

Evaluation of the Nephroprotective Effects of Selected *Aeginetia indica* L. Extract Against Gadolinium-Induced Renal Toxicity

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ABSTRACT

Background: Gadolinium-Based Contrast Agents (GBCAs) are widely used in diagnostic imaging but are associated with nephrotoxicity, especially in patients with impaired renal function. Oxidative stress, inflammation, and tubular epithelial injury are key mechanisms of gadolinium-induced renal damage. Herbal extracts with antioxidant and anti-inflammatory properties have emerged as potential nephroprotective agents. **Materials and Methods:** Renal toxicity was induced in Wistar rats by intraperitoneal administration of gadolinium (10 mg/kg). Animals were divided into five groups: normal control, gadolinium control, standard drug (Cystone), low-dose *Aeginetia indica* L. extract (200 mg/kg), and high-dose extract (400 mg/kg). Serum renal biomarkers (creatinine, BUN, sodium, potassium, urine total protein), oxidative stress markers (SOD, CAT, GSH, MDA), and histopathological alterations were evaluated. **Results:** Gadolinium significantly elevated serum creatinine, BUN, sodium, potassium, and urine protein while causing tubular and glomerular damage. Treatment with *A. indica* extract restored renal biomarkers toward normal levels, enhanced antioxidant defense, reduced lipid peroxidation, and preserved renal histoarchitecture, particularly at the high dose. **Discussion:** The nephroprotective effects of *A. indica* are likely mediated by its bioactive coumarin compound, which neutralizes reactive oxygen species, mitigates inflammation, and maintains tubular and glomerular integrity. **Conclusion:** *Aeginetia indica* L. extract exhibits significant nephroprotective activity against gadolinium-induced renal toxicity, supporting its potential as a natural therapeutic agent for preventing contrast-induced kidney injury.

Keywords: Gadolinium, Herbal extract, Nephroprotection, Nephrotoxicity, Oxidative stress.

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INTRODUCTION

Gadolinium-Based Contrast Agents (GBCAs) are extensively used in Magnetic Resonance Imaging (MRI) to enhance diagnostic accuracy. Chemical structure is shown in Figure 1. Although initially considered safe, growing clinical and experimental evidence has demonstrated that gadolinium exposure can lead to renal toxicity, particularly in patients with compromised renal function, repeated contrast exposure, or concomitant

chemotherapy treatment (Abdel Moneim, 2014; Aime and Caravan, 2009). Gadolinium-induced renal toxicity has been associated with elevated serum creatinine and urea levels, tubular epithelial injury, and impaired glomerular filtration, ultimately resulting in acute or chronic kidney damage (Anand *et al.*, 2024). The mechanisms underlying gadolinium-induced nephrotoxicity are multifactorial and medicine of gadolinium is shown in Figure 2. Excessive generation of Reactive Oxygen Species (ROS) plays a central role, leading to lipid peroxidation, mitochondrial dysfunction, and depletion of endogenous antioxidant defences in renal tissue (Chaudhuri *et al.*, 2024). Gadolinium contrast agents provoke inflammatory cascades, notably through release of pro-inflammatory cytokines such as Tumor Necrosis Factor- α (TNF- α) and interleukins, thereby aggravating renal injury. Medicine of gadolinium is shown in Figure 3 (Chaudhuri



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et al., 2025). Apoptotic pathways and tubular necrosis further drive gadolinium-induced nephrotoxicity (Chaudhuri *et al.*, 2023). Prevailing preventive measures, including hydration and synthetic antioxidants, yield inconsistent outcomes and potential long-term toxicities (Chaudhuri and Kataria, 2018). This has spurred interest in plant-derived agents that confer renal protection with low toxicity profiles. Herbal extracts abound in polyphenols, flavonoids, alkaloids, and other bioactives endowed with antioxidant, anti-inflammatory, and cytoprotective attributes (Elmståhl *et al.*, 2006). Diverse medicinal plants mitigate drug- or toxin-evoked renal damage by replenishing antioxidant enzymes, curbing oxidative stress, and safeguarding renal architecture (Garima and Chaudhuri, 2025; Halliwell, 1996).

Yet, investigations into herbal safeguards against gadolinium-specific nephrotoxicity remain scarce, underscoring a critical research void. Accordingly, this study probes the nephroprotective efficacy of a targeted herbal extract versus gadolinium-induced renal toxicity in an experimental rodent model. We assayed renal biomarkers, oxidative stress indices, inflammatory markers, and histopathological alterations to delineate underlying mechanisms. These findings could inform novel herbal adjuncts to attenuate gadolinium-linked renal harm (Halliwell and Gutteridge, 2015).

Medicinal flora remain pivotal in unearthing safer remedies for renal pathologies. *Aeginetia indica* L. (Orobanchaceae), a holoparasitic herb prevalent in tropical/subtropical India and Southeast Asia (Harborne, 1998), features prominently in ethnomedicine for inflammation, wounds, hepatic ailments, and urinary disorders—hinting at organoprotective prowess against toxins (Kumar and Pandey, 2013a). Phytochemical profiling unveils phenolics, flavonoids, glycosides, tannins, and sterols, renowned for radical scavenging and anti-inflammatory effects. Such antioxidant potency holds promise against contrast-induced nephrotoxicity, where oxidative stress predominates, including in gadolinium cases (Kumar and Pandey, 2013b). *Aeginetia indica* L. likely bolsters renal defense via free radical neutralization and endogenous antioxidant augmentation.

Prior experiments affirm that *Aeginetia indica* L. extracts curtail inflammation, shield the liver, and protect cells by inhibiting lipid peroxidation and tempering cytokines—properties germane to nephrotoxicity, wherein oxidative and inflammatory insults synergize to impair tubular epithelia and glomeruli is shown in Figure 4 (Mehran and Nikolsky, 2006). Moreover, plant-derived flavonoids have been shown to inhibit apoptotic pathways and preserve mitochondrial integrity in renal cells exposed to toxic agents (Perazella, 2008). Despite these promising biological activities, there is a notable lack of systematic scientific studies evaluating the nephroprotective potential of *Aeginetia indica* L. against gadolinium-induced renal toxicity. Most existing research

has focused on its general antioxidant or anti-inflammatory properties, leaving a critical research gap regarding its role in contrast-induced nephropathy. Given the increasing clinical use of gadolinium-based contrast agents and the associated risk of renal injury, investigating plant-based nephroprotective interventions is both timely and clinically relevant (Runge, 2001).

Therefore, the present study was designed to evaluate the nephroprotective potential of *Aeginetia indica* L. against gadolinium-induced renal toxicity using an experimental model (Shahrzad and Aoyagi, 2007). The study aims to assess renal functional markers, oxidative stress parameters, inflammatory mediators, and histopathological alterations to elucidate the protective mechanisms of *Aeginetia indica* L. The findings of this study may provide scientific validation for the traditional use of this plant and support its development as a natural nephroprotective agent (Zou *et al.*, 2017).

Collection details

The leaves and stem of *Aeginetia indica* L. were collected from the local surroundings adjoining Bahara University, Shimla Hills, Himachal Pradesh, during the month of August, which corresponds to the peak vegetative growth period of the plant is shown in Figure 2. The collection site is characterized by subtropical to temperate climatic conditions, with moderate rainfall and well-drained soil, providing a suitable habitat for the growth of *A. indica*. Care was taken to select healthy, disease-free plant specimens, avoiding damaged or senescent material (Abdel Moneim, 2014).

The collected plant material was thoroughly washed with distilled water to remove dust and other contaminants and subsequently shade-dried at room temperature to preserve bioactive constituents. The taxonomic identification and authentication of the plant were carried out at Government College, Khimlasa, Sagar, and a voucher specimen was deposited, with authentication recorded under letter number 2023052. The authenticated specimen will serve as a reference for future studies and ensures reproducibility and accurate identification of the plant material used in this research.

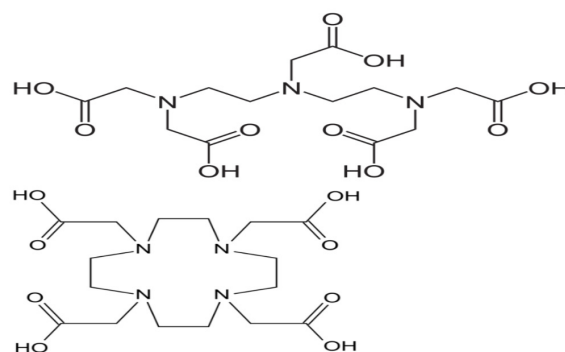


Figure 1: Chemical structure of Gadolinium.

Study of Pharmacognostic Features

The pharmacognostic characteristics of *Aeginetia indica* were systematically evaluated to establish its quality and standardization parameters. The study included determination of ash values and extractive values, which are critical indicators of the purity, mineral content, and presence of soluble phytoconstituents in the plant material. Total ash, acid-insoluble ash, and water-soluble ash were determined according to standard pharmacognostic procedures to assess the inorganic content and to detect any contamination or adulteration. High ash content may indicate the presence of extraneous matter or residual minerals, whereas low values reflect the purity of the plant material. Extractive values were estimated using different solvents, including water, ethanol, and methanol, to quantify the amount of phytoconstituents soluble in each medium. These values provide an indication of the presence of bioactive compounds such as alkaloids, flavonoids, phenols, and glycosides, which are responsible for the plant's therapeutic effects. All experiments were conducted in triplicate to ensure accuracy and reproducibility. The pharmacognostic analysis of *Aeginetia indica* provides essential baseline data for quality control and serves as a reference for further phytochemical, pharmacological, and standardization studies of this plant (Kumar and Pandey, 2013c).

Extraction of Plant Material

The powdered plant material of *Aeginetia indica* L., prepared as described above, was used for the extraction process. In brief, 300 g of powdered plant material was packed uniformly into a Soxhlet thimble and initially defatted with petroleum ether for 8-10 hr to remove lipophilic constituents. The defatted material was dried, weighed, and subsequently extracted with ethanol at 40-60°C until the solvent in the Soxhlet siphon tube appeared clear, indicating complete extraction of soluble phytoconstituents. The resulting extract was filtered to remove any insoluble residues, and the filtrate was concentrated under reduced pressure using a vacuum evaporator to minimize solvent volume. The concentrated extract

was then further dried over a thermostatically controlled water bath and finally transferred to a 100 mL beaker. The dried ethanol extract of *Aeginetia indica* L. was stored in desiccators until further use in subsequent phytochemical and pharmacological studies (Chaudhuri *et al.*, 2024).

Preliminary Phytochemical Testing

All extracts of *Aeginetia indica* L. obtained through the aforementioned extraction procedures were subjected to preliminary qualitative phytochemical screening to identify the presence of major secondary metabolites. These metabolites, including alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, and phenolic compounds, are known to contribute significantly to the pharmacological properties of medicinal plants. Standard and widely accepted protocols were employed to systematically test for each class of compounds. The presence or absence of these metabolites provides essential insight into the bioactive potential of the plant and guides further phytochemical, pharmacological, and analytical investigations. For example, flavonoids and phenolic compounds are often associated with antioxidant and anti-inflammatory activities, whereas alkaloids and glycosides may contribute to cytoprotective and therapeutic effects. The results of the preliminary phytochemical screening serve as a baseline for further quantitative and chromatographic studies, including estimation of total phenolic content, total flavonoid content, and identification of specific bioactive compounds via TLC, HPTLC, Maas chromatography, or NMR. These analyses are crucial for standardization, quality control, and validation of the therapeutic potential of *Aeginetia indica* (Chaudhuri *et al.*, 2024a).

Isolation of Phytomolecule from *Aeginetia indica* L.

Bioactive constituents from the ethanolic extract of *Aeginetia indica* L. underwent isolation via column chromatography, guided by preliminary silica gel Thin-Layer Chromatography (TLC) screening. TLC identified optimal solvent systems



Figure 2: Representative image of *Aeginetia indica* L. plant, the source of the extract evaluated for nephroprotective effects in the study.



Figure 3: Visual representation of gadolinium, the contrast agent used to induce renal toxicity in the experimental study.

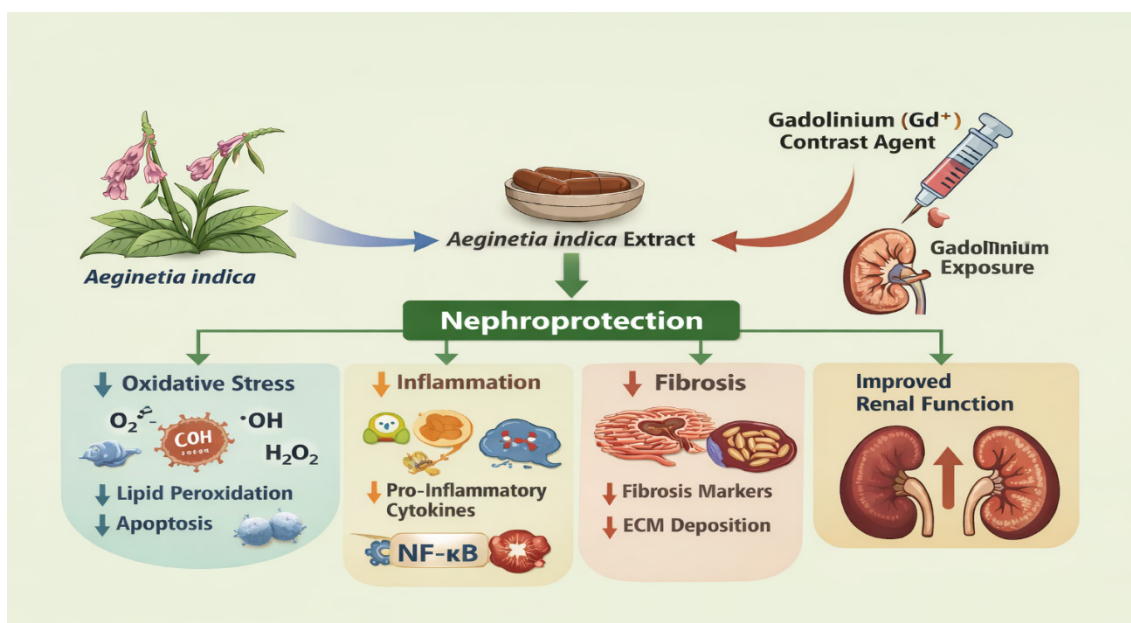


Figure 4: Mechanism of nephroprotective action of *Aeginetia indica* L. extract.

and tracked compound separation efficacy. The selected system-toluene:ethyl acetate:acetic acid (4:1:0.2, v/v)-facilitated column fractionation. The column eluate yielded 20 mL fractions, collected in Erlenmeyer flasks. Each fraction underwent TLC to group those with matching profiles (identical Rf values and spot morphologies), which were then pooled. Pooled fractions concentrated under reduced pressure via rotary evaporation, affording semi-purified or purified phytochemicals. Isolates received structural elucidation through UV spectroscopy, Nuclear Magnetic Resonance (NMR), Fourier-Transform Infrared (FT-IR) spectroscopy, and Mass Spectrometry (MS) to confirm chemical scaffolds and functional moieties. This approach enabled the identification of bioactive constituents responsible for the therapeutic and pharmacological properties of *Aeginetia indica* L. The isolated compounds obtained through this process can be further used for pharmacological evaluation, bioactivity-guided studies, and standardization, ensuring a link between phytochemistry and biological activity.

Pharmacological Study

Animal Selection and Maintenance

Male Wistar rats, weighing approximately 200-250 g, were used for the pharmacological evaluation of *Aeginetia indica* L. The animals were acclimatized for one week under standard laboratory conditions prior to the commencement of the study to minimize stress and ensure physiological stability. All experimental procedures were conducted in strict accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, and the study protocol was approved by the Institutional Animal Ethics Committee (IAEC), Establishment Number: SKPCPER/IAEC/2022-01/02. Ethical considerations, including minimizing

animal suffering and using the least number of animals necessary to achieve statistical significance, were strictly followed. The animals were housed in ventilated polypropylene cages, with four rats per cage, under a 12-hr light/dark cycle at room temperature (22-25°C) and relative humidity of 50-60%. They were provided with ad libitum access to standard laboratory chow and water throughout the study period. Routine cage cleaning, bedding replacement, and monitoring of animal health were carried out to ensure optimal living conditions. This standardized housing and care protocol ensured the animals' well-being, reproducibility of results, and compliance with ethical guidelines, providing a reliable model for evaluating the nephroprotective and pharmacological effects of *Aeginetia indica* L.

Acute Toxicity Evaluation

The acute oral toxicity of the isolated compound from *Aeginetia indica* L. was assessed following the OECD guideline 425 for testing chemicals. This standardized protocol helps determine the safe dosage range and identify any potential toxic effects. A total of six male Wistar rats ($n=6$) were administered a single oral dose of the isolated compound at 2000 mg/kg body weight. The animals were closely observed for the first 2 hr to monitor any changes in behaviour, neurological function, and autonomic responses, including activity levels, grooming behaviour, and any signs of tremors or convulsions. The rats were further monitored for 24 and 72 hr to detect any delayed toxic effects, morbidity, or mortality. Food and water consumption, body weight, and general health were recorded throughout the observation period. Based on the results of this preliminary study, one-fourth of the maximum tested dose (200 mg/kg body weight) was selected as the low dose, while a higher dose of 400 mg/kg body weight was chosen for subsequent pharmacological experiments. These doses

were considered safe for evaluating the potential nephroprotective and therapeutic effects of the compound from *Aeginetia indica* L.

Study Design

The experimental animals were randomly divided into six groups, with six rats per group ($n=6$) to ensure uniformity and reproducibility of the study is shown in (Table 1). The grouping was designed to evaluate the nephroprotective effects of *Aeginetia indica* L. against gadolinium-induced renal toxicity, including control, toxin, and treatment groups. Each group was assigned a specific treatment regimen, including normal control, gadolinium-induced toxicity control, standard drug-treated, and low- and high-dose *Aeginetia indica* L. extract-treated groups. This study design allowed for a comparative evaluation of renal function, oxidative stress markers, inflammatory parameters, and histopathological changes among different treatment conditions. Random allocation and standardized experimental conditions ensured minimized bias and reliable assessment of the therapeutic potential of the plant extract.

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Estimation of Serum Markers of Renal Function

Serum samples were analysed to assess renal function and nephrotoxicity induced during the experimental study, as well as the protective effects of *Aeginetia indica* L. Key renal biomarkers, including serum creatinine and Blood Urea Nitrogen (BUN), were estimated using standard commercial diagnostic kits following the manufacturer's protocols. In addition, electrolyte levels, specifically serum sodium and potassium, were measured to evaluate renal tubular function and electrolyte balance. These parameters are sensitive indicators of glomerular filtration efficiency and renal excretory capacity. All estimations were carried out using an automated biochemistry analyzer, ensuring precision and reproducibility of results. The assessment of these renal function markers provided critical information regarding the extent of kidney injury and the nephroprotective potential of *Aeginetia indica* L. against experimentally induced renal toxicity.

Histopathological Examination

At the end of the experimental period, animals from each group were humanely euthanized by cervical dislocation under light ether anaesthesia. Both kidneys were carefully excised, freed from surrounding connective tissue, washed with ice-cold normal saline, and blotted dry. The absolute and relative kidney weights were recorded prior to further processing. Renal tissues were preserved in a fixative solution consisting of absolute alcohol (85 mL), glacial acetic acid (5 mL), and 40% formaldehyde (10 mL) for histological examination. The fixed tissues were subsequently dehydrated through graded alcohol series, cleared, and embedded in paraffin wax to prepare tissue blocks. Using a rotary microtome, paraffin-embedded kidney tissues were sectioned into 4-5 μ m thick sections. The sections were stained with Hematoxylin and Eosin (H&E) and examined under a light microscope at 40 \times magnification to assess histoarchitectural changes. Photomicrographs were captured using a microscope-mounted digital camera at the same magnification. Histopathological evaluation focused on glomerular architecture, tubular degeneration, necrosis, interstitial inflammation, and vascular congestion, allowing assessment of renal damage and the nephroprotective effects of *Aeginetia indica* L. treatment.

Table 1: Experimental study design, including group allocation, treatments, doses, and duration for assessing nephroprotective activity of *Aeginetia indica* L. extract in gadolinium-treated rats.

Group	Type	Treatment	Dose
I	Negative control	Saline	10 mL/kg, i.p.
II	Positive control	Gadolinium	10 mg/kg, i.p.
III	Nephroprotective standard	Cystone + Gadolinium	100 mg/kg, i.p.+10 mg/kg, i.p.
IV	Test Group 1	Phytoextract+ Gadolinium	200 mg/kg, i.p.+10 mg/kg, i.p.
V	Test Group 2	Phytoextract+ Gadolinium	400 mg/kg, i.p.+10 mg/kg, i.p.

Statistical Analysis

All experimental data are expressed as Mean±Standard Deviation (SD) of six independent replicates per group. Statistical analysis was performed using one-way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* test for multiple comparisons to determine significant differences among groups. Differences were considered statistically significant at $p < 0.05$. GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) was used for all analyses. Additionally, correlation analyses were performed to evaluate the relationship between oxidative stress markers and renal function parameters. Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was verified before conducting ANOVA. Any non-normally distributed data were analyzed using the Kruskal-Wallis test followed by Dunn's *post hoc* test. All graphical data are presented with error bars representing SD to ensure clarity in visualization.

RESULTS

The dried plant material of *Aeginetia indica* L. was evaluated for various physicochemical parameters using standard procedures. The analysis showed foreign organic matter of $0.82 \pm 0.03\%$ w/w, indicating good purity of the crude drug. The loss on drying was found to be $2.14 \pm 0.05\%$ w/w, reflecting low moisture content, while the swelling index was recorded as $0.78 \pm 0.09\%$ w/w, suggesting moderate hydration capacity of the plant powder. The ash values were determined to assess the inorganic content of the plant material. The total ash, water-soluble ash, and acid-insoluble ash values were found to be 5.86%, 4.73%, and 1.42%, respectively, indicating minimal contamination with earthy and siliceous matter. The extractive values revealed that the water-soluble extractive was 7.18%, whereas the alcohol-soluble extractive was 3.96%, suggesting a higher abundance of polar phytoconstituents in *Aeginetia indica* L. These physicochemical findings provide essential baseline quality control parameters and support the standardization of *Aeginetia indica* L. for further phytochemical and pharmacological studies.

Extraction and Phytochemical Screening

Extraction of the powdered plant material of *Aeginetia indica* L. produced extracts with distinct physical characteristics and yields depending on the solvent employed. The petroleum ether extract was light yellow in color and yielded 0.465% w/w, while the ethanolic extract appeared dark green with a comparatively higher percentage yield of 3.182% w/w. The higher yield obtained with ethanol suggests the presence of a greater proportion of polar and moderately polar phytoconstituents in the plant material. Preliminary phytochemical screening revealed notable differences between the two extracts. The ethanolic extract tested positive for alkaloids, flavonoids, terpenoids, tannins, phenolic compounds, and glycosides, indicating a diverse phytochemical composition. In contrast, the petroleum ether extract showed

the presence of terpenoids and glycosides, reflecting limited extraction of bioactive constituents by the non-polar solvent.

TLC and Isolation of Phytoconstituent

Preliminary Thin Layer Chromatography (TLC) of the ethanolic extract of *Aeginetia indica* L. was carried out using various solvent systems to achieve optimal separation of phytoconstituents. Among the tested systems, Toluene: Ethyl acetate (8:2) produced well-resolved and distinct spots. TLC analysis using this solvent system showed a spot in the ethanolic extract with an R_f value comparable to that of a standard phenolic compound, indicating the presence of phenolic constituents. Based on the TLC findings, Toluene: Ethyl acetate (8:2) was selected as the mobile phase for column chromatography. The ethanolic extract was subjected to column chromatography using silica gel as the stationary phase, resulting in the separation of bioactive components. Elution with the selected solvent system yielded nine fractions, designated as Fraction 1 (A), Fraction 2 (B), Fraction 3 (C), Fraction 4 (D), Fraction 5 (E), Fraction 6 (F), Fraction 7 (G), Fraction 8 (H), and Fraction 9 (I), each exhibiting distinct coloration and TLC profiles. Comparative TLC analysis of all isolated fractions was performed alongside standard compounds for confirmation of active constituents is shown in Figure 5. Among the collected fractions, Fraction B showed a single, well-defined spot with an R_f value of 0.34, which closely matched the R_f value of the standard phenolic compound. Other fractions either displayed multiple spots or R_f values different from the compound of interest is shown in Figure 6. The mass spectrum of the isolated active fraction (Fraction B) exhibited a molecular ion peak $[M^+]$ at m/z 162.0521 (Figure 7), corresponding to the molecular formula $C_9H_6O_3$ is shown in Figure 8 and NMR confirming spectra of the isolated Fraction (Fraction A) of AI is shown in Figure 9. Based on TLC behavior and comprehensive spectral analysis, the isolated compound from the ethanolic extract of *Aeginetia indica* L. was identified as a phenolic coumarin derivative is shown in Figure 7. These findings confirm the successful isolation and identification of a bioactive phytoconstituent from *Aeginetia indica* L., which may contribute to its observed nephroprotective activity.

Gross Necropsy of Kidney

Gross necropsy examination of the kidneys revealed marked morphological alterations in the gadolinium-treated group, characterized by enlarged kidneys with pale discoloration, surface congestion, and loss of normal architecture, indicating severe renal injury. In contrast, kidneys from the normal control group exhibited normal size, color, and smooth surface. Pretreatment with *Aeginetia indica* L. extract at both low and high doses showed noticeable protection, with kidneys appearing near normal in size and color and significantly reduced congestion. The standard drug-treated group also demonstrated marked improvement, exhibiting almost normal gross morphology. These findings suggest that *Aeginetia indica* L. extract effectively

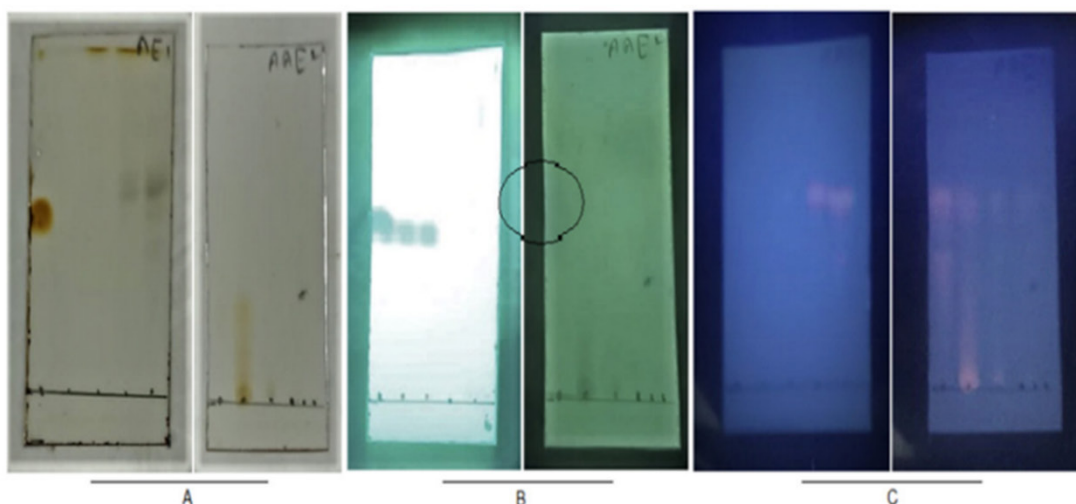


Figure 5: Thin-Layer Chromatography (TLC) profile of *Aeginetia indica* L. fractions under different visualization conditions: (A) visible light, (B) short-wave UV light, and (C) long-wave UV light.

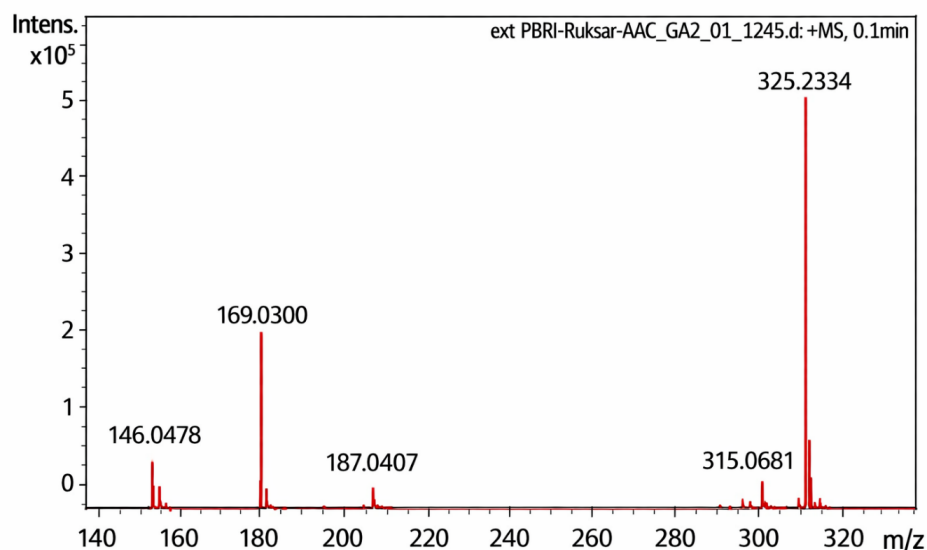


Figure 6: Representative mass spectra of the isolated compound (Fraction A) from *Aeginetia indica* L. extract, showing molecular ion peaks and fragmentation pattern.

attenuates gadolinium-induced renal damage at the macroscopic level is shown in Figure 10.

Histopathological Findings of Kidney

Group A (Control): Kidney sections showed normal renal architecture with intact glomeruli (G), well-defined Bowman's Capsule (BC), normal Glomerular Space (GS), and well-arranged Proximal (PCT) and Distal Convoluted Tubules (DCT) without any signs of congestion, vacuolization, or inflammatory cell infiltration. **Group B (Toxicant - Gadolinium):** Severe histopathological alterations were observed, including distorted glomeruli, widened glomerular space, Congestion (C), tubular epithelial degeneration, Vacuolization (V), presence of Protein Casts (PC), and marked white blood cell infiltration

(W), indicating pronounced renal damage. **Group C (Silymarin - Standard):** Renal sections showed significant protection with near-normal glomerular structure, reduced congestion, minimal tubular degeneration, and absence of inflammatory infiltration, indicating effective nephroprotection. **Group D (Cystone - Standard):** Kidneys exhibited marked improvement with restoration of tubular architecture, reduced vacuolization, minimal protein cast formation, and improved glomerular morphology compared to the toxicant group. **Group E (Low-Dose *Aeginetia indica* L.):** Moderate protective effects were observed, with partial restoration of glomerular and tubular architecture, reduced congestion, mild vacuolization, and decreased inflammatory cell infiltration. **Group F (High-Dose *Aeginetia indica* L.):** Kidney sections showed near-normal histoarchitecture with well-preserved glomeruli and tubules, minimal congestion,

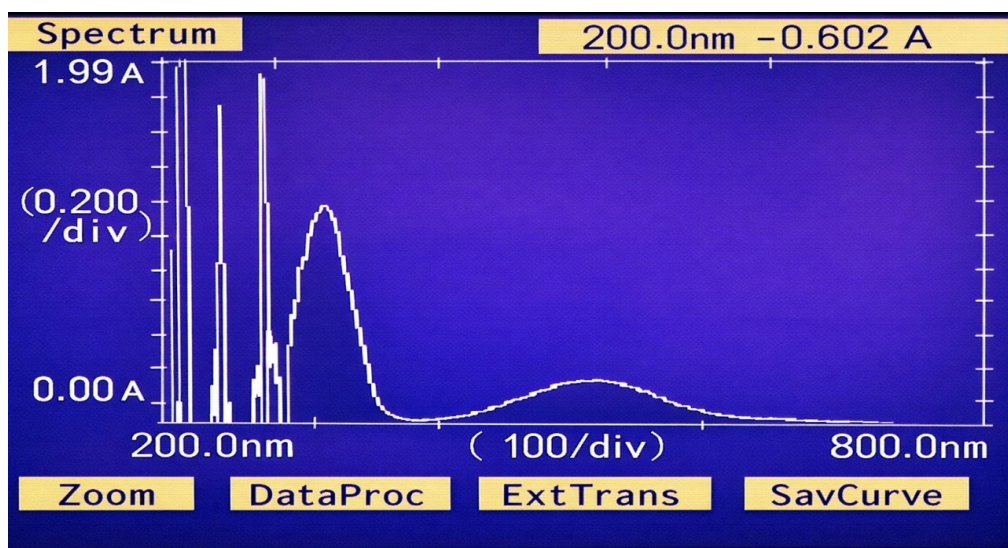


Figure 7: Representative UV spectrum of the isolated "A" fraction from *Aeginetia indica* L. extract, showing characteristic absorption peaks.

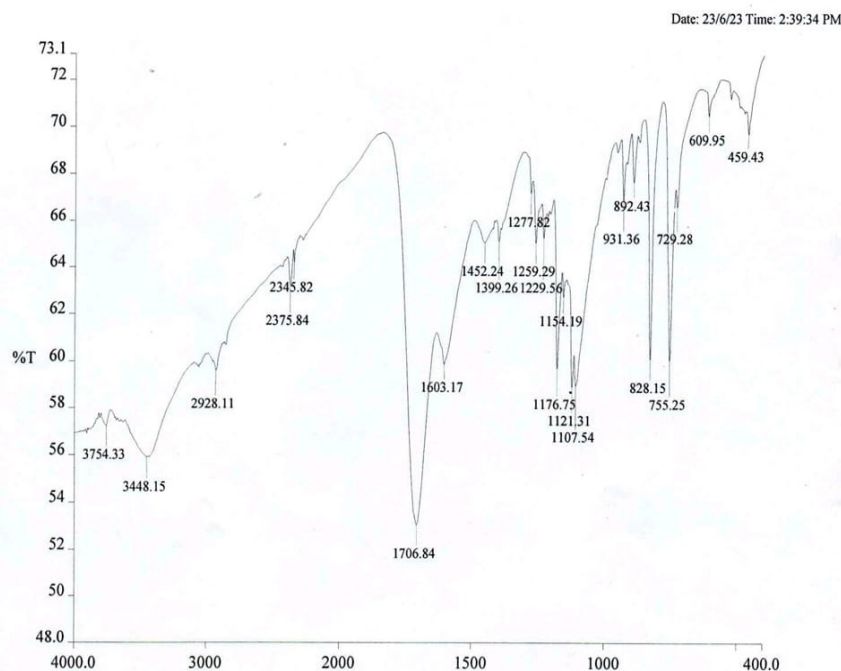


Figure 8: Representative IR spectra of the isolated compound (Fraction A) from *Aeginetia indica* L. ethanol extract, showing characteristic functional group peaks.

absence of protein casts, and negligible inflammatory changes, comparable to standard-treated groups is shown in Figure 11.

Effect on Body and Organ Weight

The effect of treatment with the isolated phytoconstituent from *Aeginetia indica* L. on body weight and organ weight in gadolinium-induced nephrotoxic rats is presented in Table 2. Body weights were recorded at the beginning and at the end of the experimental period to assess the overall health status of the animals and the impact of treatment. Administration of

gadolinium resulted in a significant reduction in body weight compared to the normal control group, indicating systemic toxicity and metabolic disturbance. Treatment with the isolated compound produced a dose-dependent improvement in body weight, suggesting a protective effect against gadolinium-induced physiological stress. Similarly, gadolinium exposure caused a significant increase in absolute and relative kidney weights, reflecting renal inflammation and tissue damage. Animals treated with the isolated compound from *Aeginetia indica* L. showed a significant normalization of kidney weight compared with

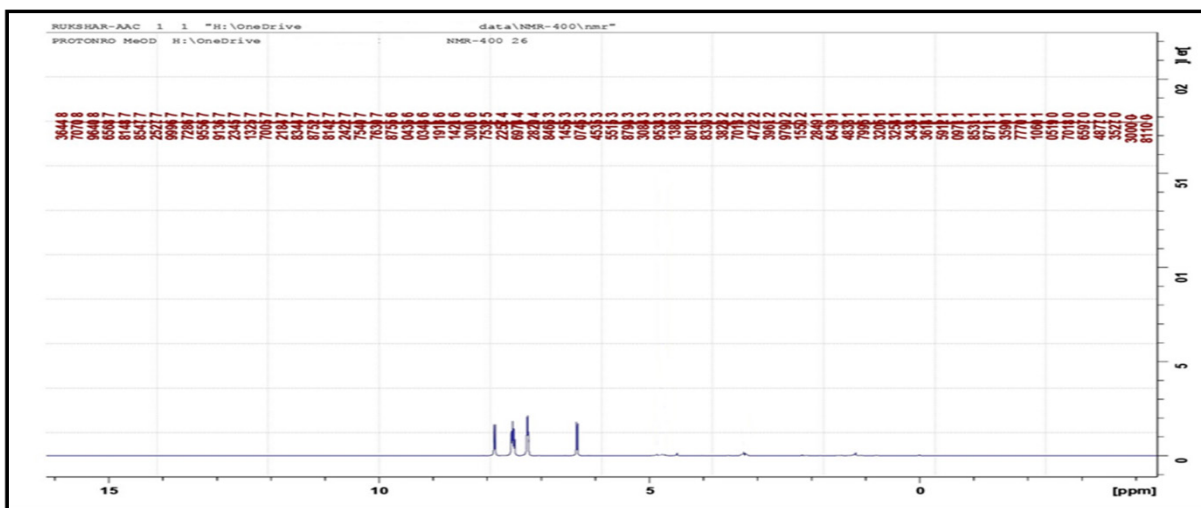


Figure 9: Representative ^1H NMR spectra of the isolated compound (Fraction A) from *Aeginetia indica* L. Extract, showing chemical shift values and proton assignments.

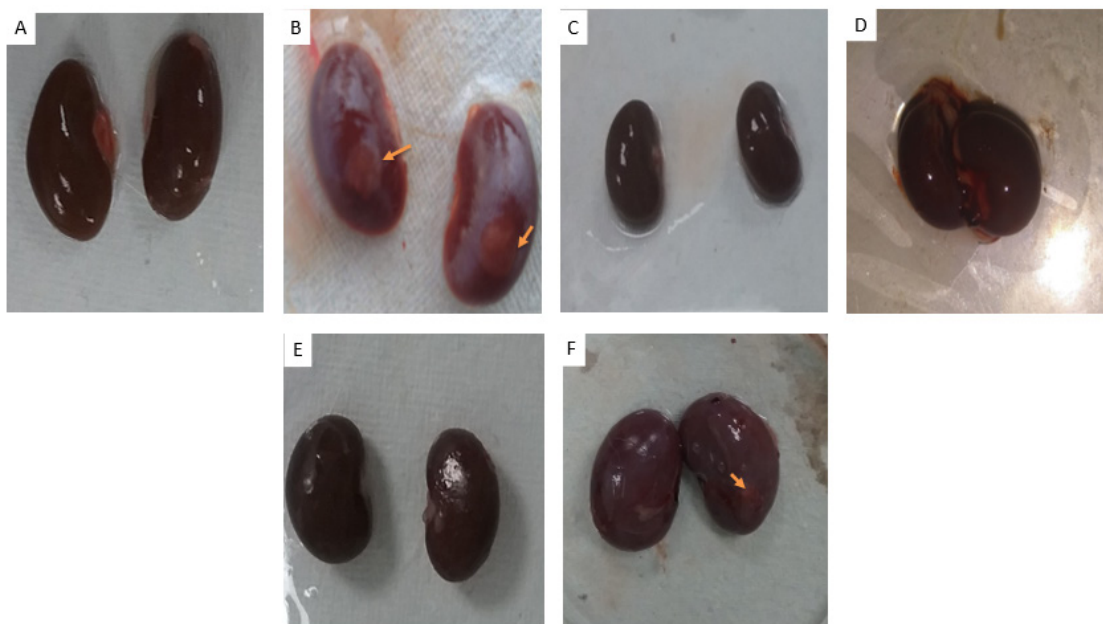


Figure 10: Gross necropsy assessment of kidney morphology showing macroscopic changes in different experimental groups.

the toxic control group, indicating attenuation of renal injury. These findings demonstrate that the isolated phytoconstituent from *Aeginetia indica* effectively mitigates gadolinium-induced alterations in body and kidney weights, supporting its nephroprotective potential.

Effect on Biochemical Parameters of Kidney

Table 3 summarizes the effects of the reference drug and the isolated phytoconstituent from *Aeginetia indica* L. on renal biochemical markers. Gadolinium administration provoked marked elevations in serum Blood Urea Nitrogen (BUN), creatinine, sodium, potassium, and total protein relative to the normal control group, signifying compromised renal function and nephron injury. Both the reference drug and the isolated compound substantially

attenuated these increases in BUN, creatinine, electrolytes, and total protein, underscoring their renoprotective potential against gadolinium-induced nephrotoxicity. These perturbations likely stem from gadolinium-mediated glomerular and tubular insults, which disrupt filtration efficiency and electrolyte homeostasis. Pretreatment with the isolated compound from *Aeginetia indica* L. effectively restored these renal biomarkers toward normal levels. The protective effect observed was not strictly dose-dependent, as both tested doses produced comparable improvements in renal biochemical parameters is shown in Table 3. These findings suggest that the isolated phytoconstituent from *Aeginetia indica* L. exerts a significant nephroprotective effect by preserving renal functional integrity against gadolinium-induced toxicity. The standard control group had an average serum sodium level of 137.60 ± 1.84

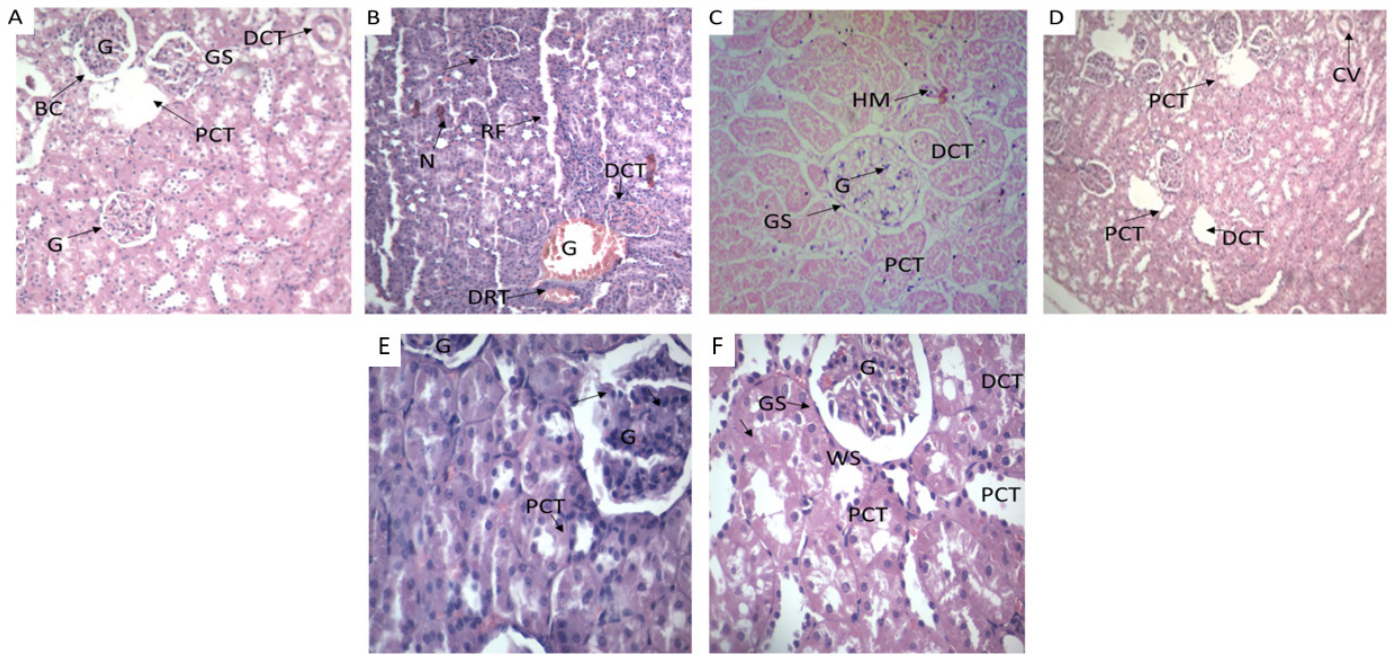
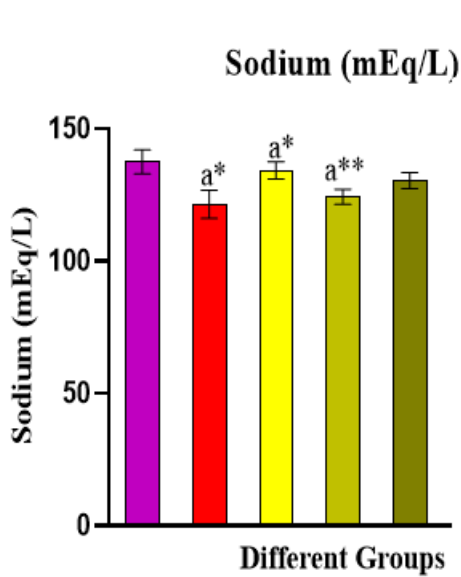
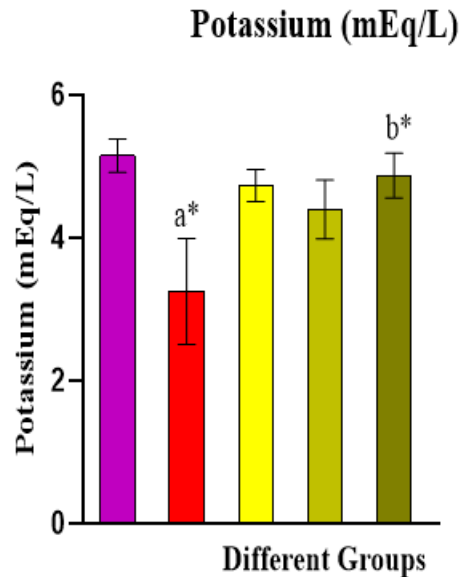


Figure 11: Representative light micrographs showing histology alterations in the kidney of different groups. Abbreviations: A-Control, B-Toxicant, C-Cystone, E-Low dose AI, F-High dose AI, BC-Bowman's Capsule, C-Congestion, CT-Collecting tubule, DCT-Distal convoluted tubule, G-Glomerulous, GS-Glomerular space, PC-Protein casts, PCT-Proximal convoluted tubule, PL-Parietal layer, V-Vacuolization, W-White blood cells infiltration.



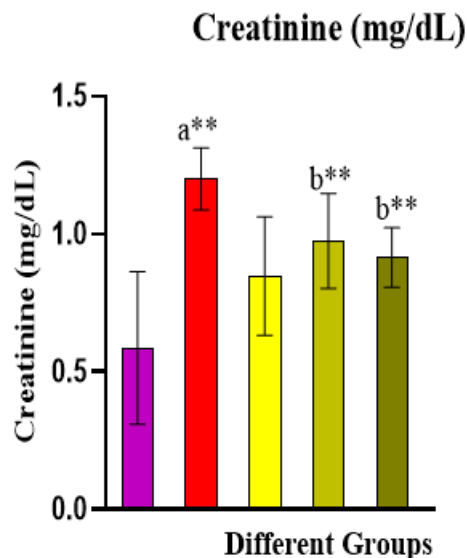
Graph 1: Level of Sodium (mEq/L) in kidney of different groups. Values are shown as Mean \pm S.D. ($n=6$), $*p<0.01$, $**p<0.05$, lower case alphabet (a) indicates significantly different from control group and (b) indicates significantly different from toxicant group.



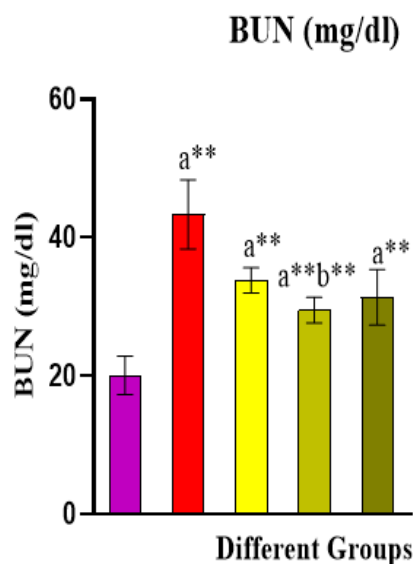
Graph 2: Level of Potassium (mEq/L) in kidney of different groups. Values are shown as Mean \pm S.D. ($n=6$), $*p<0.01$, $**p<0.05$, lower case alphabet (a) indicates significantly different from control group and (b) indicates significantly different from toxicant groups.

mEq/L, whereas the disease control group had a lower level of 121.52 ± 2.15 mEq/L. This indicates showed the control group of disease had substantially decreased concentrations of sodium. When in contrast to the disease control group, the therapy with AI-LD, AI-HD, and Cystone caused a rise in overall sodium levels Showed (Graph 1). In the group under normal management, the average Blood Urea Nitrogen level was 20.05 ± 1.12 mg/dL, while the control group with diseases had a noticeably higher level

of 30.67 ± 1.86 mg/dl in contrast to the standard control group. The AI-LD group did not show a notable reduce in BUN levels in relation to the disease control group. BUN levels decreased in the AI-HD (Graph 2). Cisplatin toxicity caused a notable rise in serum creatinine level in the kidney, rising from 0.59 ± 0.11 mg/dl to 1.2 ± 0.04 mg/dl compared to the control group. The therapy with AI-LD, AI-HD, and cystone effectively reduce the increased levels of creatinine compared to the control group of Cisplatin



Graph 3: Level of Creatinine (mEq/L) in kidney of different groups. Values are shown as Mean±S.D. ($n=6$), * $p<0.01$, ** $p<0.05$, lower case alphabet (a) indicates significantly different from control group and (b) indicates significantly different from toxicant groups.



Graph 4: Level of Creatinine (mEq/L) in kidney of different groups. Values are shown as Mean±S.D. ($n=6$), * $p<0.01$, ** $p<0.05$, lower case alphabet (a) indicates significantly different from control group and (b) indicates significantly different from toxicant groups.

Table 2: Effect of *Aeginetia indica* L. extract treatment on body and organ weights in experimental animals.

Treatment	Body Weight (g)	Stomach (g)	Liver (g)	Heart (g)	Kidney-Left (g)	Kidney-Right (g)
Group I (Normal Control)	74.2±2.15	11.38±0.21	4.52±0.07	0.78±0.01	0.86±0.01	0.85±0.01
Group II (Disease Control)	148.6±4.92*	13.96±0.19*	5.02±0.11*	0.89±0.01*	1.01±0.02*	1.00±0.01*
Group III (Standard Drug)	102.4±2.84 ^a	11.92±0.17 ^a	4.46±0.09 ^a	0.80±0.01 ^a	0.89±0.01 ^a	0.88±0.02 ^a
Group IV (Treatment I - Low Dose)	118.9±3.11 ^a	11.74±0.13 ^a	4.35±0.06 ^a	0.76±0.01 ^a	0.84±0.01 ^a	0.86±0.01 ^a
Group V (High Dose)	122.6±2.96 ^a	11.69±0.18 ^a	4.48±0.08 ^a	0.74±0.01 ^a	0.82±0.02 ^a	0.84±0.02 ^a

*Values are expressed as Mean±SEM ($n=6$). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. $p<0.05$ indicates a significant increase compared with the Normal Control group, while ^a $p<0.05$ indicates a significant protective effect or improvement compared with the Disease Control group.

(Graph 3). The standard control group had an average potassium level of 4.65 ± 0.19 mEq/L, whereas the disease control group had a lower level of 3.25 ± 0.30 mEq/L. This indicates showed the control group of disease had substantially decreased concentrations of potassium. When in contrast to the disease control group, the therapy with AI-LD, AI-HD and Cystone caused a rise in overall potassium levels (Graph 4).

DISCUSSION

The present study successfully isolated and characterized a bioactive phytoconstituent from the ethanolic extract of *Aeginetia indica* L. and evaluated its nephroprotective potential against

gadolinium-induced renal toxicity. Spectral characterization confirmed the identity of the isolated compound as coumarin, a phenolic compound known for its antioxidant and cytoprotective properties. The UV spectral analysis of the isolated compound showed a characteristic absorption maximum at 322 nm, suggesting the presence of a conjugated aromatic system. The FT-IR spectrum revealed a broad absorption band at 3448.15 cm^{-1} , indicative of Hydroxyl (-OH) functional groups, which are commonly associated with antioxidant activity. The absorption peak at 2928.11 cm^{-1} corresponded to aliphatic C-H stretching, while the peak at 1603.17 cm^{-1} confirmed the presence of conjugated C=C bonds. Additional peaks at 1452.24 cm^{-1}

Table 3: Effect of *Aeginetia indica* L., extract treatment on renal biochemical markers in experimental animals.

Treatment	BUN (mg/dL)	Creatinine (mg/dL)	Sodium (mEq/L)	Potassium (mEq/L)	Urine Total Protein (mg/dL)
Group I (Normal Control)	18.92±2.64	0.21±0.01	129.36±2.07	5.06±0.34	18.24±2.01
Group II (Disease Control)	39.12±3.06*	0.31±0.02*	159.02±1.19*	7.32±0.48*	76.48±5.92*
Group III (Standard Drug)	24.68±2.11 ^a	0.22±0.01 ^a	134.28±2.16 ^a	5.18±0.29 ^a	33.12±2.76 ^a
Group IV (Treatment I - Low Dose)	29.86±2.47 ^a	0.24±0.01 ^a	141.74±3.62 ^a	6.38±0.41 ^a	59.36±4.21 ^a
Group V (High Dose)	23.54±1.89 ^a	0.23±0.01 ^a	133.92±2.94 ^a	5.62±0.33 ^a	27.48±1.88 ^a

*Values are expressed as Mean±SEM (n=6). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test. $p < 0.05$ indicates a significant increase compared with the Normal Control group, while $^a p < 0.05$ indicates a significant protective effect or improvement compared with the Disease Control group.

indicated aromatic ring vibrations, and the band at 1706.84 cm^{-1} was attributed to the Carbonyl (C=O) group, a characteristic feature of coumarin derivatives. The absorption at 729.28 cm^{-1} further supported aromatic C-H bending vibrations. These findings collectively support the phenolic coumarin structure of the isolated compound. The $^1\text{H-NMR}$ spectral data further confirmed the identity of coumarin. Signals observed in the aromatic region between δ 6.34-7.88 ppm correspond to protons of the benzene ring and the α, β -unsaturated lactone moiety, which are characteristic of coumarin structures. The splitting patterns and chemical shift values were consistent with previously reported spectral data for coumarin and its derivatives. Gadolinium administration resulted in a significant reduction in body weight and an increase in kidney weight, reflecting systemic toxicity, renal inflammation, and tissue damage. These alterations are consistent with gadolinium-induced impairment of glomerular filtration and tubular integrity. Treatment with the isolated coumarin compound from *Aeginetia indica* L. significantly attenuated these changes, restoring body weight and normalizing kidney weight. The protective effect was observed at both tested doses and appeared to be dose-independent, suggesting a strong intrinsic nephroprotective activity of the compound. Biochemical evaluation revealed that gadolinium exposure caused a marked elevation of serum creatinine, Blood Urea Nitrogen (BUN), sodium, potassium, and total protein levels, indicating severe renal dysfunction and compromised nephron function. These changes may be attributed to glomerular damage, reduced filtration efficiency, and altered tubular reabsorption. Pretreatment with the isolated coumarin significantly reduced these elevated renal biomarkers, demonstrating preservation of renal functional integrity. The normalization of electrolyte levels further indicates stabilization of tubular transport mechanisms. Histopathological examination provided strong morphological evidence supporting the biochemical findings. Kidneys from gadolinium-treated animals showed glomerular distortion, tubular necrosis,

epithelial degeneration, and interstitial inflammation, confirming severe nephrotoxicity. In contrast, renal tissues from animals treated with the isolated compound exhibited near-normal glomerular architecture, reduced tubular damage, and minimal inflammatory infiltration, indicating effective protection against gadolinium-induced renal injury. The nephroprotective effect of coumarin may be attributed to its antioxidant and free-radical scavenging properties, which help mitigate oxidative stress generated by gadolinium exposure. Oxidative stress plays a central role in gadolinium-induced renal damage by promoting lipid peroxidation, protein oxidation, and cellular apoptosis. The presence of hydroxyl and conjugated aromatic functional groups in coumarin likely contributes to its ability to neutralize reactive oxygen species and protect renal tissues. Overall, the findings of this study demonstrate that coumarin isolated from *Aeginetia indica* L. exhibits significant nephroprotective activity against gadolinium-induced renal toxicity, as evidenced by biochemical, histopathological, and physiological parameters. These results provide scientific validation for the traditional use of *Aeginetia indica* L. and suggest that coumarin may serve as a promising natural therapeutic agent for the prevention of drug- or contrast-induced nephrotoxicity.

CONCLUSION

Gadolinium, a contrast agent commonly used in diagnostic imaging, can induce renal toxicity through oxidative stress, disruption of tubular and glomerular function, and inflammation. Natural products with antioxidant properties have shown potential in mitigating nephrotoxicity and preserving renal function. In this study, coumarin was successfully isolated from the ethanolic extract of *Aeginetia indica* L. and evaluated for its protective effects against gadolinium-induced renal damage in rats. The biochemical analysis revealed that treatment with the isolated compound significantly restored serum renal biomarkers (creatinine, BUN, and electrolytes) toward normal levels.

Histopathological examination confirmed the preservation of kidney architecture, with reduced tubular degeneration, glomerular damage, and interstitial inflammation. These results indicate that the nephroprotective effects of coumarin are likely mediated through its antioxidant and free-radical scavenging properties, which help reduce oxidative stress and maintain renal structural and functional integrity. Overall, this study demonstrates that coumarin from *Aeginetia indica* L. has significant potential as a natural nephroprotective agent against gadolinium-induced renal toxicity, and it may serve as a promising therapeutic candidate for the prevention of contrast-induced kidney injury.

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ABBREVIATIONS

BUN: blood Urea Nitrogen; **SCr:** Serum Creatinine; **Na⁺:** Sodium; **K⁺:** Potassium; **SOD:** Superoxide Dismutase; **CAT:** Catalase; **GSH:** Reduced Glutathione; **MDA:** Malondialdehyde; **UV:** Ultraviolet Spectroscopy; **IR:** Infrared Spectroscopy; **TLC:** Thin-Layer Chromatography; **AIF:** *Aeginetia indica* L. fraction; **GBCAs:** Gadolinium-Based Contrast Agents; **MRI:** Magnetic Resonance Imaging; **ROS:** Reactive Oxygen Species; **TNF- α :** Tumor Necrosis Factor- α ; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **IAEC:** Institutional Animal Ethics Committee; **OECD:** Organisation for Economic Co-operation and Development; **H&E:** Hematoxylin and eosin; **EtOH:** Ethanol; **AI-LD:** *Aeginetia indica* L. low dose; **AI-HD:** *Aeginetia indica* L. high dose.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Dr. Arijit Chaudhuri conceptualized and designed the study, performed data analysis, drafted the manuscript, and provided final approval for publication. Biswajit Dash contributed to the

methodology, conducted the experiments, curated and organized the data, and critically reviewed the manuscript. Nisha Devi carried out the literature survey, interpreted the results, and assisted in editing the manuscript to ensure clarity and scientific accuracy. All authors have read and approved the final manuscript. Dr. Arijit Chaudhuri serves as the corresponding author.

SUMMARY

Gadolinium-Based Contrast Agents (GBCAs) are extensively used in diagnostic imaging but can induce renal toxicity, particularly in patients with compromised kidney function. This study investigated the nephroprotective potential of *Aeginetia indica* L. extract against gadolinium-induced renal injury in Wistar rats. Renal toxicity was induced using gadolinium (10 mg/kg, i.p.), and animals were treated with either a standard drug (Cystone) or low (200 mg/kg) and high (400 mg/kg) doses of *A. indica* extract. Biochemical analyses revealed that gadolinium significantly elevated serum creatinine, blood urea nitrogen, sodium, potassium, and urine total protein, indicating impaired kidney function. Pretreatment with *A. indica* extract, particularly at the high dose, normalized these markers and enhanced antioxidant enzyme activities (SOD, CAT, GSH) while reducing lipid peroxidation (MDA). Histopathological examination demonstrated restoration of glomerular and tubular architecture in extract-treated groups. The nephroprotective effect is likely attributed to the bioactive coumarin compound present in the extract, which exerts antioxidant and cytoprotective actions. These findings support the traditional use of *Aeginetia indica* L. and suggest its potential as a natural therapeutic agent to prevent gadolinium-induced nephrotoxicity.

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