.02 ± 0.09 (ns)	1.11 ± 0.02 (ns)	0.98 ± 0.04 (ns)

Values are individual animals (R1–R6; marking codes H, B, T, HB, BT, HT) with mean ± SEM (n = 6). One-way ANOVA followed by Dunnett's test vs toxicant control: ####p < 0.001 toxicant vs normal control; *p < 0.05, **p < 0.01, ***p < 0.001 vs toxicant control; ns, not significant. GM, gentamicin 10 mg/kg i.p.; TE, *Tagetes erecta* hydroalcoholic leaf extract.

F. Effect on Serum Albumin

Gentamicin reduced serum albumin by 34.8%, from 4.30 ± 0.17 g/dL to 2.80 ± 0.04 g/dL (p < 0.001; Table VII). Albumin was restored to near-normal values by

silymarin (4.39 ± 0.04 g/dL), TE 50 mg/kg (4.10 ± 0.03 g/dL) and TE 100 mg/kg (4.45 ± 0.18 g/dL) (all p < 0.001 vs toxicant control).

TABLE VII EFFECT OF HYDROALCOHOLIC EXTRACT OF *Tagetes erecta* ON SERUM ALBUMIN (G/DL)

Animal	Normal control	Toxicant control	Standard	TE low dose	TE high dose
Treatment	Normal water	Gentamicin	Silymarin + GM	TE 50 + GM	TE 100 + GM
Dose (mg/kg)	—	10	100 + 10	50 + 10	100 + 10
R1	4.02	2.88	4.48	4.04	4.01
R2	4.06	2.86	4.42	4.05	4.88
R3	4.82	2.68	4.46	4.12	4.12
R4	4.84	2.88	4.46	4.02	4.88
R5	4.01	2.84	4.28	4.18	4.01
R6	4.06	2.68	4.26	4.22	4.80
Mean ± SEM	4.30 ± 0.17	2.80 ± 0.04####	4.39 ± 0.04***	4.10 ± 0.03***	4.45 ± 0.18***

Values are individual animals (R1–R6; marking codes H, B, T, HB, BT, HT) with mean ± SEM (n = 6). One-way ANOVA followed by Dunnett's test vs toxicant control: ####p < 0.001 toxicant vs normal control; *p < 0.05, **p < 0.01, ***p < 0.001 vs toxicant control; ns, not significant. GM, gentamicin 10 mg/kg i.p.; TE, *Tagetes erecta* hydroalcoholic leaf extract.

G. Effect on Serum Uric Acid

Serum uric acid increased from 7.30 ± 0.07 mg/dL in normal rats to 12.54 ± 0.36 mg/dL after gentamicin (p < 0.001; Table VIII). The extract lowered uric acid by

18.7% at 50 mg/kg (10.19 ± 0.32 mg/dL) and by 33.9% at 100 mg/kg (8.29 ± 0.15 mg/dL) (both p < 0.001). The effect of the higher dose was comparable to that of silymarin (9.03 ± 0.36 mg/dL).

TABLE VIII EFFECT OF HYDROALCOHOLIC EXTRACT OF *Tagetes erecta* ON SERUM URIC ACID (MG/DL)

Animal	Normal control	Toxicant control	Standard	TE low dose	TE high dose
Treatment	Normal water	Gentamicin	Silymarin + GM	TE 50 + GM	TE 100 + GM
Dose (mg/kg)	—	10	100 + 10	50 + 10	100 + 10
R1	7.31	11.58	10.08	10.07	8.15
R2	7.15	11.87	8.87	11.11	8.11
R3	7.18	13.75	8.17	10.11	8.88
R4	7.57	11.88	10.11	8.88	7.88
R5	7.38	13.13	8.15	10.88	8.15
R6	7.18	13.01	8.78	10.11	8.55
Mean ± SEM	7.30 ± 0.07	12.54 ± 0.36###	9.03 ± 0.36***	10.19 ± 0.32***	8.29 ± 0.15***

Values are individual animals (R1–R6; marking codes H, B, T, HB, BT, HT) with mean ± SEM (n = 6). One-way ANOVA followed by Dunnett's test vs toxicant control: ###p < 0.001 toxicant vs normal control; *p < 0.05, **p < 0.01, ***p < 0.001 vs toxicant control; ns, not significant. GM, gentamicin 10 mg/kg i.p.; TE, *Tagetes erecta* hydroalcoholic leaf extract.

H. Histopathological Findings

1) *Normal control (Group I)*: Kidney sections showed normal glomeruli with a well-preserved Bowman's space. The proximal convoluted tubules (PCT) were lined by cuboidal epithelium with a well-defined brush border, and the distal convoluted tubules and macula densa appeared normal, with no evidence of cellular injury or inflammation (Fig. 1(a)).

2) *Toxicant control (Group II; gentamicin 10 mg/kg)*: Sections showed desquamation of the tubular epithelium, marked epithelial degeneration, granular casts and lymphocytic infiltration, mainly in the cortex. The cortical convoluted tubules showed acute tubular necrosis, intraluminal desquamated cells and dilatation affecting fewer than half of the PCT, consistent with gentamicin-induced nephrotoxicity (Fig. 1(b)).

3) *Standard (Group III; silymarin 100 mg/kg + gentamicin)*: Sections showed minimal structural changes with no apoptotic features. The glomerular capillaries were intact with no disruption of the basement membrane, and the tubular lumina were free of casts, vacuoles and necrotic debris (Fig. 1(c)).

4) *TE 50 mg/kg + gentamicin (Group IV)*: Mild hyaline casts were seen in the PCT, with moderate glomerular congestion and minimal rupture of glomerular capillaries. Occasional vacuolar degeneration of the tubular epithelium was noted. Necrotic changes were modest, with partial

preservation of cytoplasmic and nuclear morphology and few pyknotic or karyolytic nuclei (Fig. 1(d)).

5) *TE 100 mg/kg + gentamicin (Group V)*: The PCT were patent and free of hyaline casts, with only minor tubular dilatation. The tubular lumina were lined by intact cuboidal epithelium, and regenerating tubular epithelium and glomerular tufts indicated substantial restoration of renal architecture towards normal (Fig. 1(e)). The findings are summarised in Table IX.

TABLE IX SUMMARY OF HISTOPATHOLOGICAL OBSERVATIONS IN KIDNEY TISSUE

Group	Treatment	Histopathological observation
I	Normal control	Normal renal architecture
II	Gentamicin 10 mg/kg	Severe tubular damage: epithelial desquamation and degeneration, granular casts, acute tubular necrosis, lymphocytic infiltration
III	Silymarin 100 mg/kg + gentamicin	Minimal histopathological changes
IV	TE 50 mg/kg + gentamicin	Mild hyaline casts, moderate glomerular congestion, minimal capillary rupture
V	TE 100 mg/kg + gentamicin	Largely restored renal architecture with minor tubular dilatation

TE, *Tagetes erecta* hydroalcoholic leaf extract.

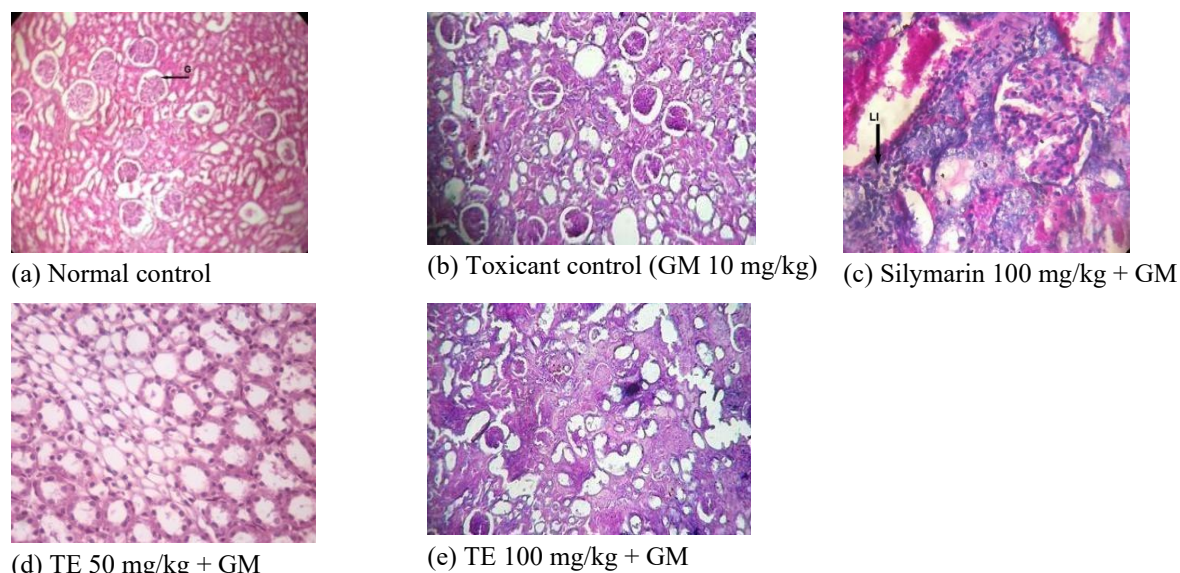


Fig. 1. Photomicrographs of kidney sections (H&E, 100×). (a) Normal control; (b) toxicant control (gentamicin 10 mg/kg); (c) silymarin 100 mg/kg + gentamicin; (d) TE 50 mg/kg + gentamicin; (e) TE 100 mg/kg + gentamicin. G, glomerulus; LI, lymphocytic infiltration; GM, gentamicin; TE, *Tagetes erecta* hydroalcoholic leaf extract.

IV. DISCUSSION

Gentamicin at 10 mg/kg/day for five days produced renal injury, with tubular degeneration and necrosis, desquamation, casts and inflammatory infiltration on histology, together with a rise in BUN and uric acid. These findings agree with the well-described pattern of gentamicin nephrotoxicity, in which the drug accumulates in proximal tubular cells and damages them through oxidative stress, phospholipidosis and mitochondrial injury, leading to reduced glomerular filtration and retention of nitrogenous waste [5], [9], [12]. Similar biochemical changes have been reported in earlier gentamicin studies in rats [30]–[32]. The increase in serum ALP is consistent with injury to the ALP-rich brush-border membrane of the PCT, although serum ALP is not specific to the kidney and also reflects hepatic and bone sources. The fall in serum albumin may reflect urinary protein loss after tubular damage and the systemic effects of gentamicin [29], but urinary protein was not measured in this study.

Serum creatinine did not increase significantly in this study. Serum creatinine is a relatively insensitive and late marker of tubular injury and may not rise appreciably until a large proportion of nephron function has been lost [12]. The high variability within the toxicant group, largely due to one animal, further

limited its value, so no conclusion about glomerular filtration can be drawn from creatinine alone. Creatinine clearance and sensitive tubular injury markers would be more informative in future work.

Co-administration of the hydroalcoholic leaf extract of *T. erecta* attenuated most of the gentamicin-induced changes. The effect on ALP and uric acid was dose-related, and at 100 mg/kg the reduction in uric acid was comparable to that of silymarin. Both doses restored serum albumin to near-normal levels. In contrast, the reduction in BUN was greater at 50 mg/kg than at 100 mg/kg, and BUN remained markedly elevated in all treated groups, indicating only partial functional protection. The histological findings supported the biochemical results, with milder casts, vacuolar degeneration and glomerular congestion at 50 mg/kg and near-normal tubular architecture at 100 mg/kg. Comparable protection by antioxidant compounds such as quercetin and diosgenin has been reported in gentamicin-treated rats [31], [33].

Phytochemical screening showed flavonoids, tannins, saponins, glycosides, alkaloids and triterpenoids in the extract. Flavonoids and other polyphenols can scavenge free radicals, chelate iron, inhibit lipid peroxidation and modulate inflammatory signalling, and have been shown to protect the kidney in several experimental models [14]. *T. erecta* extracts are rich in phenolic compounds and have demonstrated

antioxidant activity [18], [19]. The diuretic and natriuretic activity reported for *T. erecta* [20] could also favour the clearance of nitrogenous waste. These mechanisms may explain the protective effect observed here, but they remain speculative because oxidative stress markers were not measured. The protection given by silymarin, used as the reference standard, is consistent with earlier reports of its antioxidant benefit in gentamicin nephrotoxicity [15], [16].

This study has several limitations. Only two doses of the extract were tested, and the lack of a clear dose–response for BUN needs to be examined across a wider dose range. Renal oxidative stress markers (malondialdehyde, reduced glutathione, superoxide dismutase and catalase), urinary parameters, creatinine clearance and kidney weight were not measured. Histopathological changes were described qualitatively and were not scored. The extract was not standardised to any marker compound, and the active constituents responsible for the effect were not identified.

V. CONCLUSION

Oral co-administration of the hydroalcoholic leaf extract of *Tagetes erecta* partly protected Wistar rats against gentamicin-induced nephrotoxicity. The higher dose (100 mg/kg) gave the clearest benefit, lowering serum ALP and uric acid, restoring serum albumin and preserving tubular architecture, while BUN was reduced but not normalised by either dose. The flavonoid- and polyphenol-rich leaf extract may therefore be a useful source of nephroprotective agents for patients who need aminoglycoside therapy. Studies measuring renal oxidative stress and sensitive injury markers, together with bioassay-guided fractionation, are needed to confirm the mechanism and identify the active constituents.

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Committee of Malla Reddy College of Pharmacy (Approval No. MRCP/CCSEA/IAEC/2024-25/MOCOL/06, dated 09/04/2025) and were performed in accordance with CPCSEA guidelines. The authors declare no conflicts of interest.

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