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A Novel Combination Approach using Naringenin and Bromelain for the Amelioration of Gentamicin-Induced Nephrotoxicity in Rats

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Gentamicin, historically widely used and still employed in specific clinical settings, often causes nephrotoxicity primarily through oxidative stress and inflammation, among other mechanisms. This study evaluates the protective effect of Naringenin, a flavonoid antioxidant, and Bromelain, an anti-inflammatory proteolytic enzyme, against gentamicin-induced renal injury in male Wistar rats. Animals were divided into eight groups, including normal and gentamicin controls, standard treatment (N-acetyl cysteine), varying doses of Naringenin and Bromelain, and their combination. Nephrotoxicity was induced with gentamicin (80 mg/kg/day, i.p., for 8 days). Parameters such as body/kidney weight, urine output, biochemical markers, oxidative stress indicators, cytokines, and histopathology were assessed. Gentamicin caused significant renal damage, evidenced by increased serum urea (51.27 ± 6.10 mg/dL) and creatinine (6.13 ± 1.37 mg/dL) compared to control ($p < 0.001$). Treatment with Naringenin (20 mg/kg) and Bromelain (30 mg/kg) significantly reduced serum creatinine to 3.80 ± 0.71 mg/dL and 4.06 ± 0.58 mg/dL, respectively ($p < 0.01$ vs. gentamicin). The combination therapy produced the most pronounced effect, lowering serum creatinine to 2.85 ± 0.59 mg/dL and restoring total protein levels by nearly 90% compared to the control group ($p < 0.001$). Histopathological analysis further confirmed marked recovery of renal architecture, with the combination group showing minimal inflammatory infiltration and near-normal morphology. Overall, the combination therapy showed statistically significant ($p < 0.001$) superior nephroprotection compared to individual treatments, highlighting its potential as a natural, synergistic alternative for mitigating drug-induced nephrotoxicity.

Keywords: Bromelain; Combination therapy; Gentamicin; N-acetyl cysteine; Naringenin; Nephropathy; Nephroprotective agents; Oxidative stress.

Gentamicin is an aminoglycoside antibiotic still used in specific clinical settings for severe gram-negative infections, owing to its potent bactericidal activity.[1-3] However, its clinical efficacy is significantly limited by nephrotoxicity, which affects patients at variable rates, ranging from 10–25%, depending on dosage, treatment duration, and individual patient risk factors.[4-5] Gentamicin-induced nephrotoxicity is characterized by acute tubular necrosis and is primarily mediated by oxidative stress, inflammation, and apoptosis in renal tissues.[6-10] The accumulation of gentamicin in the renal cortex leads to the generation of reactive oxygen species (ROS), lipid peroxidation (LPO), nitric oxide (NO) overproduction, and the release of pro-inflammatory cytokines such as TNF-á and IL-6, contributing to progressive renal damage.[11-14]

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Given the limitations of conventional therapeutic approaches, increasing attention has been directed towards natural compounds with antioxidant and anti-inflammatory properties for the prevention and management of drug-induced nephrotoxicity.[15] Naringenin, a flavonoid mainly found in citrus fruits, shows strong antioxidant, anti-inflammatory, and free radical scavenging activities. It has been reported to modulate oxidative stress and inflammatory pathways in various experimental models of organ injury.[16-17] Bromelain, a proteolytic enzyme extracted from the stem and fruit of *Ananas comosus* (pineapple), has demonstrated significant anti-inflammatory, antioxidant, and immunomodulatory effects, with emerging evidence suggesting its role in protecting renal function.[18]

Naringenin and Bromelain have been studied alone for their pharmacological benefits,[19-20] but no data exist regarding their combined effect against nephrotoxicity. Considering their complementary mechanisms of action, this study evaluates the individual and synergistic nephroprotective effects of Naringenin and Bromelain in a gentamicin-induced rat model of renal toxicity. The study focuses on evaluating renal function markers, oxidative stress parameters, and pro-inflammatory cytokine levels to understand the protective potential of these natural agents.

MATERIALS AND METHODS

Materials

Gentamicin and N-Acetyl Cysteine was purchased from Sigma-Aldrich, USA. Naringenin (e"98% purity, HPLC grade) and Bromelain (e"97% purity, enzymatic activity grade) were purchased from Yucca Enterprises and Biolaxi Enzymes Pvt. Ltd., Mumbai, respectively. Biochemical, spectrophotometric and ELISA assay kits for serum and antioxidant parameters were obtained from Krishgen Biosystems, Mumbai. All other chemicals as well as reagents used were of analytical grade.

Experimental animals

Healthy adult male albino Wistar rats (200–250 g) were procured from a certified animal house facility. A total of 56 rats were initially acquired to account for potential losses during acclimatization or experimentation, of which 48 rats were finally included and randomized into the study groups. The animals were housed in standard polypropylene cages under controlled environmental conditions: 12-hour light/dark cycle, temperature of 22 ± 2 °C, and relative humidity of 55–65%. They had free access to a standard pellet diet and water ad libitum.[21-22] All experimental procedures were carried out in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA) for the care and use of laboratory animals. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of KBHSS Trust's Institute of Pharmacy, Malegaon (Approval No.: KBH/IAEC/2024/07-02).

Preparation of drug solutions

Gentamicin (80 mg/kg) was dissolved in normal saline and administered intraperitoneally (i.p.) as a single dose to induce nephropathy.[23] N-Acetyl Cysteine (150 mg/kg, p.o.) was dissolved in saline and administered orally.[24] Naringenin and Bromelain were suspended separately in 0.5% carboxymethylcellulose for oral administration. All solutions were administered in a standardized volume of 10 mL/kg body weight to ensure dose consistency across groups. All drug preparations were made freshly each day before administration.

Dose selection for Naringenin and Bromelain

Dose of Naringenin and Bromelain was selected from the previously reported literature. The same dose was also standardized in our laboratory in the gentamicin model. Based on this the dose of Naringenin (10 and 20 mg/kg, p.o.) and Bromelain (15 and 30 mg/kg, p.o.) was selected in the present study.[25-26] The Bromelain used in this study was derived from pineapple stem with e"97% enzymatic activity grade, ensuring reproducibility of pharmacological effects.

Induction of nephropathy

Nephropathy was induced in male albino Wistar rats by intraperitoneal administration of gentamicin at a dose of 80 mg/kg body weight, once daily for eight consecutive days. Gentamicin was administered using an insulin syringe under light anesthesia with ketamine (50 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.) to minimize animal discomfort. Following the induction, animals were monitored daily for signs of toxicity, including changes in behavior, food and water intake, and general activity level.[27-29]

Experimental protocol

A total of 48 male albino Wistar rats were randomly divided into eight groups (n = 6 per group) as follows:[25-26]

• Group I (Normal Control): Received no treatment.
• Group II (Disease Control): Received gentamicin (80 mg/kg, i.p.) once daily for eight days.
• Group III (Standard): Received N-acetyl cysteine (150 mg/kg, p.o.).
• Group IV: Received naringenin low dose (10 mg/kg, p.o.).
• Group V: Received naringenin high dose (20 mg/kg, p.o.).
• Group VI: Received bromelain low dose (15 mg/kg, p.o.).
• Group VII: Received bromelain high dose (30 mg/kg, p.o.).
• Group VIII (Combination): Received naringenin (20 mg/kg, p.o.) and bromelain (30 mg/kg, p.o.).

All treatments were administered orally once daily, given 1 hour after gentamicin injection to allow for drug absorption and to assess their protective effect following nephrotoxic insult. This sequence of administration may have influenced the pharmacodynamic interactions observed.

The number of animals per group (n = 6) was selected based on prior published nephrotoxicity studies and standard practice in preclinical pharmacology.[23] Although no formal statistical sample size calculation was performed, this group size was considered sufficient to detect biologically and statistically meaningful differences, while adhering to the principle of reduction in animal experimentation.

Initial and final body weights and urine volumes were recorded weekly to monitor nephrotoxic effects and treatment responses.[30] On Day 9, animals were euthanized to collect blood samples for biochemical estimations. The kidneys were carefully excised; one kidney from each animal was used to prepare homogenates for the estimation of antioxidant and inflammatory markers, while the other was fixed in 10% formalin for histopathological examination.[31-32]

Estimation of antioxidant parameters

Kidney tissues were excised, minced, and homogenized in phosphate buffer (pH 7.0) containing a protease inhibitor to prevent protein degradation. A 10% (w/v) tissue homogenate was prepared in accordance with the protocols provided by the respective antioxidant assay kit manufacturers. The homogenate was centrifuged at 10,000 rpm for 20 min at 4 °C, and the resulting supernatant was collected for the estimation of oxidative stress and antioxidant markers. LPO was assessed by measuring thiobarbituric acid reactive substances, and the results were expressed as malondialdehyde (MDA) equivalents. NO levels were estimated based on the accumulation of nitrite using the Griess reagent.[33] The antioxidant enzymes assessed included superoxide dismutase (SOD), evaluated based on its ability to inhibit the reduction of nitroblue tetrazolium; catalase (CAT), determined by monitoring the spectrophotometric decomposition of hydrogen peroxide at 240 nm; and reduced glutathione (GSH), estimated as protein-free sulfhydryl groups using 5,52 -dithiobis-2-nitrobenzoic acid.[34-36]

Estimation of anti-inflammatory parameters

Kidney tissues collected for ELISA were stored at “80 °C until analysis. Prior to processing, the tissues were thawed at room temperature for 15 min and homogenized in the ice-cold buffer provided with the ELISA kits. The homogenates were centrifuged at 12,000 rpm for 30 min at 4 °C, and the resulting supernatants were collected and aliquoted for cytokine estimation. Levels of pro-inflammatory cytokines, including TNF-á and IL-6, were quantified using commercially available ELISA kits, following the instructions of manufacturer. Absorbance was recorded using a microplate reader, and cytokine concentrations were calculated from the standard curves generated for each assay.[37] The ELISA kits used had a sensitivity of <2 pg/mL and specificity >95% for rat cytokines, ensuring reliable quantification.

Histopathology

For histopathological evaluation, kidney tissues were fixed in 10% neutral-buffered formalin, embedded in paraffin, and sectioned at a thickness of 5 µm. The sections were stained with hematoxylin and eosin (H&E) to visualize cellular and tissue architecture. Stained slides were examined under a light microscope for histopathological alterations, including tubular necrosis, glomerular damage, and inflammatory cell inflammation.[38-39] A blinded pathologist performed a semi-quantitative assessment of renal histological damage using a standardized 0–4 scale, where 0 indicated no abnormality detected

(NAD), 1 represented minimal changes, 2 denoted mild changes, 3 reflected moderate changes, and 4 indicated severe pathological alterations. Scoring was carried out independently for glomerular atrophy, tubular degeneration, and inflammatory infiltration.

Statistical analysis

All data are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. A p-value < 0.05 was considered statistically significant.[40] GraphPad Prism software (version 5.0) was used for data analysis and graphical representation.

RESULTS

General parameters

The effects of various treatments on body weight, kidney weight, and hypertrophy index are summarized in Table 1. Gentamicin administration (Group II) led to a statistically significant ($p < 0.001$) reduction in body weight on Day 8 compared to Day 0, along with marked increases in absolute kidney weight, relative kidney weight, hypertrophy index, and urine volume ($p < 0.001$ vs. Group I), indicating nephrotoxicity and renal hypertrophy typically associated with oxidative damage and tubular dysfunction. In contrast, rats treated with the standard antioxidant N-acetyl cysteine (Group III) showed a significant reversal of these changes ($p < 0.05$ vs. Group II), with improved body weight gain, reduced kidney hypertrophy, and normalized urine volume, demonstrating its partial nephroprotective effect.[41] Naringenin-treated groups also exhibited a dose-dependent improvement. The low-dose group (Group IV) significantly ameliorated kidney weights and hypertrophy index ($p < 0.05$ vs. Group II), while the high-dose group (Group V) showed more pronounced nephroprotection, restoring the values close to the control group. This suggests potent antioxidative and anti-inflammatory properties of Naringenin in attenuating gentamicin-induced renal damage.[42] Similarly, Bromelain treatment in both low (Group VI) and high doses (Group VII) resulted in significant decreases in kidney weight and urine output ($p < 0.05$ vs. Group II), with the high dose being more effective. The combination of Naringenin and Bromelain (Group VIII) offered the most significant protection, with almost normalized body and kidney parameters and urine volume, suggesting a synergistic effect in mitigating gentamicin-induced nephrotoxicity.[43] In Table 1, the symbol "#" denotes statistical significance compared to the normal control group (Group I), while "*" denotes significance compared to the disease control group (Group II). The hypertrophy index was calculated as the ratio of kidney weight to body weight (g/100 g), which reflects renal enlargement relative to systemic body mass.

Biochemical parameters in blood

The biochemical evaluation of serum parameters across various treatment groups (as shown in Fig. 1 (a–e) and summarized in Tables 2) highlights the nephroprotective efficacy of Naringenin and Bromelain in a gentamicin-induced nephropathy model. Gentamicin administration (Group II) induced significant nephrotoxicity in rats, as evidenced by elevated serum urea, uric acid, and creatinine levels, alongside a marked reduction in serum total protein and albumin compared with the normal control group (Group I). These changes confirm acute renal damage and impaired kidney function due to gentamicin-induced oxidative stress and tubular necrosis.[44] Treatment with the standard nephroprotective agent, N-acetyl cysteine (Group III), significantly restored serum urea, uric acid, creatinine, total protein, and albumin levels, reflecting effective renal protection.[45] Naringenin and Bromelain both showed dose-dependent nephroprotective effects. High-dose Naringenin (Group V) significantly improved serum urea, uric acid, creatinine, total protein, and albumin, outperforming the low-dose group (Group IV). Similarly, high-dose Bromelain (Group VII) led to significant improvements in serum urea, uric acid, creatinine, total protein, and albumin over its low-dose counterpart (Group VI). The combination treatment group (Group VIII) exhibited the most pronounced protective effects among all test groups, showing marked reductions in serum urea, uric acid, and creatinine, along with near-complete restoration of total protein and albumin.[46] These results align with previous studies reporting that Naringenin mitigates renal oxidative damage by enhancing antioxidant status and reducing serum creatinine and urea,[47] while Bromelain has been shown to improve biochemical markers of

Table 1. Effect of various treatments on body weight, kidney weight, and hypertrophy index in gentamicin-induced nephropathy in rats

Group	Body weight		Absolute kidney weight	Relative kidney weight	Hypertrophy index	Final urine volume (ml/24hrs)
	Day 0	Day 8				
Group I	232.5±2.68	250.4±3.44	1.73±0.07	0.65±0.03	0.006±0.001	6.53±0.39
Group II	226.1±3.56	221.0±3.58###	2.42±0.07###	1.08±0.05###	0.012±0.001###	13.03±0.77###
Group III	230.6±2.37	244.2±3.89*	1.78±0.08*	0.70±0.11*	0.007±0.001*	6.87±0.76*
Group IV	226.3±3.10	232.6±4.07	2.21±0.11*	0.92±0.07*	0.009±0.001*	11.22±0.53*
Group V	229.3±3.31	241.7±3.05*	1.90±0.13*	0.78±0.07*	0.008±0.001*	7.92±0.44*
Group VI	226.1±2.48	230.0±6.98	2.23±0.05*	0.94±0.05*	0.009±0.001*	11.27±0.62*
Group VII	228.5±3.58	237.8±2.72*	1.92±0.11*	0.78±0.10*	0.008±0.001*	7.98±0.42*
Group VIII	230.2±3.38	242.8±4.32*	1.92±0.10*	0.74±0.09*	0.007±0.001*	7.15±0.51*

Values are expressed as mean ± SEM; n = 6. One-way ANOVA followed by Tukey's post hoc test.
Significance value: ###p < 0.001 vs. Control (Group I); *p < 0.05, **p < 0.01, ***p < 0.001 vs. Disease Control (Group II).

Table 2. Effect of different experimental groups on serum urea, uric acid, creatinine, total protein and albumin levels

Group	Serum urea(mg/dL)	Uric acid(mg/dL)	Serum creatinine(mg/dL)	Total protein(g/L)	Serum albumin(g/L)
Group I	15.50 ± 2.15	22.03 ± 3.27	0.46 ± 0.04	7.77 ± 0.78	4.73 ± 0.58
Group II	51.27 ± 6.10###	52.22 ± 4.34###	6.13 ± 1.37###	2.55 ± 0.62###	1.70 ± 0.55###
Group III	19.67 ± 3.61***	25.04 ± 3.62***	2.56 ± 0.63**	6.82 ± 0.49***	4.10 ± 0.72***
Group IV	38.82 ± 4.84*	42.04 ± 6.82*	4.73 ± 0.89*	4.34 ± 0.54*	2.74 ± 0.79*
Group V	29.15 ± 2.99**	35.37 ± 3.90**	3.80 ± 0.71**	5.41 ± 0.68**	3.69 ± 0.18**
Group VI	42.70 ± 3.61*	44.32 ± 3.42*	4.96 ± 0.57*	3.94 ± 0.75*	2.65 ± 0.78*
Group VII	31.03 ± 4.52**	37.84 ± 3.49**	4.06 ± 0.58**	5.19 ± 0.58**	3.49 ± 0.77**
Group VIII	21.38 ± 3.63***	30.04 ± 3.23***	2.85 ± 0.59***	6.19 ± 0.38***	4.13 ± 0.54***

Values are expressed as mean ± SEM; n = 6. One-way ANOVA followed by Tukey's multiple comparison tests.
Significance value: ###p < 0.001 vs. Control (Group I); *p < 0.05, **p < 0.01, ***p < 0.001 vs. Disease Control (Group II).

nephrotoxicity through anti-inflammatory and proteolytic mechanisms.[48]

Estimation of antioxidant parameters

Gentamicin administration (Group II) produced a marked depletion of antioxidant defense mechanisms, as evidenced by decreased levels of GSH, SOD, and CAT, along with a significant increase in lipid peroxidation (MDA levels) ($p < 0.001$ vs. Group I), as shown in Fig. 2 (a–e) and summarized in Table 3. Treatment with N-acetyl cysteine (Group III) restored antioxidant enzyme levels and reduced LPO, confirming its nephroprotective potential.[49]

Naringenin treatment exhibited a dose-dependent restoration of antioxidant parameters.[50] High-dose Naringenin (Group V) produced a more pronounced significativamente higher increase in GSH, SOD, and CAT compared to the low-dose group (Group IV) (*$p < 0.05$ to ***$p < 0.001$ vs. Group II). Bromelain also improved antioxidant markers in a dose-dependent manner, with Group VII (30 mg/kg) showing superior effects over Group VI (15 mg/kg). Importantly, the combination therapy (Group VIII) provided the most profound antioxidant protection,[51] restoring GSH, SOD, and CAT levels close to those of the control group and

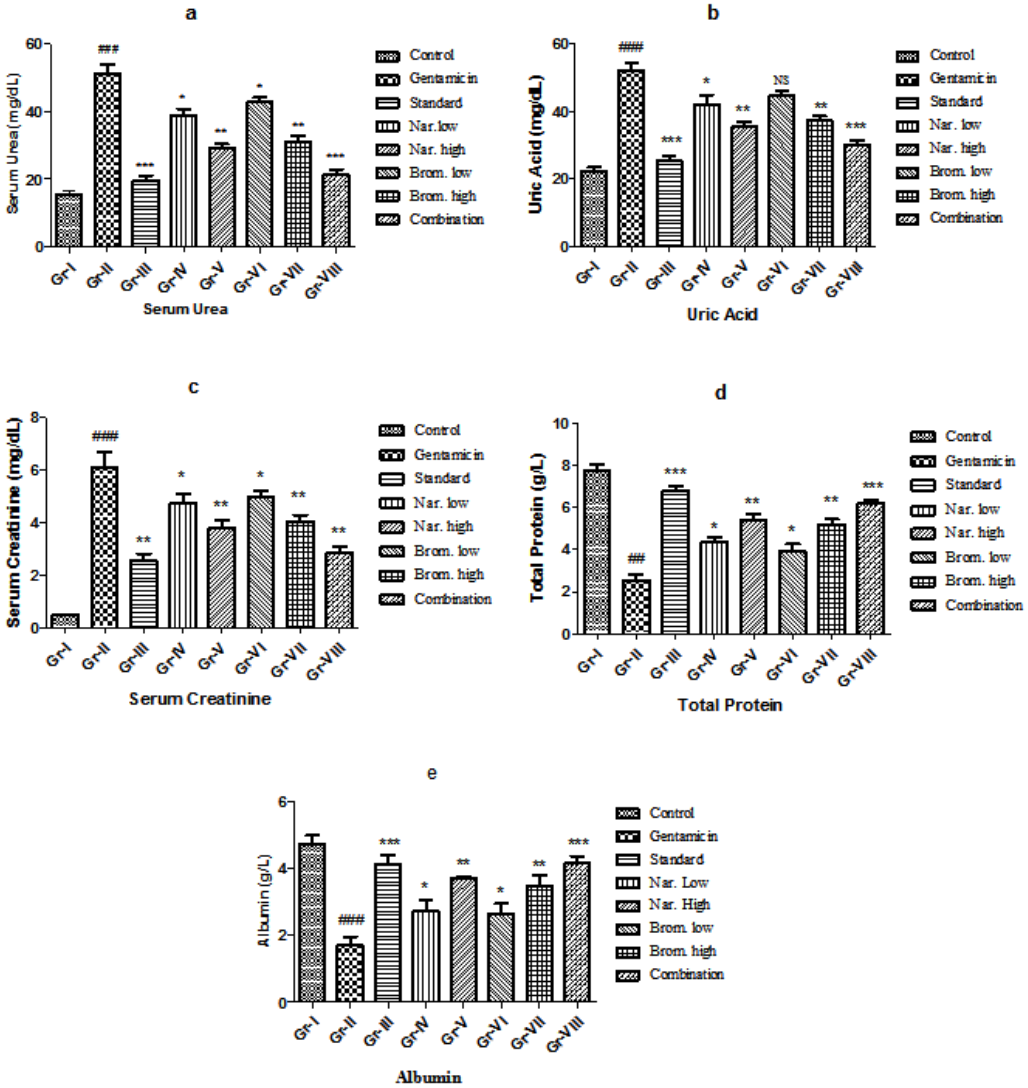

Fig. 1. Effect of various treatments on (a) serum urea, (b) uric acid, (c) creatinine, (d) total protein, and (e) albumin levels in gentamicin-induced nephropathy in rats

Table 3. Effect of different experimental groups on LPO, NO, SOD, CAT and GSH levels

Group	LPO level (nmol MDA/mg protein)	NO level (µmol/g tissue)	GSH level (µmol/g tissue)	SOD level (U/mg protein)	CAT level (U/mg protein)
Group I	6.30 ± 0.82	5.23 ± 0.96	6.54 ± 0.78	16.64 ± 2.34	15.44 ± 1.83
Group II	30.83 ± 4.34###	21.90 ± 1.87###	1.88 ± 0.24####	5.86 ± 1.44####	4.68 ± 1.27####
Group III	10.42 ± 1.56***	7.84 ± 1.98***	5.97 ± 1.27***	14.34 ± 1.94***	13.86 ± 2.15***
Group IV	23.70 ± 3.75*	16.80 ± 2.32*	3.41 ± 0.47*	9.08 ± 2.19*	9.29 ± 2.00*
Group V	19.67 ± 1.43**	12.63 ± 3.25**	4.79 ± 0.71**	11.84 ± 1.44**	11.69 ± 1.28**
Group VI	24.75 ± 3.21*	17.85 ± 1.67*	3.18 ± 0.46*	8.99 ± 1.77*	8.78 ± 1.41*
Group VII	20.08 ± 2.75**	13.60 ± 2.25**	4.09 ± 0.94**	11.24 ± 2.46**	10.65 ± 1.61**
Group VIII	12.03 ± 2.22***	9.20 ± 1.58***	5.99 ± 0.64***	14.90 ± 2.59***	13.81 ± 1.59***

Values are expressed as mean ± SEM; n = 6. One-way ANOVA followed by Tukey's multiple comparison tests.
Significance values: ###$p < 0.001$ vs. Control (Group I); *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. Disease Control (Group II).

reducing MDA levels by nearly 70% compared to gentamicin-treated animals ($p < 0.001$ vs. Group II).

Mechanistically, Naringenin enhances antioxidant capacity by activating the nuclear factor erythroid 2–related factor 2 (Nrf2)/ARE pathway, leading to the upregulation of detoxifying enzymes and direct scavenging of free radicals.[52] Bromelain reduces oxidative stress indirectly by inhibiting nuclear factor kappa B (NF-êB)–mediated inflammation and promoting the activity of endogenous antioxidant enzymes.[53] Their individual actions augment intracellular GSH replenishment and stabilize SOD and CAT activity, resulting in significant antioxidant effects. These findings align with recent studies reporting that Naringenin restores renal antioxidant status in drug-induced nephrotoxicity,[54] while Bromelain exhibits complementary antioxidant and anti-inflammatory properties.[55]

Estimation of anti-inflammatory parameters

Gentamicin administration (Group II) produced a significant elevation of pro-inflammatory cytokines TNF-á and IL-6 ($p < 0.001$ vs. Group I), confirming the role of inflammatory pathways in drug-induced nephrotoxicity.[56] Treatment with N-acetyl cysteine (Group III) significantly reduced cytokine levels, validating its nephroprotective role.[57] Both Naringenin and Bromelain produced dose-dependent decreases in TNF-á and IL-6 compared to the disease group, with high-dose groups (V and VII) showing greater efficacy. Notably, the combination group (VIII) exhibited the most profound anti-inflammatory response, with cytokine levels restored nearly to control values ($p < 0.001$ vs. Group II).[58]

Mechanistically, Naringenin is known to suppress NF-êB activation and downregulate pro-inflammatory cytokines, while Bromelain exerts anti-inflammatory effects through modulation of NF-êB and MAPK pathways, leading to reduced TNF-á and IL-6 release. Both agents individually produce inhibitory effects on renal inflammatory signaling, thereby enhancing protection against gentamicin-induced damage. These findings are in agreement with recent reports showing that Naringenin attenuates nephroinflammation via NF-êB inhibition and Nrf2 activation in nephrotoxic models,[59] while Bromelain has been demonstrated to lower renal cytokine

expression and oxidative-inflammatory markers in experimental nephropathy.[60]

Histopathology

The histopathological evaluation of kidney tissues across the experimental groups as illustrated in Fig. 4 (a-h) reveals significant insights into the nephroprotective potential of Naringenin and Bromelain against gentamicin-induced nephropathy. The control group (Group I) exhibited normal renal architecture with intact glomeruli and tubules, confirming the baseline of healthy kidney structure.[61] In contrast, the gentamicin-treated group (Group II) demonstrated extensive renal damage characterized by glomerular atrophy, tubular degeneration, and marked inflammatory infiltration, validating the induction of nephrotoxicity.[62] Treatment with the standard antioxidant, N-acetyl cysteine (Group

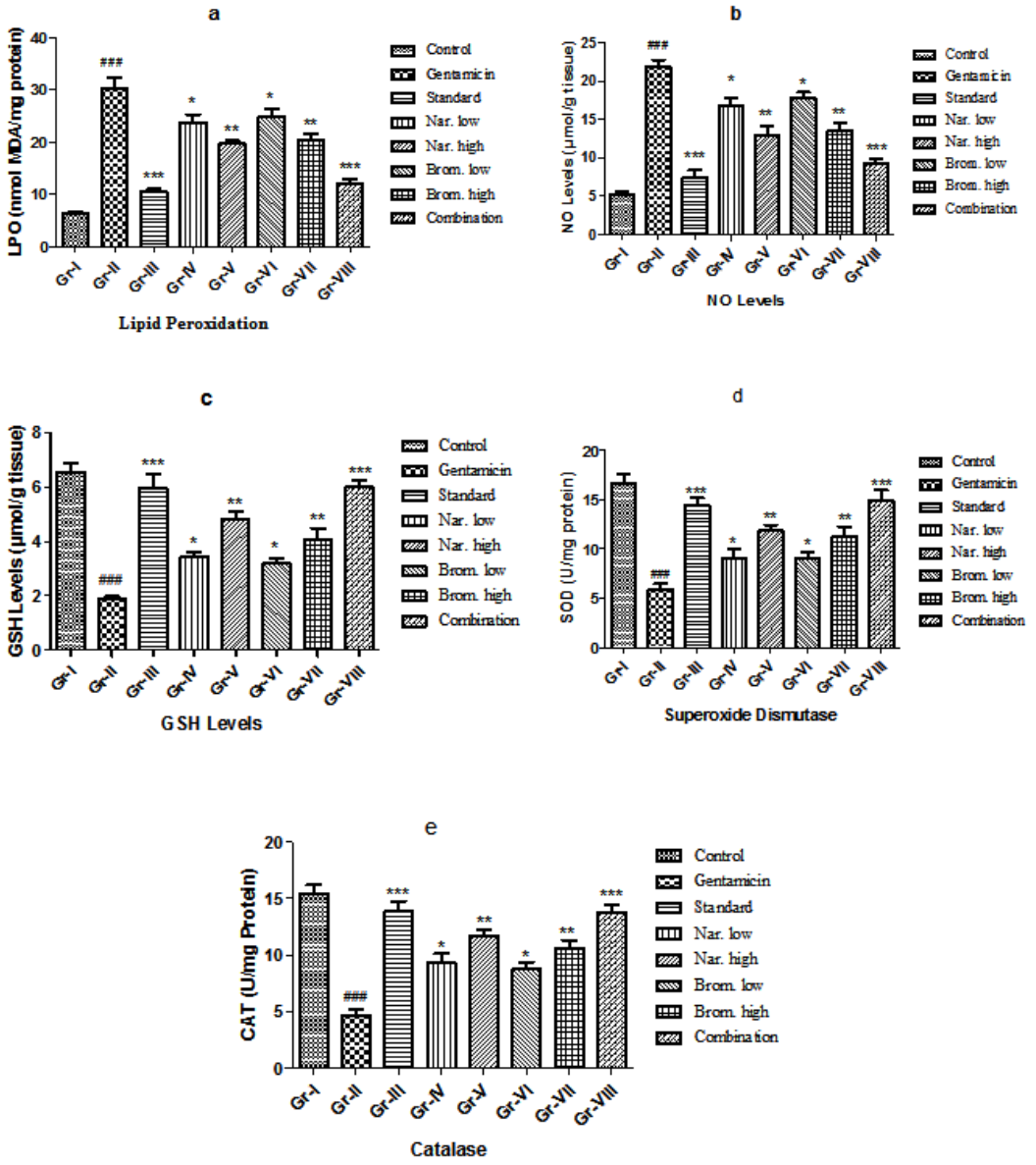

Fig. 2. Effect of various treatments on (a) LPO, (b) NO, (c) GSH, (d) SOD and (e) CAT levels in gentamicin-induced nephropathy in rats.

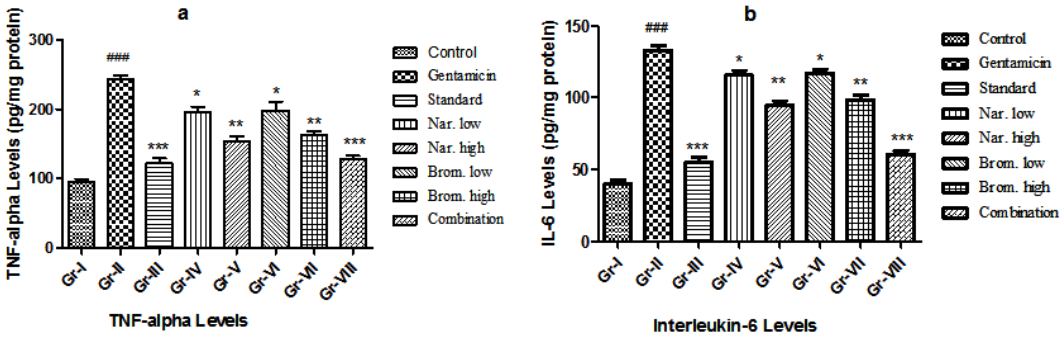

Fig. 3. Effect of different experimental groups on (a) TNF-α and (b) IL-6 levels

Table 4. Effect of different experimental groups on TNF-α and IL-6 levels.

Group	TNF-α level (pg/mL protein)	IL-6 Level (pg/mL protein)
Group I	95.79 ± 8.58	39.77 ± 7.18
Group II	242.81 ± 16.04###	132.48 ± 7.70###
Group III	122.24 ± 16.67***	54.94 ± 9.07***
Group IV	195.81 ± 18.87*	115.52 ± 7.75**
Group V	152.90 ± 21.39**	94.93 ± 6.38*
Group VI	198.32 ± 31.59*	117.37 ± 6.63**
Group VII	162.21 ± 14.93**	98.36 ± 6.47*
Group VIII	128.40 ± 11.05***	60.18 ± 6.93***

Values are expressed as mean ± SEM; n = 6. One-way ANOVA followed by Tukey's multiple comparison tests.
Significance values: ###p < 0.001 vs. Control (Group I); *p < 0.05, **p < 0.01, ***p < 0.001 vs. Disease Control (Group II).

Table 5. Semi-quantitative histopathological scores of renal tissues in different treatment groups

Group No.	Group Description	Glomerular Atrophy	Tubular Degeneration	Inflammatory Infiltration
I	Control	0	0	0
II	Gentamicin-treated	3	4	3
III	N-acetyl cysteine	1	1	2
IV	Naringenin (10 mg/kg)	3	2	2
V	Naringenin (20 mg/kg)	2	1	2
VI	Bromelain (15 mg/kg)	2	3	2
VII	Bromelain (30 mg/kg)	1	2	2
VIII	Naringenin (20 mg/kg) + Bromelain (30 mg/kg)	0	1	1

Scoring Criteria: 0 = No abnormality detected (NAD); 1 = Minimal changes; 2 = Mild changes; 3 = Moderate changes; 4 = Severe changes.

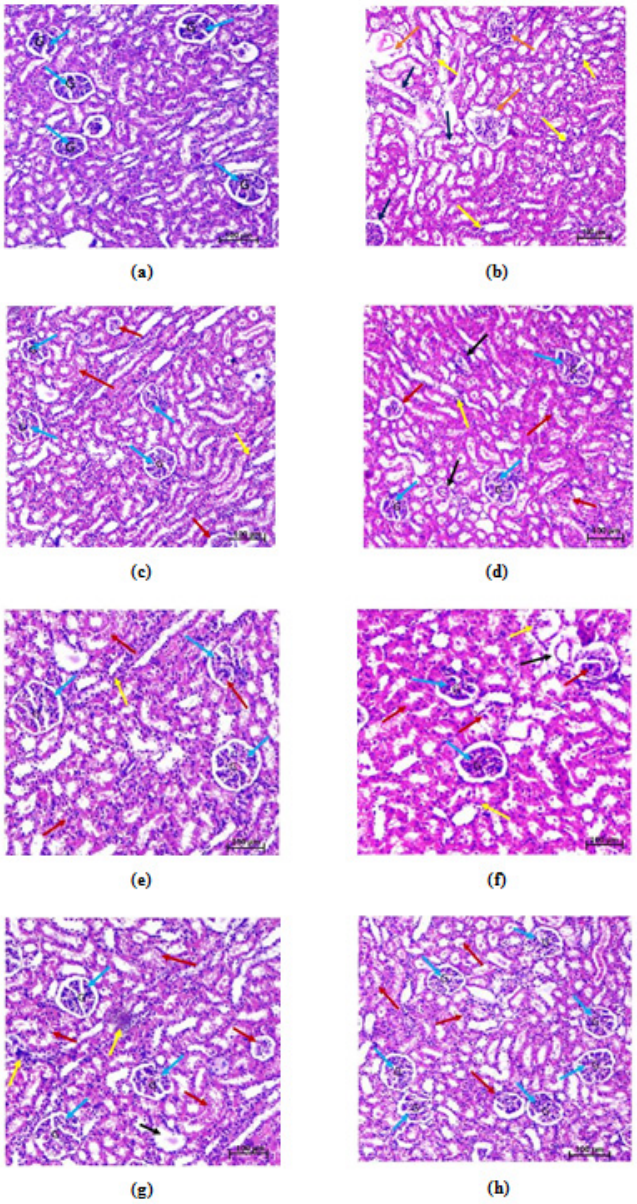

Fig. 4. Representative photomicrographs of kidney sections stained with Hematoxylin and Eosin (H&E) illustrating renal histology.

(A) Group I – Control: Normal renal architecture with intact glomerulus indicated by blue arrows. (B) Group II – Cisplatin-treated: Severe renal damage observed—orange arrows show glomerular atrophy, black arrows indicate tubular degeneration, and yellow arrows highlight areas of inflammatory cell infiltration. (C) Group III – NAC: Noticeable structural recovery—blue arrows indicate preserved glomeruli, yellow arrows indicate mild residual inflammation, and red arrows show areas of histological improvement. (D) Group IV – Naringenin (Low Dose): Partial recovery noted—blue arrows denote glomeruli, black arrows show tubular degeneration, yellow arrows indicate inflammation, and red arrows show areas of moderate recovery. (E) Group V – Naringenin (High Dose): Marked improvement—blue arrows denote glomeruli, yellow arrows indicate mild inflammation, and red arrows highlight evident recovery. (F) Group VI – Bromelain (Low Dose): Moderate protective effect—blue arrows show glomeruli, yellow arrows indicate inflammation, black arrows represent tubular regeneration, and red arrows point to recovering tissue. (G) Group VII – Bromelain (High Dose): Significant recovery—blue arrows show intact glomeruli, yellow arrows indicate mild inflammation, black arrows shows tubular degeneration, and red arrows show histological improvement. (H) Group VIII – Naringenin + Bromelain (High Dose): Near-complete restoration of renal structure—blue arrows indicate preserved glomeruli, and red arrows show minimal residual damage with substantial tissue recovery.

III), showed noticeable structural recovery with reduced inflammation and preserved glomerular integrity, though not complete restoration.[63] Administration of Naringenin and Bromelain individually at low doses (Groups IV and VI) resulted in modest protective effects with partial recovery of renal histology.[64] However, their high-dose counterparts (Groups V and VII) displayed moderate improvements in tissue architecture, with better preservation of glomeruli and tubules and reduced inflammatory signs.[65] Remarkably, the combination therapy group (Group VIII), receiving high doses of both Naringenin and Bromelain, exhibited significant improvement in kidney morphology, with minimal evidence of damage or inflammation, suggesting a synergistic protective effect.[66-68] These findings collectively indicate that both Naringenin and Bromelain possess nephroprotective properties, and their combination provides enhanced protection against gentamicin-induced renal injury. The semi-quantitative histological scores are summarized in Table 5, highlighting significant reductions in glomerular atrophy, tubular degeneration, and inflammatory infiltration, particularly in the combination group.

DISCUSSION

This study systematically evaluated the nephroprotective potential of Naringenin and Bromelain, both alone and in combination, against gentamicin-induced nephrotoxicity in a rat model. Gentamicin, although clinically valuable, is notorious for causing acute kidney injury via oxidative stress, inflammation, and tubular epithelial cell damage.[69] Our findings reinforce the role of oxidative and inflammatory pathways in gentamicin-induced renal dysfunction and demonstrate the effectiveness of Naringenin and Bromelain in mitigating these effects.

The administration of gentamicin induced characteristic renal dysfunction, as evidenced by significant weight loss, increased kidney mass, and elevated hypertrophy index, along with marked derangements in serum renal biomarkers. These outcomes align with established models of gentamicin-induced nephropathy, highlighting the clinical relevance of the model.[70] N-acetyl cysteine, a known antioxidant, offered moderate nephroprotection, confirming the important role of oxidative stress in the pathology.

Both Naringenin and Bromelain, known for their free radical-scavenging and anti-inflammatory properties, showed clear dose-dependent nephroprotective effects. Their administration improved renal function markers, decreased oxidative burden, and downregulated pro-inflammatory cytokines TNF-á and IL-6.[71] Importantly, the low- and high-dose groups demonstrated statistically significant differences, reinforcing the dose–response relationship observed in this study. High-dose treatments yielded greater benefits, indicating a dose-response relationship. Notably, the combination therapy was most effective across all evaluated parameters, indicating synergistic protective effects.

Mechanistically, Naringenin preserves mitochondrial integrity, inhibits apoptosis, activates the Nrf2 signaling pathway to upregulate antioxidant enzymes (SOD, CAT, GSH), and suppresses NF-êB–mediated inflammation.[72] Bromelain similarly inhibits NF-êB and downregulates pro-inflammatory mediators while potentially modulating TGF-â1, a key fibrogenic cytokine, thereby reducing the risk of renal fibrosis and progression to chronic kidney disease.[73]

Pharmacokinetically, Naringenin shows limited oral bioavailability due to first-pass metabolism,[74] whereas Bromelain is absorbed in its active form and retains systemic proteolytic activity after oral administration. These complementary profiles may explain the enhanced nephroprotection observed with their combination.[75]

The restoration of antioxidant enzymes (GSH, SOD, CAT), reduction in LPO and NO levels, and suppression of inflammatory cytokines collectively demonstrate the ability of these compounds to modulate redox balance and immune responses in renal tissues.[76] Histopathological findings further confirmed the protective effects, with the combination group showing the greatest preservation of renal architecture.

However, certain limitations must be acknowledged. The absence of molecular-level validation, such as Western blotting or PCR to confirm Nrf2, NF-êB, or TGF-â1 involvement, restricts mechanistic insights. Additionally, long-term toxicity and safety studies were not conducted,

which are essential for clinical translation. Future research should incorporate molecular assays, pharmacokinetic studies, and chronic toxicity models to strengthen the therapeutic relevance of this combination strategy.

CONCLUSION

The present study demonstrates that both Naringenin and Bromelain alone exhibit significant nephroprotective effects against gentamicin-induced renal toxicity through their antioxidant and anti-inflammatory properties. Gentamicin administration led to marked renal dysfunction, oxidative stress, and inflammatory responses, as evidenced by alterations in body and kidney weight, biochemical markers, cytokine levels, antioxidant defenses, and histopathological features. Treatment with N-acetyl cysteine, a known nephroprotective agent, validated the study model and offered partial protection.

Notably, the combination of high-dose Naringenin and Bromelain provided the most comprehensive improvement in renal parameters, reducing oxidative and inflammatory markers and preserving kidney architecture. However, given the preclinical nature of this work, these findings should be interpreted as a potential therapeutic approach rather than a definitive strategy. A key limitation is that the results are derived from an animal model, and further mechanistic studies as well as clinical trials will be required before translation to human use. Additionally, although no overt adverse effects were observed during the short-term study period, the long-term safety profile of Naringenin and Bromelain in combination remains to be established and must be carefully assessed in future investigations. In summary, while the findings highlight the potential of Naringenin and Bromelain as complementary agents against gentamicin-induced nephrotoxicity, their clinical applicability will depend on further validation and safety evaluation.

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The author(s) do not have any conflict of interest.

Data Availability Statement

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Ethics Statement

The study protocol was approved by the Institutional Animal Ethics Committee of KBHSS Trust's Institute of Pharmacy, Malegaon, under reference number KBH/IAEC/2024/07-02.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to reproduce material from other sources

Not Applicable.

Author Contributions

Kajal Pansare conducted the experimental work, data collection, statistical analysis, and manuscript drafting as part of her doctoral research; Dr. Vinod Bairagi, as research guide, conceptualized and supervised the study, provided critical insights into the experimental design, and revised the manuscript for intellectual content; Dr. Yogesh Ahire assisted in refining the methodology, provided guidance during data interpretation, and contributed to manuscript review and editing.

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