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ANTILITHIATIC ACTIVITY OF *GREWIA ASIATICA* IN MALE RATS

P. Veeresh Babu *, M. Vamsi Krishna, T. Ashwini and M. Ganga Raju

Department of Pharmacology, Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad, Telangana, India.

Keywords:

Grewia asiatica, Ethylene glycol, Cystone, Hyperoxaluria

Correspondence to Author:
Dr. P. Veeresh Babu

M. Pharm., Ph.D
Associate Professor,
Department of Pharmacology,
Gokaraju Rangaraju College of Pharmacy, Bachupally, Hyderabad - 500090, India.

E-mail: pratap.veeresh@gmail.com

ABSTRACT: Lithiasis is the process of stone formation in the kidney, bladder, and/or urethra. Herbal drugs are reported to be effective in the treatment of urolithiasis with no side effects. *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). Ethylene glycol (0.75% in water) feeding 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 28 days. Supplementation with EEGA significantly ($p<0.05$) restored urea, uric acid, creatinine, 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 (750 mg/kg). Histopathological examination further corroborated the antilithiatic or nephoprotective activity of the extract. The presence of triterpenoids, flavonoids and saponins in extract might be responsible for significant antilithiatic activity of the plant. From this study, we conclude that both the prophylactic and therapeutic treatment with ethanolic leaf extract of *Grewia asiatica* had an inhibitory effect on crystal growth, with improvement of kidney function as well as cytoprotective effect.

INTRODUCTION: Urolithiasis is a complex process which is a consequence of an imbalance between promoters and inhibitors in kidneys [1]. Even after the technological developments in the present medical practice, the formation and growth of renal calculi continues to afflict humankind. Approximately 80% of these calculi are composed of calcium oxalate and calcium phosphate. Urinary calculi may cause obstruction, hydronephrosis, infection and hemorrhage in the urinary tract system [2].

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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 [3]. 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 [4].

The recurrence rate without preventive treatment is approximately 10% at 1 year, 33% at 5 year and 50% at 10 years [5]. 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 [6]. Hence, the search for effective antilithiatic drugs without or minimum side effects from natural sources has gained a great potential.

In contrast, traditional medicines have offered a substitute for many diseases and also have provided some supplementary information about the pathogenesis of diseases. *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 [7]. 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 [8]. The objective of the present study was to investigate and validate the antilithiatic property of *Grewia asiatica* extract in experimentally induced urolithiasis in rats.

MATERIALS AND METHODS:

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, Government ayurvedic dispensary, 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 [9, 10].

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/ CP CSEA).

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=5). 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 [11]. The percent increase in reducing power was calculated using the following equation,

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

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 [12].

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

1. 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[13].

2. 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 10mg 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 [14].

***In vivo* antilithiatic model:**

Ethylene glycol induced urolithiasis model: Animals were divided into eight different groups

containing six animals each. Ethylene glycol (0.75% v/v) in drinking water was fed to all groups except control for induction of renal calculi till the 28th 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 (750 mg/kg) from 1st day till 28th day while in the curative study all groups except control received extract (200 & 400 mg/kg, *p.o*) and cystone (750 mg/kg) from 15th day till 28th day [15]. During the study animals were allowed free access to food.

Assessment of antilithiatic activity:

Serum analysis: The blood was collected on 0th, 7th, 14th, 21st and 28th 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 and creatinine.

Collection and analysis of urine: All the animals were kept in individual metabolic cages and urine samples were collected on 0th, 7th, 14th, 21st and 28th day of calculi induction treatment. The volume of urine and pH of the urine were measured.

Histopathological study: The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and rinsed in ice cold physiological saline. One of the kidneys was fixed in 10% neutral buffered formalin, processed in a series of graded alcohol and xylene, embedded in paraffin wax, sectioned at 5 µm and stained with haematoxylin and eosin for histopathological examination. The slides were examined under light microscope to study microscope architecture of the kidney and calcium oxalate deposits [16].

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:

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 32.5µg/mL in reducing power assay and 21.5µg/mL in H_2O_2 scavenging assay. The potential of the extract was comparable to that of standard ascorbic acid.

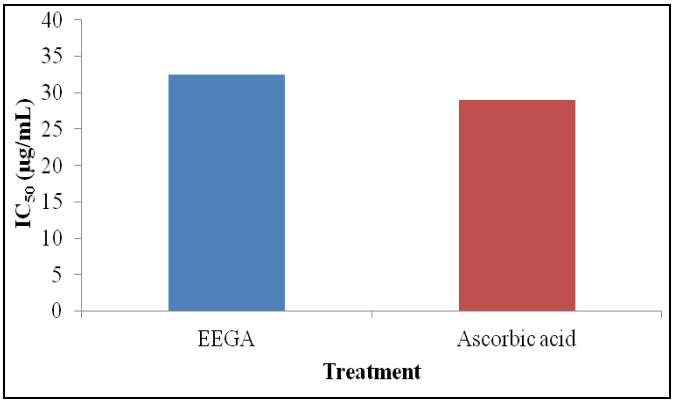

FIG. 1: REDUCING POWER ASSAY FOR ETHANOLIC EXTRACT OF *GREWIA ASIATICA*.

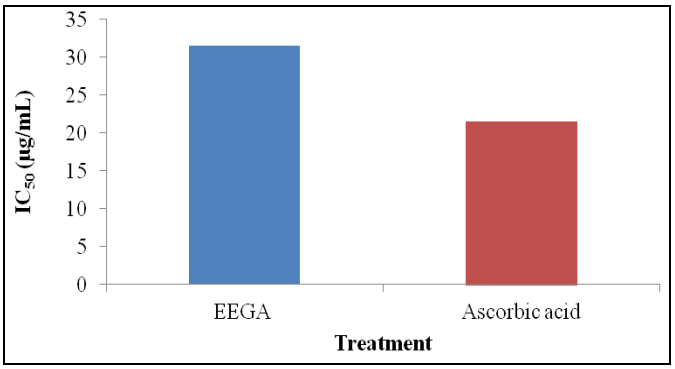

FIG. 2: HYDROGEN PEROXIDE RADICAL SCAVENGING ASSAY FOR ETHANOLIC EXTRACT OF *GREWIA ASIATICA*.

***In vitro* antilithiatic activity:** *In vitro* antilithiatic activity of *Grewia asiatica* was performed by 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: *IN VITRO* ANTILITHIATIC ACTIVITY OF ETHANOLIC EXTRACT 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%

In vitro antilithiatic activity of *Grewia asiatica* was also performed by homogenous precipitation method. In this 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: *IN VITRO* ANTILITHIATIC ACTIVITY OF ETHANOLIC EXTRACT 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

Renal function was assessed by measuring serum urea, uric acid and creatinine (**Table 3**). These levels were significantly ($p<0.05$ vs. Group-I) elevated in lithiatic control (Group-II) when compare 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 reverse these parameters closer to standard drug Cystone. The effect of EEGA in preventive regimen was found to be better than that of curative regimen.

Volume of urine and its pH were found to alter significantly ($p<0.05$ vs. Group-I) 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. Similarly, effect of EEGA was found to be better in prophylactic regimen than curative regimen.

TABLE 3: EFFECT OF *GREWIA ASIATICA* EXTRACT ON SERUM PARAMETERS IN RATS

Parameter	Day	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI	Group-VII	Group-VIII
Urea	0th	60±0.57	61±0.88	60±0.78	60±1.05	61±1.05	62±1.26	61±1.10	60±1.26
	7th	60±0.23	73±1.20	74±0.88*	72±1.02*	73±1.20 *	78+1.08 *	75+1.24 *	73±1.17 *
	14th	61±0.57	89±1.20 *	80±0.88 *	77±0.88 *	70±0.97 *	73±1.66 *	71±1.24 *	68±1.39 *
	21st	61±1.05	103±1.61 *	75±1.26 *	71±0.88 *	64±1.26 *	68±1.08 *	66±1.26 *	65±1.26 *
	28th	61±0.97	114±1.26 *	69±0.88 *	62±1.26 *	61±0.88 *	65±1.33 *	63±1.26 *	61±1.20 *
Uric acid	0th	1.87±0.23	2.09±0.06	2.53±0.09	2.63±0.01	2.13±0.05	2.88±0.02	2.06±0.01	2.16±0.01
	7th	1.93±0.03	3.43±0.09 *	2.91±0.03 *	2.27±0.02 *	2.34±0.03 *	2.63±0.02	2.61±0.01 *	2.29±0.01 *
	14th	1.95±0.01	3.59±0.03 *	2.69±0.06 *	2.71±0.02 *	2.09±0.03 *	3.09±0.02 *	2.15±0.01 *	2.60±0.01 *
	21st	1.97±0.01	3.72±0.03 *	2.86±0.03 *	2.18±0.01 *	2.46±0.02 *	2.94±0.02 *	2.55±0.01 *	2.31±0.01 *
	28th	1.98±0.02	3.72±0.03 *	2.75±0.01 *	2.44±0.02 *	2.27±0.02 *	2.70±0.01 *	2.30±0.01 *	1.99±0.01 *
Creatinine	0th	0.64±0.01	0.68±0.02	0.81±0.02	0.59±0.02	0.63±0.01	0.58±0.01	0.66±0.01	0.57±0.02
	7th	0.56±0.01	8.60±0.02 *	8.60±0.02 *	3.12±0.01 *	2.92±0.01 *	7.51±0.01	8.43±0.01 *	7.48±0.01 *
	14th	0.73±0.02	10.51±0.03 *	10.51±0.03 *	2.19±0.01 *	2.05±0.02 *	10.84±0.02 *	11.81±0.01 *	10.63±0.02 *
	21st	0.81±0.01	11.32±0.02 *	1.98±0.06 *	1.06±0.02 *	1.09±0.01 *	7.66±0.02 *	6.56±0.01 *	5.79±0.02 *
	28th	0.48±0.01	12.73±0.01 *	1.02±0.04 *	0.81±0.02 *	0.75±0.02 *	4.74±0.01 *	2.79±0.01 *	1.37±0.03 *
Volume of urine	0th	2.96±0.02	2.99±0.03	2.69±0.03	2.70±0.02	2.85±0.03	2.91±0.01	2.62±0.01	2.72±0.02
	7th	3.03±0.02	2.06±0.02 *	2.21±0.01 *	2.29±0.03 *	2.33±0.02 *	2.36±0.01	2.19±0.02 *	2.26±0.01 *
	14th	2.81±0.02	1.85±0.02 *	2.31±0.03 *	2.39±0.02 *	2.48±0.02 *	1.92±0.02 *	1.84±0.01 *	1.62±0.02 *
	21th	3.11±0.02	1.79±0.03 *	2.45±0.02 *	2.53±0.03 *	2.63±0.01 *	2.39±0.01 *	2.40±0.01 *	2.53±0.03 *
	28th	2.75±0.02	1.66±0.02 *	2.58±0.04 *	2.64±0.03 *	2.81±0.02 *	2.54±0.02 *	2.59±0.01 *	2.83±0.03 *
pH	0th	7.68±0.02	7.78±0.04	7.49±0.02	7.54±0.02	7.65±0.05	7.81±0.01	7.72±0.02	7.61±0.01
	7th	7.73±0.02	7.21±0.03 *	7.02±0.03 *	7.08±0.03 *	7.13±0.01 *	7.20±0.01	7.18±0.01 *	7.05±0.04 *
	14th	7.57±0.02	6.84±0.02 *	7.16±0.02 *	7.19±0.03 *	7.29±0.01 *	6.91±0.01 *	6.87±0.01 *	6.93±0.01 *
	21th	7.81±0.02	6.63±0.02 *	7.23±0.05 *	7.36±0.03 *	7.45±0.01 *	7.24±0.01 *	7.35±0.02 *	7.37±0.03 *
	28th	7.49±0.02	6.44±0.02 *	7.35±0.02 *	7.49±0.03 *	7.61±0.01 *	7.71±0.02 *	7.74±0.01 *	7.78±0.04 *

Histopathology: Histopathological studies of kidneys clearly revealed that the glomerulus and tubular region appeared normal without any calcium oxalate deposition.in normal control rats. Whereas the disease control rats showed severe deposition of calcium oxalate crystals along with marked periglomerular and tubular inflammation. But the kidney specimen from standard and extract treated groups showed characters similar to normal control group.

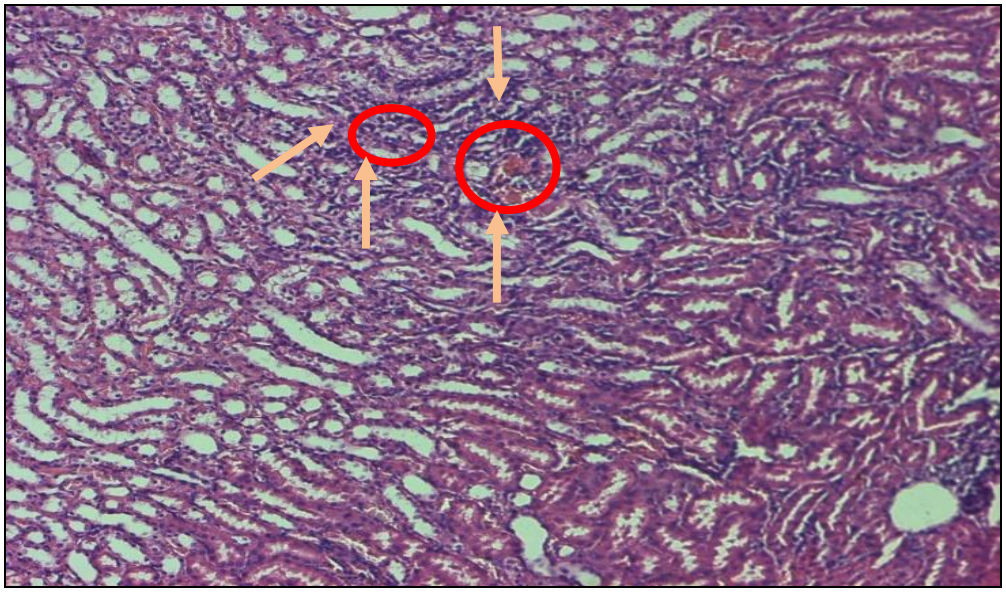

FIG. 3: NORMAL CONTROL

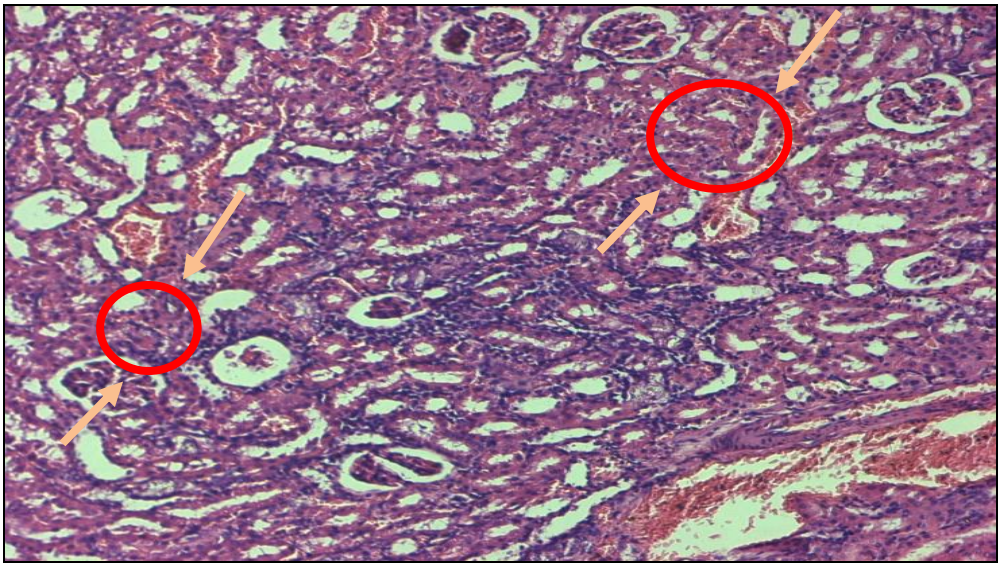

FIG. 4: DISEASE CONTROL

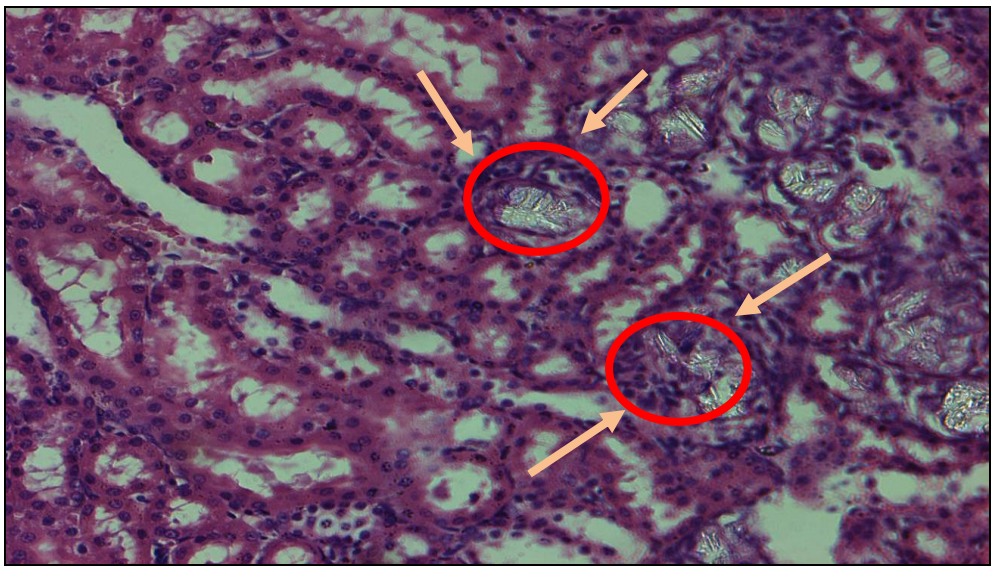

FIG. 5: PROPHYLACTIC REGIMEN 200 mg/kg bd.wt

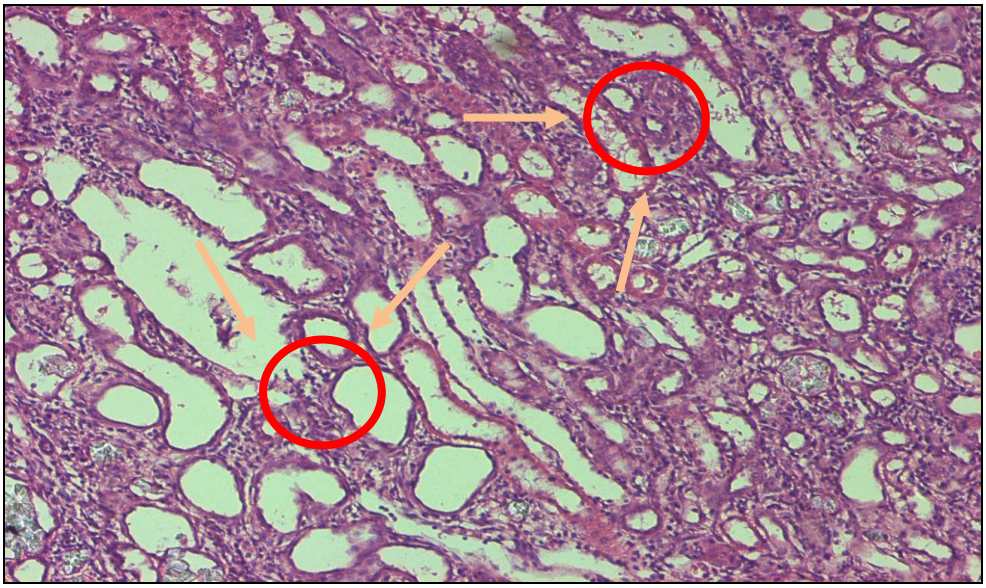

FIG. 6: PROPHYLACTIC REGIMEN 400 mg/kg bd.wt

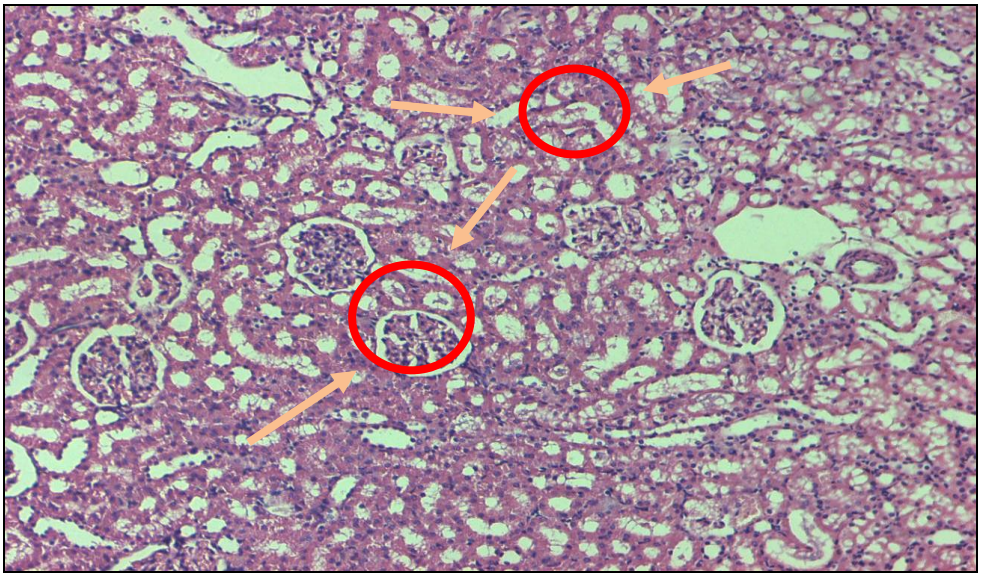

FIG. 7: PROPHYLACTIC REGIMEN CYSTONE (500 mg/kg bd.wt.)

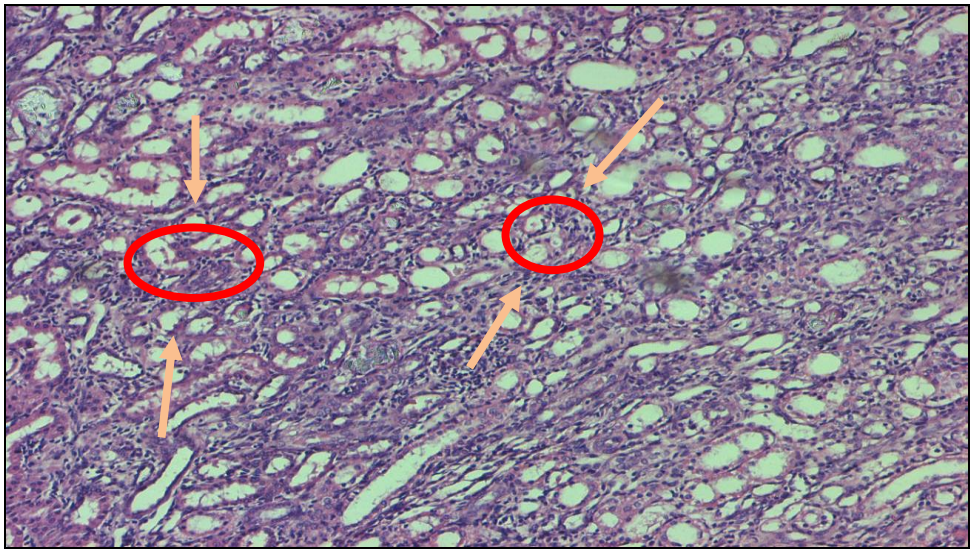

FIG. 8: THERAPEUTIC REGIMEN 200 mg/kg bd.wt

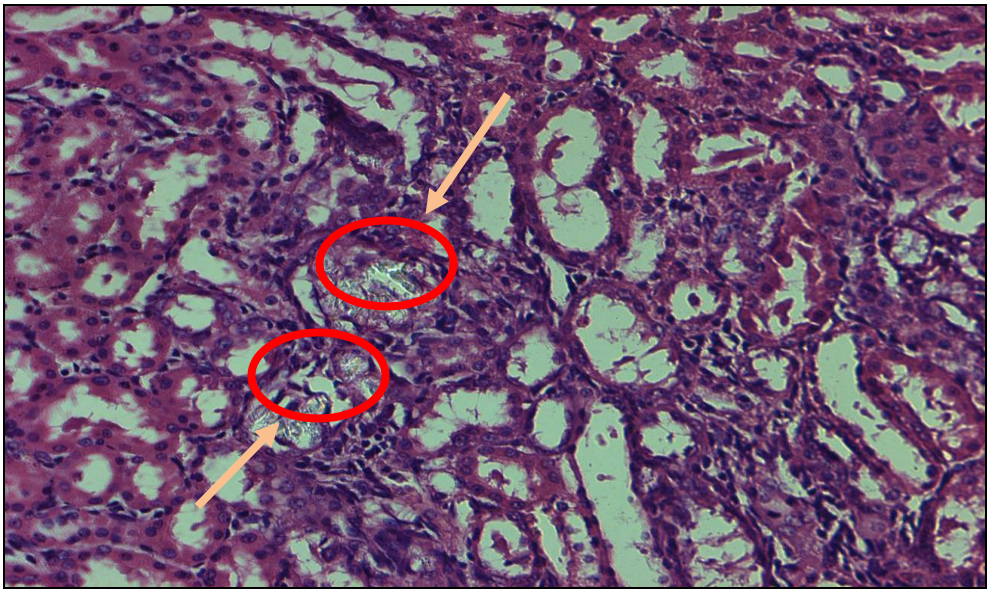

FIG. 9: THERAPEUTIC REGIMEN 400 mg/kg bd.wt

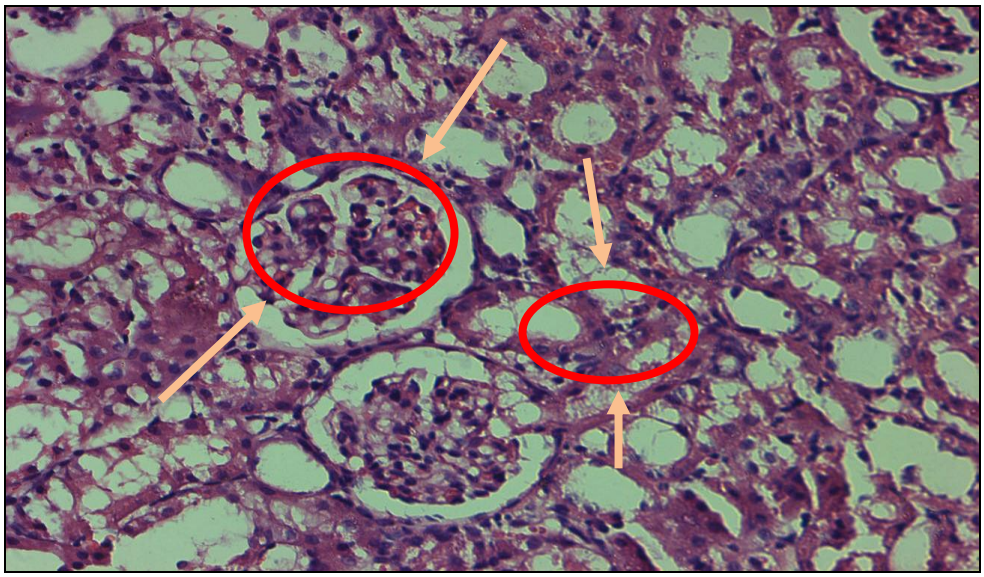

FIG. 10: THERAPEUTIC REGIMEN CYSTONE (500 mg/kg bd.wt)

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[17]. 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 [18].

On administration, ethylene glycol is rapidly absorbed and metabolized in liver via alcohol dehydrogenase or aldehyde dehydrogenase to glycolic acid. Glycolic acid is oxidized to glyoxalic acid which is further oxidized to oxalic acid/oxalate by glycolate oxidase or lactate dehydrogenase promoting hyperoxaluria [19]. Oxalate is attracted to cations like Ca^{2+} to form insoluble calcium oxalate. Calcium oxalate crystals and high oxalate levels in nephrons damages epithelial cells, including heterogenous crystal nucleation and causing aggregation of crystals [20]. Crystals in urine combine to form stone which is commonly because of super saturation of urine with crystalloids which depends on salt concentration, urinary volume and pH.

The glomerular filtration rate (GFR) is an important parameter for ensuring renal function and

it gets decreased in urolithiasis due to the obstruction to the outflow of the urine by stones in urinary system, which leads to a rise in nitrogenous waste products like urea, uric acid and creatinine [18, 21]. In disease control rats (Group-II) marked renal damage was seen by elevated serum levels of urea, uric acid and creatinine. However treatment with EEGA and Cystone restored the elevated levels of serum urea, uric acid and creatinine indicating their nephroprotective activity. However high dose of test extract (400 mg/kg) gave comparatively better effect than low dose (200 mg/kg).

In urolithiasis, urine output decreases due to the obstruction by the stones in the urinary system [22, 23]. Due to this urine volume decreases which was observed in disease control (Group-II). Urine usually acidic which is inhibitory to crystallization but, hyperoxaluria results in alkalization of urine pH as observed in disease control group[24]. Treatment with standard and test drugs improved urine output and prevented a shift of pH from acidic to alkaline suggesting that it prevents the precipitation of calcium oxalate.

Microscopic examination of the kidney sections derived from ethylene glycol induced urolithic rats showed polymorphic irregular crystal deposits inside the tubules which causes dilation of proximal tubules along with interstitial inflammation that might be attributed to oxalate. Co-treatment with the EEGA and cystone decreased number and size of calcium oxalate deposits in different parts of the renal tubules and also prevented damage to the tubules indicated by mild or absence of tubular inflammation.

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. This study affirms the assertions made regarding the effectiveness of the extract of this plant against urinary pathologies in the Indian folk medicine i.e. as an antilithiatic drug.

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