EVALUATION OF NEPHROPROTECTIVE, HEPATOPROTECTIVE AND TOXICOLOGICAL STUDIES OF SOME INDIGENOUS PLANTS

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Abstract: The phytochemical investigation's capacity to separate and submit the active ingredients in Vigna mungo (L.) Hepper seeds to further pharmacological testing can be of considerable use to researchers and the field of traditional medicine. The purpose of this study was to investigate the potential of an aqueous extract of Vigna mungo (AEVM) seeds (fabaceae) to shield rats' liver and kidneys from rifampicin-induced damage. Following selection, eight groups of six albino rats, weighing 200–250 grammes apiece, were created. Four groups were assigned hepatoprotective activity and four groups were assigned nephroprotective activity. Normal control was given to Group 2 and AEVM treatment was given to Group 3. Standard medication treatment was given to Group 2. Group 4 was given normal medication therapy. Additionally, the nephroprotective impact was investigated. The results are corroborated by function, histology, biochemistry, and physical features. The medications used for hepatoprotective action include rifampicin, silymarin, and SGPT, SGOT, ALP, and BIT diagnostic kits. Serum BUN, serum creatinine, and serum uric acid for nephroprotective effect. To extract the powdered Vigna mungo seed, water was utilised. Some preliminary phytochemical research was done to identify the phytoconstituents. The hepatoprotective and nephroprotective effects of AEVM were assessed in rats administered with rifampicin to cause hepatotoxicity and nephrotoxicity. After one-way analysis of variance (ANOVA), multiple comparison tests utilising the "Tukey-Kramer" method are conducted. The AEVM identified proteins, phytic acid, total phenolic compounds, alkaloids, carbohydrates, flavonoids, glycosides, and tannins. Rifampicin caused significant alterations in the following areas: biochemical (increase in serum glutathione pyruvate transaminase (SGPT), oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP) and total bilirubin (BIT) level, increase in blood urea nitrogen (BUN), serum creatinine, and serum uric acid level), functional (barbiturates-induce sleeping time), physical (increased liver weight, decreased body weight), and histological (damage to hepatocytes, nephrons). Pretreatment with AEVM significantly reduced the physical, biochemical, and histological changes that rifampicin caused in the liver and kidney, respectively.

Keywords: Nephroprotective, Hepatoprotective, *Vigna mungo* , Herbal, Liver, Kidney, etc**.**

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Introduction

Recently, herbal medicines have gained a lot of attention as complementary therapies that can be used to treat or prevent illnesses connected to lifestyle choices. However, relatively little is known about how these medications work. Research on plant products' possible health benefits has been intense due to increased interest in their analysis [1]. Vigna mungo seeds are used extensively in medicine, both topically and topically. The seeds are used in dropsy, rheumatism, paralysis, and affections of the nervous system. They are also used in fever, piles, liver affections, cough, and dropsy. The seeds are also said to be hot and tonic. Another species uses green gram seeds to treat kidney illnesses [2]. Total phenolic compounds, tannins, saponins, flavonoids, proteins, amino acids, lipids, ascorbic acid, and enzymes are abundant in the seeds [3]. There have been reports of the hepatoprotective and antioxidant properties of Vigna mungo seeds [4–5]. But no further models were employed to check for its hepatoprotective properties. Furthermore, no rigorous, scientific research on their effects on the kidney has been documented in the literature too far.

The seeds of the Fabaceae family plant Vigna mungo are used to make black gramme. It is really nourishing. Moisture (10.9 %), protein (24.0 %), fat (1.4 %), fibre (0.9 %), minerals (3.2 %), and carbs (59.6%) make up its principal constituents. It is used to treat CNS diseases, diabetes, hair disorders, and as a demulcent and aphrodisiac [6].A essential pulse crop, black gramme (Vigna mungo (L.) Hepper) is grown in summertime under a variety of Agro-ecological zones, primarily those that get rain. Numerous South Asian nations, including Thailand, the Philippines, Bangladesh, India, Pakistan, Nepal, and Korea, cultivate it. Among pulses, it is the least studied crop, and no international CGIAR centre includes this crop on its list of approved crops. While it is acknowledged as a promising crop in several nations, there is a dearth of comprehensive research data on pharmacognosy other than a few publications from around the globe [7].The major reason black grammes are grown is for their high protein seeds. Its dietary protein level is comparable to that of soybeans. While India is the primary producer of this crop, several biotic and abiotic stressors have a negative impact on output. It is quite vulnerable to fungal diseases, insects, dehydration, and yellow mosaic virus, all of which cause large output losses. Due to the lack of sufficient and acceptable genetic variety in the available germplasm, traditional breeding efforts to increase black gram's resistance to insects and disease have been less successful [8]. Contiguous and mineral composition, protein fractions, ascorbic acid and niacin, the fatty acid and amino acid profiles of seed lipids, in vitro protein digestibility, and other antinutritional components were all examined in relation to black grammes. Minerals including Na, K, Mg, and P were abundant in the seed sample. The main proteins in the examined Vigna mungo types were discovered to be albumins and globulins. Among the three types of Vigna mungo, Raffinose was the main oligosaccharide. Human health advantages derived from the nutritional content of Vigna mungo are numerous [9].

The liver, the most complex but adaptable internal organ, is essential to the body's metabolic processes. Its significance also stems from its role in promoting the regulation of the internal environment and the biochemical transformation of foreign and endogenous substances into safe and excretable forms. Its preservation therefore has a particular status in treatments because it is an essential organ [10]. The primary organ involved in the metabolism of pharmaceuticals and biological poisons is the liver. These substances are invariably linked to hepatocyte disruption, which produces reactive oxygen species (ROS) [11]. Two important hepatic illnesses that cause a significant fatality rate are jaundice and hepatitis [12].

Additionally, the kidney is a key target organ for the harmful effects of xenobiotics, medications, and oxidative stress. Numerous cellular processes that may be crucial in glomerular disorders have been linked to oxygen-free radicals [13, 14].

Rifampicin over dosage may result in "red man syndrome" in which brownish orange discoloration of the skin, urine, sweat, feces, tears, and saliva occurs and is proportional to the amount ingested. Rifampicin produces hepatic dysfunction and elevation of liver enzymes and some fatalities have occurred. Rifampicin also reported to produce nephrotoxicity. Acute renal failure.

In addition to the drug's strong diuretic action, which results in the evacuation of sodium, potassium, and other metabolites, etc., certain plants with antioxidant qualities also demonstrated nephroprotective effect against gentamicin and cisplatin. Aqueous extract of Vigna mungo (Linn.) Hepper seeds was used in this investigation to assess the hepatoprotective

and nephroprotective effects against rifampicin-induced toxicities.

Material and Method

Material

Shreya Life Science Pvt Ltd. in Roorkee provided a complimentary sample of rifampicin. The silymarin was donated by Micro Labs in Bangalore. We purchased the necessary kits from Pathozyme Diagnostics Kagal, District Kolhapur, India, for each biochemical estimation. The solvents and other chemicals were supplied by standard sources and were made available by the MTR Institute of Pharmaceutical Sciences, Gulbarga store of HKES.

Plant collection, identification and extraction

The Vigna mungo (L.) Hepper seeds were obtained in August from Maliyana, which is located in the Meerut District of Uttar Pradesh, India. Its validity was confirmed by Dr. A. K. Gupta (Reader), Botany Department, Meerut College, Meerut (U.P.), India. In the interest of future research, the plant bearing voucher specimen number MCM/Bot-2 was deposited at Meerut College's Department of Botany in Meerut, Uttar Pradesh, India. After being dried in the shade, the seeds were ground into a fine powder using a pestle and mortar. For these little powders, Pharmacognostic criteria are investigated.

Pharmacognostic evaluation of the plant

Determination of Extractive values

To ascertain the organoleptic characteristics, such as colour, 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 the increasing order of polarity for 24 hours. The resultant liquid extracts were evaporated until they were entirely dry under reduced pressure. The yield of the extracts was calculated using the following formula [15].

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

Alcohol soluble extractive value

It was precisely 5 grammes of air-dried powdered medicine, which was then macerated in a stoppered flask containing 100 millilitres of 99% ethanol for 24 hours while being shaken frequently. After that, it was rapidly filtered through filter paper, taking care not to lose too much of the ethanol in the process. The capacity was increased to 100 millilitres by adding ethanol. After being weighed and dried at 105 0C, the remaining material was evaporated in a shallow dish with a flat bottom and kept in desiccators. The average extractive value in percentage w/w (on a dry basis) for medication that has been air-dried was calculated (Table 1) [16].

Water soluble extractive value

Five grammes of air-dried and coarsely crushed drug material were macerated in one hundred millilitres of water for a full day in a stoppered flask, with frequent shaking during the first six hours. The extract was quickly filtered using filter paper, being careful not to lose too much solvent in the process. The remaining material was weighed, dried at 1050C, and then placed in a desiccator to evaporate in a shallow dish with a flat bottom. The average extractive value in percentage w/w (on a dry weight basis) for medication that has been air-dried was calculated (Table 1) [16].

Chloroform soluble extractive value

The medicine weighed exactly five grammes after it had been air-dried and powdered. It was macerated in a stoppered flask with 100 millilitres of chloroform for 24 hours while being shaken frequently. Following that, it was rapidly filtered through filter paper, taking care not to lose too much chloroform in the process. The capacity was increased to 100 ml by adding chloroform. After being weighed and dried at 105 0C, the remaining material was evaporated in a shallow dish with a flat bottom and kept in desiccators. The average extractive value in percentage w/w (on a dry basis) was calculated using air-dried medication as a guide (Table 1).

Petroleum ether soluble extractive value

100 ml of petroleum ether was used to macerate 5 g of coarsely powdered and air-dried drug material for 24 hours, shaking frequently during the first 6 hours, in a stoppered flask. The extract was quickly filtered using filter paper, being careful not to lose too much solvent in the process. Weighing and drying at 105 0C, the residue was then evaporated in a shallow dish with a flat bottom and kept in a desiccator. The average extractive value in percentage w/w (on a dry weight basis) for medication that has been air-dried was calculated (Table 1).

Determination of ash value

Total ash value

Two grammes of dried and powdered plant material were placed into the clean, sintered silica crucibles that had been previously weighed. The temperature in the muffle furnace was then steadily increased (from 400 to 500 0C) until white ash was produced and the ash weight stayed constant. Using the air-dried sample of the crude pharmaceutical (Table 2), the crucible was cooled to room temperature in a desecrator, and the ash was weighed. The percentage of total ash was then calculated.

$$\text{Total ash Value (\%)} = \frac{Z - X}{Y} \times 100$$

Where, X= Weight of the crucible; Z = Weight of the crucible with ash; Y = Weight of the powder taken (g).

Acid insoluble ash value

The plant material with the highest ash content was boiled for 15 minutes and then allowed to cool after 25 ml of 25% (v/v) HCl was added to a 100 ml beaker. After going through an Ash-Less Whatman filter paper No. 44, hot water was used three times to wash the residue away. The insoluble ash that was so retained on filter paper and paper were then fired at 1000 0C in a preweighed sintered crucible. After that, the residue and crucible were weighed, and the formula in Table 2 [17] was used to calculate the amount of acid insoluble ash.

$$\text{Acid insoluble ash Value (\%)} = \frac{a \text{ X } 100}{Y}$$

Where, a= weight of the residue; Y= Weight of powder taken (g)

Water soluble ash value

Total ash value was computed using two grammes of the air-dried, powdered sample. Twenty-five millilitres of distilled water were used to boil the entire ash for five minutes. Following a thorough cleaning with hot distilled water, the insoluble material was gathered onto ash-free filter paper and burned for fifteen minutes at a temperature that did not climb over 450 0C. The weight difference that remains after deducting the insoluble matter's weight from the overall ash weight is the water-soluble ash. The percentage of the water-soluble ash was calculated using the powdered plant sample that had been air-dried as a reference. It was computed using the following formula (Table-2) [18].

$$\text{Water insoluble ash Value (\%)} = \frac{a \text{ X } 100}{Y}$$

Where, a= Weight of the residue; Y= Weight of powder taken (g)

Water soluble ash Values (%) = Total ash value ─ Water insoluble ash value.

Fluorescent studies of powder drugs

Many herbs exhibit fluorescence when exposed to UV light on their powdered or chopped surfaces, which can aid in identification. The fluorescence of plant powders (40 mesh) was examined in daylight and UV light (254 nm and 366 nm), as well as after being exposed to a variety of substances, including sodium hydroxide, ferric chloride, nitric acid, and hydrochloric acid (Table 3) [18].

Phytochemical Screening

After being collected, the seeds were sun-dried and coarsely crushed. The powdered seeds were extracted using a Soxhlet apparatus with petroleum ether, chloroform, ethanol, methanol, and distilled water. Reduced pressure distillation was used to filter and remove the solvent from the extracts. Following the computation of percentage yields, additional phytochemical testing was performed on the extracts to determine the content of flavonoids, glycosides, alkaloids, carbohydrates, and tannins (Table 4) [17].

Animals and housing parameters

200–250 g of mature, healthy albino wistar rats of both sexes were used in this study. Male and female adult rats of normal weight were supplied by the MTR Institute of Pharmaceutical Sciences of H.K.E.S. Standard environmental conditions were upheld during the quarantine, which included a 12-hour light and dark cycle, a temperature of 26 ± 2°, and a relative humidity of 45–55 percent. Standard pelletized feed and water were provided freely under hygienic conditions. The animals were acclimated to laboratory conditions 48 hours before to the experiment in order to minimise any nonspecific stress. The experimental protocol was approved by the Institutional Animal Ethics Committee of H.K.E.S's College of Pharmacy, India, in accordance with the guidelines established by the Committee for the purpose of controlling and supervising animal experiments (CPCSEA).

Acute toxicity studies

According to earlier research, a hepatoprotective dose of 500 mg/kg body weight p.o. of Vigna mungo seed extract was used. Given that Vigna mungo seeds are a widely consumed food, the recommended dosage was determined to be non-toxic, and this study employed the same dosage.

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.

The investigation was conducted over ten days. On the eleventh day, thiopentone sodium (40 mg/kg, i.p.) was injected, and the duration of sleep was recorded. After each animal had fully recovered, blood samples were obtained using the retro-orbital puncture procedure. Following serum separation by centrifugation at 2500 rpm for 15 minutes, biochemical indicators such as ALP, BIT (total bilirubin), SGPT, SGOT, and ALP were analysed. Shortly after the blood was drawn, the animals were given an overdose of ether to kill them. Their livers were then removed, cleansed in saline, and their moist weight and volume were measured before being preserved in 10% formalin for histological examination.

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 of six animals each.

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.

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

The study lasted for two weeks. The body weight was measured both before and after the two-week period. After being overfed ether, all of the animals were sacrificed and put to sleep on the sixteenth day. After the blood samples were taken using the heart puncture procedure, the kidneys were quickly removed and put in 10% formalin for histological analyses. After centrifuging the blood samples for 15 minutes at 2500 rpm, biochemical markers such as blood urea nitrogen, serum creatinine, and serum uric acid were measured.

Histopathological studies

In the histopathology lab, a consultant histopathologist performed a histological examination of the kidneys and livers.

Statistical analysis

The experiment's results were expressed as mean ± SEM. One-way analysis of variance (ANOVA) was used to assess the statistical significance of the data. For post-hoc analysis, "Tukey-Kramer" multiple comparison tests were employed. P values less than 0.05 were used to identify significant results. The positive control group and all other treatment groups were compared, as well as the normal control group and the positive control group.

Result and Discussion

Table 1 presents the findings of the organoleptic study, including the extraction yield of Hepper's seeds extract from Vigna mungo (L.). Aqueous extracts > ethanol > chloroform > petroleum ether is a ranking of the extraction yields of different solvents, which ranged from 0.9% to 24.7% in the cold maceration process and from 0.35% to 41.17% in the hot extraction process. The ratio of solvents, temperature, and sample extraction will all improve the percentage of extraction yield. Hepper's Vigna mungo (L.) crude extracts have shown a broad spectrum of colour. The petroleum ether and chloroform extracts have yellowish green and greenish brown colours, respectively, whereas the ethanol and aqueous extract are dark brown and brown. In addition, the tastes of petroleum ether and chloroform extract are sharply bitter, the ethanol extract is harsh, and the aqueous extract is pleasant. The character of these extracts also varies; they are oily (petroleum ether), sticky (aqueous), waxy (chloroform), and resinous (ethanol).

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

Determining the medicine's physico-chemical constant is essential for spotting drug adulteration or improper administration. The total ash content is particularly important for determining the drug's purity, or whether it contains metallic salts or other foreign organic matter like silica. Although the overall ash content of the Vigna mungo (L.) Hepper seed was 2.5%, Table 2 shows that the water insoluble ash content was larger than the acid insoluble ash content (1.5% and 0.5%, respectively). A considerable amount of total ash was discovered in the seed in the current investigation. In the event of adulteration, the results can be utilised as a quality criterion to evaluate the biomass of Vigna mungo (L.) Hepper.

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

The fluorescence analysis is sufficiently sensitive to allow for an exact and accurate determination across a suitable concentration range. Each chemical fluoresces in a different colour. A non-fluorescent chemical can fluoresce when it is mixed with fluorescent impurities. The colour of the extracts from organic and inorganic solvents was discernible under both normal and UV light. The findings of the fluorescence study of Vigna mungo (L.) Hepper seeds treated with different chemical reagents are tabulated in Table 3.

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

Sr.no.	Solvents Treatment	Visible light	Short UV (252 nm)	Long UV (366nm)
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

Water, ethanol, methanol, petroleum ether, and chloroform were used in a phytochemical screening process for Vigna mungo (L.) Hepper seed extracts. The investigation found the presence of alkaloids, tannin, flavonoids, saponins, glycosides, terpenoids, and ascorbic acid. The main attraction of phytochemical screening is the presence of flavonoids, saponins, phenols, ascorbic acid, tannins, and tannin-containing compounds in many extracts. The phytochemical screening of chemical contents of Vigna mungo (L.) Hepper seeds revealed high levels of flavonoids, saponins, ascorbic acid, tannins, and phenols (Table 4). They were

reported to exhibit both physiological activity and therapeutic efficacy.

Table 4: 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	-	+	+	-	+

Parameters assessed for liver functions

The livers of the rats in group 2 who were treated with rifampicin were heavier and larger. In contrast, the liver weight and volume of the rats who received pretreatment with silymarin and AEVM were considerably lower than those of the group that Table 5 shows as the positive control. AEVM also produced a faster onset time (in seconds) and a shorter sleeping length (in minutes) than the positive control when thiopentone was used to induce sleep.

Table 5: 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

The rifampicin treatment markedly increased the levels of SGPT, SGOT, ALP, and BIT (Tot. Bilirubin) in comparison to the normal control group. Table 6 shows how AEVM and silymarin pretreatment significantly reduced the metabolic changes caused by rifampicin.

Table 6: 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	54.68 ±	75.91 ±	189.29 ±	0.96 ±

		mg/kg	2.90	2.26	6.27	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

The hepatocytes in the normal control group had normal liver histology. The livers of the rifampicin-treated group showed loss of lobular architecture, focal hepatocyte dropout, focal necrosis, and extensive portal vein inflammation. However, the silymarin and AEVM-pretreated groups had significantly averted the rifampicin-induced histological changes shown in Figure 1.

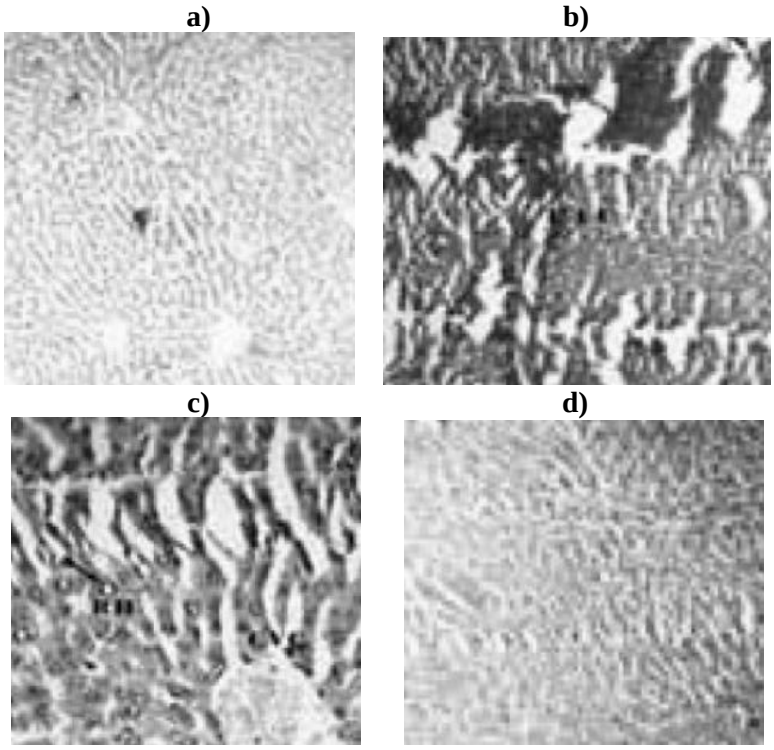

Fig. 1: Impact of the aqueous extract from the seeds of Vigna mungo (AEVM) on the histological examination of the rat liver in a state of hepatotoxicity caused by rifampicin (stained cells with H and E). Group 1 (Control) rats were found to have normal liver histology. Group 2 (Positive Control) consists of N-Focal Necrosis, PTI (Extensive Portal Triad Inflammation), and CVC (Central Vein Congestion). Central vein congestion (CVC) and regenerating hepatocytes (RH) make up the third category, or Standard. Group 4 (AEVM) (d): minimal central vein dilatation (MCD) and very mild inflammation (VMI).

Parameters assessed for kidney functions

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

Table 7: 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 +	1 g/kg + 500	3.14 ± 0.19	23.27 ± 0.62	0.59 ± 0.04	3.52 ± 0.15

	CYS	mg/kg				
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 Biochemical parameters such as 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.

Rat kidney histology was found to be normal in the normal control group, but the rifampicin (group 2) group displayed cortical glomerular, peritubular, blood vessel congestion, and interstitial inflammation. It was demonstrated that in the groups treated with cystone and AEVM, the rifampicin-induced kidney histological abnormalities diminished (see fig. 2).

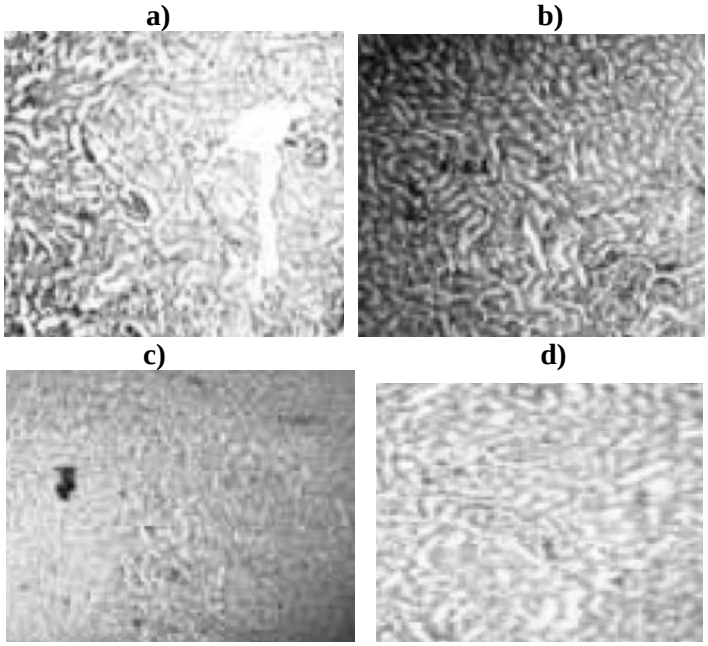

Fig. 2: Effects of Vigna mungo seed aqueous extract (AEVM) on rifampicin-induced nephrotoxicity in rat kidney histopathology (H and E-stained cells) (A) The kidney histology of rats in Group 1 (Control) is normal. (b) Group 2 (Positive control) is represented by congestion and inflammation (C&I). (c) Group 3: Standard MCC-Mild Cortical Congestion (d). Group 4 (AEVM): MI&C: Mild inflammation and congestion

Hepatoprotective activity

Numerous drugs and substances can damage the liver. In this study, the hepatotoxicant employed

to destroy liver tissue was rifampicin. Rifampicin is a widely used, effective, and safe antitubercular drug; nonetheless, overdosing on it can seriously harm the liver in both humans and laboratory animals. When exposed to high dosages of rifampicin, adult human hepatocytes cultivated in culture can express the enzymes CYP3A4 and CYP3A7 mRNAs. Studies reveal that when rifampicin is bio transformed into its active metabolite, 25-desacetyl rifampicin, it specifically binds to RNA polymerase to inhibit the production of proteins and nucleic acids that cause hepatotoxicity and decreases the amounts of enzymes involved in the drug's metabolism. Serum levels of SGPT (ALT), SGOT (AST), ALP, and BIT (total bilirubin), which are markers for assessing the effects of toxins and hepatoprotective drugs, were higher in rats with hepatic damage. During hepatic damage, certain enzymes that are exclusive to liver cells escape into the serum, resulting in increased levels. According to reports, the ancient Indian medical system uses Vigna mungo (L.) Hepper to cure a variety of liver disorders.

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.

The rifampicin-treated (positive) control group showed histological alterations such as loss of lobular architecture, extensive dilatation of the central vein, focal hepatocyte drop out, focal necrosis, and severe inflammation of the portal tract. 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

The metabolic activation of highly reactive free radicals, such as superoxide and oxygen reactive species, can result in severe organ toxicities caused by a variety of environmental toxicants and clinically helpful medications, such as paracetamol and gentamicin. 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. A robust correlation has been documented between the antioxidant activity and the total phenolic content of fruits, vegetables, grain products, and plant subjects receiving ethnopharmacological treatments. 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.

AEVM-pretreated animals had significantly increased body weight and prevented the elevation of these enzyme levels compared to the positive control group, indicating their nephroprotective effect against rifampicin-induced kidney damage. In the current study, rifampicin administration for 14 days significantly decreased body weight but increased serum enzymes such as 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

Using albino wistar rats, the AEVM showed adequate hepatoprotective and nephroprotective effect as indicated by the functional, histological, biochemical, and physical properties of the liver and kidney, respectively. Strong antioxidants such as flavonoids, phytic acid, tannins, and total phenolic compounds may be responsible for the nephroprotective and hepatoprotective effects. To further protect the kidneys and liver from the negative effects of rifampicin, the extract contains potent diuretics called saponins that encourage the excretion of salt, potassium, drug metabolites, pollutants, etc. Research has demonstrated that the aqueous extract of Vigna mungo (Linn.) Hepper's seeds (AEVM) have hepatoprotective and nephroprotective activities against rifampicin-induced hepatotoxicity and nephrotoxicity, respectively. The effects of AEVM on hepatoprotection and nephroprotection were statistically significant.

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