



Effect of *Macrotyloma uniflorum* seeds in ethylene glycol induced urolithiasis in rats

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Abstract : *Macrotyloma uniflorum* Linn. (Fabaceae) seeds are widely used for their diuretic and urolithiatic effects in India. The present study investigated the effect of aqueous extract of *Macrotyloma uniflorum* seeds (AEMU) on ethylene glycol induced urolithiasis in rats. To induce urolithiasis, 0.75% v/v ethylene glycol was administered orally for 14 days. The curative doses of 400 and 800 mg/kg were administered from 15th to 28th day. On 28th day, 24 hr urine, serum was collected and various biochemical parameters were estimated in urine, serum and kidney homogenate along with histology of kidney. Co-administration of AEMU with ethylene glycol has significantly ($p < 0.001$) increased the urine volume and the level of calculus inhibitors like magnesium, citrate and decreased the level of calculus promoters like calcium, oxalate, uric acid and urea also decreased in crystalluria in urine. AEMU supplement also prevented the pathological changes in kidney and increased the glomerulus activity of the kidney. These results indicate that AEMU showed significant activity in urolithiasis which might be due to its diuretic, calcium oxalate crystal formation inhibitory effects and its ability to increase the levels of inhibitors and decrease the level of promoters of urolithiasis.

Keywords : *Macrotyloma uniflorum*, Urolithiasis, Ethylene glycol, Diuresis, Kidney stone.

Introduction

Urolithiasis commonly known as kidney stone or renal stone and causes a major impact on public health in last two decades. There are various types of calcareous (Calcium oxalate monohydrate and calcium oxalate dehydrate, apatite) and noncalcareous (uric acid, struvite, cystine, uric acid, and others) stones, among which over 80% cases of calcium oxalate, where 5-10 % cases of uric acid stone are found in population. As per the survey of National Health and Nutrition Examination, 7.1 % of women and 10.6 % of men were affected by renal stone disease¹. In India, approximate 12 % population is suffered with renal stone every year with the high incidence states like Gujarat, Rajasthan, Punjab, Maharashtra, Delhi and Haryana². Renal stone formation is a

highly unpredictable with complex etiology. Various endogenous and exogenous factors and multivariate pathogenesis are involved in renal stone formation. Some endogenous factors like improper metabolism of calcium, oxalic acid, phosphorus and uric acid. Nitrogenous waste products like urea also contribute in renal stone formation. Peoples food habits, dehydration, hot climate, hard water usage involves in exogenous factors³. Supersaturation of urine with component like calcium, oxalate, phosphate which initiate renal stone formation which is followed by nucleation, crystal growth and crystal aggregation process. Many stone inhibitors are available in urine like magnesium, citrate which make the soluble complex with calcium ions and reduce the supersaturation level of calcium oxalate ions, but its inhibition capacity varies person to person⁴. Treatment of renal stone are depends on stone size and location. Many therapy like diuretics, probiotics, citrate, chelating agents given but they have own pharmacological limitation, side effect on long use and not remove stone. So, in majority cases renal stone are removed by surgical treatment like ESWL, ureteroscopy and percutaneous nephrolithotomy, but unfortunately stone recurrence rate was observed in about 50% cases after removal of stone by surgical treatment⁵. Surgical treatment causes side effect such as hypertension, tubular necrosis, hemorrhage and fibrosis of the kidney⁶. So in renal stone treatment needs preventive as well as curative therapy for better relief. But, there are not any proper effective drugs in current therapy which completely remove the stones. In Ayurveda, it has been mentioned that many herbal plants have been used in treatment of urolithiasis. Herbal plants have complex spectrum of action, like anti-oxidant, diuretic, antimicrobial, anti-inflammatory, analgesic, antispasmodic properties, litholytic and anti-calcifying activities without any side effects⁷.

Macrotyloma uniflorum [Lam] verde. commonly known as Horse gram found extensively cultivated in the state of Karnataka, Orissa, Tamil Nadu, Andhra Pradesh, M. P., Bihar, W.B. and in foot hills of Uttaranchal in India. It is also cultivated in other countries like Burma, Sri Lanka, and Australia. It is one of the highly nutritious vegetable pulse crop with ethno-medicinal values in India, which is commonly known as Kulattha (Sanskrit), Gahot (Kumaon and Garhwal), Gahot means which destroys stone in initial stage⁸. According to Ayurveda the seeds are bitter, hot, acrid, and dry. Its decoction is used traditionally in leucorrhoea and menstrual dysfunctions. In Indian traditional medicine, horse gram seeds are used for treatment of urinary stones⁹, piles and urinary diseases, act as astringent, tonic, regulate the abnormal menstrual cycle in women¹⁰. Furthermore, the cooked liquor of the horse gram seeds with spices is considered to be a potential remedy for the common cold, throat infection, fever and the soup said to generate heat¹¹. Seeds of *M. uniflorum* contain proteins, carbohydrates, amino acids, phenolic acids (caffeic acid, 3,4-dihydroxy benzoic acid, p-coumaric acid, vanillic acid, sinapic acid, chlorogenic acid, ferulic acid and syringic acid), lipids, flavonoids (kaempferol, quercetin and myricetin), fatty acids (hexanoic acid and hexadecanoic acid), tannins, phytosterols (stigmasterol and β -sitosterol), saponins, anthocyanidins (cyanidin, petudin, delphinidin and malvidin), and minerals like iron, calcium and molybdenum. Phenolic acids of *M. uniflorum* seeds are considered to be the most potent antioxidants which act by scavenging free radicals and ROS. The principal phenolic compounds of horse gram seed are flavonoids like quercetin, kaempferol, and myricetin, vanillic, *p*-hydroxybenzoic and ferulic acids¹². However, the potential bioactive components and the underlying mechanisms associated with treat urolithiasis are still unknown. The results of recent studies have shown that methanolic extract of the plant could inhibit the formation of calcium oxalate stones *in vivo*, correlating with their antioxidant and other protective effects. Thus, the objective of the present study is to evaluate the methanolic extract of *M. uniflorum* on experimentally induced calcium oxalate crystallization *in vivo* methods.

Materials & Methods

Materials

Analytical grades chemicals were used in experiments. Cystone was purchased from local market of Ahmedabad. Calcium, uric acid, urea, phosphorus, creatinine, uric acid estimation kits were procured from Lab-Care Diagnostics Pvt Ltd. Gujarat, India.

Plant material and preparation of the extract

Dried seeds of *M. uniflorum* were purchased from local market of Ahmedabad, Gujarat, India. Seeds were authenticated by Ethnobotanist, Smt. S. M. Panchal Science College, Talod, Gujarat, India. The specimen was submitted to the Pharmacognosy department of Institute of Pharmacy, Nirma University (Ref No. IPNSAVPMU2015). Dry seeds were grounded into the fine powdered by electric grinder. Powder was stored in

the air-tight container at ambient temperature. Powder of *M. uniflorum* (100 gm) seeds was refluxed with water (500 ml) for 24 hr using soxhlet extraction. The extract was filtered and evaporated at 50° C in vacuum oven, dry extract (8.0 % w/w) was labeled as aqueous extract.

Phytochemical screening and quantitative estimation of phytoconstituents

Phytochemical screening was carried for identify the nature present phyto-constituents of AEMU. Total flavonoid content was measured using colorimetric assay using aluminum chloride method¹³ and results were expressed as milligram of quercetin equivalent per gram of the extract. Total saponins were measured according to the methods described by of Obadoni and Ochuko¹⁴.

Animal study

Adult Male Albino Wistar rats (180-250g) were housed at the animal house of Institute of Pharmacy, Nirma University, under controlled environmental condition (temperature of 22-25°C, relative humidity (55±5%) and 12 hr light-dark cycle) and Animals have received food pellet and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee of Institute of Pharmacy, Nirma University, Gujarat, India. (IP/PCOG/PHD/19/015, dated 28th July 2016)

Acute toxicity study

The acute toxicity study was carried out in wistar male rats as per the guideline by OECD. Zero mortality observed upto extract dose of 6000 mg/kg.

Determination of diuretic activity

The diuretic activity of AEMU was determined by method described by Lipschitz et al.¹⁵ Twenty Four healthy rats (200-250g) were selected and randomly divided into 4 groups of 6 animals each. All experimental animals were fasted for 18 hr prior to the experiment and allocated only for water during the fasting period. Normal and standard groups were given saline (20 ml/kg) and hydrochlorothiazide (10 mg/kg, as standard) respectively, while treated groups received the same ml of saline containing 400 mg/kg & 800 mg/kg of AEMU, p.o. as a single dose. The rats were kept separately in metabolic cages. The urine was collected in cylinders at an interval of one hr for 6 hr. Total collected urine volume was measured.

Ethylene glycol (EG) model of urolithiasis

In EG model animals were divided into five groups of 6 animals in each. Group I (Normal Group) animals received regular food and drinking water and Group II (Disease control group), III (Standard group), IV & V (Treatment group) received calculi inducing agent ethylene glycol (0.75% w/v) in drinking water till 28th days. Group III served as a standard group (cystone drug given 750 mg/kg, orally), Group IV & V received AEMU (400 mg/kg & 800 mg/kg, orally respectively) from 15th day to 28th day.

Urine collection and analysis

On 28th day, animals were kept in individual metabolic cages and 24 hr urine samples were collected. The urine volume, pH and crystalluria was determined. Urine was acidified by addition of drop of concentrated hydrochloric acid and stored at -20°C for determination of calcium, uric acid, magnesium, urea and phosphate using standard kits. The citrate and oxalate were estimated by the method described by Rajagopal¹⁶ and Hodgkinson¹⁷ respectively.

Serum collection and analysis

Blood was collected under light anesthetic condition from retro orbital plexes. It centrifuged at 10,000 g for 10 min and Serum was separated for the analysis of calcium, magnesium, uric acid, creatinine, and blood urea nitrogen (BUN) using diagnostic kits.

Kidney histopathology and homogenate analysis

At the end of the study, on 28th day animals were scarified, abdomen was incised and opened, and both kidneys were removed from every animal. Extraneous tissue was cleaned from isolated kidneys, weighed and rinsed with ice-cold normal saline. The left kidney was fixed with 10% *v/v* neutral formalin solution and after harvesting, sliced horizontally and sent to histology services for Hematoxylin and Eosin staining. The section of kidney was observed under light microscope. The right kidney was finely chopped and 20% homogenate prepared in Tris-HCl buffer (pH 7.4). Kidney homogenate was used for determination of calcium, uric acid, phosphate, oxalate, urea and catalase.

Statistical analysis

Results data were expressed as mean \pm SEM. The results among the groups were analysed by one-way ANOVA followed by Dunnett's test using Graphpad Prism version 6. Results were considered significant when value of $p < 0.05$ or $p < 0.001$.

Results

Phytochemical screening and quantitative estimation of phytoconstituents

The AEMU was qualitatively analyzed for various phytoconstituents and result revealed presence of carbohydrate, alkaloids, flavonoid, saponin, carbohydrates, phytosterols and phenolic compounds. Total flavanoid and saponin content of the powdered drug was found to be 4.72 ± 0.12 mg quercetin equivalents/g of extract and 36.44 ± 0.78 mg diosgenin equivalent/g of extract respectively.

Effect on diuresis

AEMU has showed a significant diuretic activity at the dose of 400 & 800 mg/kg (10.97 ± 0.06 ml & 16.06 ± 0.09 ml/100 gm/6 hr) as compared to normal group (8.51 ± 0.26 ml/100 gm/6 hr), furthermore, the effect of AEMU at dose of 800 mg/kg was also comparable with the standard diuretic agent, furosemide (14.08 ± 0.39 ml/100 gm/6 hr) (Fig. 1)

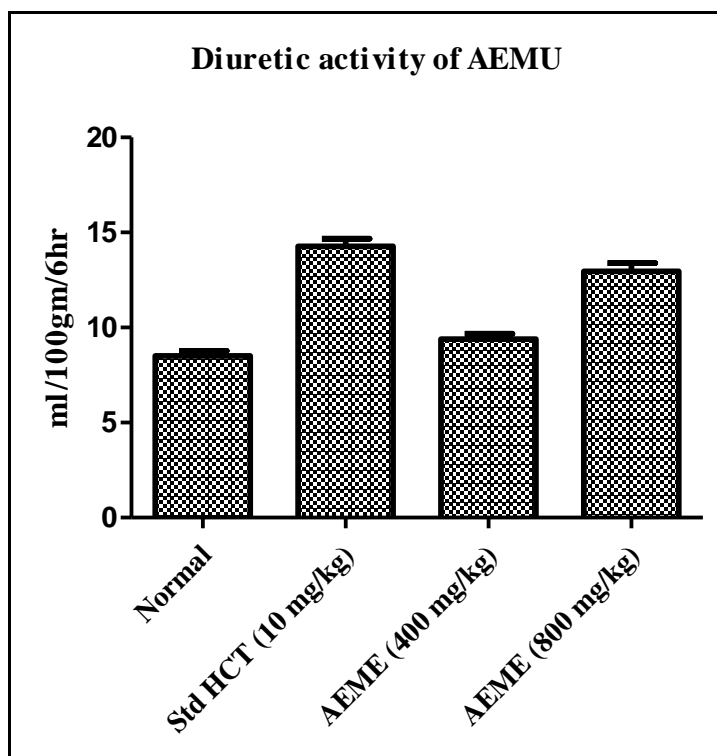


Figure 1: Diuretic effect of AEMU in rats.

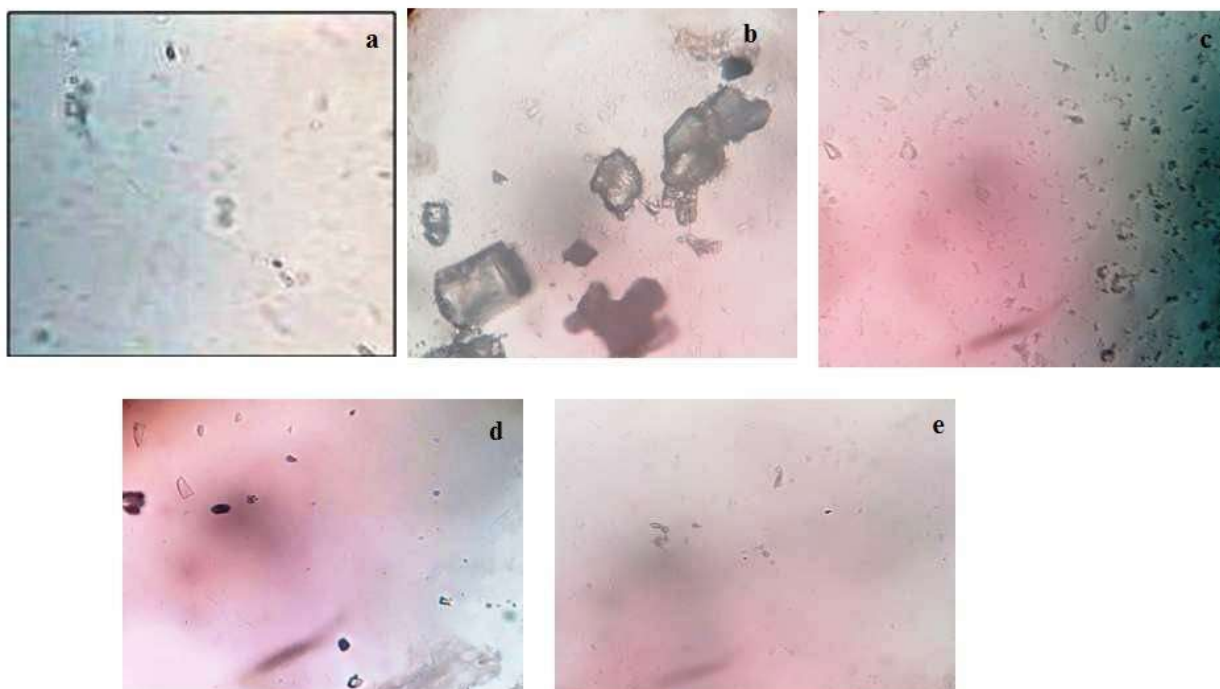


Figure 2: Calcium oxalate crystal observed under microscope in 24 hr urine of rat. (a) Normal control group showed absence of crystal (b) Disease control group showed large crystal and (c) Standard group (cystone 750 mg/kg) (d) Treatment group I (400 mg/kg, AEMU) & (e) Treatment group II (800 mg/kg, AEMU) showed less number of crystals in urine

Effect on urine volume and urinary pH

EG (0.75% v/v in drinking water) administration in rat for 14 days caused significant ($p < 0.001$) decreased in urine volume in all groups as compared to the normal group. On 28th day in disease group urine volume was decreased as compared the 14th day urine volume, while AEMU (400 and 800 mg/kg) and cystone (750 mg/kg) treated group showed significant ($p < 0.001$) increase in urinary output as compared to disease control group on 28th day. Furthermore, significant ($p < 0.001$) decrease in urinary pH was observed in disease group, which was significantly ($p < 0.001$) increased in AEMU (400 and 800 mg/kg) and cystone (750 mg/kg) group. The data are listed in Table 1. In crystalluria study of urine, calcium oxalate crystals were absent in normal group animals (Fig.2(a)), whereas large size and more number of crystals were observed in disease control group animal urine. (Fig.2(b)). In EAFMU and cystone treated animals urine showed very less number and small size of calcium oxalate crystals. (Fig.2(d) &2(e) &2(c)).

Table 1 Effect of AEMU on Urinary parameters in ethylene glycol induced urolithiasis in rats.

	Days	Group I (Normal Control)	Group II (Disease Control)	Group II (Standard Control)	Group IV (Treatment Group I)	Group V (Treatment Group II)
Urine Volume (ml/24 hr)	14	9.12±0.42	5.27±0.45**	5.37±0.35**	5.45±0.35**	5.62±0.36**
	28	9.22±0.45	5.00±0.26**	11.75±0.32##	13.05±0.24##	16.05±0.20##
Urinary pH	14	6.76±0.047	5.32±0.09**	5.4±0.082**	5.55±0.13**	5.85±0.22**
	28	6.74±0.047	5.12±0.08**	6.4±0.029##	6.37±0.06##	6.52±0.08##
Calcium	14	3.05±0.11	5.79±0.36**	6.08±0.13**	6.17±0.22**	6.37±0.27**
	28	3.07±0.12	5.64±0.32**	3.49±0.10##	3.97±0.05##	2.93±0.04##
Oxalate	14	4.67±0.28	9.98±0.81**	9.54±0.77**	9.38±0.74**	9.85±0.71**
	28	4.65±0.26	11.66±0.69**	8.06±0.36##	7.62±0.14##	5.41±0.15##
Phosphate	14	4.91±0.20	8.34±0.92**	8.43±0.40**	8.56±0.52**	8.86±0.68**
	28	5.03±0.15	8.98±0.31**	5.31±0.07##	6.34±0.11##	4.82±0.11##
Uric acid	14	1.90±0.07	4.64±0.40**	4.71±0.20**	4.77±0.15**	4.94±0.28**
	28	2.18±0.07	4.48±0.25**	2.34±0.06##	2.73±0.06##	1.92±0.02##
Urea	14	0.61±0.02	1.24±0.11**	1.25±0.05**	1.27±0.04**	1.32±0.07**
	28	0.67±0.04	1.25±0.08**	0.82±0.04##	1.03±0.03#	0.84±0.03##
Citrate	14	21.22±0.34	8.42±0.84**	8.56±0.53**	8.66±0.42**	8.99±0.72**
	28	21.25±0.26	8.46±0.10**	15.86±0.15##	12.88±0.26##	17.52±0.14##
Magnesium	14	3.24±0.13	1.30±0.12**	1.32±0.08**	1.34±0.08**	1.39±0.09**
	28	3.23±0.13	1.24±0.07**	2.58±0.12##	2.45±0.07#	3.08±0.23##
Creatinine Clearance	14	37.52±4.08	15.12±2.85**	16.35±3.32**	10.41±0.70**	11.55±1.40**
	28	40.72±2.18	11.39±2.33**	47.73±2.81##	53.35±7.06#	64.40±5.86##

All values are mean±SEM (n = 6), one-way ANOVA followed by Dunnett's test.

**P<0.001 and *P<0.05 versus Normal group,

##P<0.001 and #P<0.05 versus disease control group.

Table 2 Effect of AEMU on serum parameters in ethylene glycol induced urolithiasis in rats.

	Days	Group I (Normal Control)	Group II (Disease Control)	Group II (Standard Control)	Group IV (Treatment Group I)	Group V (Treatment Group II)
Calcium	14	10.11±0.11	12.87±0.23**	12.37±0.21**	12.40±0.16**	12.24±0.19**
	28	10.25±0.17	14.05±0.49**	10.98±0.25##	11.83±0.23##	10.74±0.13##
Phosphate	14	5.05±0.15	7.88±0.18**	7.7±0.17**	7.52±0.24**	7.88±0.16**
	28	5.01±0.19	8.39±0.21**	5.74±0.17##	5.47±0.11##	4.86±0.16##
Uric acid	14	2.65±0.12	6.53±0.20**	6.42±0.14**	6.46±0.15**	6.34±0.21**
	28	2.68±0.13	6.98±0.32**	3.47±0.17##	3.89±0.22##	3.08±0.08##
Urea	14	14.77±2.17	28.40±2.17**	27.27±1.85**	29.54±1.3**	29.54±2.9**
	28	14.58±1.20	32.29±1.04**	17.70±1.1##	27.08±1.20#	15.62±1.04##
Magnesium	14	3.18±0.02	1.93±0.02**	1.95±0.03**	1.86±0.02**	1.91±0.02**
	28	3.12±0.05	1.88±0.03**	2.83±0.10##	2.88±0.10##	3.08±0.05##

All values are mean±SEM (n = 6), one-way ANOVA followed by Dunnett's test.

**P<0.001 and *P<0.05 versus Normal group,

##P<0.001 and #P<0.05 versus disease control group.

Effect on serum and urinary parameter

All the parameters of urine and serum were recorded at 0 day, which was similar between all the groups. Tables 1 and 2 summarise the changes in urinary and serum parameter of different groups at an interval of 14th & 28th day of study, respectively. EG treatment caused significant increase in the calcium, phosphorus, oxalate, urea and uric acid level and significant decrease in the magnesium and citrate level in all group except

normal group on 14th day. Treatment with AEMU (400 and 800 mg/kg) and cystone (750 mg/kg) significantly ($p < 0.001$) reduced the levels of calcium, phosphorus, oxalate, urea and uric acid along with increased magnesium and citrate level when compared to the disease control group.

Effect on kidney parameter

Urolithiatic promoters like oxalate, calcium, phosphate and uric acid level were significantly ($p < 0.001$) increased in renal tissue of disease control group as compared to the normal group. However, those promoters were found to be significantly ($p < 0.001$) decreased in the renal tissue of AEMU and cystone treated groups as compared to the disease control group. Stone inducing agent significantly decreased the activity of antioxidant enzyme catalase ($p < 0.001$) in the disease control group while in treatment group increased the level of catalase enzyme; provide protection against oxidative change in tissue. The data are listed in Table 3.

Table 3 Effect of AEMU on Kidney homogenate parameters in ethylene glycol induced urolithiasis in rats.

	Group I (Normal Control)	Group II (Disease Control)	Group II (Standard Control)	Group IV (Treatment Group I)	Group V (Treatment Group II)
Calcium (mg/gm tissue)	5.18±0.10	8.75±0.17**	6.44±0.10##	6.90±0.18##	5.61±0.22##
Oxalate (mg/gm tissue)	1.63±0.14	5.47±0.27**	2.30±0.17##	2.57±0.22##	2.10±0.26##
Uric acid (mg/gm tissue)	2.94±0.06	5.19±0.11**	3.39±0.08##	3.96±0.16##	3.61±0.25##
Phosphate (mg/gm tissue)	3.21±0.15	5.10±0.16**	3.46±0.15##	4.30±0.20#	3.49±0.25##
Catalase (nmoles of H ₂ O ₂ utilized/ min/ mg Protein)	1.70±0.02	0.88±0.03**	1.54±0.01##	1.24±0.12#	1.49±0.08##

All values are mean±SEM (n = 6), one-way ANOVA followed by Dunnett's test.

**P<0.001 and *P<0.05 versus Normal group,

##P<0.001 and #P<0.05 versus disease control group.

Histopathology of kidney

The microscopy section of kidney showed normal architecture in normal group (Fig. 3(a)), while in disease control group kidney revealed the presence of calcium oxalate crystal, severe damage to the glomeruli, medulla, interstitial spaces, tubules, mononuclear cell infiltration. (Fig. 3(b)) while in AEMU (400 & 800 mg/kg) received animal kidney section major damage was recovered and prevention of crystal deposition in intratubular space. (Fig. 3(d) & 3(e)). In cystone treated group section crystal deposition was not observed and renal damage was almost recovered. (Fig. 3(c))

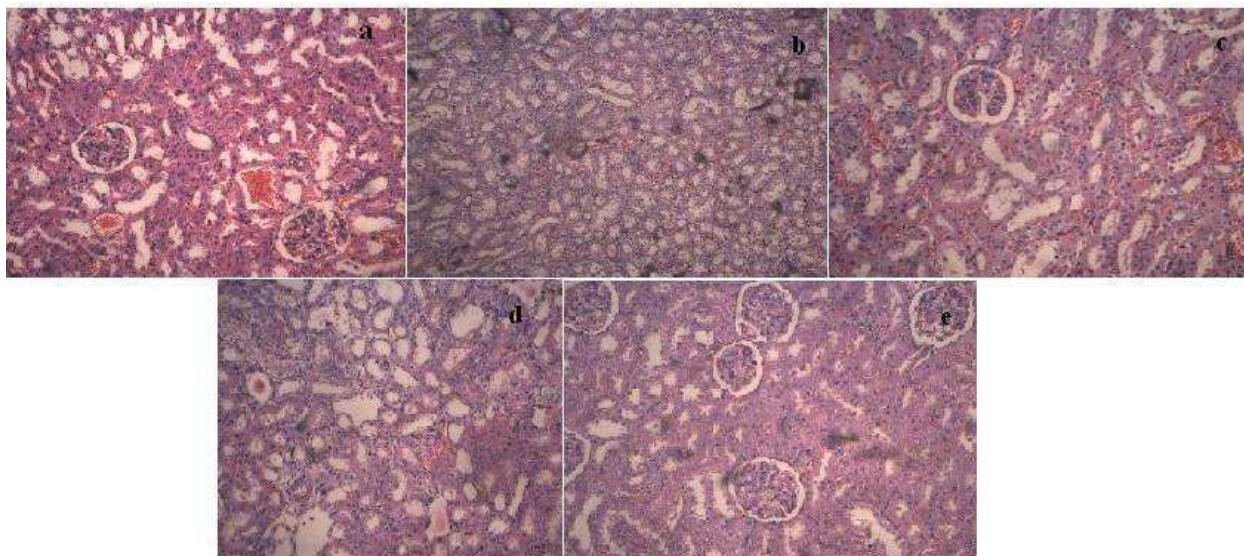


Figure 3: Histology of kidney(a) Normal group, (b) Disease control group showed crystal deposition having large size, (c) Standard group (cystone (750 mg/kg) treated), (d) Treatment group I (400 mg/kg AEMU), (e) Treatment group II (800 mg/kg AEMU) showed less number of crystal and normal structure of kidney

Discussion

Stones formed in kidneys of rats and humans are identical at the ultra structural level in nature and composition of their matrix, thus rat models of urolithiasis are helpful experimental tools for exploring the pathophysiology of the disease. In the present study, male albino wistar rats were selected for induction study because the urinary system of male rats resembles to human¹⁸ and earlier studies have shown that the amount of stone deposition in male rats was significantly high as compared to female rats¹⁹. Spontaneous formation of calcium oxalate is found very rare in rats, & so the stones are induced experimentally to make the animal hyperoxaluric either by administration of excessive amount of oxalate or by exposure to the toxin EG, or various nutritional manipulations in the rat food²⁰.

Many studies showed that administration of EG in animals caused decreased urine output, hyperoxaluria and subsequent hypercalciuria, which further lead to the increased retention and excretion of oxalate²¹. Increased urinary levels of calcium and oxalate favors the nucleation and precipitated calcium oxalate attached to renal tubules and create more nucleation centres for new calcium oxalate crystals. Decrease in calcium and oxalate excretion upon treatment with extract was might be attributed to the ability to interfere with the oxalate metabolism and reducing supersaturation level of calcium and oxalate ions in urine and by preventing the stone formation in kidney. Decrease in urine volume led to increase in the saturation level of oxalate and thus the calcium oxalate crystal formation was initiated. Upon treatment with AEMU which increased urine volume eventually decreased the saturation of oxalate and calcium ions and thus prevented the crystal formation of calcium oxalate in kidney. Thus, diuretic effect of AEMU has played role in flushing out the excessive ions and helping in mechanical expulsion of stone.

Urinary pH is the important factor in formation of kidney stone. At low urinary pH solubility of calcium oxalate stone decreases in urine and it promote stone formation. In present study urinary pH of disease group was significantly found decreased on 14th and 28th days, which indicates that calcium oxalate solubility is minimum and hence urine get supersaturated with oxalates and calcium ions, initiating the stone formation event. pH of urine in AEMU treated rats was found significantly increased, which indicate that treatment with AEMU may be increased the solubility of calcium oxalate stone and decreased the supersaturation level of ions in urine.

It has been reported that, level of inorganic phosphate and uric acid has been increased in stone formers and toxin agents induced urolithiatic rats. Increased urinary phosphate level along with oxalate provides an appropriate environment for formation of calcium phosphate crystals, which further induces calcium oxalate

deposition in renal¹⁹. Uric acid which interfere calcium oxalate solubility, induces the nucleation of calcium oxalate and reduces the inhibitory activity of glycosaminoglycans²². There was significant increase in excretion of inorganic phosphate & uric acid level in serum and urine in all groups on 14th days as compared to normal group. Upon treatment with AEMU levels of phosphate and uric acid was significantly decreased on 28th day, which indicate that treatment with AEMU may be prevent the crystal deposition and increase the solubility of calcium oxalate stone.

Magnesium and citrate are considered as urolithiatic inhibitors. Magnesium makes complexes with oxalate and reduces the supersaturation of calcium oxalate, as a consequence calcium oxalate crystals growth and rate of nucleation were also reduced²³ while citrate makes complexes with calcium ions to form soluble complex and decreased the supersaturation levels of ions and as a consequence, crystal aggregation and growth was reduced¹⁹. In disease group level of magnesium and citrate weresignificantly reduced as compared to normal group. Magnesium and citrate level in AEMU treated rats were found significantly increased in biological samples as compared to disease control group, so AEMU may be prevents the crystallization of calcium oxalate by affect in supersaturation level of ions via increased in urolithiatic inhibitors level and its activity in body.

In renal stone patients and urolithitic rats, there was decreased in urinary output due to decreases in glomerular filtration rate, this leads to the accumulation of waste products in the blood like nitrogenous substance such as urea, creatinine and decrease in creatinine clearance²⁴. Treatment with AEMU which lowered the serum urea and increased the creatinine clearance levels as compared to disease control group. This effect can be attributed due to diuresis effect of AEMU.

It has been reported that reactive species play an important role in renal stone formation as a signaling molecule and induced the cell injury. Reactive oxygen species damage to the renal cell which leads to cell death and formation of vesicles in membrane which provides the support for crystal nucleation as a result increase in crystal growth and crystal aggregation²⁵. In the present study, there was significantly decreased in the activity of catalase, in stone induced rats as compared to normal rats which might be due to production of Reactive oxygen species. However, upon treatment with AEMU and cystone which showed significant increased in catalase activity which indicates that AEMU and cystone have antioxidant activity. This activities of extract was due to presences of flavonoids, which have remarkable anti inflammatory and antioxidant activities²⁶.

Histopathology of kidney of calculi induced rats showed polymorphic irregular calcium oxalate crystal deposition inside the tubules which cause dilation of the proximal renal tubules along with interstitial inflammation that might be attributed to oxalate. Many studies showed that adenosine A1 receptor (AA1R) antagonist induce diuresis and provide renal protection. Karton et al²⁷ reported that a number of flavonoids and its derivatives have ability to compete for adenosine A1 receptor binding and found the strong antagonist. Co-treatment with AEMU decreased calcium oxalate deposits in different parts of the renal tubules and also prevented damages in the tubules and calyces. Our phytochemical analysis was indicated the presence of flavonoids in extract, it might be inhibit the AA1R receptor and protect renal tissues.

The lithotriptic effect of AEMU may be due to presence of saponin and flavonoids which identified by phytochemical analysis in the present study. The free radical scavenging, anti inflammatory, anti microbial activity²⁶ and AA1R antagonist activity of flavonoids play remarkable role in the prevention of further formation and dissolution of crystals.

Conclusion

On the basis of above discussion it can be concluded that the, AEMU has antiurolithiatic activity in EG induced urolithiasis by promoting various inhibitors like magnesium, citrate and suppressing various promoters like calcium, oxalate, phosphate in serum, urine and kidney tissue. Furthermore, AEMU has a significant diuretic action that can help to flush out promoters in urine and increases the dissolution of calcium oxalate stones and this prevents new stone formation. Thus, the present finding emphasizes that the AEMU possess potential medicinal value and beneficial in the prevention and cure in stone formation in renal. Further studies need to explain detail mechanism(s) of *M. uniflorum* seeds.

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