RESEARCH ARTICLE

Nephroprotective, Hepatoprotective and Toxicological Studies of *Vigna mungo* Aqueous Extract

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ABSTRACT

Hepatitis and nephropathy are significant global public health issues. The liver plays a crucial role in the body's glucose, protein, and lipid metabolism while also facilitating the elimination of waste products and harmful metabolites, thereby preventing toxicity. Similarly, the kidneys, being key components of the excretory system, are particularly vulnerable to a wide array of toxic agents, chemicals, and drug-related injuries. About 80% of people worldwide use traditional medicine to treat a variety of illnesses, according to the World Health Organisation (WHO). However, scientific studies reveal that only about 1% of these plants exhibit therapeutic potential when consumed in extract form by humans. Given the absence of stable liver and kidney protective drugs in conventional allopathic medicine, herbal remedies play a vital role in managing liver and kidney disorders. Herbal medicines are often considered safe and free from significant side effects due to their natural origin and widespread availability. The seeds of *Vigna mungo* are traditionally utilised in treatments for conditions such as paralysis, rheumatism, nervous system disorders, fever, and as a diuretic and tonic. These seeds are rich in bioactive compounds, including phenolic compounds, tannins, saponins, flavonoids, carbohydrates, proteins, amino acids, lipids, ascorbic acid, and enzymes. However, the hepatoprotective effects have not been extensively studied using additional experimental models. Moreover, there is a lack of systematic and scientific analysis regarding their potential renal effects in the existing literature.

Keywords: Nephroprotective, Hepatoprotective, Toxicological, Kidney, Liver, etc.

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INTRODUCTION

The most adaptable and sophisticated internal organ, the liver, is crucial to the body's metabolic processes. Its significance also stems from the thrust it gives to the control of the body's environment and the biochemical transformation of harmful and excretable substances from both endogenous and external sources. Since it is a crucial organ, therapy accords special significance to its protection[1]. The primary organ associated with the metabolism of natural poisons and therapeutic substances is the liver. Reactive oxygen species (ROS) are always produced by such substances because of hepatocyte disruption[2]. There is a substantial fatality rate due to two major hepatic disorders: jaundice and hepatitis[3].

The kidney is a major target organ for the negative effects of medications, oxidative stress and xenobiotics. Numerous biological mechanisms that may be essential in glomerular diseases have been connected to free radicals without oxygen[4,5].

Since ancient times, humans have used medicinal plants to treat their basic medical needs under the Indian medical system known as Ayurveda. Plant-based therapies have been utilised for thousands of years to prevent and treat illnesses, including liver disorders[6]. The use of natural items like herbs to give the liver the support it requires daily is now being pursued in conventional medicine[7]. Investigating appropriate herbal medications that could substitute chemical ones is crucial. Nephrotoxicity, which involves alterations to the nephrons, is critically dependent on renal cell death. Millions of nephrons make up a typical kidney, which serves as both the organ's structural and functional unit.

In traditional medical systems all around the world, numerous plants have been utilised to treat renal failure. Plant preparations did in fact constitute the foundation of the disease's therapy up until the advent of allopathic medicine, along with dietary changes. Traditional knowledge will act as a potent search engine and, more crucially, greatly facilitate deliberate, targeted, and secure natural product research to rediscover the drug discovery process. As a result, finding Nephroprotective herbs from medicinal plants has become crucial and necessary[8].

Hepatitis and nephropathy are important public health concerns all over the world. The liver's role in glucose, protein, and lipid metabolism, as well as waste and hazardous metabolite elimination, assists the body in avoiding toxicity. A variety of toxic agents, compounds, and drug-induced harm target the kidney because of its major role as a blood filter throughout the excretory process. Traditional medicine is used by nearly 80% of people around the world to treat a variety of illnesses, according to the WHO. Only about 1% of these plants have

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therapeutic potential when consumed in extract form by humans, according to scientific studies. Due to the lack of dependable liver and kidney defensive drugs in allopathic medical processes, herbs play crucial role in the treatment of many liver and kidney problems. Herbal medications are thought to be safe and free of major side effects because they come from nature and are widely available[9,10].

Vigna mungo seeds are commonly used in medicine for paralysis, rheumatism, nervous system devotion, heat and tonic, and diuretic. Total phenolic compounds, tannins, saponins, flavonoids, proteins, amino acids, lipids, ascorbic acid, and enzymes are all abundant in the seeds. There were no additional models employed in the hepatoprotective impact investigation. Furthermore, there is no scientific or methodical evaluation of their renal action in the literature[11].

MATERIALS AND METHOD

Drugs and Chemicals

A gift sample of rifampicin was received from Shreya Life Science Pvt Ltd. in Roorkee. Micro Labs in Bangalore provided the silymarin. We bought the kits for every biochemical estimation from Pathozyme Diagnostics Kagal, District Kolhapur, India. The MTR Institute of Pharmaceutical Sciences, Gulbarga, store of HKES provided the solvents and other chemicals, which were supplied by standard suppliers.

Plant Collection, Identification and Extraction

The legitimacy of the *Vigna mungo (L.)* Hepper seeds was confirmed in august 2022 after they were purchased from the Botany Department of Science College, Nanded, MH, India. The plant bearing voucher specimen number MCM/Bot-2 was stored for future use at Meerut College's

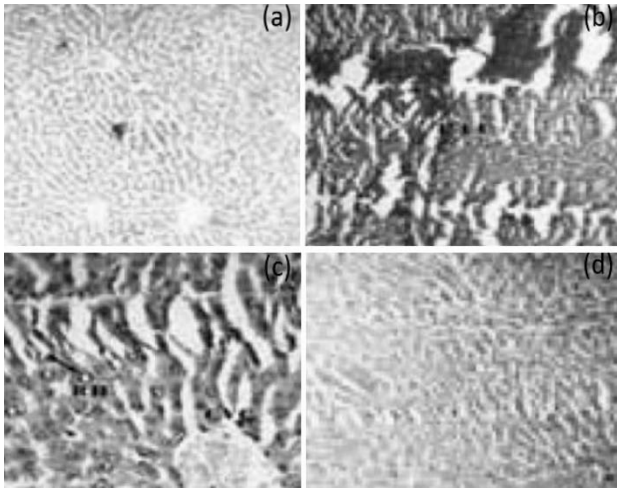

Figure 1: Influence of the *Vigna mungo* seed aqueous extract (AEVM) on the histological analysis of the rat liver in a hepatotoxic rifampicin-induced state (Stained cells with H and E): (a) The liver histology of Group 1 (Control) rats was found to be normal; (b) N-Focal Necrosis, PTI-Extensive Portal Triad Inflammation, and CVC-Central Vein Congestion make up Group 2 (Positive Control); (c) The third group (Standard) consists of regenerating hepatocytes (RH) and CVC (central vein congestion); (d) Group 4 (AEVM): VMI (very mild inflammation), MCD (mild central vein dilatation)

Table 1: Ash value of *Vigna mungo (L.)* Hepper seeds

S. 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

Botany Department in Meerut, Uttar Pradesh, India. After drying in the shade, the seeds were crushed using a pestle and mortar into a fine powder. These little particles are analyzed using pharmacognostic criteria.

Pharmacognostic Evaluation of the Plant Determination of Extractive Values

To ascertain the organoleptic characteristics, including color, nature, taste, and yield of the extracts, 100 g of dried and powdered plant material was successively extracted in the soxhlet extract or using petroleum ether, chloroform, ethanol (99%, v/v), and distilled water solvents in increasing order of polarity for 24 hours. The resultant liquid extracts were evaporated until they were totally dry under reduced pressure. The yield of the extracts was calculated using the following formula[12].

$$\text{Extractive value (\%)} = \frac{\text{Residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

Hepatoprotective Activity

Agrawal et al.'s description of the hepatoprotective activity study was followed. Four groups of six albino rats, each of any sex, were chosen and assigned to the 200–250 g weight range. Group 1: Gum acacia (5 mg/kg p.o.) was the only treatment given as a normal control group.

Group 2: Rifampicin (1 g/kg p.o.) was given one hourly.

Group 3: Standard; silymarin (25 mg/kg p.o.) was given after 30 minutes, and rifampicin (1 g/kg p.o.) was given every 72 hours.

Group 4: AEVM (500 mg/kg p.o.) was administered 30 minutes later, and rifampicin (1 g/kg p.o.) was given every 72 hours.

Ten days were spent doing the study. The 11th day saw the injection of thiopentone sodium (40 mg/kg, i.p.), and the amount of time spent sleeping was noted. All animals had blood samples taken using the retro-orbital puncture technique once they had fully recovered. ALP, BIT (total bilirubin), SGPT, SGOT, and ALP were among the biochemical markers that were examined after the serum was separated by centrifugation at 2500 rpm for 15 minutes. The animals were put to death with an overdose of ether shortly after the blood was collected, and their livers were removed, cleaned in saline, and their wet weight and volume measured before being placed in 10% formalin for histological analysis.

Nephroprotective Activity

According to Shelke et al., a nephroprotective activity investigation was conducted. Albino rats weighing between 200 and 250 g, regardless of sex, were chosen and split into four groups each group have six animals.

Group 1: Standard control group; equal amounts of distilled water were given to them.

Group 2: Positive control; rifampicin (1 g/kg p.o.) was administered every 72 hours.

Group 3: Standard; rifampicin (1 g/kg p.o.) every 72 hours and cystone (500 mg/kg p.o.) after 30 minutes were given.

Table 2: Influence of aqueous extract of seeds of *Vigna mungo* (AEVM) on selected physical and functional parameters in rifampicin-induced hepatotoxic rats

Group	Treatment	Dose	Mean liver weight (g/100 g)	Mean liver volume (ml/100 g)	Thiopentone induced sleeping time	
					Onset (s)	Duration (min)
1	Normal control	5 mg/kg (Gum acacia)	4.88 ± 0.05	4.91 ± 0.05	173.61 ± 0.99	84.85 ± 2.36
2	Positive control	1 g/kg (RIF)	8.39 ± 0.39	8.42 ± 0.39	95.41 ± 3.07	147.61 ± 2.06
3	RIF + SIL	1 g/kg + 25 mg/kg	5.68 ± 0.12	5.70 ± 0.12	163.01 ± 0.99	96.29 ± 0.92
4	RIF + AEVM	1 g/kg + 500 mg/kg	5.72 ± 0.12	5.80 ± 0.07	164.48 ± 0.98	98.67 ± 2.59

RIF- Rifampicin, SIL- Silymarin, AEVM- Aqueous extract of seeds of *Vigna mungo*

Table 3: Influence of aqueous extract of seeds of *Vigna mungo* (AEVM) on selected serum biochemical parameters in rifampicin- induced hepatotoxic rats

Group	Treatment	Dose	SGPT (IU/L)	SGOT (IU/L)	ALP (IU/L)	BIT (mg/dL)
1	Normal control	5 mg/kg (Gum acacia)	42.13 ± 2.28	63.84 ± 2.91	144 .08 ± 2.02	0.85 ± 0.11
2	Positive control	1 g/kg (RIF)	164.79 ± 4.95	363.39 ± 6.75	618.59 ± 10.99	3.18 ± 0.22
3	RIF + SIL	1 g/kg + 25 mg/kg	54.68 ± 2.90	75.91 ± 2.26	189.29 ± 6.27	0.96 ± 0.06
4	RIF + AEVM	1 g/kg + 500 mg/kg	54.64 ± 0.78	75.56 ± 0.55	192.54 ± 6.06	0.98 ± 0.05

RIF- Rifampicin, SIL- Silymarin, AEVM- Aqueous extract of seeds of *Vigna mungo*

Group 4: rifampicin (1 g/kg p.o.) every 72 hours and AEVM (500 mg/kg p.o.) after 30 minutes.

Two weeks were dedicated to the study. Before and after two weeks, the body weight was recorded. Every animal was put to sleep on the fifteenth day after being given too much ether, and they were all sacrificed. The kidneys were promptly removed and placed in 10% formalin for histological examinations after the blood samples were obtained using the heart puncture technique. The blood samples were subjected to the determination of biochemical markers, including blood urea nitrogen, serum creatinine, and serum uric acid, after being centrifuged for 15 minutes at 2500 rpm.

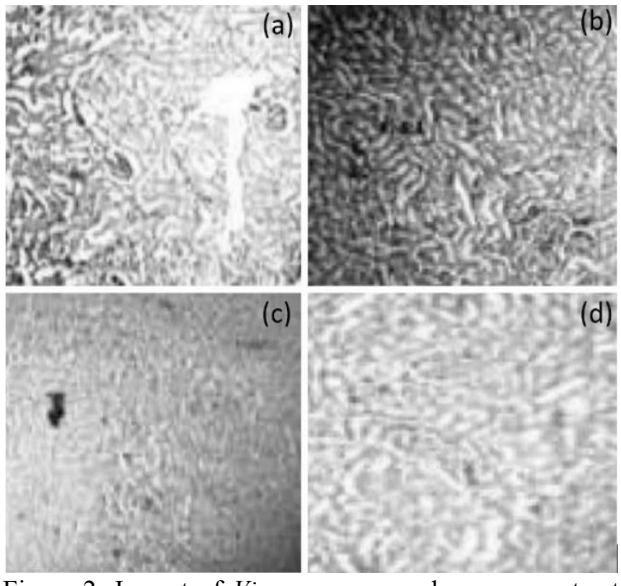

Figure 2: Impact of *Vigna mungo* seed aqueous extract (AEVM) on rat kidney histology in rifampicin-induced nephrotoxicity (H and E-stained cells): (a) Group 1 (Control): Rat kidney histology is normal; (b) Congestion and inflammation (C&I) represent Group 2 (Positive control); (c) Group 3: MCC-Mild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

Histopathological Studies

A consultant histopathologist examined the livers and kidneys histopathologically in the histopathology laboratory.

Statistical Analysis

The mean ± SEM was used to express the experiment's results. The data's statistical significance was evaluated using the application of one-way analysis of variance (ANOVA). Multiple comparison tests known as "Tukey-Kramer" were used for post-hoc analysis. Significant results were defined as P values <0.05. Comparisons were made between the normal control group and the positive control group, as well as between the positive control group and all other treatment groups.

RESULTS AND DISCUSSION

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. The color of the organic and inorganic solvent extracts was observed under both normal and ultraviolet light. The fluorescence analysis findings of *Vigna mungo (L.)* Hepper seeds treated with different chemical reagents are tabulated in Table 3.

Parameters Assessed for Liver Functions

Rats in group 2 that received rifampicin treatment had larger and heavier livers. Rats who were pretreated with silymarin and AEVM, on the other hand, had significantly lower liver weight and volume than the group that was shown as the positive control in Table 5.

When thiopentone was used to induce sleeping time, AEVM also resulted in a shorter sleeping time (in minutes) and a faster onset time (in seconds) than the positive control.

Table 4: 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 (mg/dl)	Serum creatinine (mg/dl)	Serum uric acid (mg/dl)
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*

When compared to the normal control group, the administration of rifampicin significantly raised the levels of SGPT, SGOT, ALP, and BIT (Tot. Bilirubin). Table 6 illustrates how pretreatment with AEVM and silymarin greatly inhibited the biochemical alterations brought on by rifampicin.

The liver histology of the control group's hepatocytes was normal. The liver of the rifampicin-treated group displayed focal necrosis, widespread portal vein inflammation, focal hepatocyte dropout, loss of lobular architecture, and focal necrosis. However, the histological alterations caused by rifampicin that are depicted in Figure 1 had been considerably avoided by the silymarin and AEVM-pretreated groups.

Parameters Assessed for Kidney Functions

Rats given cystone with AEVM had significantly greater body weights than the positive control group, but rats given rifampicin alone had significantly lower body weights than the normal control group (Table 7).

Biochemical parameters like blood urea nitrogen, serum creatinine, and serum uric acid were found to be significantly higher in the group treated with only rifampicin than in the normal control group when comparing the groups treated with cystone and AEVM to the positive control group in Table 7.

The rifampicin (group 2) group showed cortical glomerular, peritubular, blood vessel congestion, and interstitial inflammation, while the normal control group's rat kidney histology was normal. In the groups treated with cystone and AEVM, it was demonstrated that the kidney histological abnormalities brought on by rifampicin diminished (Figure 2).

Hepatoprotective Activity

Many substances and medications can harm the liver. Rifampicin was used as the hepatotoxicant in this investigation in order to cause liver damage. Although rifampicin is a commonly used, safe, and efficient antitubercular medication, overdosing on it can cause serious liver damage in both humans and experimental animals.

Adult human hepatocytes cultured in culture can express the enzymes CYP3A4 and CYP3A7 mRNAs when given excessive doses of rifampicin. Research has shown that the biotransformation of rifampicin into its active metabolite, 25-desacetyl rifampicin, lowers the levels of enzymes involved in the drug's metabolism and selectively binds to RNA polymerase, preventing the creation of proteins and nucleic acids that cause hepatotoxicity. Rats with hepatic injury had elevated serum levels of SGPT (ALT), SGOT (AST), ALP, and BIT (total bilirubin), which are indicators for evaluating the effects of toxins and hepatoprotective medications. These liver-cell-resident enzymes leak into the serum during hepatic injury, leading to elevated amounts. *Vigna mungo (L.)* Hepper is said to be utilised in the traditional Indian medical system to treat a range of liver diseases.

In the current investigation, rifampicin treatment for ten days caused morphological alterations, including liver enlargement, scratches, dark brown colouring, and increased volume. One type of xenobiotics that undergoes substantial hepatic metabolism are barbiturates. Liver dysfunction causes a delay in the clearance of barbiturates, which prolongs the hypnotic effect. In this work, thiopentone sodium treatment prolonged the amount of time rats receiving rifampicin-treated thiopentone-induced sleep.

In contrast, the liver morphology of the AEVM-pretreated mice was identical to that of the healthy control animals, and they demonstrated a significant reduction in both volume and thiopentone-induced sleeping time—indirect proof of the treatment's hepatoprotective effects. Administration of rifampicin also results in substantial elevations in SGPT, SGOT, ALP, and BIT (Tot. Bilirubin) in the serum. In contrast, the rats that received AEVM pretreatment showed a significant reduction in the elevation of these enzyme levels, suggesting that AEVM has a hepatoprotective effect against liver cell damage caused by rifampicin.

Histological changes, including loss of lobular architecture, central vein dilation, localized hepatocyte drop out, focal necrosis, and severe portal tract inflammation, were observed in the rifampicin-treated (positive) control group. The hepatoprotective efficacy of AEVM was further demonstrated by the considerable prevention of these histological alterations in the rats treated with it. Every histological alteration that was seen was correlated with the liver's physical, biochemical, and functional characteristics. Significant hepatoprotective effects were seen in the extract of *Vigna mungo* successive water extract.

It has been discovered that AEVM can stop the biochemical alterations in liver damage brought on by rifampicin. By measuring blood SGPT, SGOT, ALP, and BIT (total bilirubin), which provides a useful indication of the liver's functioning status, the hepatoprotective effect of AEVM was seen. ALP was a definite sign of cellular leakage and loss of functional integrity of the cell membrane, and the

rise in serum bilirubin levels indicated the degree of jaundice and severity of hepatic necrosis.

It was discovered that rifampicin causes hepatotoxicity. The evaluation of the *Vigna mungo* extract has been done in the current study. It is possible that AEVM's capacity to increase antioxidative enzyme activity explains its capacity to shield the liver from harm caused by rifampicin. Our study demonstrated that the subsequent AEVM had good hepatoprotective efficacy.

Nephroprotective Activity

Many environmental toxicants and therapeutically useful drugs, such gentamicin and paracetamol, can cause severe organ toxicities by metabolically activating highly reactive free radicals, such as superoxide and oxygen reactive species. Numerous experimental animal models have clearly shown a connection between oxidative stress and nephrotoxicity. Adriamycin-induced nephrotoxic effects were much lessened when vitamin E was administered. The use of medicinal plants as antioxidants to lessen tissue damage brought on by free radicals has garnered more attention in recent years.

Pedalium murex has been shown to have nephroprotective properties against nephrotoxicity induced by cisplatin, as well as diuretic and antioxidant properties. High-level antioxidants, such as phenolic compounds, have the capacity to both quench reactive oxygen species and absorb and neutralise free radicals. Antioxidants in vivo are also produced by plant flavonoids that exhibit antioxidant action *in vitro*. The total phenolic content of fruits, vegetables, grain products, and plant subjects undergoing ethnopharmacological treatments has been shown to be strongly correlated with antioxidant activity. *In vitro* and in vivo stabilising effects on the lysosomes of experimental animals have been described for flavonoids, tannins, and saponins. Through their ability to bind cations and other biomolecules, tannins and saponins stabilise the erythrocyte membrane. Because Vigna contains saponins, it has been observed that the seeds of *Vigna mungo* have diuretic and antioxidant properties.

In contrast to the positive control group, AEVM-pretreated mice exhibited a substantial increase in body weight and inhibited the elevation of these enzyme levels, suggesting a nephroprotective action against kidney injury caused by rifampicin. Rifampicin treatment for 14 days in the current study resulted in a substantial drop in body weight but an increase in serum enzymes, including blood urea nitrogen, serum creatinine, and serum uric acid.

The rifampicin-treated (positive) control group showed histological alterations such as cortical glomerular, peritubular blood vessel congestion, and interstitial inflammation. Further evidence of the AEVM pretreatment animals' Nephroprotective activity comes from their considerable prevention of these histological alterations. The physical and biochemical characteristics of the kidney correlated with every histological alteration that was seen. According to the reports, *Vigna mungo* seeds have diuretic and antioxidant properties. Furthermore, it has been demonstrated that P. murex contains Nephroprotective properties against nephrotoxicity induced by cisplatin in addition to diuretic and antioxidant properties. Our findings align with those in this report. We infer from the experimental investigation that the AEVM has sufficient Nephroprotective action in albino wistar rats. It had strong Nephroprotective effects.

CONCLUSION

The AEVM demonstrated sufficient hepatoprotective and Nephroprotective action on albino wistar rats in the current investigation, as demonstrated by the liver and kidney's functional, histological, biochemical, and physical characteristics, respectively. The Nephroprotective and hepatoprotective effects could be attributed to potent antioxidants like flavonoids, phytic acid, tannins, and total phenolic compounds. Furthermore, the extract contains strong diuretics called saponins that promote the excretion of salt, potassium, drug metabolites, toxins, etc., shielding the kidneys and liver from the harmful properties of rifampicin. The aqueous extract of *Vigna mungo (Linn.)* Hepper's seeds (AEVM) have been shown in the research to have hepatoprotective and Nephroprotective properties against hepatotoxicity and nephrotoxicity caused by rifampicin, respectively. Hepatoprotective and Nephroprotective effects of the AEVM were statistically significant.

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