

ANTIUROLITHIATIC ACTIVITY OF ETHANOLIC EXTRACT OF *EICHHORNIA CRASSIPES* (MART.) SOLMS WHOLE PLANT ON ETHYLENE GLYCOL-INDUCED UROLITHIASIS IN WISTAR RATS

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Received: 20 February 2021, Revised and Accepted: 10 January 2022

ABSTRACT

Objective: In India Indigenous system of medicine, the plant *Eichhornia crassipes* (Mart.) Solms is claimed to be useful for different ailments. The purpose of this study is to investigate the significant activity of the ethanolic extract of *E. crassipes* (Mart.) Solms whole plant in the treatment of renal stone by ethylene glycol (EG)-induced urolithiasis in Wistar rats.

Methods: Wistar rat will be divided into four groups. All the animals received EG 0.75%v/v by orally for 28 days. Group I was received 0.75%v/v EG for 28 days. Groups II, III, and IV were received cystone, ethanolic extract of *E. crassipes* (Mart.) Solms (200 mg/kg, and 400 mg/kg), respectively, administered orally from 15th to 28th day for curative regimen. Animals were housed in metabolic cages and collect the 24 h urine samples and serum samples on the 7th, 14th, 21th, and 28th day of the experiments. Under the microscopical study to observe the crystals in urine sample. The biochemical parameters monitored in the present study are calcium, oxalate, magnesium, phosphorus, urea, creatinine, uric acid, blood urea nitrogen (BUN), and lactate dehydrogenase (LDH) in urine and serum samples.

Results: The EG feeding resulted in elevated renal excretion of calcium, phosphate, oxalate, and decreased renal excretion of magnesium as well-elevated serum BUN, creatinine, uric acid, urea, LDH levels, and decreased the urine pH and urine volume. Treatment of the ethanolic extract of *E. crassipes* (Mart.) Solms significantly reduced the elevated calcium, phosphate, oxalate in urine as well as BUN, creatinine, uric acid, urea, and LDH level in serum. It also restores the normal urine pH and promotes the urine output.

Conclusion: Ethanolic extract of *E. crassipes* (Mart.) Solms exhibited significant anti-urolithiatic activity evident from urine calcium, phosphate, oxalate levels and serum BUN, creatinine, uric acid, urea and LDH levels.

Keywords: Urolithiasis, *Eichhornia crassipes* (Mart.) Solms, Ethylene glycol, Urine volume.

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INTRODUCTION

Today urological practice, urinary stone continues to occupy an important place. Urinary stones mostly affect 10–12% of the population in industrialized countries. Epidemiological studies revealed that nephrolithiasis is highly present in men (12%) than in women (6%). Urinary stone is more prevalent between the ages of 20 and 40 in both sexes [1]. Especially, calcium-containing stones such as calcium oxalate monohydrate, calcium oxalate dihydrate and basic calcium phosphate are the most commonly occur ones to an extent of 75–90%. Most commonly occurring stones are calcium oxalate or magnesium ammonium phosphate type [2]. Ethylene glycol (EG)-induced hyperoxaluria in animal model to use the experimental stone formation [3]. EG-induced kidney toxicity occurs within 24–72 h of post-ingestion. The glycolic acid is then metabolized to glyoxylic acid and finally to oxalic acid. Oxalic acid binds with calcium to form calcium oxalate crystals which may deposit in the kidney [4]. This can result in hematuria and proteinuria, increased creatinine, and renal failure [5]. Open surgical procedures for the treatment of ureteric stones have gradually disappeared in the past 30 years and have been replaced by minimal invasive techniques such as extracorporeal shock wave lithotripsy (ESWL) and ureteroscopy (URS). ESWL might show some significant side effects such as tissue damage to the kidney [6]. ESWL has been associated with long-term medical effects such as diabetes mellitus and hypertension. URS produced ureteric perforation and stricture formation remains around 2–4% [7].

According to the WHO estimate, 80% of population living in the developing countries almost exclusively uses herbal medicines. Herbal medicines are in great demand in the developed world for primary health-care

system. Herbal medicines are better compatible with human, because of their efficacy, safety, and lesser side effects. In India, Ayurvedic system of medicine "Pashanabheda" (Pashana = stone; Bheda = break) group of plants break up the urinary stones and increase the urine output. This information on the present trends in research on plants accredited with antiurolithiatic activity [8]. *Eichhornia crassipes* (Mart.) Solms is a free-floating perennial aquatic plant (or hydrophyte), herbaceous monocotyledon member of the pickerelweed family (*Pontederiaceae*) is native to tropical and subtropical South America. The plant is locally known as "aahaya thamarai or vengaya thamarai." The nutritionally important compounds such as phenolic, flavonoids, alkaloid, sterols, terpenoids, glycosides, and many other metabolites have been isolated from different parts of *E. crassipes* (Mart.) Solms and possess various pharmacological activities such as sedative, central nervous system depressant, analgesic, anti-epileptic, antidepressant, memory enhancing agents, herbicidal, wound healing, anti-cancer, larvicidal, anti-inflammatory, immunostimulant, antibacterial, and cytotoxic activity. It can be used in agriculture as a fertilizer, feed, biomanure, and anti-diarrheal-induced urination [9–18]. The antiurolithiatic activity of the ethanolic extract of *E. crassipes* (Mart.) Solms whole plant on EG-induced urolithiasis in Wistar rats was evaluated presently.

METHODS**Plant material**

The whole plant of *E. crassipes* was collected from their natural habitats in Kooduthurai River, Bhavani, Erode District, Tamil Nadu, in the month of August 2017. It was authenticated by Dr. K.N. Sunil Kumar. RO and HOD, Pharmacognosy, Siddha Central Research Institute, Arumbakkam, Chennai-600106, Tamil Nadu.

Preparation of ethanolic extract

The whole plants of *E. crassipes* were cleaned and chopped into small pieces and dried under shade. The coarse powder of plant was obtained by mechanical grinding. The powdered material (40 g) was subjected to continue hot extraction in Soxhlet apparatus at a temperature of 60–70°C using ethanol (95% v/v) as solvent. After complete extraction, the extract was dried. The extract was suspended in distilled water using 0.5% acacia as suspending agent for oral administration to animals [19].

Experimental animals

The present study was conducted as per CPCSEA/IAEC approval no: 1917/ReBi/S/16/CPCSEA/25.10.2016 and 5/AEL/IAEC/MMC, Date: 12.9.2017. The Wistar albino rats (150–200 g) used for this study were produced from Animal Experimental Laboratory, Madras Medical College, Chennai, India. The animals are kept in polypropylene cages (each cage contains six animals) under the standard laboratory condition (12 h light/dark cycle) and had free access to commercial pellet diet with water *ad libitum*. The animal house temperature was maintained at 25±2°C with relative humidity at 50±15%.

Acute toxicity study [20]

The acute toxicity study has been carried out already. The non-toxic nature of the ethanol extract of *E. crassipes* is evident from the acute oral toxicity conducted as per OECD guidelines (Organization of Economic Co-operation and development) 423 (Acute Toxic Class Method). The normal behavior of the test animals during a period of 14 days suggests the non-toxic nature of the extracts. Hence, *E. crassipes* should be safe up to the dose of 2000 mg/kg body weight of the animal.

Pharmacological screening for antiurolithiatic activity [21,22]

Animals were divided into four groups, each containing six animals. Groups I–IV animals were fed with 0.75% EG in water to induce renal calculi till the 28th day. Group II animals were received standard antiurolithiatic drug cystone (750 mg/kg body weight) from 15th to 28th day. Groups III and IV animals served as curative regimen and received ethanolic extract of *E. crassipes* at a dose of 200 mg/kg and 400 mg/kg body weight from 15th day to 28th day. The extracts are administered once daily by oral route.

Assessment of antiurolithiatic activity

Collection and analysis of urine

Urine samples were collected on the 7th, 14th, 21th, and 28th day for 24 h by keeping the animals in individual propylene metabolic cages. Animals have free access to drinking water during the urine collection period.

The collected urine was analyzed for calcium, oxalate, phosphate, and magnesium using standard methods. The volume of urine collected from all groups was recorded and urine pH was also recorded.

Serum analysis

After the experimental period, the blood was collected from the retro orbital puncture of rat eye under ether anesthesia. Serum was separated by centrifugation of the blood samples at 10,000 rpm for 10 min and analyzed for creatinine, uric acid, urea, and blood urea nitrogen (BUN) using standard methods.

Statistical analysis [19]

Results were expressed as Mean±Standard Error of Mean (SEM). $p < 0.05$ was considered as statistically significant. Data obtained were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test using GraphPad Prism version 7.

RESULTS AND DISCUSSION

In the present study, chronic induction of EG (0.75% v/v) to Wistar rats were resulted significant ($p \leq 0.0001$) increase in the 7th and 14th day of urinary excretion of calcium, phosphate, and oxalate and reduction of magnesium levels in Groups I, II, III, and IV rats. On day 21 and 28, whereas the cystone-treated Group II animals were shown significant ($p \leq 0.0001$) reduction in calcium, phosphate, and oxalate and increase magnesium levels in urine. Similarly, treatment with ethanolic extract of *E. crassipes* whole plant at the dose of 200 mg/kg and 400 mg/kg was administered for Groups III and IV, respectively, and then significant ($p \leq 0.0001$) lowered the elevated levels of calcium, oxalate, phosphate, and increase magnesium level in urine, when curative regimens compared to EG-induced Group I animals (Table 1 and Figs. 1-4).

On the day 7 and 14, urine volume and pH were significantly ($p \leq 0.0001$) reduced in Groups I, II, III, and IV animals, while the urine volume and pH were significantly ($p \leq 0.0001$) increase in cystone-treated Group II animals. However, on days 21 and 28, urine volume and pH were significantly ($p \leq 0.0001$) increased in curative regimen groups (III and IV). However, on days 21 and 28, urine volume and pH were significantly ($p \leq 0.0001$) increased in curative regimen groups (III and IV) when compared to EG-induced Group I animals (Table 2, Figs. 5 and 6).

On the day 7 and 14, BUN, serum creatinine, urea, uric acid levels, and lactate dehydrogenase (LDH) level were significantly ($p \leq 0.0001$)

Table 1: Estimation of calcium, oxalate, phosphate, and magnesium in urine

Urine parameters (mg/dl)	Group	Ethylene glycol induction				
		Before	After			
			7 th day	Curative regimens		
0 th day	14 th day	21 th day	28 th day			
Calcium	Disease control	7.66±0.22	8.18±0.24	8.61±0.33	9.02±0.13	11.13±0.36
	Standard	8.18±0.24	8.73±0.04*	9.02±0.14 ^{ns}	8.52±0.04*	8.16±0.06****
	Low dose	7.42±0.06	8.23±0.04 ^{ns}	8.71±0.06 ^{ns}	8.36±0.08**	8.18±0.25****
	High dose	7.22±0.06	8.04±0.16 ^{ns}	8.43±0.10 ^{ns}	8.32±0.16****	7.78±0.08****
Oxalate	Disease control	1.36±0.05	2.20±0.25	3.03±0.07	4.41±0.06	5.35±0.05
	Standard	1.57±0.12	2.35±0.19 ^{ns}	2.59±0.28 ^{ns}	2.15±0.23****	1.88±0.20****
	Low dose	1.46±0.08	1.86±0.07 ^{ns}	2.05±0.06***	1.74±0.07****	1.43±0.06****
	High dose	1.55±0.06	1.94±0.08 ^{ns}	2.13±0.11**	1.82±0.08****	1.62±0.09****
Phosphate	Disease control	4.87±0.14	6.16±0.13	7.22±0.18	7.96±0.10	8.46±0.11
	Standard	4.4±0.15	4.93±0.07****	5.34±0.09****	4.83±0.06****	4.46±0.08****
	Low dose	4.4±0.12	4.90±0.14****	5.23±0.09****	4.95±0.07****	4.65±0.10****
	High dose	4.67±0.19	5.05±0.14****	5.31±0.14****	4.96±0.13****	4.58±0.15****
Magnesium	Disease control	3.07±0.01	2.97±0.01	2.88±0.01	2.66±0.05	1.75±0.04
	Standard	3.06±0.01	2.89±0.01****	2.66±0.05****	2.79±0.02*	2.86±0.03****
	Low dose	3.08±0.01	2.97±0.01 ^{ns}	2.88±0.01 ^{ns}	2.9±0.01****	2.94±0.02****
	High dose	3.08±0.01	2.98±0.01 ^{ns}	2.79±0.02*	2.94±0.02****	3.02±0.02****

Values were expressed as Mean±SEM, n=6. Comparisons were made between: Group-I versus Group II, Group-III, and Group- IV. The data were statistically analyzed by one-way ANOVA, followed by Dunnett's multiple comparison test. P values **** $p \leq 0.0001$, *** $p \leq 0.001$, ** $p \leq 0.01$, and * $p \leq 0.05$. ns: Non-significant $p > 0.05$

Table 2: Estimation of pH and volume in urine

Parameters	Group	Ethylene glycol induction				
		Before	After			
			0 th day	7 th day	Curative regimens	
				14 th day	21 th day	28 th day
Urine volume (ml)	Disease control	2.53±0.19	2.38±0.21	2.06±0.17	1.67±0.16	1.25±0.22
	Standard	2.38±0.05	2.20±0.12 ^{ns}	1.96±0.09 ^{ns}	5.23±0.19 ^{****}	5.67±0.16 ^{****}
	Low dose	2.35±0.04	2.33±0.07 ^{ns}	2.16±0.10 ^{ns}	4.75±0.20 ^{****}	5.21±0.03 ^{****}
	High dose	2.55±0.31	2.36±0.08 ^{ns}	1.96±0.08 ^{ns}	5.98±0.18 ^{****}	6.23±0.15 ^{****}
Urine pH	Disease control	7.18±0.03	6.82±0.16	6.63±0.08	6.5±0.07	6.47±0.08
	Standard	7.15±0.02	6.97±0.20 ^{ns}	6.9±0.11 ^{ns}	7.27±0.08 ^{****}	7.13±0.07 ^{****}
	Low dose	7.23±0.02	7.03±0.08 ^{ns}	7.17±0.08*	7.25±0.03 ^{****}	7.2±0.04 ^{****}
	High dose	7.25±0.03	7.08±0.16 ^{ns}	6.73±0.16 ^{ns}	7.23±0.06 ^{****}	7.33±0.02 ^{****}

Values were expressed as Mean±SEM, n=6, Comparisons were made between: Group-I versus Group II, Group-III, and Group-IV. All the data were statistically analyzed by one-way ANOVA, followed by Dunnett's multiple comparison test. P values ****p<0.0001 ***p<0.001, **p<0.01, and *p<0.05. ns: Non-significant-p>0.05

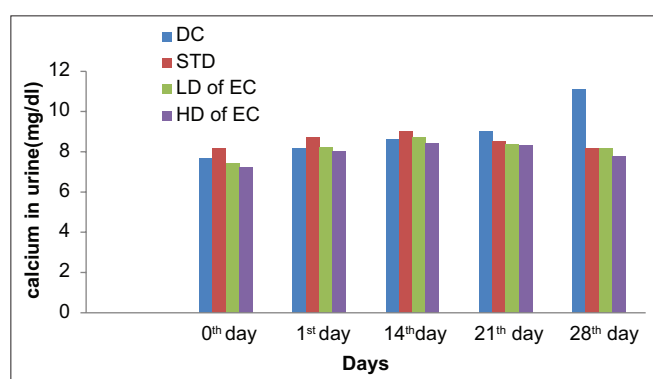


Fig. 1: Estimation of calcium in urine

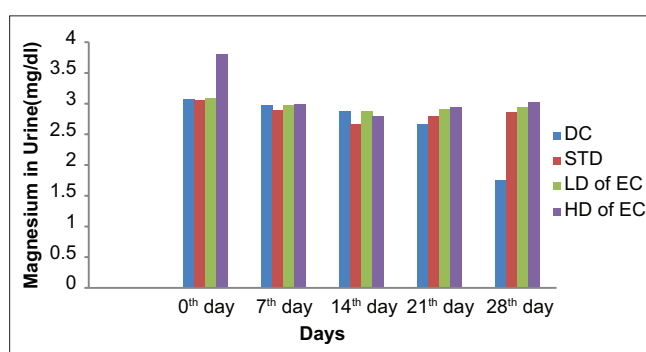


Fig. 4: Estimation of magnesium in urine

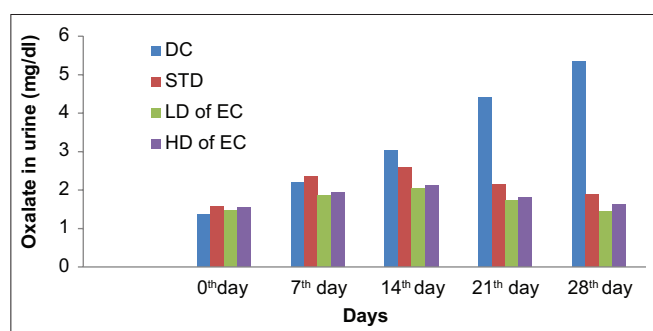


Fig. 2: Estimation of oxalate in urine

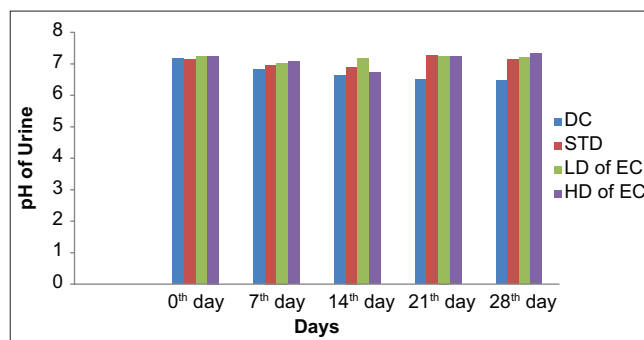


Fig. 5: Estimation of pH

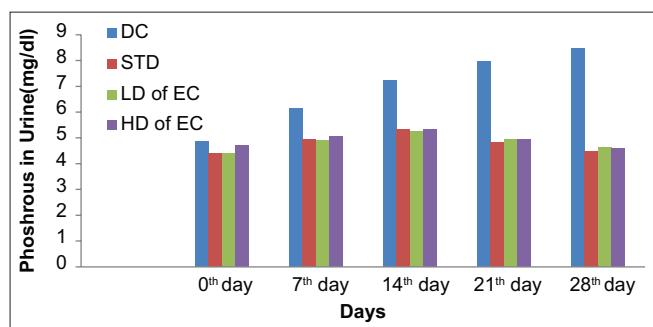


Fig. 3: Estimation of phosphate in urine

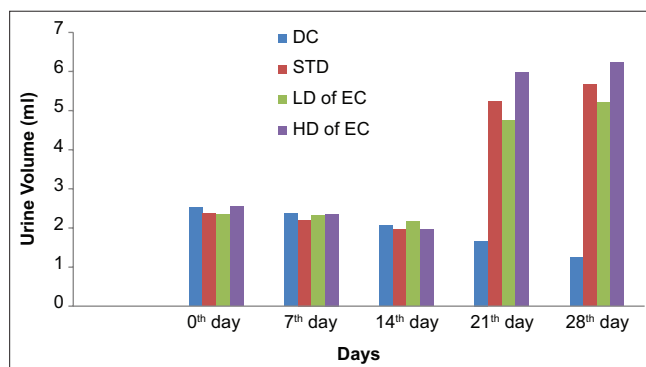


Fig. 6: Estimation of urine volume

Table 3: Estimation of serum urea, uric acid, creatinine, and BUN

Serum parameters (mg/dl)	Group	Ethylene glycol induction				
		Before	After			
			0 th day	7 th day	Curative regimens	
				14 th day	21 th day	28 th day
Urea	Disease control	9.51±0.12	10.21±0.07	11.54±0.25	13.57±0.26	15.05±0.14
	Standard	9.74±0.21	10.04±0.21 ^{ns}	10.95±0.18 ^{***}	10.39±0.19 ^{****}	9.90±0.12 ^{****}
	Low dose	9.50±0.91	9.84±0.06 ^{ns}	10.22±0.06 ^{****}	9.76±0.06 ^{****}	9.54±0.10 ^{****}
	High dose	9.64±0.10	9.97±0.07 ^{ns}	10.13±0.04 ^{****}	9.9±0.047 ^{****}	9.5±0.14 ^{****}
Uric acid	Disease control	2.40±0.03	2.87±0.03	3.13±0.03	3.54±0.07	4.14±0.19
	Standard	2.39±0.06	2.57±0.06 ^{**}	2.82±0.04 ^{****}	2.56±0.04 ^{****}	2.32±0.04 ^{****}
	Low dose	2.47±0.08	2.77±0.03 ^{ns}	3.07±0.05 ^{ns}	2.85±0.08 ^{****}	2.48±0.06 ^{****}
	High dose	2.46±0.03	2.81±0.03 ^{ns}	3.19±0.01 ^{ns}	2.98±0.05 ^{****}	2.58±0.08 ^{****}
Creatinine	Disease control	0.59±0.02	0.66±0.01	0.72±0.01	0.75±0.01	0.77±0.01
	Standard	0.64±0.01	0.64±0.01 ^{ns}	0.68±0.01 ^{ns}	0.61±0.06 ^{****}	0.54±0.01 ^{****}
	Low dose	0.57±0.01	0.60±0.01 ^{**}	0.63±0.01 ^{***}	0.59±0.01 ^{****}	0.53±0.01 ^{****}
	High dose	0.58±0.02	0.60±0.01 ^{**}	0.63±0.01 ^{****}	0.57±0.01 ^{****}	0.52±0.01 ^{****}
BUN	Disease control	4.87±0.14	6.16±0.13	7.22±0.18	7.96±0.10	8.46±0.11
	Standard	4.4±0.15	4.93±0.07 ^{****}	5.34±0.09 ^{****}	4.83±0.06 ^{****}	4.46±0.08 ^{****}
	Low dose	4.4±0.12	4.90±0.14 ^{****}	5.23±0.09 ^{****}	4.95±0.07 ^{****}	4.65±0.10 ^{****}
	High dose	4.67±0.19	5.05±0.14 ^{****}	5.31±0.14 ^{****}	4.96±0.13 ^{****}	4.58±0.15 ^{****}

Values were expressed as Mean±SEM, n=6, Comparisons were made between: Group-I versus Group II, Group-III, and Group-IV. The data were statistically analyzed by one-way ANOVA, followed by Dunnett's multiple comparison test. P values ****p<0.0001, ***p<0.001, **p<0.01, and *p<0.05. ns: Non-significant) p>0.05, BUN: Blood urea nitrogen

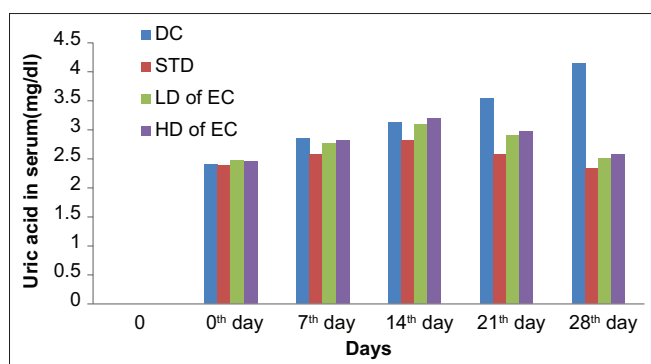


Fig. 7: Estimation of serum uric acid

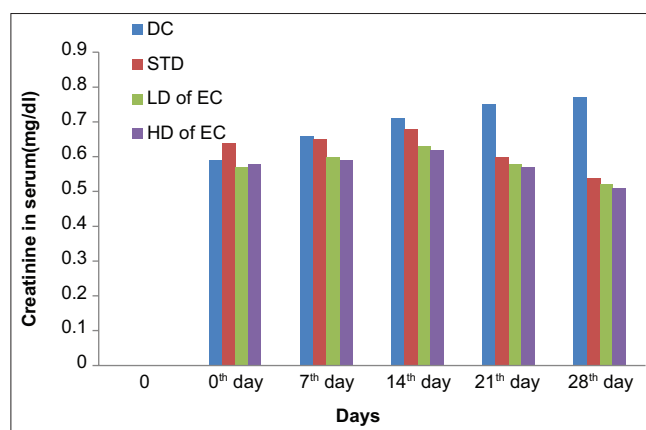


Fig. 9: Estimation of serum creatinine

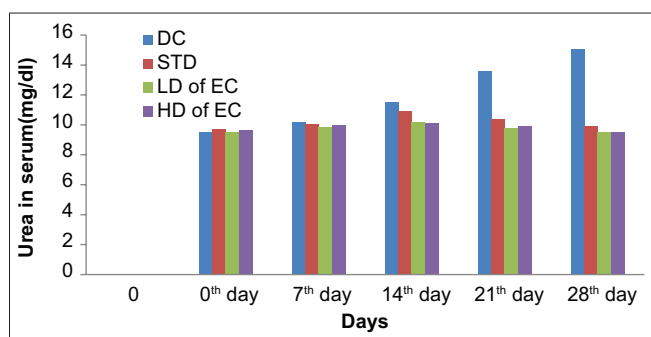


Fig. 8: Estimation of serum urea

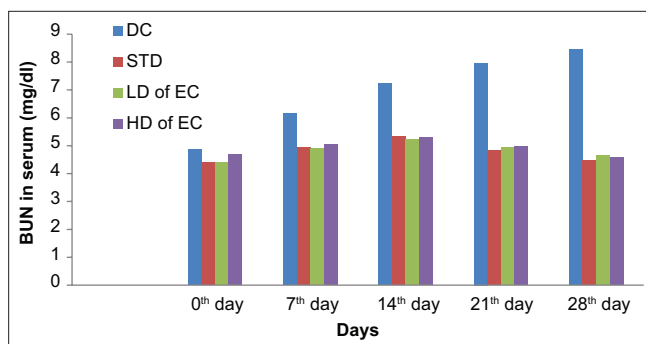


Fig. 10: Estimation of serum blood urea nitrogen

increased in Groups I, II, III, and IV animals. On the day 7 and 14, BUN, serum creatinine, urea, uric acid levels and lactate dehydrogenase (LDH) level were significantly (p<0.0001) increased in Groups I, II, III, and IV animals, while the BUN, serum creatinine, uric acid, urea and lactate dehydrogenase (LDH) levels were significantly (p<0.0001) decreased in cysteine-treated Group II animals. However, on days 21

and 28, the BUN, serum creatinine, urea, uric acid levels, and LDH level were significantly (p<0.0001) decreased in curative regimen groups (III and IV). However, on days 21 and 28, the BUN, serum creatinine, urea, uric acid levels, and LDH level were significantly (p<0.0001) decreased in curative regimen groups (III and IV) when compared to EG-induced Group I animals (Tables 3 and 4, Figs. 7-11).

Table 4: Estimation of serum LDH

Serum parameter ($\mu\text{mole/L}$)	Group	Ethylene glycol induction					
		Before	After				
			0 th day	7 th day	Curative regimens		
					14 th day	21 th day	28 th day
LDH	Disease control	174 \pm 0.58	182 \pm 0.60	199 \pm 1.08	231 \pm 1.58	259 \pm 2.06	
	Standard	173 \pm 0.61	179 \pm 0.60 ^{ns}	185 \pm 0.96 ^{****}	181 \pm 0.86 ^{****}	176 \pm 0.76 ^{****}	
	Low dose	173 \pm 0.61	181 \pm 0.85 ^{ns}	185 \pm 0.97 ^{****}	179 \pm 0.60 ^{****}	174 \pm 0.67 ^{****}	
	High dose	174 \pm 0.57	182 \pm 0.60 ^{ns}	185 \pm 0.66 ^{****}	177 \pm 0.33 ^{****}	170 \pm 0.59 ^{****}	

Values were expressed as Mean \pm SEM, n=6. Comparisons were made between: Group-I versus Group II, Group-III, and Group- IV. The data were statistically analyzed by one-way ANOVA, followed by Dunnett's multiple comparison test. P values ****p \leq 0.0001, ***p \leq 0.001, **p \leq 0.01, and *p \leq 0.05. ns: Non-significant-p>0.05, LDH: Lactate dehydrogenase

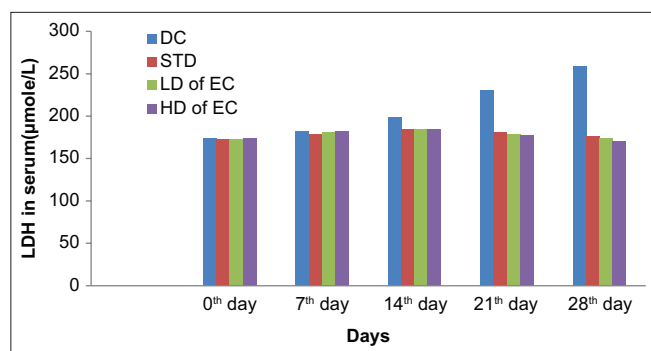


Fig. 11: Estimation of serum lactate dehydrogenase

CONCLUSION

The study was revealed that the ethanolic extract of *E. crassipes* (Mart.) Solms whole plant was found to possessed potent antiurolithiatic activity. However, further evaluation of the mechanism of action of isolated compound from this plant and clinical trials may yield a solution for this urinary problem.

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