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Hepato and Nephro-Protective Effect of Methanolic Extract of *Vigna mungo* (Linn.) Hepper on Rifampicin Induced Toxicity in Albino Rats.

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ABSTRACT

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The present study was conducted to investigate the hepatoprotective and nephroprotective activity of methanolic (MEVM) extract of seeds of *Vigna mungo* (fabaceae) against rifampicin-induced liver and kidney toxicity in rats. The seed powder of *Vigna mungo* was successively extracted with methanol and preliminary phytochemical tests were done. The hepatoprotective and nephroprotective activity of the MEVM were assessed in rifampicin-induced hepatotoxic and nephrotoxic rats. The MEVM showed presence of amino acids, alkaloids, carbohydrates, flavonoids, glycosides, proteins, phytic acid, total phenolic compounds, saponins and tannins. Rifampicin produced significant changes in physical; (increased liver weight, decreased body weight), biochemical; (increased serum glutathione pyruvate transaminase, oxaloacetate transaminase, alkaline phosphatase and total bilirubin level, increased biood urea nitrogen, serum creatinine, and uric acid level), histological; (damage to hepatocytes, nephrons) and functional; (thiopentone-induced sleeping time) induced by rifampicin in liver and kidney parameters respectively. Pretreatment with MEVM significantly prevented the physical, biochemical, and histological changes induced by rifampicin in the liver and kidney respectively. The present study indicates that MEVM possessed hepatoprotective and nephroprotective activity, though MEVM was found to exhibit greater hepatoprotective activity than Silymarin.

Keywords: Hepatoprotective activity, Nephroprotective activity, Rifampicin, *Vigna mungo*.

INTRODUCTION

Liver is the key organ of the body playing major role in maintaining homeostasis. It is involved with almost all the biochemical pathways related to growth, fight against diseases, nutrient supply, energy provision and reproduction.[1] Liver is the main organ involved in the metabolism of biological toxins and medicinal agents. Such agents are always associated with the disturbance of hepatocytes resulting in generation of Reactive Oxygen Species (ROS).[2] Liver damage ranging from subclinical icteric (jaundice) hepatitis to necro-inflammatory hepatitis, cirrhosis, and carcinoma has been proved to associate with the redox imbalance and Oxidative Stress (OS).[3] In absence of reliable liver-protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief.[4]

Kidney is another important target for the toxic effects of drugs, xenobiotics and oxidative stress. Oxygen free radicals have been implicated in several biological processes potentially important in glomerular diseases, [5,6] and also have a role in neutrophil mediated glomerular diseases.[7,8]

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Rifampicin overdosage may result in "red man syndrome" in which brownish orange discoloration of the skin, urine, sweat, faeces, tears and saliva occurs and is proportional to the amount ingested.[9] Rifampicin produces hepatic dysfunction and elevation of liver enzymes and some fatalities have also occurred with it.[10] Rifampicin also has been reported to produce nephrotoxicity,[11] acute renal failure,[12,13] and renal papillary necrosis. [14]

Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relative low cost.[15] *Vigna mungo* (Linn.) Hepper commonly called as black gram is a member of family- Fabaceae. The seeds are black with a white hilum protruding from the seeds. The seeds are rich in total phenolic compounds, tannins, saponins, flavonoids, carbohydrates, proteins, amino acids, lipids, ascorbic acid and enzymes.[16] The seeds are much used in medicine, both internally and externally in paralysis, rheumatism, affections of the nervous system, in fever, considered hot and tonic, useful in piles, affections of liver and cough, the seeds are considered diuretic and is used in dropsy. It`s another species green gram seeds are used in kidney diseases.[17]

Vigna mungo (Linn.) have been reported for its hepatoprotective activity in acetaminophen and CCl_4 model,[18] antioxidant activity.[19] However, no other models were used for screening of its hepatoprotective activity. And also there is no scientific and methodical investigations have so far been reported in literature regarding their actions on kidney.

Several plants containing antioxidant properties exhibited nephroprotective activity against gentamicin and cisplatin.[20] and also the drug is found to be potent diuretic which causes excretion of sodium and potassium. These observations made us to investigate the plant material for its hepatoprotective and nephroprotective activity against rifampicin-induced toxicity in rats.

MATERIALS AND METHODS

Drugs and chemicals

Rifampicin was obtained as a gift sample from Shreya life Science Pvt Ltd. Roorkee. Silymarin was obtained from Micro Labs. Bangalore. The kits for all biochemical estimation were purchased from Pathozyme diagnostics, Kagal, Dist. Kolhapur, India. The solvents and other chemicals were procured from reputed manufacturers.

Plant materials and extraction

The seeds of *Vigna mungo* (Linn.) Hepper were purchased from the local market of Gulbarga, Karnataka; were authenticated at Pharmacognosy department of HKES's MTR Institute of Pharmaceutical Sciences, Gulbarga. The seeds are powdered and defatted with petroleum ether and then subjected to successive extraction with methanol (95%) using soxhlet apparatus for 36 h. the solvent was evaporated at 60^{o} on a water bath to have thick pasty mass referred to as seed extract, the percentage yield was 13 g.

Phytochemical screening

Preliminary phytochemical screening of MEVM was carried out as described by Khandelwal.[21]

Animals

Healthy adult albino Wistar rats of either sex weighing 200-230 g were used for the study. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles, at 25 ± 5 ^{0} and 35 – 60% humidity) standard pelletised feed and tap water were provided *ad libitum*. The animals were habituated to laboratory conditions for 48 h prior to the experimental protocol to minimize any nonspecific stress. The Institutional Animal Ethics Committee of H.K.E.S`s College of pharmacy, Gulbarga, India, approved the experimental protocol in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) with registration no. 142/1999 CPCSEA 5 TH July 1999.

Hepatoprotective activity

According to earlier reports the dose of *Vigna mungo* seed extract 500mg/kg body weight p.o. was used as hepatoprotective in the present study same dose was used.[18]

The hepatoprotective activity study was carried out as described by Agrawal *et al*.[22] Albino rats of either sex (200-230 g) were selected and divided in to four groups of six animals in each.

Group 1: Normal control; received only vehicle with gum acacia (5 mg/kg, p.o.),

Group 2: Rifampicin control; received rifampicin (1g/kg, p.o.) every 72 h for 10 days.

Group 3: Standard control; received silymarin (25mg/kg, p.o.) after 30m, treated with rifampicin (1g/kg, p.o.) every 72 h.

Group 4: received MEVM (500mg/kg, p.o.) after 30 m. treated with rifampicin (1g/kg, p.o.) every 72 h.

Sleeping time

After completion of 10 days, on 11th day thiopentone sodium (40 mg/kg, i.p.) was injected and the sleeping time was recorded.[22]

After complete recovery, the blood samples were collected from all animals by retro orbital puncture method. Serum was separated by centrifugation at 2500 rpm for 15 m. and analyzed for various biochemical parameters such as serum glutathione pyruvate transaminase (SGPT), oxaloacetate transaminase (SGOT), alkaline phosphatase(SALP), and total bilirubin (SBIT). Immediately after collection of blood the animals were euthanized with an over dosage of ether. The livers were removed, washed in saline and the wet weight and volume was determined then transferred into 10% formalin for its histopathological studies.[23]

Nephroprotective activity

Nephroprotective activity study was carried out as described by Shelke *et al*.[24] Albino rats of either sex (200- 230 g) were selected and divided in to four groups of six animals each.

Group 1: Normal control; received equivalent volumes of vehicle (distilled water).

Group 2: Rifampicin control; received rifampicin (1g/kg, p.o.) every 72 h. for 14 days.

Group 3: Standard control; received cystone tablet (500mg/kg, p.o.) after 30 m treated with rifampicin (1g/kg, p.o.) every 72 h.

Group 4: received MEVM (500mg/kg, p.o.) after 30 m treated with rifampicin (1g/kg,p.o.)

The study was carried out for 2 weeks.[11] Body weight was noted before and after 2 weeks. On 15th day all the animals were anaesthetized by overdosage of ether and sacrificed. The blood samples were collected by cardiac puncture method and kidneys were dissected out immediately and transferred into

10% formalin for its histopathological studies.[25] The blood samples were centrifuged at 2500 rpm for 15 m. and then subjected for the estimation of biochemical parameters such as blood urea nitrogen, serum creatinine, and serum uric acid.

Histopathological studies

A histopathological study of both livers and kidneys was performed in histopathology laboratory by consultant pathologist.

Statistical analysis

The data obtained in the experiment were expressed in terms of mean ± SEM. Statistical significance of data was assessed by one way analysis of variance (ANOVA) followed by a comparison between different groups using "Tukey-Kramer" multiple comparison test. A value of $P<0.05$ was considered to be statistically significant. The rifampicin control group was compared with the normal control group and all other treatment groups were compared with the rifampicin control group.

RESULTS

Preliminary phytochemical studies revealed the presence of total phenolic compounds, tannins, saponins, flavonoids, carbohydrates, proteins, amino acids and phytic acid.

Parameters assessed for liver functions

Rats treated with rifampicin (group 2) showed significantly ($P < 0.001$) increased liver weight and volume as compared to normal control. Rats pretreated with silymarin and MEVM showed significant ($P < 0.001$) decrease in wet liver weight and volume compared to rifampicin control group [Table 1]. In thiopentone induced sleeping time studies, MEVM also increased onset time (in s) and decreased duration (in minutes) of sleeping time as compared to rifampicin control [Table 1].

Rifampicin administration resulted in significant ($P < 0.001$) elevation of SGPT, SGOT, ALP, and BIT levels compared to normal control group. Pretreatment with silymarin and MEVM significantly ($P < 0.001$) prevented the biochemical changes induced by rifampicin. The hepatoprotective effect offered by MEVM was found to be significantly greater than silymarin treatment [Table 2].

Hepatocytes of the normal control group showed a normal histology of the liver. In the rifampicin treated group the liver showed loss of lobular architecture, extensive central vein dilation, focal hepatocytes drop out, focal necrosis and extensive portal traid inflammation. Silymarin-and MEVM-pretreated groups showed mild central vein dilation, architecture of the liver was maintained, and hepatocytes showed regeneration. (Fig. 1)

Parameters assessed for kidney functions

The body weight of the rats treated with only rifampicin were found to be significantly ($P < 0.001$) reduced as compared to normal control group and the groups treated with cystone and MEVM showed significant elevation in body weight compared to rifampicin control group (Table 3).

Biochemical parameters such as blood urea nitrogen, serum creatinine, and serum uric acid were found to be significantly ($P < 0.001$) increased in the group treated with only

Table 1: Influence of methanolic extract of seeds of *Vigna mungo* (MEVM) on selected physical and functional parameters in rifampicin-induced hepatotoxic rats.

Group	Treatment	Dose	Mean liver weight (g/100g)	Mean liver volume (ml/100g)	Thiopentone induced sleeping time	
					Onset (s)	Duration (m)
1.	Normal control	5mg/kg (Gum acacia)	3.87±0.04	3.90±0.04	172.60±.98	83.84±1.35
2.	RIFcontrol	1g/kg (RIF)	7.38±0.38***	7.41±0.38***	94.40±2.06***	146.60±1.05***
3.	SIL + RIF	25mg/kg + 1g/kg	4.67±0.11***	4.69±0.11***	162.00±0.98***	95.28±0.91***
4.	MEVM + RIF	500mg/kg + 1g/kg	4.44±0.16***	4.56±0.05***	161.17±0.81***	93.09±0.92***

Values are expressed as mean ± SEM; n = 6; ***P<0.001 Rifampicin control Vs Normal control; ***P<0.001 (SIL + RIF) and (MEVM + RIF) Vs Rifampicin control ; RIF-Rifampicin, SIL-Silymarin, MEVM-Methanolic extract of seeds of *Vigna mungo.*

Table 2: Influence of methanolic extract of seeds of *Vigna mungo* (MEVM) 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	5mg/kg	41.12±1.27	62.83±1.90	143.07±1.01	0.84±0.10
2.	RIFcontrol	1g/kg	163.78±3.94***	363.38±5.74***	617.58±9.98***	3.17±0.21***
3.	SIL + RIF	25mg/kg + 1g/kg	53.67±1.89***	74.90±1.25***	188.28±5.26***	0.95±0.05***
4.	MEVM + RIF	500mg/kg + 1g/kg	51.58±0.67***	73.33±0.47***	187.33±5.24***	0.86±0.04***

Values are expressed as mean ± SEM; n = 6; ***P<0.001 Rifampicin control Vs Normal control; ***P<0.001 (SIL + RIF) and (MEVM + RIF) Vs Rifampicin control ; RIF-Rifampicin, SIL-Silymarin, MEVM-Methanolic extract of seeds of *Vigna mungo.*

rifampicin as compared to the normal control group, whereas groups treated with cystone and MEVM showed significantly ($P < 0.001$) low when compared with the rifampicin control group [Table 3].

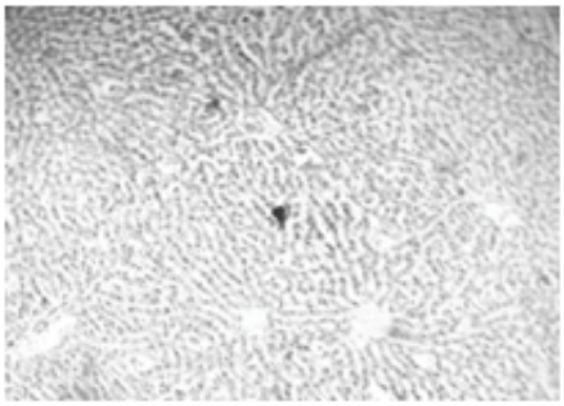
Normal control group showed normal histology of rat kidney, whereas rifampicin control group showed cortical glomerular, peritubular blood vessels congestion and interstitial inflammation. Groups treated with cystone and MEVM was found to reduce such changes in kidney histology induced by rifampicin (Fig. 2).

DISCUSSION

Hepatoprotective activity

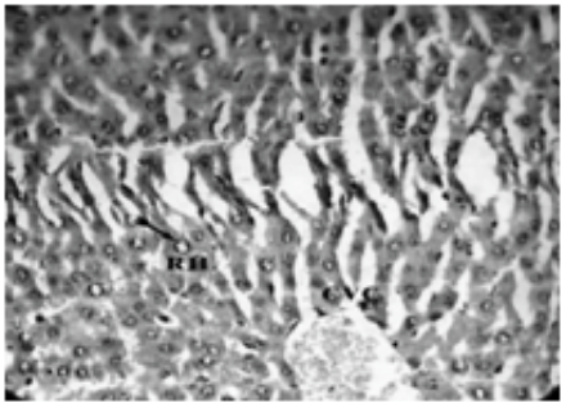
The liver gets injured by many chemicals and drugs. In the present study rifampicin was selected as a hepatotoxicant to induce liver damage. Rifampicin is one of the most common drugs used in the treatment of tuberculosis however an

Fig. 1: Effect of methanolic extract of seeds of *Vigna mungo* (MEVM) on histopathological examination of rat liver in rifampicin-induced hepatotoxicity, Photographs showing histopathology of liver sections (5-10µ), 40X, Haematoxylin-eosin stained.

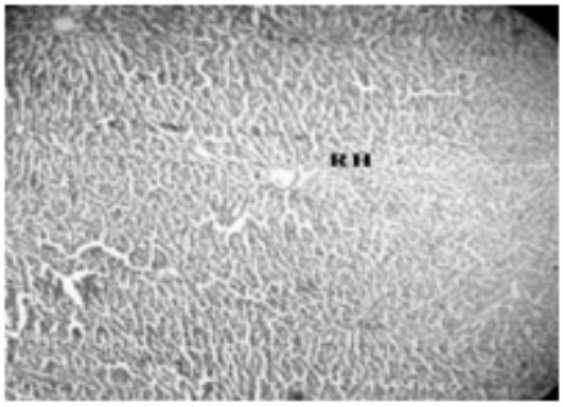


a. Group 1 (Normal control): Showing normal histology of rat liver.

b. Group 2 (Rifampicin control): N-Focal necrosis, PTI-Extensive portal triad inflammation, CVC-Central vein congestion.



c. Group 3 (Standard): CVC-Central vein congestion, RH-Regenerating hepatocytes.



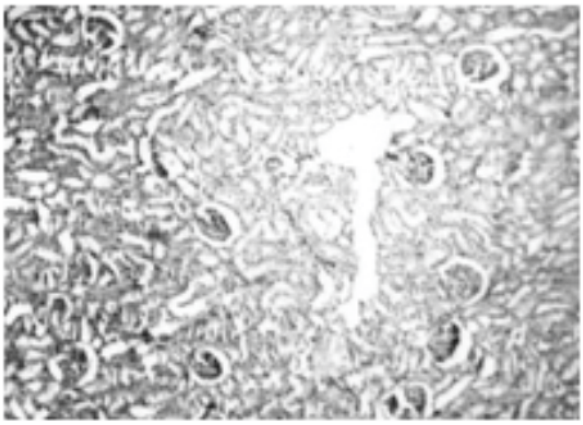
d. Group 4 (MEVM): RH-Regenerating hepatocytes

Table 3: Influence of methanolic extract of seeds of *Vigna mungo* (MEVM) on selected physical and serum biochemical parameters in rifampicin-induced nephrotoxic rats.

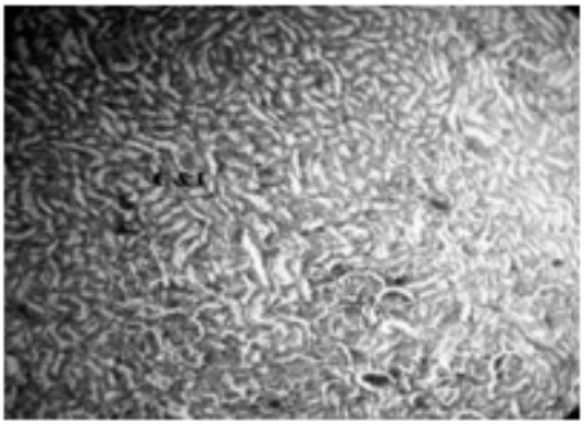
Group	Treatment	Dose	Physical Parameter	Biochemical parameters		
			Body weight Change (g)	Blood urea nitrogen (mg/ dl)	Serum creatinine (mg/ dl)	Serum uric acid (mg/ dl)
1.	Normal control	Eq. Volumes	10.17±0.40	21.95±1.11	0.53±0.04	2.43±0.14
2.	RIF control	1g/kg	-19.67±0.71***	38.88±0.69***	1.91±0.07***	6.54±0.30***
3.	CYS + RIF	500mg/kg + 1g/kg	4.67±1.89***	22.26±0.61***	0.58±0.03***	2.51±0.14***
4.	MEVM + RIF	500mg/kg + 1g/kg	5.00±0.51***	22.30±0.61***	0.57±0.03***	2.50±0.1***

Values are expressed as mean ± SEM; n = 6; ***P<0.001 Rifampicin control Vs Normal control; ***P<0.001 (CYS + RIF) and (MEVM + RIF) Vs Rifampicin control; DW-Distilled water, RIF-Rifampicin, CYS-Cystone, MEVM-Methanolic extract of seeds of *Vigna mungo*.

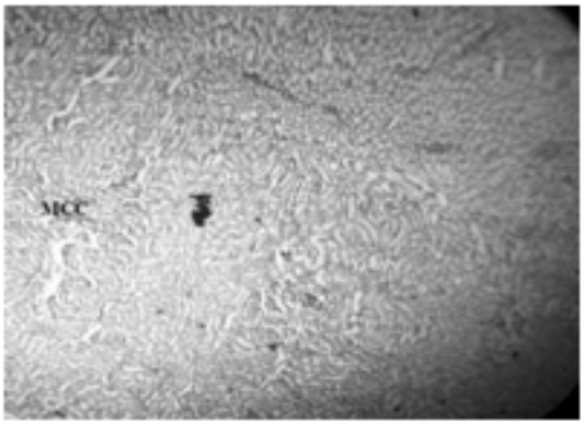
Fig. 2: Effect of methanolic extract of seeds of *Vigna mungo* (MEVM) on histopathological examination of rat kidney in rifampicin-induced nephrototoxicity, Photographs showing histopathology of kidney sections (5-10µ), 40X, Haematoxylin-eosin stained.



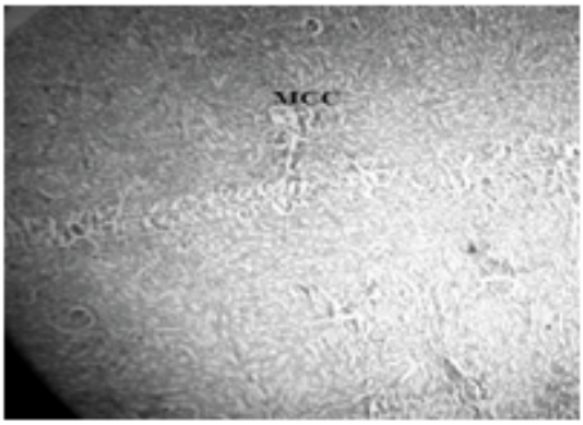
a. Group 1 (Normal control): Showing normal histology of rat kidney.



b. Group 2 (Rifampicin control): C&I-Congestion and inflammation.



c. Group 3 (Standard): MCC-Mild cortical congestion



d. Group 4 (MEVM): MCC-Mild cortical congestion.

overdose can induce hepatotoxicity in experimental animals and humans. Excessive administration of rifampicin can induce enzymes CYP3A4 and CYP3A7 mRNAs in adult`s human hepatocytes in culture.[26] Rifampicin strongly induces cytochrome P-450 3A-dependant enzyme and UDP-glucosyltransferase activities in rat liver microsomes.[27] The hepatic injury leads to elevation of serum levels of SGPT (ALT), SGOT (AST), ALP, and BIT in rats and are used as markers for assessing toxicant effect and also hepatoprotective agents. During hepatic damage these enzymes present in the liver cells leak in to the serum, resulting in increased concentrations.[28] Rifampicin also alters the metabolic activity of hepatocytes.[29] *Vigna mungo* (Linn.) Hepper was reported to be used in a variety of disease conditions of liver in Indian traditional system of medicine.[30]

In the present study rifampicin administration for 10 days resulted in morphological changes such as enlargement of liver, scratches, dark brown coloration and increased volume. Barbiturates are a class of xenobiotic that are extensively metabolized in the liver, deranged liver function leads to delay in the clearance of barbiturates, resulting in a longer duration of hypnotic effect.[31] In the present study, administration of thiopentone sodium to rats treated with rifampicin resulted in an increased duration of thiopentone-induced sleeping time. Whereas the MEVM pretreated animals showed similar morphology of livers compared to that of normal control animals that were healthy in appearance and significantly decreased volume and also decreased thiopentone-induced sleeping time, an indirect evidence of their hepatoprotective effect.

Rifampicin administration also produces significantly increased serum marker enzymes such as SGPT, SGOT, ALP and BIT in rifampicin control group compared to normal control group. Treatment with MEVM significantly decreased the enzymes SGPT, SGOT, ALP and BIT levels as compared to rifampicin control group. All the above parameters indicating their hepatoprotective effect against rifampicin-induced liver cell damage.

Histological changes such as loss of lobular architecture, extensive central vein dilation, focal hepatocyte drop out, focal necrosis and extensive portal traid inflammation were observed in rifampicin control group. MEVM pretreated animals had significantly prevented these histological changes, further indicating their hepatoprotective effect. All the histological changes observed were in correlation with the physical, biochemical and functional parameters of the liver.

Nephroprotective activity

Various environmental toxicants and clinically useful drugs, like acetaminophen and gentamicin, can cause severe organ toxicities through the metabolic activation to highly reactive free radicals including the superoxides and oxygen reactive species.[32] A relationship between oxidative stress and nephrotoxicity has been well demonstrated in many experimental animal models. In recent times, there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing tissue injuries caused by free radicals.[33] It has been proved that *pedalium murex* has a diuretic and antioxidant activity and also possesses a nephroprotective activity against cisplatin induced nephrotoxicity.[24] Phenolic compounds function as high-level antioxidants because they possess the ability to absorb and neutralize free radicals as well as quench reactive oxygen species. Plant flavonoids which show an antioxidant activity *in vitro* also function as antioxidants *in vivo.* Again, a strong relationship between the total phenolic content and antioxidant activity in fruits, vegetables, grain products and plant subjects of ethnopharmacological treatments has also been reported.[34] Flavonoids, tannins and saponins have been reported to exert profound *in vitro* and *in vivo* stabilizing effect on the lysosomes in experimental animals.[35] Tannins and saponins stabilize the erythrocyte membrane by binding cations and other biomolecules.[36] It has been reported that *Vigna mungo* seeds possess an antioxidant activity[19] and diuretic activity of saponins in *Vigna* species has also been reported.[37]

In the present study rifampicin administration for 14 days significantly decreased body weight however increased serum enzymes such as blood urea nitrogen, serum creatinine and serum uric acid whereas MEVM pretreated animals significantly prevented these changes indicating their nephroprotective effect against rifampicin-induced kidney damage.

Histological changes such as cortical glomerular, peritubular blood vessel congestion and interstitial inflammation were observed in rifampicin control group. The MEVM pretreated animals had significantly prevented these histological changes, further indicating their nephroprotective activity. All the histological changes observed were in correlation with the physical and biological parameters of the kidney.

The nephroprotective activity observed was may be due to the presence of strong antioxidants such as total phenolic compounds, phytic acid, tannins, and flavonoids. And the diuretic activity can be due to the presence of saponins. There by it protects the kidney from harmful oxidative reactions and accumulation of sodium, potassium and other metabolites which may cause severe kidney damage.

It can be concluded from the above results that methanolic extract of seeds of *Vigna mungo* (Linn.) Hepper possess hepatoprotective as well as nephroprotective activity against rifampicin-induced hepatotoxicity and nephrotoxicity respectively.

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