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Original Research Article

Acute toxicity, renoprotective evaluation, and histopathological assessment of natural flavonoids against gentamicin-induced nephrotoxicity

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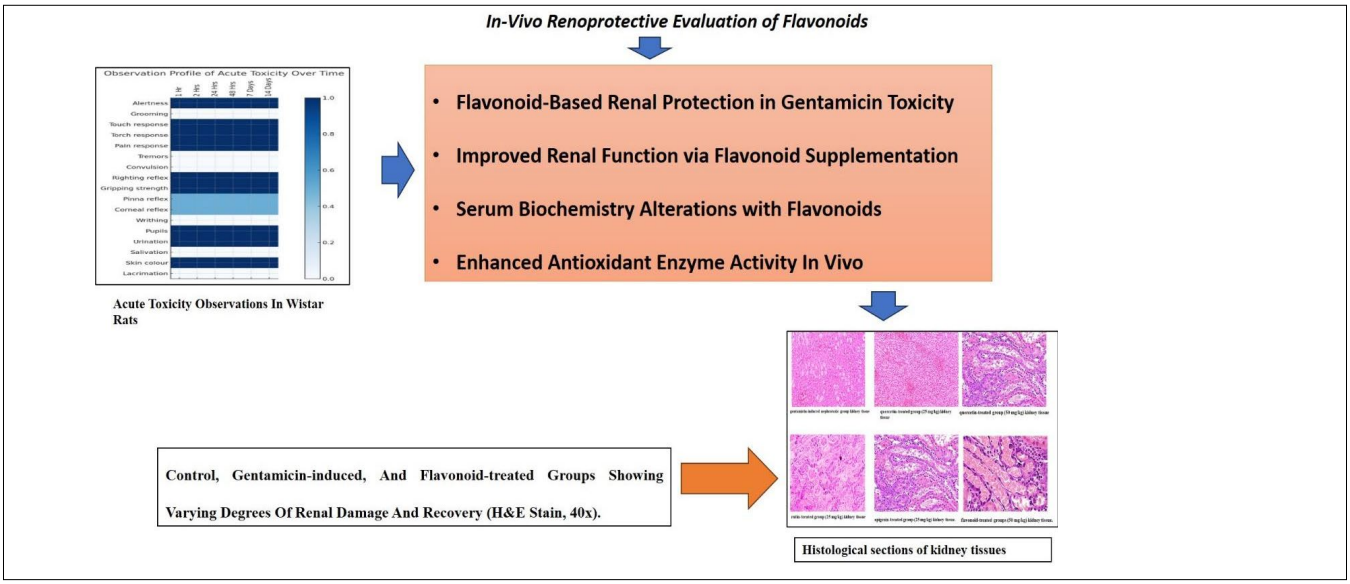
Abstract



Background: Gentamicin is a widely used aminoglycoside antibiotic associated with nephrotoxicity due to oxidative stress and inflammation. Natural flavonoids have shown potential antioxidant and anti-inflammatory properties that may mitigate such renal damage.

Objective: This study aimed to investigate the acute toxicity, renoprotective efficacy, and histopathological effects of three natural flavonoids quercetin, rutin, and apigenin against gentamicin-induced nephrotoxicity in rats.

Materials and Methods: Acute oral toxicity of quercetin, rutin, and apigenin was assessed in Wistar rats at doses up to 2000 mg/kg. Nephrotoxicity was induced using gentamicin (100 mg/kg, intraperitoneally) for 7 days. Treatment groups received flavonoids at 50 mg/kg orally. Renal function markers (serum creatinine and urea), oxidative stress parameters, and histopathological changes were evaluated.

Results: Flavonoids were found to be safe up to 2000 mg/kg with no mortality or adverse effects. Gentamicin caused significant elevations in serum creatinine, urea, and oxidative stress markers, along with tubular necrosis, glomerular thickening, and inflammation. Flavonoid treatment, especially with quercetin, significantly improved biochemical parameters and histological architecture, indicating renal protection and tissue regeneration.

Conclusion: Quercetin, rutin, and apigenin exhibited dose-dependent renoprotective effects through antioxidant and anti-inflammatory mechanisms. Quercetin showed the highest efficacy, highlighting its therapeutic potential in managing drug-induced nephrotoxicity.

Keywords: Gentamicin-induced nephrotoxicity, Flavonoids, Renoprotection, Histopathology, Oxidative stress

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1. Introduction

Nephrotoxicity, a major clinical challenge, often arises as an adverse effect of therapeutic drugs, particularly aminoglycoside antibiotics such as gentamicin. Gentamicin-induced nephrotoxicity is primarily characterized by oxidative stress, inflammation, and tubular necrosis, significantly compromising renal function. Despite its widespread clinical use for treating severe gram-negative bacterial infections, gentamicin is known to accumulate in the renal cortex, causing functional and structural damage that limits its long-term use.[1,2]

Recent pharmacological advancements have directed attention toward natural bioactive compounds with potent antioxidant and anti-inflammatory properties. Flavonoids—polyphenolic compounds abundantly found in fruits, vegetables, and medicinal plants—have demonstrated significant therapeutic potential in mitigating drug-induced organ toxicities. Among them, quercetin, rutin, and apigenin are well-studied for their ability to scavenge free radicals, inhibit lipid peroxidation, and modulate signaling pathways involved in cell survival and inflammation.[3,4]

The present study aims to evaluate the acute oral toxicity and renoprotective effects of these selected natural flavonoids in a well-established gentamicin-induced nephrotoxicity model in rats. Additionally, histopathological assessment of renal tissues was performed to correlate biochemical findings with tissue-level alterations. This research provides insight into the therapeutic relevance of flavonoids as nephroprotective agents and supports their safe usage for potential clinical applications in renal disorders.[5,6]

2. Materials and Methods

2.1. Materials

High-purity natural flavonoids quercetin, rutin, and apigenin (≥98%) were procured from Sigma-Aldrich (USA) for experimental use. Gentamicin sulfate, used to induce nephrotoxicity, was obtained from a certified pharmaceutical vendor. All solvents and reagents employed were of analytical grade and purchased from Merck and HiMedia Laboratories (India). Biochemical assay kits for serum creatinine, blood urea nitrogen, uric acid, total protein, and albumin were sourced from Span Diagnostics (India). Equipment utilized included a precision weighing balance (Palak), homogenizer and magnetic stirrer (Remi), digital pH meter (Eqip–Tronic), Brookfield viscometer, and a thermostatic hot plate (Remi). Biochemical analyses were conducted using a Shimadzu UV-1800 spectrophotometer, and histological examinations were performed with a Labomed optical microscope. All materials were stored and handled according to manufacturer guidelines to ensure experimental integrity.

2.2. Acute toxicity studies

The acute toxicity study was conducted in accordance with OECD Guideline 423 to determine the safety profile and median lethal dose (LD_{50}) of three natural flavonoids quercetin, rutin, and apigenin in healthy adult Wistar rats. Animals selected were 8–10 weeks old, weighing 150–200 g, and clinically normal. They were housed under standard laboratory conditions (12-h light/dark cycle, 22 ± 3°C temperature, and 50–60% relative humidity). Rats were randomly assigned to three groups (n=3 per group), with each group receiving a single oral dose of one flavonoid at 2000 mg/kg body weight. The selection of this dose was based on OECD recommendations for limit testing, and the aim was to monitor mortality, behavioral changes, and signs of systemic toxicity over a 14-day observation period.[7,8]

Comprehensive evaluations were carried out at multiple intervals 1 h, 2 h, 24 h, 48 h, and daily thereafter including behavioral observations (arousal, grooming, locomotion, and coordination), neurological reflexes (pupillary light reflex, pinna response, and corneal blink reflex), and pain sensitivity assessments. Physiological parameters such as skin coloration, salivation, urination, lacrimation, and grooming behavior were also monitored, alongside motor and grip strength tests (righting reflex and grasp reflex). Responses were documented as Normal (N), Present (P), or Absent (A) in a structured format. No mortality or adverse clinical signs were observed in any group, suggesting an LD_{50} value greater than 2000 mg/kg for all three compounds. These results affirm the non-toxic nature of quercetin, rutin, and apigenin at both high and therapeutic doses, justifying their use in subsequent nephroprotective evaluations at 200 and 400 mg/kg.[9,10]

2.3. In-Vivo renoprotective evaluation

The nephroprotective effects of quercetin, rutin, and apigenin were evaluated using a gentamicin-induced nephrotoxicity model in healthy adult Wistar rats. The study was conducted following approval from the Institutional Animal Ethics Committee (IAEC), Loknete Dr. J. D. Pawar College of Pharmacy (Reg. No. 1929/PO/Re/S/16/CPCSEA). Rats were randomly divided into nine groups (n = 6): normal control (saline), negative control (gentamicin 80 mg/kg, i.p. for 8 days), positive control (gentamicin + silymarin 50 mg/kg, oral), and six treatment groups receiving gentamicin followed by quercetin, rutin, or apigenin at 25 mg/kg and 50 mg/kg doses orally for 7 days.[11,12]

Renal function was assessed by measuring serum and urine creatinine, blood urea, uric acid, total protein, and albumin levels on days 5, 10, and 15 using standard biochemical kits. Body weights were recorded on days 1, 3, 5, 7, 11, and 14, and kidney weights were measured post-sacrifice to evaluate changes related to nephrotoxicity and treatment response. Antioxidant status was evaluated by measuring superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels in kidney tissue homogenates. Data were expressed as mean ± SEM and analyzed using one-way ANOVA followed by Dunnett's test, with $p < 0.05$ considered statistically significant. [13]

2.4. Histopathological examination

Kidney tissues from all experimental groups were collected at the end of the treatment period and immediately fixed in 10% neutral buffered formalin to preserve tissue structure. After routine processing—including dehydration, clearing, and paraffin embedding thin sections (4–5 µm) were cut and stained with hematoxylin and eosin (H&E) to examine microscopic changes. [14,15]

The stained sections were analyzed under a light microscope to assess structural alterations caused by gentamicin-induced nephrotoxicity and the protective effects of flavonoid treatments. The control group showed normal kidney architecture with intact glomeruli and tubules. In contrast, the gentamicin-treated group displayed marked tubular damage, necrosis, glomerular basement membrane thickening, and inflammatory cell infiltration.[16,17]

Flavonoid treatment resulted in dose-dependent improvement in renal histology. At 25 mg/kg, partial recovery was observed with reduced tubular necrosis and inflammation. Higher doses (50 mg/kg) of quercetin, rutin, and apigenin demonstrated significant renal protection, including near-complete tubular regeneration and restored glomerular structure. Among these, quercetin showed the most pronounced protective effects. These histopathological findings support the nephroprotective potential of flavonoids against gentamicin-induced kidney injury.[18,19]

3. Result

3.1. Acute toxicity studies

Acute toxicity evaluation of quercetin, rutin, and apigenin was performed in Wistar rats following OECD 423 guidelines to establish their safety profiles. A single oral dose of 2000 mg/kg was administered, and animals were monitored for 14 days for behavioral, physiological, and neurological changes.

Throughout the observation period, no signs of distress, altered activity, or neurological impairment were detected. Behavioral parameters such as alertness, locomotion, grooming, and reflex responses remained within normal limits, indicating no adverse effects on central nervous system functions. Physiological assessments also showed no abnormalities in skin color, pupil reflexes, salivation, urination, or body weight changes, confirming the compounds' non-toxic nature.

No mortality or toxic signs such as convulsions, tremors, or respiratory distress were observed, suggesting an LD50 value greater than 2000 mg/kg for all three flavonoids, consistent with low toxicity classification under OECD standards. These findings indicate that quercetin, rutin, and apigenin possess a high safety margin suitable for further therapeutic and nephroprotective investigations. The detailed observations are summarized in **(Table 1)**, while **(Figure 1)** presents a heatmap illustrating the behavioral and physiological responses over time, further supporting the compounds' low toxicity profile.

Table 1: Acute oral toxicity screening general observations of flavonoids in Wistar rats as per OECD 423 guidelines

Parameter	Time Point	Quercetin	Rutin	Apigenin
Locomotion	0h, 1h, 24h, 7d, 14d	Normal	Normal	Normal
Grooming behavior	0h, 1h, 24h, 7d, 14d	Normal	Normal	Normal
Pupil reflex	0h, 1h, 24h, 7d, 14d	Normal	Normal	Normal
Salivation	0h, 1h, 24h, 7d, 14d	Normal	Normal	Normal
Mortality	14 days	0/6	0/6	0/6
Body weight change	Day 0–14	Stable	Stable	Stable

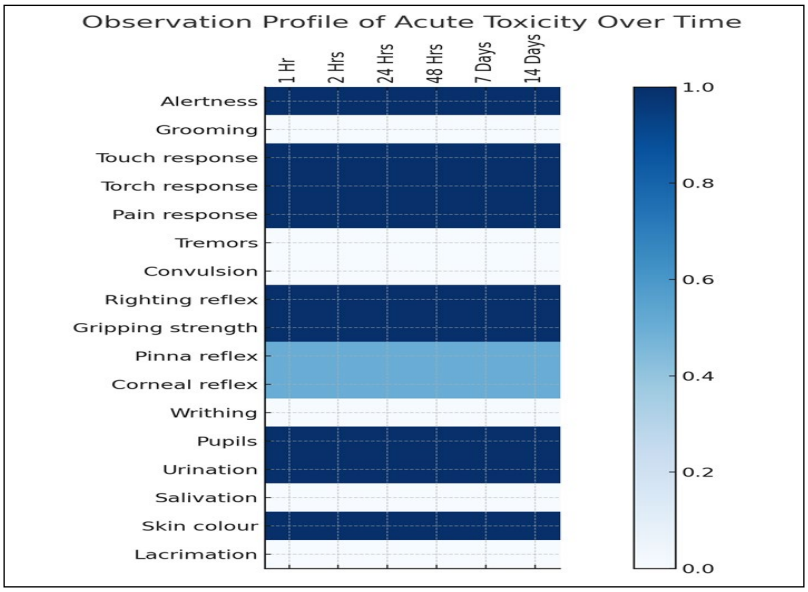

Figure 1: Heatmap of acute toxicity observations in wistar rats at various time points after flavonoid administration, showing behavioral and physiological responses indicating a low toxicity profile

3.2. In-Vivo renoprotective evaluation of flavonoids

3.2.1. Flavonoid-Mediated renal protection in gentamicin toxicity

Gentamicin administration (80 mg/kg, intraperitoneally, eight days) induced marked renal toxicity, shown by significant elevation in serum creatinine, blood urea, and uric acid levels compared to controls ($p < 0.05$), confirming nephrotoxicity **(Table 2)**. Flavonoid treatment with quercetin, rutin, and apigenin at 25 and 50 mg/kg doses significantly attenuated these biochemical disruptions in a dose-dependent manner, with quercetin at 50 mg/kg demonstrating the most pronounced normalization of renal markers. Additionally, gentamicin-induced body weight loss, indicative of systemic toxicity, was significantly reversed by flavonoids, particularly with high-dose quercetin, correlating with improved biochemical profiles **(Table 3)** and **(Figure 2)**. Silymarin (50 mg/kg) as a positive control exhibited comparable nephroprotection, validating flavonoids as potential therapeutic agents. The dose-dependent efficacy of flavonoids highlights their capacity to restore renal function and ameliorate systemic effects of gentamicin toxicity. These findings underscore the promising nephroprotective role of quercetin, rutin, and apigenin in mitigating drug-induced renal impairment.

Table 2: Effect of flavonoid treatments on biochemical parameters (creatinine, blood urea, and uric acid) in gentamicin-induced nephrotoxicity in rats

Sr. No.	Animal groups & dose	Biochemical parameter's		
		Creatinine (mg/dl)	Blood urea (mg/dl)	Uric acid (mg/dl)
1.	Normal control group	0.684± 0.21	53.20± 2.34	1.634± 0.26
2.	Negative control (gentamycin 80 gm/kg i.p.)	1.972± 0.42	74.46± 2.44	2.78 ±0.52
3.	Positive control silymarin 50 mg/kg oral	0.932± 0.24***	55.23 ±4.32***	1.54 ±0.33***
4.	Quercetin 25 mg/kg oral	1.128 ±0.08*	65.54±2.24*	2.12±0.16*
5.	Quercetin 50 mg/kg oral	1.833± 0.14**	63.26 ±3.43**	1.44 ±0.07**
6.	Rutin 25 mg/kg oral	1.828 ± 0.35*	66.42±3.42*	2.12± 0.26*
7.	Rutin 50 mg/kg oral	1.265± 0.04**	61.78 ±2.82**	1.77 ±0.05**
8.	Apigenin 25 mg/kg oral	1.721 ± 0.14*	62.71±3.65*	2.59± 0.13*
9.	Apigenin 50 mg/kg oral	1.705± 0.24**	67.33 ±3.32**	2.47 ±0.14**

Values are expressed as Mean ± SD (n = 6). $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with negative control group.

Table 3: Observations and results of body weights in both normal and experimental rats during the study protocol

Sr. No.	Animal groups	Before gentamicin injection	After gentamicin injection	Body weight of nephrotoxic animals treated with standard drug and plant sampless			
		Day 1	Day 3	Day 5	Day 7	Day 11	Day 14
1.	Normal control (Saline)	184.4 ± 2.17	188.31 ± 1.04	193.6 ± 2.01	197.1 ± 1.24	198.5 ± 1.12	201.3 ± 1.19
2.	Gentamicin induced Nephrotoxicity 80 mg/kg i.p.	182.2 ±1.61	168.6 ± 1.21	170.3 ± 2.01	171.6 ± 1.18	173.3 ± .14	175.6 ± 1.34
3.	Silymarin	186.8 ± 2.12	190.31 ± 1.04	196.6 ± 2.03	199.1 ± 1.21	199.8 ± .17	200.3 ± 1.14
4.	GEN 80 mg/kg Standard Quercetin 25 mg/kg oral	182.6 ± 1.21	186.6 ± 1.51	188.2 ± 1.01	191.8 ± 1.23	195.2 ± 1.14	199.7 ± 1.17
5.	GEN 80 mg/kg + Quercetin 50 mg/kg oral	183.1 ± 1.17	185.2 ± 1.34	187.1 ± 1.61	188.4 ± 1.32	189.6 ± 1.41	193.5 ± 1.03
6.	GEN 80 mg/kg + Rutin 25 mg/kg oral	183.4 ± 1.32	185.1 ± 1.37	185..3 ± 1.21	188.6 ± 1.11	192.6 ± 1.21	197.6 ± 1.71
7.	GEN 80 mg/kg + Rutin 50 mg/kg oral	182.5 ± 2.05	184.2 ± 1.32	187.7 ± 1.45	189.4 ± 1.37	191.6 ± 1.22	193.52 ± 1.23
8.	GEN 80 mg/kg + Apigenin 25mg/kg oral	184.41 ± 1.03	182.6 ± 1.21	185.3 ± 1.53	187.1 ± 1.41	194.3 ± 1.46	196.1 ± 1.62

Values are expressed as Mean ± SD (n = 6). Body weights were recorded at specified time intervals.

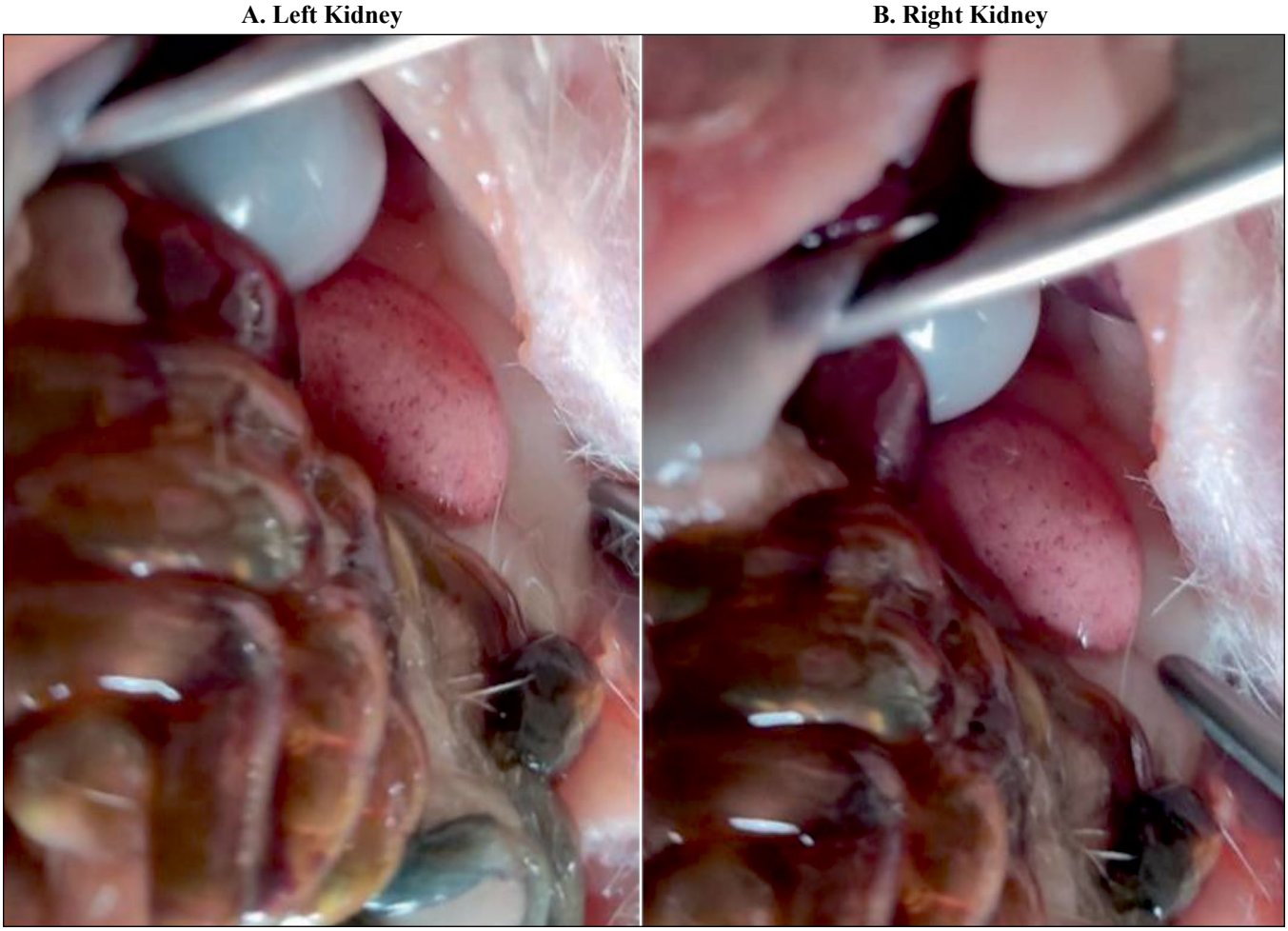

Figure 2: Images of rat kidney in gentamycin-induced group

3.2.2. Effects of flavonoid supplementation on renal function in gentamicin-induced nephrotoxicity

Gentamicin administration (80 mg/kg) caused a significant decline in serum total protein and albumin levels compared to basal and positive control values across days 5, 10, and 15, reflecting renal dysfunction likely due to impaired protein synthesis or reabsorption from nephrotoxic renal damage. This confirms gentamicin's effect in disrupting kidney filtration and protein retention.

Treatment with flavonoids at 25 and 50 mg/kg significantly improved serum total protein, albumin, and albumin/globulin ratios versus the gentamicin group. High-dose quercetin (50 mg/kg) notably restored protein levels to near-normal by day 5 (total protein 6.74 ± 0.028 g/dL; albumin 4.46 ± 0.002 g/dL; $p < 0.0001$). Rutin and apigenin also improved these parameters dose-dependently, indicating partial renoprotection **(Table 4)**.

Table 4: Results of flavonoid supplements on serum total protein and albumin levels

Sr. No.	Group and treatment	Serum biochemical parameters day 15	
		Total protein (g/dL)	Albumin (g/dL)
1.	Normal control	$7.31 \pm 0.007^{****}$	$4.37 \pm 0.003^{****}$
2.	Disease control gentamycin 80mg/kg	5.64 ± 0.044	4.43 ± 0.003
3.	Standard control Silymarin 50mg/kg	$7.47 \pm 0.025^{****}$	$4.27 \pm 0.003^{****}$
4.	Quercetin 25 mg/kg	$6.24 \pm 0.033^{****}$	4.54 ± 0.003^{ns}
5.	Quercetin 50 mg/kg	$6.74 \pm 0.028^{****}$	$4.46 \pm 0.002^{***}$
6.	Rutin 25mg/kg	$5.66 \pm 0.045^{**}$	4.54 ± 0.003^{ns}
7.	Rutin 50mg/kg	$6.64 \pm 0.028^{****}$	$4.55 \pm 0.003^{**}$
8.	Apigenin 25 mg/kg	$5.81 \pm 0.045^{**}$	4.44 ± 0.003^{ns}
9.	Apigenin 50mg/kg	$6.41 \pm 0.028^{****}$	$4.53 \pm 0.003^{**}$

Values are expressed as Mean ± SD (n = 6). ns = non-significant; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared with disease control group.

The renoprotective effect was dose-dependent, with 50 mg/kg outperforming 25 mg/kg. Quercetin showed the highest efficacy, followed by rutin and apigenin. Compared to silymarin (50 mg/kg), flavonoid treatments achieved comparable restoration of serum protein and albumin by days 10 and 15, reinforcing their therapeutic potential.

Evaluation of renal biomarkers—serum creatinine, blood urea, and uric acid—showed significant reductions in flavonoid-treated groups at all time points. Quercetin 50 mg/kg consistently yielded the greatest improvements, with rutin and apigenin also demonstrating significant nephroprotective effects **(Table 5), (Table 6), (Table 7)**.

Statistical analysis using one-way ANOVA with Dunnett's posttest confirmed these results ($p < 0.0001$, < 0.001, < 0.01). Data are mean ± SEM (n=6). Overall, flavonoid supplementation exhibited a dose-dependent renoprotective effect by restoring serum protein and albumin levels and normalizing renal function markers, suggesting promising efficacy against gentamicin-induced nephrotoxicity.

Table 5: Effects of flavonoid supplements on serum biochemical parameters on day 5

Sr. No.	Group and treatment	Serum biochemical parameters day 5		
		Creatinine (mg/dL)	Blood Urea (mg/dL)	Uric acid (mg/dL)
1.	Normal control	0.80 ± 0.0191****	26.53 ± 0.170****	2.52 ± 0.029****
2.	Disease control gentamycin 80 mg/kg	1.94 ± 0.0183	34.30 ± 0.196	3.56 ± 0.021
3.	Standard control Silymarin 50 mg/kg	1.23 ± 0.065***	25.47 ± 0.210**	2.19 ± 0.009****
4.	Quercetin 25 mg/kg	1.75 ± 0.0191**	28.93 ± 0.091***	3.34 ± 0.020***
5.	Quercetin 50 mg/kg	1.43 ± 0.0352****	28.24 ± 0.152**	3.09 ± 0.036***
6.	Rutin 25 mg/kg	1.93 ± 0.029 4^{ns}	29.23 ± 0.162***	3.32 ± 0.019**
7.	Rutin 50 mg/kg	1.44 ± 0.064**	28.46 ± 0.117**	3.31 ± 0.016**
8.	Apigenin 25 mg/kg	1.63 ± 0.029 4^{ns}	29.03 ± 0.162***	3.12 ± 0.019**
9.	Apigenin 50 mg/kg	1.38 ± 0.064**	28.36 ± 0.117**	3.26 ± 0.016**

Values are expressed as Mean ± SD (n = 6). ns = non-significant; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared with disease control group.

Table 6: The effects of flavonoid supplements on serum biochemical parameters on day 10

Sr. No.	Group and treatment	Serum biochemical parameters day 10		
		Creatinine (mg/dL)	Blood Urea(mg/dL)	Uric acid (mg/dL)
1.	Normal control	0.74 ± 0.005****	26.54 ± 0.137****	2.35 ± 0.021****
2.	Disease control gentamycin 80 mg/kg	1.83 ± 0.020	33.73 ± 0.202	3.38 ± 0.026
3.	Standard control Silymarin 50 mg/kg	1.11 ± 0.014****	24.77 ± 0.230****	2.12 ± 0.010****
4.	Quercetin 25 mg/kg	1.63 ± 0.018**	28.47 ± 0.135****	3.15 ± 0.026**
5.	Quercetin 50 mg/kg	1.24 ± 0.027***	27.68 ± 0.075****	3.05 ± 0.030**
6.	Rutin 25 mg/kg	1.79 ± 0.019 ns	28.75 ± 0.038****	3.27 ± 0.017 ^{ns}
7.	Rutin 50 mg/kg	1.28 ± 0.027***	28.39 ± 0.042****	3.24 ± 0.020*
8.	Apigenin 25 mg/kg	1.69 ± 0.019 ^{ns}	28.45 ± 0.038****	3.13 ± 0.017 ^{ns}
9.	Apigenin 50 mg/kg	1.23 ± 0.027***	28.19 ± 0.042****	3.11 ± 0.020*

Values are expressed as Mean ± SD (n = 6). ns = non-significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared with disease control group.

Table 7: The effect of flavonoid supplements on serum biochemical parameters on day 15

Sr. No.	Group and treatment	Serum biochemical parameters day 15		
		Creatinine (mg/dL)	Blood urea(m g/dL)	Uric acid (mg/dL)
1.	Normal control	0.66 ± 0.020****	26.38 ± 0.090****	2.26 ± 0.0367****
2.	Disease control gentamycin 80 mg/kg	1.76 ± 0.0358	33.39 ± 0.074	3.28 ± 0.020
3.	Standard control Silymarin 50 mg/kg	0.87 ± 0.048****	24.24 ± 0.034****	2.076 ± 0.020****
4.	Quercetin 25 mg/kg	1.55 ± 0.054**	28.28 ± 0.0419****	3.05 ± 0.029****
5.	Quercetin 50 mg/kg	0.91 ± 0.017****	27.33± 0.052****	2.91 ± 0.021****
6.	Rutin 25 mg/kg	1.63 ± 0.051^{ns}	28.29 ± 0.0732***	3.20 ± 0.023^{ns}
7.	Rutin 50 mg/kg	0.97± 0.028****	27.51± 0.062****	3.10 ± 0.045***
8.	Apigenin 25 mg/kg	1.57 ± 0.051^{ns}	28.09 ± 0.0732***	3.01 ± 0.023^{ns}
9.	Apigenin 50 mg/kg	0.89± 0.028****	27.71± 0.062****	3.13 ± 0.045***

Values are expressed as Mean ± SD (n = 6). ns = non-significant; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared with disease control group.

Table 8: The effects of flavonoid supplements in-vivo antioxidant enzymes

Sr. No.	Group and treatment	Serum biochemical parameters *in vivo* enzymes (Unit)		
		SOD U/min/mg of protein	GSH µM/mg protein	CAT µmoles of H_2O_2consumed/ min/mg
1.	Normal control	8.52 ± 0.175****	25.16 ± 0.055****	22.12± 0.069****
2.	Disease control gentamicin 80 mg/kg	3.23 ± 0.0377	21.36 ± 0.144	18.24 ± 0.069
3.	Standard control Silymarin 50 mg/kg	7.94 ± 0.075****	25.36 ± 0.052****	21.30 ± 0.039****
4.	Quercetin 25 mg/kg	6.24 ± 0.046****	22.26 ± 0.066****	20.37 ± 0.055****
5.	Quercetin 50 mg/kg	6.72 ± 0.056****	23.38 ± 0.057****	21.12± 0.053****
6.	Rutin 25mg/kg	6.37 ± 0.066****	22.45 ± 0.090****	20.25 ± 0.080****
7.	Rutin 50mg/kg	6.51 ± 0.063****	23.36 ± 0.050****	21.29 ± 0.054****
8.	Apigenin 25 mg/kg	6.17 ± 0.066****	22.15 ± 0.090****	21.11 ± 0.080****
9.	Apigenin 50mg/kg	6.33 ± 0.063****	23.06±0.050****	21.39 ± 0.054****

Values are expressed as Mean ± SD (n = 6). **** $p < 0.0001$ compared with disease control group.

3.2.3. In-vivo antioxidant enzyme activity in flavonoid-treated groups

In the gentamicin-only group, antioxidant enzyme levels (SOD, GSH, and CAT) were notably reduced, indicating oxidative stress in the renal tissues. Flavonoid treatments elevated these enzyme levels, with high-dose quercetin and rutin displaying the most significant restoration. SOD and CAT activities were significantly improved in the high-dose flavonoid groups, which demonstrated a comparable or superior effect to the silymarin control, underscoring the antioxidant efficacy of these compounds in mitigating oxidative renal damage **(Table 8)**. The effects of flavonoid supplements *in-vivo* antioxidant enzymes).

3.3. Histopathological evaluation of renal tissue

Histopathological examination of kidney sections revealed marked differences among groups, reflecting the extent of gentamicin-induced damage and flavonoid-mediated protection. Normal control kidneys exhibited intact renal architecture, with well-defined glomeruli, clear Bowman's spaces, and healthy tubular epithelium devoid of necrosis or inflammatory infiltration.

Gentamicin administration caused severe renal damage characterized by tubular degeneration and necrosis, thickened glomerular basement membranes, and prominent mononuclear cell infiltration, indicating inflammation and disrupted tissue integrity.

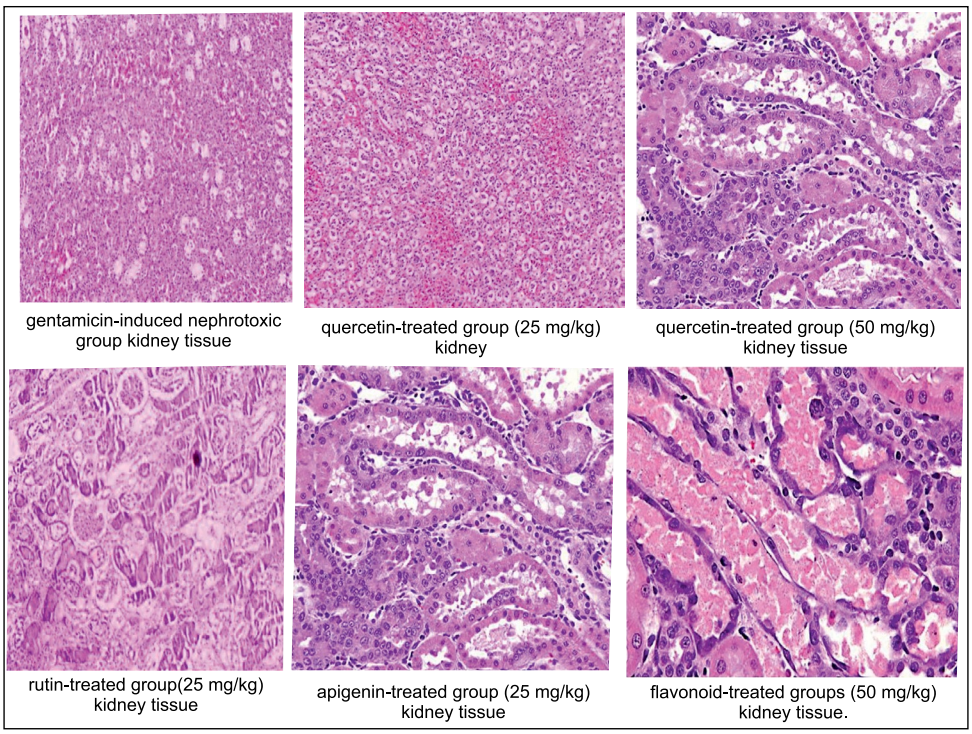

Figure 3: Histological sections of kidney tissues from control, gentamicin-induced, and flavonoid-treated groups showing varying degrees of renal damage and recovery (H&E stain, 40x)

Flavonoid treatment demonstrated dose-dependent nephroprotection. At 25 mg/kg, quercetin-treated kidneys showed moderate regeneration with reduced necrosis and inflammation, while rutin and apigenin groups exhibited partial tubular recovery but persistent cellular damage. At 50 mg/kg, all flavonoids significantly restored renal histology, with quercetin yielding near-complete recovery characterized by healthy tubular epithelium, restored glomerular structure, and minimal inflammatory infiltrates. Rutin and apigenin similarly improved renal morphology, approaching normal tissue architecture. Comparatively, quercetin displayed the most pronounced histological recovery, followed by rutin and apigenin. These results support the dose-dependent antioxidative and anti-inflammatory effects of flavonoids in mitigating gentamicin nephrotoxicity and facilitating renal tissue regeneration **(Figure 3)**.

4. Discussion

The present study aimed to assess the nephroprotective potential of natural flavonoids—quercetin, rutin, and apigenin—against gentamicin-induced nephrotoxicity, with a focus on biochemical, antioxidant, and histopathological outcomes.

Acute toxicity tests confirmed the safety of all three flavonoids up to 2000 mg/kg, aligning with OECD 423 guidelines, suggesting their suitability for therapeutic use. The absence of adverse effects, behavioral changes, or mortality underscores their high safety margins. Gentamicin-induced nephrotoxicity was evident through elevated serum creatinine, blood urea, and uric acid levels, as well as weight loss and reduced total protein and albumin levels. These changes reflect significant renal impairment and systemic toxicity, consistent with previous studies highlighting gentamicin's oxidative and inflammatory renal damage mechanisms.

Flavonoid treatments, especially at 50 mg/kg, significantly ameliorated these biochemical disturbances. Quercetin showed the most potent nephroprotective activity, followed by rutin and apigenin. This hierarchy of effectiveness supports prior findings on the superior antioxidant and anti-inflammatory capacity of quercetin in renal injury models. The restoration of renal function markers and protein levels indicates improved glomerular and tubular function. Flavonoid-treated groups also exhibited enhanced antioxidant enzyme activity (SOD, CAT, GSH), signifying a strong defense against oxidative stress. These findings correlate with reports that flavonoids enhance endogenous antioxidant systems and reduce ROS-mediated cellular injury.

Histopathological analyses revealed severe structural damage in gentamicin-treated rats, including tubular necrosis and inflammatory infiltration. In contrast, flavonoid administration markedly restored normal renal architecture in a dose-dependent manner. Quercetin-treated kidneys showed nearly complete tissue regeneration, corroborating its high therapeutic efficacy. Overall, the results demonstrate that quercetin, rutin, and apigenin confer renoprotection primarily through antioxidative, anti-inflammatory, and tissue-regenerative mechanisms. The effects were dose-dependent and comparable to silymarin, a known hepatoprotective and nephroprotective agent.

5. Conclusion

The present study demonstrates that natural flavonoids—quercetin, rutin, and apigenin—exhibit significant nephroprotective effects against gentamicin-induced renal toxicity. Acute toxicity evaluation confirmed their safety at therapeutic doses. Flavonoid supplementation effectively improved renal function biomarkers, reduced oxidative stress, and mitigated inflammatory responses. Histopathological analysis further validated their capacity to preserve and restore kidney architecture, with quercetin showing superior efficacy compared to rutin and apigenin. These findings underscore the potential of flavonoids as promising candidates for preventing and managing drug-induced nephrotoxicity through their antioxidant, anti-inflammatory, and tissue regenerative properties. Further clinical studies are warranted to translate these protective effects into therapeutic applications.

6. Abbreviations

GM: Gentamicin, QC: Quercetin, RT: Rutin, AP: Apigenin, ROS: Reactive Oxygen Species, MDA: Malondialdehyde, SOD: Superoxide Dismutase, CAT: Catalase, GSH: Glutathione, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, BUN: Blood Urea Nitrogen, Cr: Creatinine

7. Ethical No.

1929/PO/Re/S/16/CPCSEA

8. Authors Contribution

1. **Mr. Vijaykumar Yelwantge:** Conceptualization, Methodology, Investigation, Data Curation, Writing Original Draft Preparation, Visualization,
2. **Dr. Vivek Chauhan:** Supervision, Validation, Writing—Review & Editing, Project Administration, Final Approval of Manuscript.

9. Source of Funding

None

10. Conflict of Interest

None

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