AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Antilithiatic activity of *Grewia asiatica* by Sodium oxalate induced Urolithiatic model

P. Veeresh Babu[1]*, M. Vamsi krishna[1], M. Ganga Raju[1], P. Venkanna[1]

1.Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, Telangana

ABSTRACT

Grewia asiatica is a plant commonly used as a traditional herbal medicine and possesses the wide range of pharmacological applications. The present study investigated the antilithiatic activity of an ethanolic leaf extract of *Grewia asiatica* Linn (EEGA). Sodium oxalate (7 mg/100 gm *i.p*) administration resulted in hyperoxaluria as well as increased urinary volume and its pH. EEGA (200 & 400 mg/kg) was given orally in curative and preventive regimens over a period of 14 days. Supplementation with EEGA significantly ($p<0.05$) restored urea, uric acid, creatinine, sodium, potassium, chloride, volume of urine and pH levels. The preventive regimen was found to be better than the curative regimen. The results were comparable with the standard drug, Cystone (500 mg/kg). The presence of triterpenoids, flavonoids and saponins in extract might be responsible for significant antilithiatic activity of the plant. These findings affirm assertions made regarding the effectiveness of the extract of this plant against urinary pathologies in the Indian folk medicine.

Keywords: *Grewia asiatica*, Sodium oxalate, Cystone, Hyperoxaluria

*Corresponding Author Email: pratap.veeresh@gmail.com

Received 14 June 2016, Accepted 25 June 2016

Please cite this article as: Babu VP *et al.*, Antilithiatic activity of Grewia asiatica by Sodium oxalate induced urolithiatic model. American Journal of PharmTech Research 2016.

INTRODUCTION

Urolithiasis is one such disease that after extensive research in the field of urology has remained incurable in allopathy. It is a process of stone formation which occurs either in the kidney (commonly known as nephrolithiasis) and or in any part of urinary tract, including the ureters (known as ureteral stone) and bladder (bladder stone). Urolithiasis has an important effect on the health care system with a prevalence of >10% and an expected recurrence rate of ~50%[1]. Approximately 80% of these calculi are composed of calcium oxalate and calcium phosphate. Epidemiological studies revealed that the nephrolithiasis is more prevalent in men (12%) than in women (6%) and is more prevalent between the ages of 20-40 in both sexes[2].

Urinary calculi may cause obstruction, hydronephrosis, infection and hemorrhage in the urinary tract system[3]. Kidney stone formation is a complex process that results from a succession of several physicochemical events including supersaturation, nucleation, growth, aggregation and retention within the renal tubules[4]. Surgical operation, lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. However, in these procedures recurrence is quite common[5]. Moreover, they cause side effects such as hemorrhage, hypertension, tubular necrosis and subsequent fibrosis of the kidney[6]. Various therapies including thiazide diuretics and alkali-citrate are being used in attempt to prevent recurrence but scientific evidence for their efficacy is less convincing[7]. Various therapies including thiazide diuretics and alkali-citrate are being used attempt to prevent recurrence but scientific evidence for their efficacy is less convincing[8]. Noteworthy, traditional herbal medicines are efficacious and have lesser side effects compared to modern drugs and also reduce the recurrence rate of the renal stone. The vast Ayurvedic literature claims a number of plants to useful in the treatment of urinary stones; still many plants need to be explained for their pharmacological actions[9].

Grewia asiatica is a shrub or small tree from the family Tiliaceae commonly known as Phalsa or Falsa. *Grewia asiatica* is food plant and can also be used as herbal medicine for the treatment of various diseases such as cancer, ageing, fever, rheumatism and diabetes[10]. A literature survey revealed that ethanolic leaf extract of *Grewia asiatica* is endowed with various chemical components such as triterpenoids, sterols, flavonoids, saponins and tannins which possibly contribute to its vast uses in folkloric medicine[11]. Hence, in the present study, the ethanolic extract of *Grewia asiatica* evaluated against sodium oxalate induced renal calculi in wistar albino rats.

MATERIALS AND METHOD

Plant material and preparation of extract

The plant *Grewia asiatica* was collected from the Nallamalla forest region, near Atmakur, Kurnool Dist. The botanical identity was confirmed by Dr. D. Saritha, Medical officer, Governament ayurvedic dispensery, Pamulapadu, Kurnool Dist.

The leaves were dried in shade at room temperature and extracted with ethanol by simple distillation technique. The solvent was completely removed under reduced pressure and a semisolid mass was obtained and stored for further study.

Preliminary Phytochemical Screening

The ethanolic extract of *Grewia asiatica* was screened by different chemical tests for identifying the basic chemical constituents present in the extract. The standard chemical tests for alkaloids, tannins, flavonoids, terpenoids steroids and saponins were performed to get a preliminary idea of the chemical constituents[12,13].

Animals

Healthy Wistar-albino male rats weighing about (150-200 gm) were procured from Gentox Bioservices, Hyderabad. The animals were housed in specific standard laboratory conditions. They were kept in a temperature controlled environment (25±1 ^{o}C) and with a regular 12h light/12h dark cycle. All animals were fed with commercial diet & water *ad libitum* during the experiment. Present study was carried out in CPCSEA approved animal house of Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, India (Reg. No.1175/PO/Ere/S/08/CPCSEA).

Acute toxicity studies

The acute toxicity was determined on Swiss albino mice by up-and-down procedure of OECD Guideline No. 425 given by CPCSEA. The ethanolic leaf extract of *Grewia asiatica* 2000 mg/kg was administered orally to mice (n=6). The animals were observed for behavioural and physiological variations initially continuously for 4 hours, followed by 4th hourly for 12 hours and there after once daily for 14 days.

***In vitro* antioxidant activity:**

The *in vitro* antioxidant activity was carried out by reducing power assay and hydrogen peroxide scavenging assay.

Reducing power assay

Fe^{3+} reducing power of *Grewia asiatica* extract was determined by modified method of Oyaizu. Reducing power was determined by taking different concentrations of the plant extract. Ascorbic acid was used as reference standard. To 1 mL of test (10-50 µg/mL) and standard compounds added 2.5 mL of potassium ferricyanide (1 % w/v), 2.5 mL of phosphate buffer pH 6.6 and incubated at 50 ^{0}C for 30 min. To 2.5 mL of above supernatant liquid added 2.5 mL of distilled

water and 0.5 mL of $FeCl_3$ solution (0.1% w/v). The absorbance of ferric ferrous complex was measured using phosphate buffer pH 6.6 as control at 700 nm using UV-Visible spectrophotometer and estimated the increase in absorbance[14].

The percent increase in reducing power was calculated using the following equation:

$$\text{Percentage increase in reducing power (\%)} = \frac{\text{Abs}_{\text{test}} - \text{Abs}_{\text{blank}}}{\text{Abs}_{\text{blank}}} \text{ X } 100$$

Where 'Abs $_{test}$' is absorbance of test solution; 'Abs $_{blank}$' is absorbance of blank.

Hydrogen peroxide scavenging assay

The scavenging activity of extract towards hydrogen peroxide radicals was determined by modified method of Dehpour. Solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer pH 7.4 and its concentration was determined by measuring the absorbance at 560 nm using UV spectrophotometer. 0.1 mg/mL (10-50 µg/M) of extract was added to hydrogen peroxide solution and absorbance measured at 560 nm using UV spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extract and standard compound was calculated by using the given formula:

Percentage scavenged (H_2O_2) = 1- Absorbance standard/Absorbance control X 100

Where, Abs control was the absorbance of the control (without extract) at 560 nm; Abs sample was the absorbance in the presence of the extract at 560 nm[15].

***In vitro* antilithiatic activity:**

The *in vitro* antilithiatic activity was carried out by inhibition of mineralization and homogenous precipitation method.

Inhibition of mineralization method:

Inhibition of calcium oxalate and calcium phosphate mineralization was measured by simultaneous flow static model. The procedure was carried out in two sets in which one served as blank set and other as experimental set. In blank set 0.1 M sodium oxalate (25 mL) was taken in two separate burettes. Whereas experimental set extract (25 mL) was taken in a 3rd burette. In both the sets, chemicals were allowed to fall simultaneously slowly at the speed into a 250 mL beaker for 30 minutes. The mixture was kept in hot water bath for 10 minutes, cooled to room temperature and collected into a preweighed centrifuge tube. Centrifugation of mixture was done at 3000 rpm for 15 minutes. Supernatant liquid was discarded and precipitate was obtained. The tubes were then dried in a hot air oven at 120 ^{0}C and cooled to room temperature and weighed. Similar procedure was repeated using 0.1 M sodium phosphate (25 mL) and 0.1 M calcium acetate (25 mL) for inhibition of calcium phosphate mineralization[16].

Homogenous precipitation method:

Step-1: Preparation of experimental kidney stones (Calcium oxalate stones) by homogenous precipitation

Equimolar solution of calcium chloride dihydrate (AR) in distilled water and sodium oxalate (AR) in 10 ml of 2 N H_2SO_4 was allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium oxalate. Equimolar solution of calcium chloride dihydrate (AR) in distilled water and disodium hydrogen phosphate (AR) in 10 ml of (2 N H_2SO_4), were allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium phosphate. Both precipitates freed from traces of sulphuric acid by ammonia solution. Washed with distilled water and dried at 60 ^{0}C for 4 hours.

Step -2: Preparation of semi-permeable membrane from farm eggs

The semi -permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin & yolk. Shell was removed chemically by placing the eggs in 2 M HCl for an overnight, which caused complete decalcification. Further, washed with distilled water, and carefully with a sharp pointer a hole was made on the top and the contents squeezed out completely from the decalcified egg. Then egg membrane washed thoroughly with distilled water, and placed it in ammonia solution, in the moistened condition for a while & rinsed it with distilled water. Stored in refrigerator at a pH of 7- 7.4.

Step-3: Estimation of calcium oxalate by Titrimetry

Weighed exactly 1mg of the calcium oxalate and 10 mg of the extract/compound/standard and packed it together in semi evaluation permeable membrane by suturing. This was allowed to suspend in a conical flask containing 100 mL of 0.1 M TRIS buffer. One group served as negative control (contained only 1mg of calcium oxalate). Place the conical flask of all groups in an incubator, preheated to 37 ^{0}C for 2 hours, for about 7-8 hours. Remove the contents of semi-permeable membrane from each group into a test tube. Added 2 mL of 1 N sulphuric acid and titrated with 0.9494 N $KMnO_4$ till a light pink colour end point obtained.1ml of 0.9494 N $KMnO_4$ equivalent to 0.1898 mg of 4 Calcium. The amount of undissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate actually test substance(s) could dissolve[17].

***In vivo* antilithiatic model:**

Sodium oxalate induced urolithiasis model

Animals were divided into eight different groups containing six animals each. Sodium oxalate (7 mg/100 gm *i.p*) was fed to all groups except control for induction of renal calculi till the 7th day.

Two types of study were performed, viz. prophylactic study and curative study. In the prophylactic study all the groups except control received extract (200 & 400 mg/kg *p.o*) and standard cystone (500 mg/kg) from 1st day till 7th day while in the curative study all groups except control received extract (200 & 400 mg/kg, *p.o*) and cystone (500 mg/kg) from 8th day till 15th day. During the study animals were allowed free access to food.

Assessment of antilithiatic activity:

Serum analysis

The blood was collected on 0th, 7th and 14th day from the retro-orbital sinus under anaesthetic condition and serum was separated by centrifugation at 5000 rpm for 10 min and analyzed for urea, uric acid, creatinine, sodium, potassium and chloride.

Collection and analysis of urine

All the animals were kept in individual metabolic cages and urine samples were collected on 0th, 7th and 14th day of calculi induction treatment. The volume of urine and pH of the urine were measured[18,19].

Statistical analysis

The data were expressed as mean ± SEM. The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's test and $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening

The preliminary phytochemical screening of the *Grewia asiatica* extract showed the presence of alkaloids, tannins, flavonoids, terpenoids, steroids and saponins.

Acute toxicity studies

Administration of EEGA at the dose of 2000 mg/kg resulted in no mortality or evidence of adverse effects implying that *Grewia asiatica* is nontoxic. No changes were observed in behavioral pattern, clinical signs and body weight of mice throughout 14 days of the study.

***In vitro* antioxidant activity**

EEGA showed dose dependent inhibition of free radicals in both the reducing power and H_2O_2 scavenging assays. Its IC_{50} value was found to be 32.5 µg/mL in reducing power assay and 21.5 µg/mL in H_2O_2 scavenging assay. The potential of the extract was comparable to that of standard ascorbic acid (Figure 1 & 2).

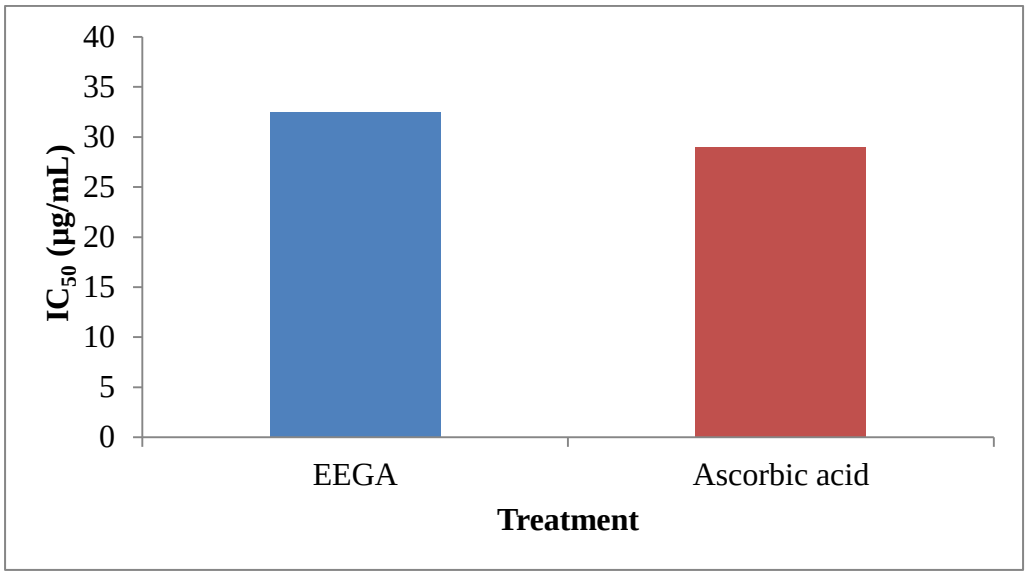

Figure 1: Reducing power assay of ethanolic extract of *Grewia asiatica.*

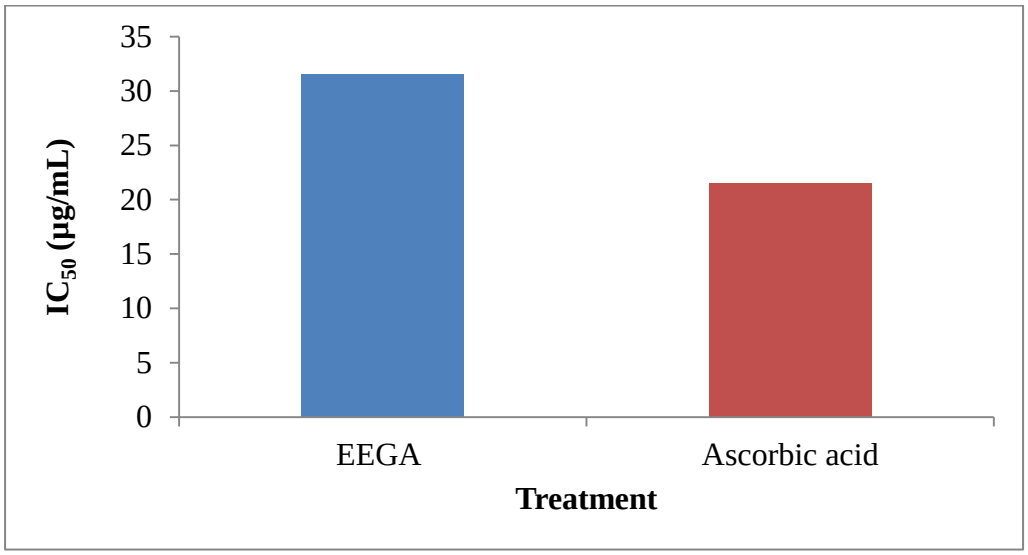

Figure 2: Hydrogen peroxide radical scavenging assay of ethanolic extract of *Grewia asiatica.*

***In vitro* antilithiatic activity:**

Inhibition of mineralization method

In inhibition of mineralization method, the test extract EEGA exhibited 43.05% inhibition of calcium oxalate and 47.67% inhibition of calcium phosphate. It produced better inhibitory activity on calcium phosphate rather than calcium oxalate. The activity of the EEGA was comparable to that of standard (Table 1).

Table 1: Antilithiatic activity of *Grewia asiatica* by inhibition of mineralization.

S. No	Compound	% of inhibition	
		Calcium oxalate	Calcium phosphate
1.	Test	43.05%	47.67%
2.	Standard/Cystone	50.60%	53.44%

precipitation method

In homogenous precipitation method, the standard drug cystone showed 52.7±0.07 % dissolution of calcium oxalate crystals whereas the test extract EEGA showed 46.5±0.03 % of calcium oxalate crystals dissolution. The activity of the EEGA was comparable to that of standard (Table 2).

Table 2: Antilithiatic activity of *Grewia asiatica* by Homogenous precipitation method.

S. No	Group	% dissolution of Calcium oxalate
1.	Blank	0
2.	Extract	46.5±0.03
3.	Standard (Cystone)	52.7±0.07

***In vivo* antilithiatic activity:**

Renal function was assessed by measuring serum urea, uric acid and creatinine. These levels were significantly ($p<0.05$) elevated in lithiatic control (Group-II) when compared with Group-I indicating renal damage. While treatment with EEGA significantly ($p<0.05$ vs. Group-II) reduced the levels of these NPN substances excreted by kidneys. However, EEGA 400 mg/kg significantly reversed these parameters and comparable to standard drug Cystone. The effect of EEGA in preventive regimen was found to be better than that of curative regimen.

In lithiatic control (group-II), sodium and chloride levels were reduced and potassium level was increased significantly ($p<0.05$) compared to normal control. In prophylactic regimen both the doses of EEGA i.e. 200 and 400 mg/kg produced significant elevation of sodium and chloride levels and reduction of potassium levels. The activity of 400 mg/kg dose was found to be better than that of 200 mg/kg dose and it was comparable to that of standard cystone. The same pattern of response was observed even in therapeutic regimen with quantitative differences.

Volume of urine and its pH were found to alter significantly ($p<0.05$) in lithiatic control (Group-II) when compared with Group-I. Treatment with EEGA significantly ($p<0.05$ vs. Group-II) and dose dependently restored these parameters (Table 3).

Table 3: Effect of *Grewia asiatica* on serum and urinary parameters

Parameter	Day	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Group-VII	Group-VIII
Urea	0th	60±0.88	61±0.89	60±1.10	59±1.52	62±1.20	58±1.26	61±1.20	60±1.20
	7th	60±0.89	84±0.88*	75±1.17*	68±0.97*	67±1.10*	72±0.88*	73±0.78*	70±3.10*
	14th	61±1.08	109±1.37*	68±1.10*	63±0.88*	63±1.58*	66±0.88*	64±1.20*	62±1.20*
Uric acid	0th	1.99±0.17	1.96±0.10	1.98±0.08	1.97±0.07	1.99±0.10	1.95±0.13	1.96±0.12	1.92±0.19
	7th	1.95±0.19	3.24±0.21*	2.49±0.20*	2.43±0.12*	2.39±0.25*	2.97±0.13	2.75±0.13*	2.71±0.12*
	14th	1.97±0.06	4.08±0.25*	2.21±0.10*	2.03±0.15*	2.10±0.11*	2.53±0.88*	2.25±0.12*	2.09±0.14*
Creatinine	0th	0.73±0.12	0.54±0.05	0.62±0.09	0.58±0.06	0.81±0.11	0.64±0.12	0.77±0.13	0.82±0.12
	7th	0.81±0.07	4.06±0.09*	1.03±0.10*	1.04±0.07*	1.03±0.13*	3.02±0.11	2.71±0.13*	2.33±0.10*
	14th	0.68±0.06	4.27±0.40*	0.97±0.08*	0.89±0.12*	0.83±0.10*	1.63±1.02*	1.01±0.08*	0.95±0.17*
Sodium	0th	145.39±3.16	146.62±3.69	147.46±2.79	148.17±2.39	148.17±2.39	149.23±1.50	145.84±2.41	145.13±2.21
	7th	148.85±3.35	109.93±2.34*	129.92±2.05*	127.33±2.64*	123.96±1.63*	116.21±2.41	120.15±2.16*	123.41±2.34*
	14th	147.21±3.02	96.34±2.85*	141.37±2.99*	144.36±1.62*	146.10±2.48*	138.17±1.60*	142.63±2.53*	146.12±2.10*
Potassium	0th	213.50±0.90	221.02±3.53	216.44±3.01	214.33±2.16	217.48±2.19	215.55±2.81	218.33±2.74	214.15±2.79
	7th	215.42±2.54	250.40±2.50*	229.86±1.96	223.25±2.06*	220.88±1.27*	234.60±1.60*	229.29±2.46*	228.36±2.81*
	14th	216.30±4.01	241.50±2.69*	220.09±2.19*	215.34±1.70*	212.33±2.91*	221.15±1.98*	219.69±2.18*	216.11±2.84*
Chloride	0th	232.65±3.27	230.93±3.93	228.74±2.60	230.22±2.46	228.44±2.21	232.26±2.09	229.39±2.50	232.96±2.66
	7th	238.18±2.83	177.50±2.64*	210.42±2.52*	217.15±2.66*	219.35±1.84*	205.13±1.80	207.40±2.09*	210.37±2.66*
	14th	241.60±4.52	156.34±2.97*	221.09±2.76*	226.82±2.24*	228.47±2.35*	223.54±1.93*	226.11±2.46*	230.49±2.13*
Volume of urine	0th	2.80±0.10	2.81±0.10	2.27±0.11	2.80±0.26	2.79±0.23	2.80±0.20	2.82±0.10	2.81±0.11
	7th	2.82±0.08	2.09±0.29*	2.52±0.06*	2.59±0.26*	2.62±0.12*	2.39±0.07	2.48±0.15*	2.51±0.15*
	14th	2.83±0.10	1.90±0.11*	2.64±0.09*	2.77±0.13*	2.79±0.21*	2.39±0.07*	2.77±0.15*	2.80±0.11*
pH of urine	0th	7.74±0.12	7.74±0.22	7.71±0.07	7.72±0.23	7.73±0.19	7.71±0.09	7.74±0.16	7.73±0.17
	7th	7.74±0.16	6.42±0.18*	7.34±0.23*	7.42±0.17*	7.53±0.20*	7.32±0.20	7.45±0.20*	7.52±0.13*
	14th	7.77±0.21	6.42±0.18*	7.63±0.16*	7.68±0.16*	7.72±0.24*	7.60±0.28*	7.66±0.15*	7.72±0.11*

Data were expressed as mean ± SEM (n=6). Statistical analysis was performed by using ANOVA followed by Dunnett's T-test. Comparisons were made against group-I and group-II and *p<0.05 was considered significant

DISCUSSION

Urolithiasis is a common disease with an increasing incidence worldwide that appears even more pronounced in industrialized countries. Kidney stone formation is a complex that results from a succession of several physicochemical events including supersaturation, nucleation, growth, aggregation and retention within the kidneys[20].

Male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans and earlier studies shown that the amount of stone deposition in female rats was significantly less[21].

Sodium oxalate administration results in rapid formation of calcium oxalate crystals in renal tubules in experimental animals. The major reason for this instantaneous crystal formation after a sodium oxalate challenge is the rapid increase in urinary excretion of oxalate[22]. The speed with which crystal appears in renal tubules is an index of efficiency of transport to kidney of this added oxalate and its eventual excretion in the urine. After administration of sodium oxalate, the crystalline deposition first reaches to the cortex, then to the medulla and then renal tubules[23,24]. It was proved by increased levels of nitrogenous waste products like urea, uric acid and creatinine in disease control group. However, the curative and prophylactic treatment with EEGA and cystone significantly reduced the above parameters which might be due to hastening the process of dissolving the preformed stones and prevention of new stone formation in urinary system. High dose of EEGA (400 mg/kg) showed prominent activity which was comparable to that of standard.

Changes in ionic pattern of urine are the major determinant of stone formation. In this study, the ionic pattern was found disturbed by treatment with sodium oxalate indicated by reduced sodium and increased potassium and chloride levels. However, treatment with EEGA and cystone restored the altered levels of above mentioned electrolytes or ions which indicates their nephroprotective effect.

Reduction in volume of urine and increased pH was observed in sodium oxalate treatment group (Group-II) due to the stone formation[25]. Restoration of volume of urine and its pH was observed after the treatment with EEGA and cystone.

The ethanolic extract of *Grewia asiatica* was proved to possess prominent antioxidant activity by reducing power assay and hydrogen peroxide radical scavenging assay. On the basis of phytochemical research, *Grewia asiatica* found to contain steroids, flavonoids, triterpenoids, saponins, carbohydrates, alkaloids, tannins and glycosides. Hence the antioxidant potential of the plant extract is correlated with its triterpenoids, flavonoids, saponins. Therefore, EEGA might

prevented hyperoxaluria induced perioxidative damage to the renal tubular membrane surface (lipid peroxidation), which inturn can prevent calcium oxalate crystal attachment and subsequent development of kidney stones.

Results indicate that administration of EEGA reduced and prevented the growth of urinary stones. It also seems that the prophylactic regimen is more effective than its therapeutic regimen. Therefore, the leaf extract of *Grewia asiatica* is helpful to prevent the reoccurrence of the disease as it showed its effect on early stages of stone development.

The mechanism underlying this effect is mediated possibly through an antioxidant, nephroprotective properties and lowering the concentration of urinary stone forming constituents.

CONCLUSION

The antilithiatic activity of ethanolic leaf extract of *Grewia asiatica* is mediated possibly through inhibition of calcium oxalate crystal formation and its effect on the urinary concentration of stone-forming constituents. Thus, the present finding emphasizes that the leaves of *Grewia asiatica* possess potential medicinal value and beneficial in the prevention of renal stone. Further studies need to be undertaken to explain detail mechanism of action of *Grewia asiatica* leaves.

ACKNOWLEDGMENTS

The authors are grateful to the Principal and Management of Gokaraju Rangaraju College of Pharmacy for providing all necessary facilities to carry out the research work and their constant support.

REFERENCES

1. Knall T. Stone disease. Eur urol Suppl 2007; 6: 717-722.
2. Worcester EM, Coe FL. Nephrolithiasis. Prime core 2008; 35: 369-391.
3. Hadjizadesh M, Khoei A, Hadjzadesh Z, PParizady M. Ethanolic extract of *Nigella sativa* seeds on ethylene glycol induced kidney calculi in rats. Urol . J 2007; 4: 86-90.
4. Khan SR, Finlayson B, Hackelt RL. Experimental calcium oxalate nephrolithiasis in rats role of renal papilla. American journal of Pathology 1982; 107: 59-69.
5. Pietvow P, Preminger GM. Campbell-Walsh urology. 9th ed., vol 2, WB Saunders, Philadelphia; 2007.
6. Doddamametikurke RB, Biyani CS, Browning AJ, Cartledge JJ. The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. EAU-EBU update series 2007; 5: 126-136.

7. Bashir S, Gilani AH. Antiurolithiatic effect of *Bergenia ligulata* rhizome an explanation of the underlying mechanisms. Journal of Ethnopharmacology 2009; 122: 106-116.
8. Prasad K, Sujatha D, Bharathi K. Herbal dugs in urolithiasis a review. Ph cog; 1: 175-179.
9. Butterweck V, Khan SR. Herbal medicines in the management of urolithiasis. Alternative and complmentary? Planta Med 2009; 75: 1095-1103.
10. Zia-ul-Haq M, Shahid SA, Ahmed S, Qayum M, Khan I. Antiplatelet activity of methanol extract of *Grewia asiatica* leaves and *Terminalia chebula* fruits. Journal of Medicinal plants and Research 2012; 6: 2029-2032.
11. Patil P, Patel MM, Bhavsar CJ. Preliminary phytochemical and hypoglycemic activity of leaves of *Grewia asiatica*. Res. J. Pharm. Biol. Chem Sci 2011; 2: 516-520.
12. Trease GE, Evans WC. Pharmacognosy. London: WB. Saunders; 2008: 15.
13. Harbone JB. Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall London 2007; 3: 207-208.
14. Khanam S, Shivaprasada HN, Devi K. *In vitro* antioxidant screening models: a review. Indian Journal of Pharmaceutical Education and Research 2004; 38 (4): 180–183.
15. Serhat K, Sait C, Semra T, Ökkes Y, Ismail T. Hydrogen peroxide radical scavenging and total antioxidant activity of hawthorn. Chemistry Journal 2012; 2(1): 9-12.
16. Shukla AB, Mandavia DR, Barvaliya MJ, Baxi SN, Tripathi CB. Anti-urolithiatic effect of cow urine Ark on ethylene glycol-induced Renal Calculi. IJBU 2013; 39(4): 565-571.
17. Atodariya U, Roshni B, Siddhi U, Umesh U. Anti-Urolithiatic Activity of *Dolichos biflorus* Seeds. Journal of Pharmacognosy and Phytochemistry 2013; 2(2): 209-21.
18. Mert I, Burcin E, Ipek S, Serkan O, Gulcin SC, Ayse DM, Hikmet K, Levent A, Esra KA. Preclinical evaluation of Anti-Urolithiatic Activi ty of *Viburnum opulus* L. on Sodium Oxalate Induced Urolithiasis Rat model. Hindawi Publishing Corporation 2014; 3: 1-5.
19. Pankaj G, Naimesh P, Lokesh B, Zambare GN, Bodhankar SL, Jain BB, Nandakumar K. Anti-urolithiatic effects of Petroleum ether extract stem bark of *Crataeva adansonii* in rats. Pharmaceutical Biology 2006; 44(3): 160-165.
20. Kalyani Divakar, Pawae AT, Chandrasekhar SB, Goli Divakar. Protective effect of the hydro-alcoholic extract of *Rubia cordifolia* roots against ethylene glycol induced urolithiasis in rats. Food and Chemical Toxicology 2010; 48: 1013-1018.
21. Karadi RV, Gadge N, Alagawadi KR, Savadi RV. Effect of *Moringa olerera* Lam. root-wood on ethylene glycol induced urolithiasis in rats. Journal of Ethnopharmacology 2006; 105: 306-311.

22. Bijarnia RK, Kaur T, Agsgarwal K, Singla SK, Tandon C. Modulatory effects of N-acetylcysteine on hyperoxaluric manifestations in rat kidneys. Food and Chemical Toxicology 2008; 46(6): 2274-2278.
23. Joshi PC, Patil SA, Sambrekar SN, Evaluation of the Anti-urolithiatic activity of ethonolic extract of *Celosia argentea* (Seeds) in rats. Universal Journal of Pharmacy 2012; 1(1): 52-60.
24. Khan SR, Finlayson B, Hackett RL. Experimental calcium oxalate nephrolithiasis in the rat, role of renal papilla. American Journal of Pathology 1982; 107: 59-69.
25. Takawale RV, Mali VR, Kapase CU, Bodhakar. Effect of *Lagenaria siceraria* fruit powder on sodium oxalate induced urolithiasis in wistar rats. Journal of Ayurveda and Integrative Medicine 2012; 3(2):75-79.

AJPTR is

- **Peer-reviewed**
- **bimonthly**
- **Rapid publication**

Submit your manuscript at: editor@ajptr.com