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Research Article

PHYTOCHEMICAL INVESTIGATION AND ANTI LITHIATIC ACTIVITY OF *ABELMOSCHUS MOSCHATUS* MEDIKUS

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ABSTRACT

Aim: This study investigated the protective effect of the hydro-alcoholic extract of *Abelmoschus moschatus medikus* (HAEAM) against Ethylene glycol induced urolithiasis and its possible underlying mechanisms using male Wistar albino rats.

Materials and methods: Urolithiasis is induced by the administration of 0.75% of ethylene glycol in drinking water for four weeks, and was manifested by high urinary calcium, phosphate, oxalate and low urinary magnesium content.

Results: Simultaneous administration of hydro alcoholic extract of *Abelmoschus moschatus medikus* (HAEAM 200mg/kg and 400mg/kg body weight) orally for 28 days along with ethylene glycol (0.75%) the urinary calcium, oxalate, phosphate level were decreased and increased urinary magnesium level. It also increased the urinary volume there by reducing the tendency for crystallization. The histopathological studies confirmed the induction of lithiasis as micro crystal deposition was observed in the sections of kidney from animals treated with ethylene glycol. The mechanism underlying this effect is mediated possibly through an antioxidant, nephroprotection and its effect on the urinary concentration of stone-forming constituents and risk factors.

Conclusion: These observations enable us to conclude that hydro alcoholic extract of the *Abelmoschus moschatus medikus* is effective against ethylene glycol induced nephrolithiasis.

Keywords*:* Ethylene glycol, Hyperoxaluria, *Abelmoschus moschatus medikus,* Nephrolithiasis, Renal calculi.

INTRODUCTION

Herbs are used as medicine since time immemorial. Many of the natural products in plants of medicinal value offer us new sources of drugs which have been used effectively in traditional medicine. There is an increased consciousness regionally and globally in production and use of plants with healing property. *Abelmoschus moschatus Medikus* (Malvaceae) [1,2,3],[4]is a prostate herb rooting at nodes, internodes elongate creeping, flowering and fruiting time is September and January. Propagation by seeds. Renal lithiasis defined as the consequence of an alteration of the normal crystallization condictions of urine in the urinary tract.[5]Urine composition factors are important in crystal formation as urine is a metastable liquid containing several coexisting substance that can crystallize to generate renal calculi. These substances are present at super saturation levels. The ease of crystallization depends on the degree of super saturation, the presence of performed particles (heterogeneous nucleants that act as promoter substances) and the level of crystallization inhibitors (inhibits crystal nucleation and growth)[6]. Some of the substances found in the urine are able to crystallize and in a concentrated form these chemicals can precipitate into a solid deposit attach to the kidney walls. These crystals can grow through a process of accretion to form a kidney stone.[7] Nephrolithiasis has afflicted mankind since antiquity and can persist, with serious medical consequences, throughout patient's life time, with a recurrence rate of 70-80% in males and 47-60%in females. The present day medical management of nephrolithiasis is either costly or not without side effects. Hence the search for antilithiatic drugs from natural sources has assumed greater importance. Many Indian plants have been quoted to be useful as anti lithiatic agents. They are effective with fewer side effects and are also inexpensive. In the present study, an effort has been made to establish the scientific validity for the anti urolithiatic property of hydro alcoholic extract of *Abelmoschus moschatus* using ethylene glycol induced hyperoxaluria model in rats. *Abelmoschus moschatus* is used in treating spasms of digestive tract, muscle cramps, poor circulation and aching joints. The seeds are much more valuable medicinally due to their diuretic, demulcent, stomachic, stimulant, cooling, tonic, carminative, aphrodisiac, and antiseptic properties. When seed are chewed they act as nerve tonic, stomachic and sweeten the breath.

MATERIALS AND METHODS

Plant material

Abelmoschus moschatus was collected from the Alagar hills Madurai, India and identified by the department of botany, The Americian college (Madurai,India).A vocher specimen was deposited in the KMCP herbarium under the number KMCP/AM/68 for further reference.

Preparation of the extract

The whole plants were dried in the shade. Then the shade dried plants were pulverized to get coarse powder sieved under mesh no 40. About 500gm of whole plant powder was extracted with petroleum ether at 70-80^{0}C by continuous hot percolation using for soxhlet apparatus for 48 hours. The extraction was continued 72 hrs with alcohol and distilled water. Both extract were filtered and concentrated to dry mass by using vacuum distillation. Dark greenish residue was obtained. The residue was subjected to phytochemical evaluation and pharmacological screening.

Pharmacological screening of antilithiatic activity

Animals

Male albino wistar rats (180-200gm) were obtained from chellamuthu Trust (Madurai, India). They were housed in well ventilated cages (3 to4 per cage) maintained at 25±2^{0}C under 12hour dark/light cycle. They were fed with standard pellet diet and had free accesses to water. The animals were maintained in these conditions for 1 week before the experimental session. Our institutional animal ethical committee (IACE) approved this study.

Ethylene glycol induced Antilithiatic activity

The acclimatized animals were divided into 2 main groups one is prophylactic group and the other curative group each containing 4 sub groups of each 6 animals.

Prophylactic group

Group 1: Served as normal control and receive normal diet.

Group 2: Served as lithiatic control and received 0.75% ethylene glycol in drinking water for 28 days and normal diet.

Group3: Received 1% ethylene glycol in drinking water along with HAEAM 200mg/kg for 28days.

Group 4: Received 1% ethylene glycol in drinking water along with HAEAM 400mg/kg for 28 days.

Curative group

Group 1: Served as curative control and receive normal diet.

Group 2: Served as lithiatic control and received 1% ethylene glycol in drinking water for 28 days and normal diet.

Group3: Received 1% ethylene glycol in drinking water for 28days then treated with HAEAM 200mg/kg for next 15 days.

Group 4: Received 1% ethylene glycol in drinking water for 28 days then treated with HAEAM 400mg/kg for next 15 days.

Assessment of antilithiatic activity

Urine sampling

For prophylactic treatment on the 24th hour urine samples were collected from rats, housed in metabolic cages, on the 14th day and 28th day urine volume noted. Urinary and serum parameters like calcium, Phosphate, Magnesium, Oxalate, Protein, Uric acid and creatinine concentration were estimated using standard methods.

For curative treatment on the 24th hour urine samples were collected from rats, housed in metabolic cages, on the 28th day urine volume noted. Urinary and serum parameters like calcium, Phosphate, Magnesium, Oxalate, Protein, Uric acid and creatinine concentration were estimated using standard methods.

Blood sampling

All rats were anaesthetized with diethyl ether; blood was collected from retro orbital puncture of the animal. Then centrifuged for 10 minutes at 3000 r.p.m. to separate the serum. The serum of each animal of all the groups was estimated for calcium, magnesium, oxalate, Phosphate creatinine and uric using their respective kits in the laboratory.

Histopathological studies

The kidney was carefully removed, washed in ice cold 0.15M Kcl. The kidney was fixed in formaldehyde (10%) for H.E (Hematoxylin Eosin) stain. The crystal deposit was visually examined under light microscope.

RESULT

In the present study chronic administration of 0.75% ethylene glycol aqueous solution to male wistar rats resulted in hyperoxaluria. The preliminary phytochemical analysis [8,9] were carried out to find out the phytoconstituents present in the crude extracts Table 1.

Table 1: Phytochemical screening of *Abelmoschus moschatus Medikus*

Extract	Pet.Ether	Chloroform	Ethanol
Sterols	+	+	+
Terpenoids	_	_	_
Carbohydrate	_	+	+
Flavanoids	_	+	_
Proteins	_	_	+
Alkaloids	_	_	_
Glycosides	_	_	_
Tannins	_	_	+
Saponins	_	_	_
Phenolic Compounds	_	_	+
Fixed oil & fats	+	+	+

(+) Presence of Constituents; (-) Absence of Constituents

Urinary concentration of various ions was investigated for prophylactic treatment at the 14th day and 28th day of the study varied drastically, following ethylene glycol treatment. The values of urinary protein, calcium and uric acid at 14th day were 64.82±3.76mg/dl, 5.66±0.58mg/dl, 3.26±0.76mg/dl respectively. On the 28th day the values of urinary protein, calcium and uric acid was 72.95±3.95mg/dl, 6.75±0.72 and 3.55±0.60mg/dl respectively in G1 rats. These values increased significantly in the animals treated with ethylene glycol in G 2. The values of urinary protein, calcium and uric acid at 14th day was 140.58±8.38mg/dl 20.36±1.78mg/dl and 14.62±1.56 respectively and the 28th day values are 160.45±8.34mg/dl, 19.03±1.55mg/dl, 13.60±1.38mg/dl ($p<0.001$) respectively. The animals when treated with HAEAM 200mg/kg and 400mg/kg body weight respectively in G3 and G4 respectively, the values are restored to near normal values as shown in table 2 and 3.

Table 2: Effect of haeam on urinary biochemical parameters on the day 14

GP	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
G1	64.82±3.76	4.23±0.56	5.66±0.58	3.26±0.76	0.80±0.10	14.82±1.80	32.75±2.25
G2	140.58±8.38**(a)	0.98 ±0.27**(a)	20.36±1.78**(a)	14.62 ±1.56**(a)	1.65 ±0.18**(a)	33.45±3.40**(a)	74.68 ±4.32**(a)
G3	82.30±6.30**(b)	2.80 ±0.55**(b)	7.78 ±0.62**(b)	5.15 ±0.91**(b)	0.92 ±0.08**(b)	20.10±1.85**(b)	36.26 ±2.50**(b)
G4	74.35±5.23**(b)	3.10±0.65**(b)	6.57±0.45**(b)	4.10±0.65**(b)	0.87±0.07**(b)	17.56±1.47**(b)	33.34±2.36**(b)

Values are expressed as Mean± SEM

Values were find out by using ONE WAY ANOVA Followed by Newman keul's multiple range test

**(a)values were significantly different from normal control G1 at P< 0.01

**(b) values were significantly different from Lithiatic control G2 at P<0.01

Table 3: Effect of haeam on urinary biochemical parameters on the day 28

Group	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
G1	72.95±3.95	4.55±0.62	6.75±0.72	3.55±0.60	0.90±0.10	16.30±1.55	34.56±2.35
G2	160.45±8.34**(a)	1.66±0.34**(a)	19.03±1.55**(a)	13.60±1.38**(a)	1.82±0.26**(a)	48.22±4.52**(a)	76.60±4.25**(a)
G3	86.63±3.65**(b)	2.95±0.35**(b)	9.52±0.45**(b)	7.92±0.74**(b)	0.95±0.09**(b)	24.05±2.65**(b)	40.25±2.75**(b)
G4	76.89±2.89**(b)	3.25±0.67**(b)	7.58±0.63**(b)	5.10±0.87**(b)	0.88±0.07**(b)	19.32±1.98**(b)	35.62±2.32**(b)

Values are expressed as Mean± SEM

Values were find out by using ONE WAY ANOVA Followed by Newman keul's multiple range test

**(a)values were significantly different from normal control G1 at P< 0.01

**(b) values were significantly different from Lithiatic control G2 at P<0.01

The values of urinary creatinine, oxalate, phosphate increased significantly in the animals treated with ethylene glycol in G 2 when compared with the normal control animals in G1 in the prophylactic study. The values at 14th day was 1.65±0.18mg/dl, 33.45±3.40mg/dl and 74.68±4.32 respectively and the 28th day values are 1.82±0.26mg/dl, 48.22±4.52mg/dl, 76.60±4.25mg/dl (p<0.001) respectively. The animals when treated with HAEAM 200mg/kg and 400mg/kg body weight respectively in G3 and G4 respectively, the values are restored to near normal values as shown in table 2 and 3.

The values of urinary magnesium in the prophylactic study at 14th day were 4.23±0.56mg/dl, on the 28th day the value was 4.55±0.62mg/dl, in G1 rats. These values decreased significantly in the animals treated with ethylene glycol in G 2. The value urinary magnesium at 14th day was 0.98±0.27mg/dl and the 28th day value was 1.66±0.34mg/dl, (p<0.001). The animals when treated with HAEAM 200mg/kg and 400mg/kg body weight respectively in G3 and G4 respectively, the values are restored to near normal values as shown in table 2 and 3.

In the prophylactic group the serum uric acid, creatinine, phosphate, calcium, and oxalate were increased in the ethylene glycol alone treated rats and the values were regained to the near normal values in HAEAM treated rats as shown in the table 4.

The serum magnesium level is decreased in the ethylene glycol alone treated rats and the values were regained to the near normal values in HAEAM treated rats as shown in the table 4. In the curative group the urine and serum parameters like uric acid, creatinine, phosphate, calcium, and oxalate were increased in the ethylene glycol alone treated rats and the values were regained to the near normal values in HAEAM treated rats as shown in the table 5 and 6.

Table 4: Effect of haeam on serum parameters in prophylactic treatment of animals

GP	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
G1	4.85±0.70	9.45±1.33	3.55±0.42	0.62±0.08	6.8±0.65	13.22±1.50
G2	1.30±0.40**(a)	18.22±2.50**(a)	9.9±1.22**(a)	1.08±0.22**(a)	13.55±1.82**(a)	28.15±3.25**(a)
G3	*3.42±0.42**(b)*	*12.26±1.42**(b)*	*4.40±0.55**(b)*	*0.82±0.10**(b)*	*8.05±0.65**(b)*	21.05±2.12**(b)
G4	4.10±0.54**(b)	10.74±1.34**(b)	3.90±0.54**(b)	0.68±0.08**(b)	7.23±0.12**(b)	16.29±0.87**(b)

Values are expressed as Mean± SEM

Values were find out by using ONE WAY ANOVA Followed by Newman keul's multiple range test

**(a)values were significantly different from normal control G1 at P< 0.01

**(b) values were significantly different from Lithiatic control G2 at P<0.01

Table 5: Effect of haeam on urinary biochemical parameters in curative treatment of animals

GP	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
G1	73.45±3.84	4.66±0.72	6.40±0.65	3.80±0.78	14.95±2.15	19.21±1.76	35.05±2.90
G2	170.83±7.97**(a)	1.51±0.22**(a)	17.96±2.11**(a)	15.00±2.52**(a)	81.88±4.14**(a)	49.98±3.45**(a)	80.28±4.75**(a)
G3	90.66±3.82**(b)	3.50±0.45**(b)	10.28±1.05**(b)	8.6±1.08**(b)	32.93±3.30**(b)	26.05±2.25**(b)	41.33±2.83**(b)
G4	80.34±2.98**(b)	4.03±0.38**(b)	7.87±0.88**(b)	5.10±0.56**(b)	25.63±2.67**(b)	22.95±2.54**(b)	38.43±2.03**(b)

Values are expressed as Mean± SEM

Values were find out by using ONE WAY ANOVA Followed by Newman keul's multiple range test

**(a)values were significantly different from normal control G1 at P< 0.01

**(b) values were significantly different from Lithiatic control G2 at P<0.01

Table 6: Effect of haeam on serum parameters in curative treatment of animals

GP	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
G1	5.16±0.92	10.38±1.29	4.12±0.89	0.98±0.19	8.8±0.96	15.62±2.01
G2	1.92±0.48**(a)	24.06±2.42**(a)	12.06±2.38**(a)	2.82±0.62**(a)	17.46±1.20**(a)	29.90±3.06**(a)
G3	4.05±0.79**(b)	13.36±1.10**(b)	6.65±0.95**(b)	1.39±0.45**(b)	10.53±1.10**(b)	17.03±2.42**(b)
G4	4.76±0.83**(b)	11.89±1.34**(b)	5.43±0.90**(b)	1.10±0.22**(b)	9.78±1.08**(b)	16.48±2.10**(b)

Values are expressed as Mean± SEM

Values were find out by using ONE WAY ANOVA Followed by Newman keul's multiple range test

**(a)values were significantly different from normal control G1 at P< 0.01

**(b) values were significantly different from Lithiatic control G2 at P<0.01

The urine and serum magnesium levels were decreased in the ethylene glycol alone treated rats and the values were regained to the near normal values in HAEAM treated rats as shown in the table 5 and 6.

Histopathological Studies

In stone induced models, the following changes were noted.

1. Damaged epithelial cells at the inner layer of the tubules.
2. Dilatation of the tubules
3. Presence of crystals in the tubules

Histopathological studies of kidney of rats

Scores were given according to the severity of changes in the tubules. Sections of kidney from animals treated with ethylene glycol (GP-2) in Figure: 2.

Showed large quantity of microcrystal deposition and severe dilation of most tubules and mass tubulointerstitial inflammatory infiltration with lesion area > 40% (score3). However, kidney sections of animals treated with hydro alcoholic extracts of *Abelmoschus moschatus both doses* shows obvious dilation of many

tubules and tubulointerstitial inflammatory infiltration with lesion area < 40% (score 2) in figure: 3-4 and curative study also showed improvement in the above symptoms and reduced crystal deposition.(score 1) in figure:5,6,7&8.

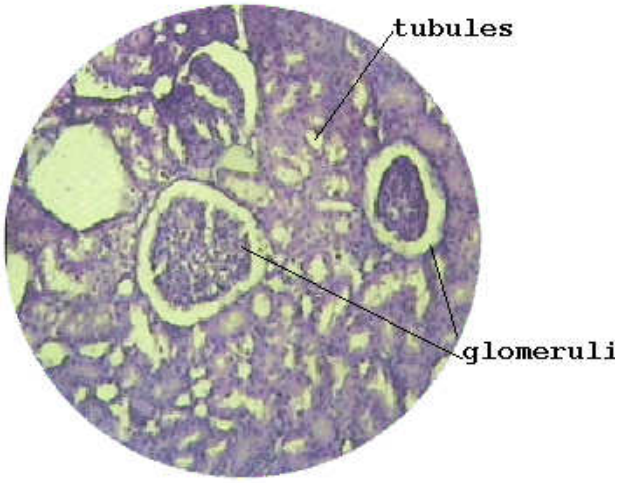

Fig. 1: Kidney Section of GP-1(Normal Control) Rats

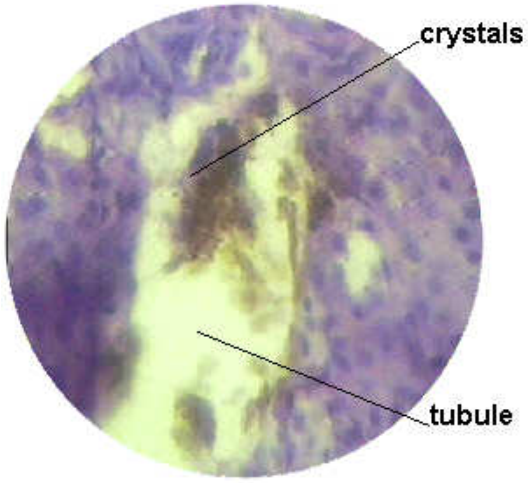

Fig. 2: Kidney section of GP-2 (Lithiatic control)

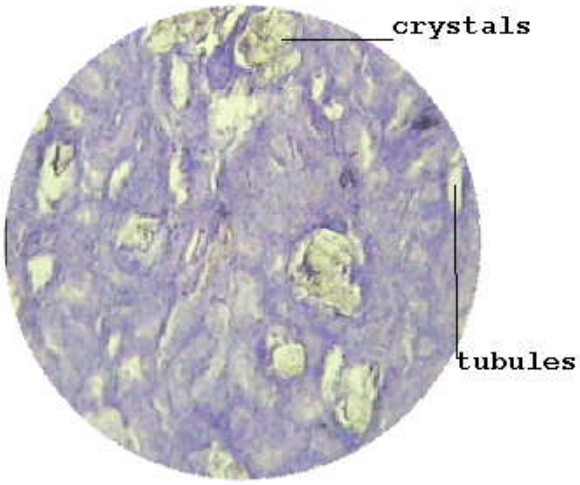

Fig. 3: Kidney section of GP- 3 (Prophylatic treatment, HAEAM 200mg/kg)

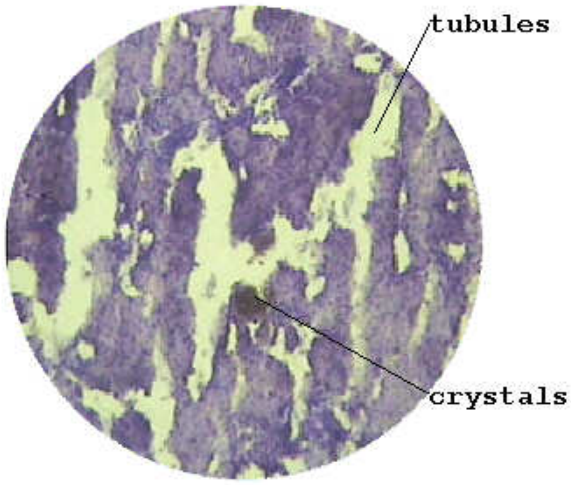

Fig. 4: Kidney section of GP- 4 (Prophylatic treatment, HAEAM 400mg/kg)

Curative Treatment

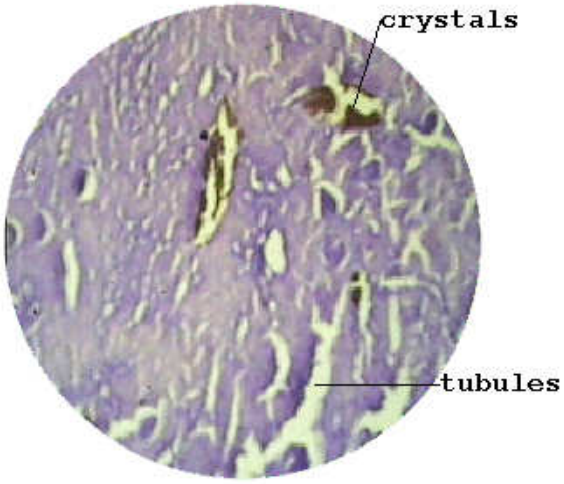

Fig. 5: Kidney section of GP- 5 (Curative treatment, Normal control)

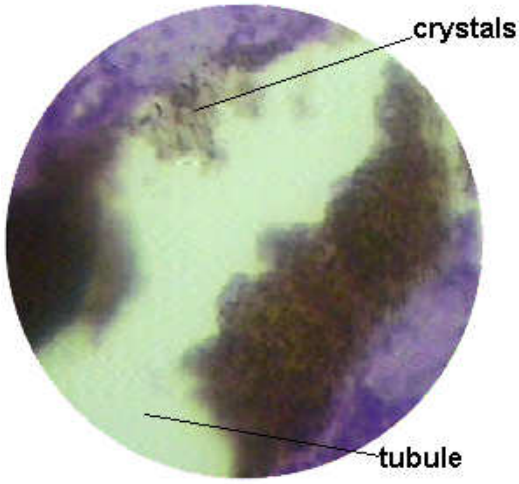

Fig. 6: Kidney section of GP- 6 (curative treatment, Lithiatic control)

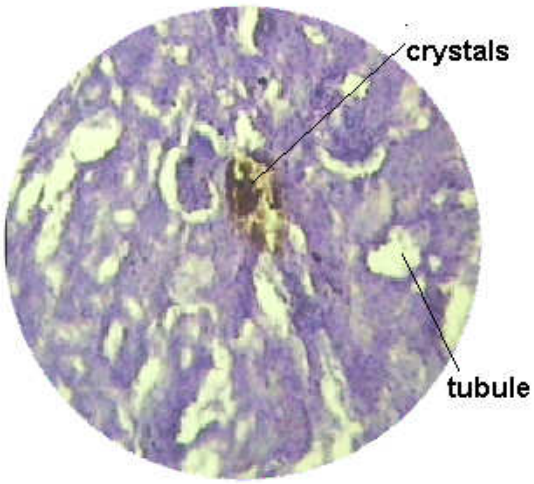

Fig. 7: Kidney section of GP- 7 (curative treatment, HAEAM 200mg/kg)

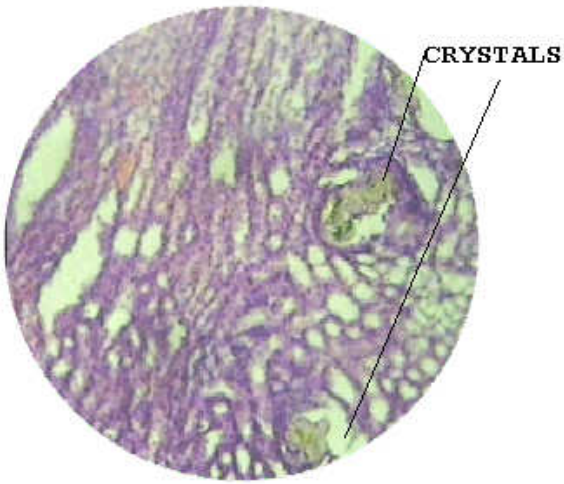

Fig. 8: Kidney section of GP- 8(Curative treatment, HAEAM 400mg/kg)

DISCUSSION

In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans and also earlier studies have shown that the amount of stone deposition in female rats was significantly less. Urinary super saturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidence in previous studies indicated that in response to 14 day period of ethylene glycol (0.75%, v/v) administration, young male albino rats form renal calculi composed mainly of calcium oxalate [10,11].The biochemical mechanisms for this process are related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate. Similar results have been obtained when rats were treated with ethylene glycol and ammonium oxalate [12,13]. In the present study, oxalate and calcium excretion are progressively increased in calculi-induced animals (Group II). Since it is accepted that hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria [14] the changes in urinary oxalate levels are relatively much more important than those of calcium [15]. Increased urinary calcium is a factor favoring the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine and subsequent crystal growth. [16] However, HAEAM lower the levels of oxalate as well as calcium excretion.

An increase in urinary phosphate is observed in calculi induced rats (Group II). Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition [17]. Treatment of HAEAM restores phosphate level, thus reducing the risk of stone formation. In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid get accumulated in blood [18]. Also, increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi-producing diet [19,20]. In this context, oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in cell membrane [21]. In calculi-induced rats (Group II), marked renal damage was seen as indicated by the elevated serum levels of creatinine and uric acid. However, the curative and prophylactic treatment with HAEAM causes diuresis and hastens the process of dissolving the preformed stones and prevention of new stone formation in urinary system.

CONCLUSION

In conclusion, the presented data indicate that administration of the HAEAM to rats with ethylene glycol induced lithiasis, reduced and prevented the growth of urinary stones, supporting folk information regarding anti urolithiatic activity of the plant. The mechanism underlying this effect is still unknown, but is apparently related to increased diuresis and lowering of urinary concentrations of stone forming constituents. These effects could conclude the anti urolithiatic property of ***Abelmoschus moschatus*.**

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