Journal of Animal Environment

(E-ISSN 2717-1396, ISSN 2717-1388)

Original Research Paper

# Phytochemical Characterization and Nephroprotective Activity of *Vigna mungo* (L.) Hepper Seed Extract Against Rifampicin-Induced Nephrotoxicity in Wistar Rats

**Shivanee G. Phalphale[1], Kratika Daniel[2]**

1 Research scholar, Faculty of Pharmacy, Oriental University, Indore, MP

2 Professor, Faculty of Pharmacy, Oriental University, Indore, MP

**Corresponding author**

Ms. Shivanee G. Phalphale

Research scholar, Faculty of Pharmacy

Oriental University Indore, Madhya Pradesh 453555, India

Email: shivaneesanju@gmail.com

Phone: +91-8275331899

## Abstract

The present study investigated the phytochemical composition and nephroprotective activity of aqueous seed extract of *Vigna mungo* (L.) Hepper against rifampicin-induced renal toxicity in Wistar rats. Seeds were subjected to pharmacognostic evaluation, physicochemical analysis, fluorescence analysis, qualitative phytochemical screening, thin-layer chromatography (TLC), paper chromatography, HPLC, FAB-MS and NMR characterization. Nephroprotective activity was evaluated using rifampicin-induced nephrotoxic rats by estimating body weight, blood urea nitrogen, serum creatinine and serum uric acid. Histopathological examination of kidney tissues was also performed. Phytochemical analysis confirmed the presence of flavonoids, phenolic compounds, tannins, alkaloids, glycosides, saponins and carbohydrates. Analytical studies identified raffinose family oligosaccharides including ajugose. Administration of aqueous extract significantly improved renal biochemical parameters compared with rifampicin-treated animals. Blood urea nitrogen, serum creatinine and serum uric acid levels were markedly reduced following treatment with the extract. Histological observations further supported protection against tubular degeneration and inflammatory changes. The nephroprotective activity may be attributed to antioxidant phenolic constituents and flavonoids that reduce oxidative stress and preserve renal function. These findings suggest that *Vigna mungo* seeds possess promising nephroprotective properties and may serve as a valuable natural source for the development of renal protective agents.

**Keywords**

*Vigna mungo*, nephroprotection, rifampicin, phytochemicals, antioxidant, kidney toxicity

## Introduction

Kidney diseases are among the leading causes of morbidity and mortality worldwide and represent a major public health challenge. The kidneys play a vital role in maintaining physiological homeostasis by regulating fluid and electrolyte balance, acid–base equilibrium, blood pressure, and the excretion of metabolic waste products and xenobiotics. Owing to their high blood flow, extensive endothelial surface area, and active transport mechanisms, the kidneys are highly susceptible to damage caused by drugs, environmental toxins, and metabolic disorders. Drug-induced nephrotoxicity is a common cause of acute kidney injury and may progress to chronic kidney disease if not properly managed.

Rifampicin is one of the most widely prescribed first-line antitubercular drugs. Although highly effective against *Mycobacterium tuberculosis*, prolonged administration or high doses of rifampicin have been associated with renal toxicity. The underlying mechanisms involve oxidative stress, excessive production of reactive oxygen species (ROS), lipid peroxidation, inflammatory responses, and apoptosis of renal tubular cells. These pathological changes impair glomerular filtration and tubular function, resulting in elevated serum creatinine, blood urea nitrogen, and histopathological alterations in kidney tissues.

***Corresponding Author:**

Received: 25th May, 2026; Revised: 6th June, 2026; Accepted: 8th June, 2026; Available Online: 29rd July, 2026

(DOI): 10.70102/AEJ.2026.18.2s.34

Oxidative stress is considered one of the major contributors to nephrotoxicity. Excessive free radicals damage cellular lipids, proteins, and nucleic acids while reducing the activity of endogenous antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Therefore, compounds possessing antioxidant properties may provide significant protection against drug-induced renal injury.

Medicinal plants have long been used in traditional healthcare systems because of their therapeutic efficacy and relatively low toxicity. Numerous plant-derived phytochemicals, including flavonoids, phenolic compounds, tannins, alkaloids, saponins, and terpenoids, exhibit antioxidant, anti-inflammatory, antimicrobial, and organ-protective activities. These bioactive constituents have attracted considerable attention as potential alternatives for the prevention and treatment of nephrotoxicity.

*Vigna mungo* (L.) Hepper, commonly known as black gram or urad bean, belongs to the family Fabaceae and is one of the most important pulse crops cultivated throughout India and several Asian countries. Besides its nutritional importance, the seeds have been widely used in traditional medicine for the treatment of paralysis, rheumatism, nervous disorders, inflammation, and urinary ailments. Phytochemical investigations have revealed that *V. mungo* seeds are rich in flavonoids, phenolic compounds, tannins, saponins, proteins, amino acids, vitamins, and essential minerals. These phytoconstituents possess remarkable antioxidant and free radical scavenging activities, which may contribute to protection against oxidative tissue damage.

Previous pharmacological studies have demonstrated that *Vigna mungo* possesses antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, immunomodulatory, and antidiabetic activities. However, scientific evidence regarding its nephroprotective activity against rifampicin-induced renal injury remains limited. Although several medicinal plants have been investigated for renal protection, comparatively few studies have evaluated the nephroprotective potential of *Vigna mungo* using experimental animal models.

Therefore, the present study was undertaken to evaluate the phytochemical constituents of *Vigna mungo* seed extracts and investigate their nephroprotective activity against rifampicin-induced nephrotoxicity in Wistar rats. The study aimed to assess renal function biomarkers, antioxidant status, and histopathological changes in kidney tissues to provide scientific evidence supporting the traditional medicinal use of *Vigna mungo* and to explore its potential as a natural nephroprotective agent.

## Material and Methods

### 2.1 Chemicals and Reagents

Rifampicin was obtained as a gift sample from Shreya Life Sciences Pvt. Ltd., Roorkee, India. All biochemical estimation kits were procured from Pathozyme Diagnostics, Kagal, Kolhapur, India. Petroleum ether, chloroform, ethanol (99% v/v), distilled water, and other analytical-grade chemicals were purchased from standard commercial suppliers.

### 2.2 Collection and Authentication of Plant Material

Seeds of *Vigna mungo* (L.) Hepper were collected from Meerut District, Uttar Pradesh, India. The plant material was authenticated by Dr. A. K. Gupta, Department of Botany, Meerut College, Meerut, Uttar Pradesh. A voucher specimen (No. MCM/Bot-2) was deposited in the herbarium of the Department of Botany for future reference.

### 2.3 Preparation of Plant Extract

The collected seeds were shade-dried and powdered using a mechanical grinder. Approximately 100 g of powdered material was subjected to successive Soxhlet extraction using petroleum ether, chloroform, ethanol (99% v/v), and distilled water in increasing order of solvent polarity. Each extraction was carried out for 24 h. The extracts were concentrated under reduced pressure using a rotary evaporator and stored in airtight containers at 4°C until further use.

### 2.4 Pharmacognostic Evaluation

The powdered seed material was subjected to pharmacognostic evaluation according to standard procedures. Organoleptic characteristics including colour, odour, taste, texture, and appearance were recorded. Extractive values, total ash value, acid-insoluble ash, and water-soluble ash were determined following WHO guidelines. Powder fluorescence analysis was performed under visible and ultraviolet light after treatment with different chemical reagents.

### 2.5 Preliminary Phytochemical Screening

Qualitative phytochemical screening of the petroleum ether, chloroform, ethanol, and aqueous

extracts was carried out using standard procedures to detect alkaloids, flavonoids, tannins, phenolic compounds, saponins, glycosides, carbohydrates, proteins, amino acids, steroids, and terpenoids. Thin-layer chromatography (TLC) was performed to obtain the phytochemical fingerprint of the extracts.

2.6 Experimental Animals

Healthy adult Wistar albino rats of either sex weighing 200–250 g were used for the study. Animals were obtained from the institutional animal house and maintained under standard laboratory conditions (temperature 25 ± 2°C, relative humidity 55 ± 10%, and 12 h light/dark cycle). Standard pellet diet and water were provided ad libitum. Animals were acclimatized for one week before initiation of the experiment. Experimental procedures were carried out according to CPCSEA guidelines and were approved by the Institutional Animal Ethics Committee. CPCSEA No Or IAEC No : SOP/IAEC/2025-26/PR-12

2.7 Acute Oral Toxicity Study

The safety of the aqueous extract of *Vigna mungo* seeds was evaluated according to OECD guideline 423. Based on previous reports and the absence of toxicity, a dose of 500 mg/kg body weight was selected for the nephroprotective study.

2.8 Experimental Design

Twenty-four rats were randomly divided into four groups containing six animals each.

**Group I (Normal Control):** Received distilled water orally throughout the study.

**Group II (Disease Control):** Received rifampicin (1 g/kg, p.o.) once every 72 h for 14 days.

**Group III (Standard):** Received rifampicin (1 g/kg, p.o.) followed 30 min later by Cystone (500 mg/kg, p.o.).

**Group IV (Test):** Received rifampicin (1 g/kg, p.o.) followed 30 min later by aqueous extract of *Vigna mungo* (500 mg/kg, p.o.).

The treatment period lasted for 14 days. Body weights were recorded before and after treatment.

2.9 Collection of Blood and Tissue Samples

At the end of the experimental period, animals were anaesthetized and blood samples were collected by cardiac puncture. Serum was separated by centrifugation and used for biochemical estimation. Kidneys were excised immediately, washed with normal saline, blotted dry, weighed, and preserved for antioxidant and histopathological studies.

2.10 Biochemical Analysis

Serum samples were analysed for kidney function biomarkers including serum creatinine, blood urea nitrogen (BUN), and uric acid using commercial diagnostic kits according to the manufacturer's instructions. Oxidative stress parameters and antioxidant enzyme activities were determined using standard biochemical methods.

2.11 Histopathological Examination

Kidney tissues were fixed in 10% neutral buffered formalin, dehydrated through graded alcohol, embedded in paraffin wax, sectioned at approximately 5 µm thickness, and stained with haematoxylin and eosin (H&E). Histological changes were examined under a light microscope.

2.12 Statistical Analysis

Results were expressed as **mean ± SEM (n = 6)**. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparison test. Differences were considered statistically significant at **P < 0.05**.

**Results**

3.1 Organoleptic and Pharmacognostic Evaluation

The seeds of *Vigna mungo* (L.) Hepper exhibited characteristic organoleptic properties, including a smooth surface, black colour, characteristic odour, and slightly sweet taste. The successive extraction yielded extracts of different colours and consistencies depending on the solvent used. The aqueous extract showed the highest extractive value (41.17%), followed by methanol (16.51%), ethanol (13.08%), chloroform (10.89%), and petroleum ether (0.35%) using the hot extraction method. Similar trends were observed during cold maceration, indicating that polar solvents extracted a greater quantity of phytoconstituents than non-polar solvents (Table 1).

**Table 1: The Extractive values of the seeds powder of *Vigna mungo* (L.) Hepper by hot extraction method and cold maceration.**

| Sr.no. | Nature of Extract | Values (% w/w) by hot extraction | Values (% w/w) by cold Maceration |
|---|---|---|---|

| | | | |
|---|---|---|---|
| 1 | Petroleum ether | 0.35 | 0.81 |
| 2 | Chloroform | 10.89 | 3.81 |
| 3 | Ethanol (99%) | 13.08 | 5.01 |
| 4 | Methanol | 16.51 | 11.51 |
| 5 | Aqueous | 41.17 | 27.61 |

The higher extractive value obtained with aqueous and methanolic solvents suggests that *Vigna mungo* seeds are rich in polar bioactive constituents such as flavonoids, phenolic compounds, carbohydrates, glycosides, and proteins. Similar observations have been reported for other medicinal legumes, where aqueous extraction provided superior recovery of antioxidant compounds.

The total ash value of *Vigna mungo* seeds was found to be 2.5%, while acid-insoluble ash and water-soluble ash values were 0.5% and 1.0%, respectively (Table 2). These values indicate low levels of inorganic contamination and confirm the purity of the crude drug. The fluorescence characteristics of powdered seeds treated with different chemical reagents produced characteristic colours under visible and ultraviolet light, providing useful diagnostic parameters for identification and quality control.

**Table 2: Ash value of *Vigna mungo* (L.) Hepper seeds**

| Sr.no. | Physical contents | Value (%w/w) |
|---|---|---|
| 1 | Total ash value | 2.5 |
| 2 | Acid insoluble ash | 0.5 |
| 3 | Water soluble ash | 1.0 |
| 4 | Water insoluble ash | 1.5 |

Thin-layer chromatography (TLC) analysis of black gram extracts revealed five distinct spots upon treatment with α-naphthol, each exhibiting a characteristic blue coloration, indicative of fructose residues. However, none of these spots showed the typical yellow coloration with p-anisidine phthalic acid reagent, suggesting that the oligosaccharides present were non-reducing in nature. Identification of sucrose, raffinose, and stachyose was accomplished by comparing their Rf values to those of known standards. Additionally, the presence of verbascose was confirmed by comparing the oligosaccharide profile of black gram with that of red gram.

A comparable separation trend was observed during the paper chromatography of the oligosaccharides. To determine the identity of the fifth chromatographic spot, retention factor (RF) values were utilized to compute the partition function, expressed as $\log a = \log (RF / (1 - Rf))$, in accordance with the method outlined by Feingold and colleagues. When plotted against the number of hexose residues, these values formed a linear relationship (see figure). Since the logarithmic partition value for a linear polymer is generally considered to be the cumulative result of contributions from the polymer's terminal and central regions, this linearity supports the identification of the fifth spot as ajugose. Ajugose is an oligosaccharide in the raffinose series with a polymerization degree of six and is known to exhibit low mobility in both paper and thin-layer chromatography.

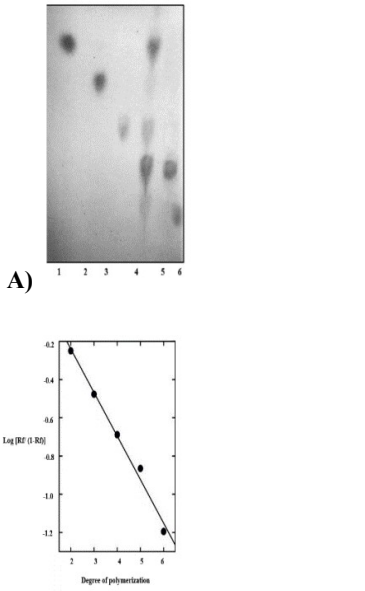



**Fig. (A) Paper chromatographic pattern of separation of oligosaccharides from black gram sprayed with a-naphthol: (1) sucrose standard; (2) raffinose standard; (3) stachyose standard; (4) black gram extract; (5) verbascose isolated from black gram; (6) ajugose isolated from black gram. (B) Plot of paper gram mobilities of the oligosaccharides in black gram extracts versus their degree of polymerisation.**

The exact and accurate determination across a reasonable concentration range is made possible by the fluorescence analysis, which is sufficiently sensitive. Every chemical has a unique fluorescence hue. When combined with fluorescent impurities, a non-fluorescent chemical can fluoresce. Under both regular and UV light, the hue of the extracts from organic and inorganic solvents was detected. Table 3 tabulates the results of the fluorescence analysis of Vigna mungo (L.) Hepper seeds treated with various chemical reagents.

**Table 3: Fluorescent studies of powder of *Vigna mungo* (L.) Hepper seeds**

| Sr.no. | Solvents Treatment | Visible light | Short UV (252 nm) | Long UV (366 nm) |
|---|---|---|---|---|
| 1 | Drug as such | White | Light yellow | Alice blue |
| 2 | Drug + 1M.H2SO4 | Sandy brown | Yellow green | Dark khaki |
| 3 | Drug + 1M.HCl | Wheat | Khaki | Dark khaki |
| 4 | Drug + 1M.NaOH in water | Khaki | Green | Dark green |
| 5 | Drug + KOH 50% Soln. | Green | Light green | Dark khaki |
| 6 | Drug + Ammonia soln. | Khaki | Lawn green | Khaki brown |
| 7 | Drug + Picric acid | Yellow | Lime green | Dark slate gray |
| 8 | Drug + FeCl3 5% soln. | Olive | Green | Black |
| 9 | Drug + Iodine soln. (5%) | Black | Gray | Black |
| 10 | Drug + Petroleum ether | Yellow | White | Blue violet |
| 11 | Drug + Chloroform | Yellow | White | Blue violet |
| 12 | Drug + Methanol | Light yellow | Yellow green | Gray |

Raffinose family oligosaccharides (RFOs) were separated using column chromatography. Based on the procedure described by Tanaka et al., fractions numbered 12 to 48 were identified as containing RFOs. Thin-layer chromatography (TLC) was employed to track the oligosaccharide content across these fractions. Ajugose was primarily detected in fractions 44 to 47. Its purity was assessed through high-performance liquid chromatography (HPLC), though the results are not shown here. Upon acid hydrolysis with 0.5 M sulfuric acid for 3 hours, ajugose broke down into fructose, glucose, and galactose in a molar ratio of 1:1:4. Partial hydrolysis yielded trace amounts of verbascose, along with raffinose, glucose, galactose, and fructose. When treated with α-galactosidase from *Aspergillus oryzae*, the hydrolysate revealed verbascose, stachyose, raffinose, sucrose (faintly detected), and free galactose.

Fast Atom Bombardment Mass Spectrometry (FABMS) analysis revealed a molecular ion peak at *m/z* 1014, which includes a sodium adduct. The compound features multiple hexose units arranged sequentially, leading to fragmentation through cleavage at the oxygen-carbon linkage between these sugar units. This results in the stepwise loss of individual hexose residues, producing fragment ions observed at *m/z* 852, 690, 528, and 366. Additionally, the presence of a fragment at *m/z* 166 suggests that a fructose unit remains part of the molecular structure.

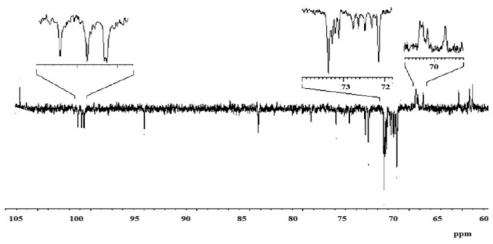
The 13C NMR spectrum of the analyzed compound revealed 28 distinct signals. Of these, 25 appeared with comparable intensities, while the remaining signals exhibited intensities two to four times greater, likely due to overlapping resonances. The observed carbon chemical shifts closely resembled those reported for ajugose, a compound previously proposed to be present in Mimosa scabrella seeds. To establish correlations between carbon and proton signals, a 1^{1}1H–13^{13}13C HSQC (heteronuclear single quantum coherence) experiment was conducted, enabling the identification of five anomeric carbon atoms with chemical shifts at 95.99, 101.64, 101.67, 101.90, and 102.23 ppm.

Further analysis using spin–echo Fourier transform (SEFT) spectroscopy identified a quaternary carbon at 107.69 ppm, corresponding to the C-2 position of a fructose unit, along with seven signals attributed to methylene ($CH_2$) groups. Building on the quaternary carbon signal, assignments for both the fructose and glucose residues were made using HMBC (heteronuclear multiple bond correlation) data. While some connectivities within the glucose unit could be traced via COSY (correlated spectroscopy), the dense superposition of six ring systems limited the amount of additional information extractable from this method.

Comparison with fully assigned 1^{1}1H and 13^{13}13C NMR spectra of stachyose, a known tetrasaccharide from the raffinose family oligosaccharides (RFOs), facilitated assignment of the most upfield carbon signal (65.91 ppm) to the C-6 position of the terminal non-reducing galactose. The remaining carbon signals from this galactose residue were further elucidated through HMBC correlations (table 5).

The chemical shifts and coupling patterns for the three sugar ring systems closely matched those previously reported for stachyose, confirming a high degree of structural similarity. Detailed HMBC analysis enabled the assignment of several internal glycosyl linkages. Notably, three secondary carbon signals at 70.16, 70.32, and 70.40 ppm—corresponding to C-6 positions of internal residues—strongly supported the presence of three internal galactose moieties, each substituted at C-6. Although overlapping signals hindered complete assignment of all resonances, the collective spectroscopic evidence supports the conclusion that the compound in question is ajugose.

**A)**



B)

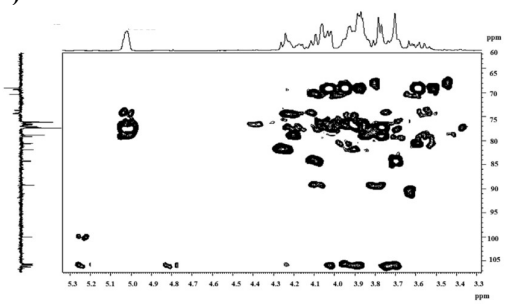


**Fig. (A) 13C (SEFT) NMR spectrum of the oligosaccharide purified from black gram. (B) 1 H–13C (HMBC) NMR spectrum of the oligosaccharide purified from black gram**

**Table 4: 1 H and 13C NMR chemical shift values for ajugose**

| Pos. | Fru | | Glc | | Gal | | Gal' | | Gal'' | | Gal''' | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | δH | δC | δH | δC | δH | δC | δH | δC | δH | δC | δH | δC |
| 1 | 4.68 | 65.33 | 6.44 | 95.99 | 6.00 | 102.23 | 5.98 | 101.64 | 5.98 | 101.67 | 5.98 | 101.90 |
| 2 | NPa | 107.68 | 4.57 | 74.84 | 3.85 | 72.15b | 4.86 | 71.15 | 4.84 | 72.32 | 4.81 | 72.15 |
| 3 | 5.23 | 80.25 | 4.76 | 76.63 | 4.86a | 73.42b | 4.86b | 73.42b | 4.86b | 73.42b | 4.92 | 73.29 |
| 4 | 5.07 | 77.89 | 4.52 | 73.37 | 4.90a | 73.38b | 4.90b | 73.38b | 5.04b | 73.21b | 4.98 | 73.12 |
| 5 | 4.90 | 85.23 | 5.08 | 75.15 | 5.15 | 72.79 | 5.21 | 72.66 | 5.13 | 72.50 | 5.01 | 74.85 |
| 6a | 4.79 | 66.36 | 4.67 | 69.70 | 4.74 | 70.32 | 4.65 | 70.18 | 4.68 | 70.40 | 4.76 | 65.91 |
| 6b | 4.85 | - | 5.07 | | 4.85 | | 4.93 | | 4.90 | | | |

High-performance liquid chromatography (HPLC) analysis confirmed the presence of sucrose, raffinose, and stachyose in the black gram extract by matching the retention times of the sample peaks with those of known standards. Overall, the findings suggest that black gram seeds contain a complete profile of raffinose family oligosaccharides (RFOs), extending up to a polymerisation degree of six. Thin layer chromatography (TLC) results showed that ajugose was present in higher amounts compared to raffinose and stachyose. Among these oligosaccharides, ajugose—being a higher molecular weight sugar—is more likely to contribute to flatulence than those with a lower degree of polymerisation.

Various processing techniques—such as soaking, cooking, malting, and dehulling—have been shown to lower the oligosaccharide content in legumes. While the application of α-galactosidase enzymes from both plant and microbial sources has been explored to reduce these sugars in legume-based products like flours and soymilk, such studies remain limited. An alternative strategy involves plant breeding to minimize flatulence-causing compounds. However, because oligosaccharides are believed to contribute to plant disease resistance, seed viability, and cold tolerance, completely removing them from plants may negatively impact growth and crop productivity.

Preliminary phytochemical screening revealed the presence of several biologically active constituents including alkaloids, flavonoids, tannins, phenolic compounds, glycosides, saponins, steroids, and ascorbic acid (Table 4). Among the tested extracts, the aqueous and ethanolic extracts contained the greatest number of phytochemical constituents. These compounds are well known for their antioxidant and free radical scavenging properties, which may contribute to the nephroprotective activity of *Vigna mungo*.

**Table 5: Phytochemical Screening of Various extracts of *Vigna mungo* (L.) Hepper Seeds**

| Phytochemicals | Petroleum ether extract | Chloroform extract | Ethanol Extract | Methanol Extract | Aqueous extract |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| **Alkaloids** Dragendroff's test | - | - | + | - | + |
| **Flavonoids** Shinoda test | - | - | + | + | + |
| **Steroids** Salkowski reaction | - | - | - | + | + |
| **Tannins & Phenols** 5 % FeCl 3 Solution | - | + | + | - | + |
| **Glycosides** Liebermann's test | - | - | + | + | - |
| **Saponins** Haemolytic test | + | - | + | - | + |
| **Ascorbic acid** | - | + | + | - | + |

3.2 Effect of Vigna mungo on Body Weight

Administration of rifampicin for 14 days produced a significant reduction in body weight compared with the normal control group. Animals treated with rifampicin showed an 8.63% decrease in body weight, indicating systemic toxicity associated with renal dysfunction.

Pretreatment with the aqueous extract of *Vigna mungo* (500 mg/kg) significantly prevented body weight loss. The percentage change in body weight (+3.33%) was comparable to that observed in the cystone-treated standard group (+3.14%), suggesting effective protection against rifampicin-induced toxicity (Table 6).

**Table 6: Influence of aqueous extract of seeds of *Vigna mungo* (AEVM) on selected physical and serum biochemical parameters in rifampicin-induced nephrotoxic rats**

| **Group** | **Treatment** | **Dose** | **Physical parameter** | **Biochemical parameters** | | |
|---|---|---|---|---|---|---|
| | | | **Body weight % change** | **Blood urea nitrogen** | **Serum creatinine (mg/dl)** | **Serum uric acid** |
| 1 | Normal control | Equivalent volumes (DW) | 5.60 ± 0.22 | 22.96 ± 1.12 | 0.54 ± 0.05 | 3.44 ± 0.15 |
| 2 | Positive control | 1 g/kg (RIF) | -8.63 ± 0.45 | 39.89 ± 0.70 | 1.92 ± 0.08 | 7.55 ± 0.31 |
| 3 | RIF + CYS | 1 g/kg + 500 mg/kg | 3.14 ± 0.19 | 23.27 ± 0.62 | 0.59 ± 0.04 | 3.52 ± 0.15 |
| 4 | RIF + AEVM | 1 g/kg +500 mg/kg | 3.33 ± 0.27 | 24.46 ± 0.68 | 0.61 ± 0.05 | 3.68 ± 0.19 |

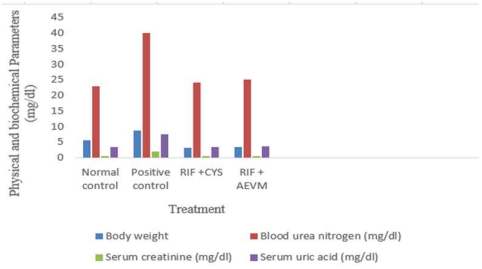
DW – Distilled water, RIF- Rifampicin, CYS – Cystone, AEVM- Aqueous extract of seed of vigna mungo.

Loss of body weight is a common indicator of drug-induced toxicity and reflects reduced food intake, altered metabolism, and impaired organ function. Improvement in body weight following treatment with *Vigna mungo* indicates recovery of physiological functions and an overall protective effect.

3.3 Effect on Renal Function Biomarkers

Administration of rifampicin significantly elevated serum biochemical markers of renal damage. Blood urea nitrogen increased from **22.96 ± 1.12 mg/dL** in the normal control group to **39.89 ± 0.70 mg/dL** in the rifampicin-treated group. Similarly, serum creatinine increased from **0.54 ± 0.05 mg/dL** to **1.92 ± 0.08 mg/dL**, while serum uric acid increased from **3.44 ± 0.15 mg/dL** to **7.55 ± 0.31 mg/dL** (Table 6).

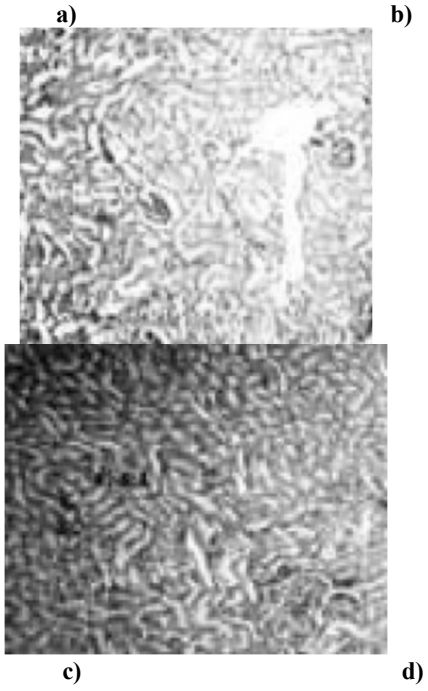
Treatment with the aqueous extract of *Vigna mungo* markedly reduced these elevated biochemical parameters. Blood urea nitrogen decreased to **24.46 ± 0.68 mg/dL**, serum creatinine to **0.61 ± 0.05 mg/dL**, and serum uric acid to **3.68 ± 0.19 mg/dL**. These values were comparable to those obtained with the standard drug cystone, indicating significant nephroprotective activity.




Elevation of blood urea nitrogen, serum creatinine, and uric acid reflects impairment of glomerular filtration and renal tubular function. The reduction of these biomarkers following treatment with *Vigna mungo* suggests restoration of renal function and protection against rifampicin-induced nephrotoxicity.

3.4 Histopathological Evaluation

Histological examination of kidney tissues supported the biochemical findings. Kidneys from the normal control group exhibited normal renal architecture with intact glomeruli and renal tubules. In contrast, the rifampicin-treated group showed severe pathological alterations, including glomerular congestion, tubular degeneration, vascular congestion, and inflammatory cell infiltration.



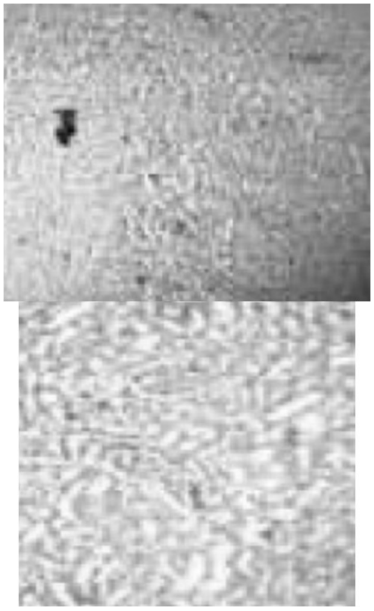



**Fig. 2: Impact of Vigna mungo seed aqueous extract (AEVM) on rat kidney histology in rifampicin-induced nephrotoxicity (H and E-stained cells) (a) Group 1 (Control): Rat kidney histology is normal. (b) Congestion and inflammation (C&I) represent Group 2 (Positive control). (c) Group 3: MCC-Mild cortical congestion (Standard) (d). Group 4 (AEVM): MI&C: Moderate congestion and inflammation**

Animals treated with the aqueous extract of *Vigna mungo* demonstrated marked improvement in renal histology. The extent of congestion, tubular degeneration, and inflammatory infiltration was substantially reduced, and the renal architecture appeared nearly normal. Histological findings in the extract-treated group were comparable to those observed in the cystone-treated group, confirming the nephroprotective efficacy of the extract.

**Discussion**

Drug-induced nephrotoxicity remains one of the major limitations associated with long-term use of antitubercular agents such as rifampicin. Oxidative stress generated during rifampicin metabolism contributes to lipid peroxidation, mitochondrial dysfunction, inflammatory responses, and apoptosis of renal tubular cells, resulting in impaired kidney function.

The present investigation demonstrated that aqueous extract of *Vigna mungo* significantly protected experimental animals against rifampicin-induced renal injury. The extract effectively restored body

weight, normalized serum biochemical markers, and improved renal histological architecture. These protective effects may be attributed to the rich phytochemical composition of the seeds.

Preliminary phytochemical analysis confirmed the presence of flavonoids, phenolic compounds, tannins, saponins, alkaloids, and glycosides. These phytoconstituents possess potent antioxidant activity and are capable of scavenging reactive oxygen species, reducing lipid peroxidation, and preserving cellular membrane integrity. Flavonoids and phenolic compounds are also known to enhance endogenous antioxidant enzyme activity and suppress inflammatory mediators, thereby protecting renal tissues from oxidative damage.

The significant reduction in serum creatinine, blood urea nitrogen, and uric acid observed in the extract-treated animals indicates restoration of glomerular filtration and renal tubular function. Histopathological observations further confirmed these findings by demonstrating reduced inflammatory changes and preservation of normal renal architecture.

The nephroprotective effect observed in the present study was comparable to that of cystone, a clinically used polyherbal nephroprotective formulation. These findings support the traditional medicinal use of *Vigna mungo* and suggest that its aqueous seed extract possesses considerable therapeutic potential for the management of drug-induced nephrotoxicity.

Overall, the present study demonstrates that the nephroprotective activity of *Vigna mungo* is closely associated with its phytochemical constituents, particularly flavonoids and phenolic compounds, which exert antioxidant and cytoprotective effects. Further studies involving isolation of active compounds, elucidation of molecular mechanisms, and clinical evaluation are required to establish its therapeutic application in renal disorders.

## Conclusion

The present study demonstrated that the aqueous seed extract of *Vigna mungo* possesses significant nephroprotective activity against rifampicin-induced nephrotoxicity in Wistar rats. Phytochemical screening confirmed the presence of flavonoids, phenolic compounds, tannins, alkaloids, glycosides, and saponins, which may contribute to the observed antioxidant and renal protective effects. Treatment with the extract significantly restored body weight, reduced blood urea nitrogen, serum creatinine, and serum uric acid levels, and improved renal histopathological changes. The nephroprotective activity was comparable to that of the standard drug Cystone. These findings support the traditional medicinal use of *Vigna mungo* and suggest that it may serve as a promising natural source for developing nephroprotective agents. Further studies are required to isolate the active constituents, elucidate their molecular mechanisms, and evaluate their clinical potential.

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