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RESEARCH ARTICLE

Evaluation of Antiurolithiatic activity of Ethanolic Extract of *Alphonsea Sclerocarpa*on Ethylene Glycol Induced Urolithiasis in Rats

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ABSTRACT

Kidney stones are one of the most painful of the urologic disorders. Renal stone affect 5% to 15% of adults. In the present study the roots of *Alphonsea sclerocarpa* Linn were used forantiurolithiatic activity. *In vivo* study the effect of an ethanolic root extract of *M. esculenta*(150 mg/kg, p.o**.** and 300 mg/kg, p.o.) was studied in experimentally inducedrenal stone in rats. Ethylene glycol model (0.75 % in drinking water, for 28 days) was used for renal stone induction. After completion of experimental studyblood, urine and kidney sample was used for various parameters. In preliminary phytochemical screening and TLC analysis identifies presence of saponinsand flavanoids in ethanolic extract of *A. sclerocarpa*roots. Inethylene glycol treated animal modelethanolic extract of *A. sclerocarpa*rootsshowed significant results on stone promoters (Calcium Oxalate, Inorganic Phosphate and Sodium), Kidney function parameters (Uric acid, BUN, Creatinine and LDH) and antioxidant parameters (Lipid peroxidation, catalase and glutathione).Conclusion of this investigation was ethanolic extract of *A. sclerocarpa*roots shown promising antiurolithiatic activity and support folklore claims of theseplants as antiurolithiatic. The mechanism of action of these plants for antiurolithiatic isapparently related to increased diuresis and lowering of urinary concentrations of stoneforming constituents, though it should be confirmed by the extensive exploratory studies.

Keywords: Kidney stones, *Alphonsea sclerocarpa,* Ethylene glycol, anti-urolithiatic activity.

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CONTENTS

1. Introduction

Urolithaisis or nephrolithiasis represents the clinical condition of kidney stone disease. Stone formation in the urinary tract has been recognized for thousands of years, but during the last few decades the pattern and incidence of the disease have changed markedly. Urinary stones affect 10–12% of the population in industrialized countries. The incidence of urinary stones has been increasing over the last years while the age of onset is decreasing. With a prevalence of > 10% and an expected recurrence rate of ~ 50%, stone disease has an important effect on the healthcare system. Once recurrent, the subsequent relapse risk is raised and the interval between recurrences is shortened. Features associated with recurrence include a young age of onset, positive family history, infection stones and underlying medical conditions. Epidemiological studies revealed that nephrolithiasis is more common in men (12%) than in women (6%) and is more prevalent between the ages of 20 to 40 in both sexes (Worcester & Coe, 2008). The etiology of this disorder is multifactorial and is strongly related to dietary lifestyle habits or practices.

Herbal Treatment:

Urolithiasis is still a mysterious disease even after extensive research in Urology. Sophisticated instruments, investigations etc., have failed to trace cause and mechanism of urolithiasis. But, few researches concluded in recent times revealed various factors, which are responsible in manifesting this condition. The treatment for this condition in modern medicine is not only expensive but also not easily affordable to the needy poor. Actually, there are no satisfactory drugs in modern medicine which can dissolve the stone and the physicians' remains to be depends on alternative systems of medicine for better relief.

Plant Profile:

Alphonsea sclerocarpa is a species of flowering plant of Annonaceae that is native to Mexico, Central America and Northern South America. It is introduced and extensively naturalized in the Caribbean, Florida, Guam and south East Asia like Philippines. In india is is extensively distributes over Andhra Pradesh, Tamil Nadu.

Plant profile : *Alphonsea Sclerocarpa*
Synonym : Thaluku mamidi
Family : Annonaceae
Parts used : Leaves, Root, Bark, Flower

Fig 1: Whole plant of *A. sclerocarpa*

2. Experimental work

Plant collection:

The leaves of *Alphonsea sclerocarpa* was collected from nearby forest Thalakona, Tirupati, India.

Preparation of plant extraction:

The roots ware washed and dried room temperature (35^0C) for 7 days and then crushed in to coarse powder using a mixer grinder. Particle sizes were separated using sieve number 24 38.

Extraction procedure:

The coarse powder was extracted using 100 % ethanol at $(45\text{-}50)^0$C. Coarse powder was packed in muslin cloth and then packed in soxhlet apparatus, heat was applied to the round bottom flask using through heating mantel .The process was continued for 120 hrs. The Ethanolic extract was concentrated and allowed to evaporate the solvent. The wet mass was kept in dessicator to remove the moisture content. The powder was weighed and kept aside for phytochemical screening and pharmacological evaluation.

Priliminary Phytochemical analysis:

The ethanolic extract is subjected to Phytochemical analysis using conventional protocol like alkaloids, flavonoids, carbohydrate and glycosides gums and mucilage, fixed oils, saponins and proteins.

Determination of physicochemical parameters:

Determination of physicochemical parameters such as total ash, acid insoluble ash, water-soluble ash, foreign organic matter and moisture content of both drugs were determined according to WHO guidelines on quality control methods for medicinal plant materials (WHO, 1992).

Pharmacological Studies

Experimental Animals:

Healthy adult male Wistar Albino rats (150-200gm) were obtained from the animal house of Mahaveer enterprises, Hyderabad. for study of antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions (temperature: 25 ± 5°C), humidity (55 ± 5%) and maintained on 12-h light: 12-h dark cycle. They were provided with regular rat chow and drinking water ad libitum.

Acute toxicity studies:

Acute oral toxicity study for the test extract of the *Alphonsea sclerocarpa* was carried out as per the guidelines set by Organization for Economic Co-operation and, revised draft (OECD) 425 and by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The study revealed that the administration of ethanolic extract of *Alphonsea sclerocarpa* was safe up to a dose of 2000 mg/kg. No death was observed up to this dose, and the experimental animals were physically active. By keeping $1/10^{th}$ (200 mg/kg) dose as highest, the doses of 100 mg/kg, and 200 mg/kg were selected as working doses for the present study.

Preparation of suspension of extracts:

The extracts were suspended in a dilute solution of Tween-80 before the dosing.

Induction of experimental urolithiasis:

Calcium oxalate urolithiasis was induced in experimental animals by administering ethylene glycol 0.75% (0.75 ml of

ethylene glycol in 100ml of drinking water) to rats for a period of 28 days for the production of calcium oxalate stone in rats.

Experimental Design:

Animals were divided into seven groups, each containing 6 animals.

- Group I - normal control.
- Group II to VII were fed with 0.75% ethylene glycol (EG) in water for induction of renal calculi till 28th day.
- Group III received standard antiurolithiatic drug Cystone (750 mg/kg body weight) from 15th to 28th day.
- Group IV - Ethanolic extract of *Alphonsea sclerocarpa* roots at a dose of 250 mg/kg body weight from 15th day to 28th day respectively.
- Group V Ethanolic extract of *Alphonsea sclerocarpa* roots at a dose of 500 mg/kg body weight from 15th day to 28th day respectively.

Study of Biochemical Parameters

Urine collection and analysis:

Rats were kept separately in metabolic cages and urine samples of 24 h were collected on 28th day. The urine samples were acidified with HCl. The acidified samples were titrated with 0.9494 N of $KMNO_4$ till a light pink colour end point was obtained for estimation of calcium and oxalate.

Serum collection and analysis:

Blood samples were collected from the retro-orbital plexus under light ether anesthesia and Serum was separated by centrifugation at 10,000rpm for 10minand analyzed for Urea, Uric acid, Creatinine and Calcium using commercially available diagnostic kits.

Histopathological Study of Rat Kidney:

To confirm the incidence of lithiasis, the animals were sacrificed and their kidneys were subjected to histopathological studies. The abdomen was incised and opened, and both kidneys were removed from each animal. Isolated kidneys were cleaned off extraneous tissue, weighed and rinsed with ice-cold normal saline. The left kidney was fixed with 10%v/v neutral formalin and after harvesting, sliced horizontally and sent to histology services (Light care Diagnostics, Vijayawada) for Hematoxylene and Eosin staining.

Data analysis: The data obtained by the various parameters was statistically evaluated by one way analysis of variance (ANOVA). The mean values ± SEM were calculated for each parameter. P<0.05 was considered significant.

3. Results and Discussion

Physicochemical Parameters:

The determination of physicochemical parameter is important in determination of adulterants and improper handling of drugs. Table 1 and table 2 show the result of various physicochemical parameter of powdered drug carried out using standard methods.

***In vivo* antiurolithiatic activity**

Ethylene glycol induced renal stone: There was significant decrease in animal weight and significant increase in dry and wet kidney weight in ethylene glycol induced model control as compared to control animals. Similarly significant decrease in urine output was observed in model control animals. These changes were significantly prevented as well as reversed by the treatment of *Alphonsea sclerocarpa* and Cystone respectively. In normal control animals, urinary pH was found acidic. But in model control animals, urine was found alkaline in nature. Treatment of test and standard drugs significantly reduced urinary pH level.

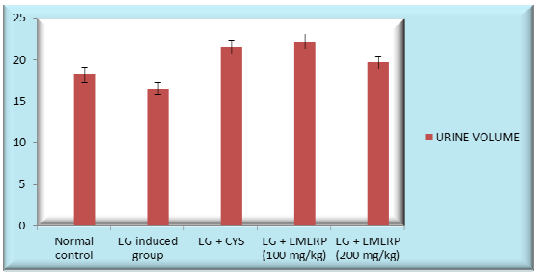

Fig 2: Effect of EeAs on urine Volume

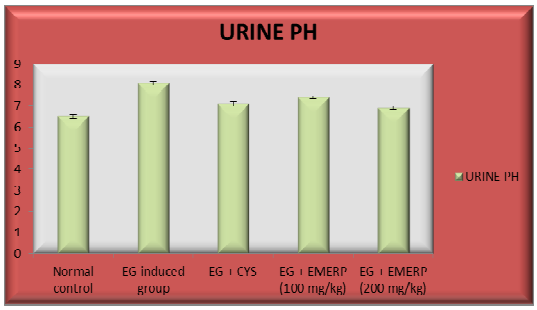

Fig 3: Effect of EeAs on urine pH

All values are expressed as mean ± SEM (n = 6). Significance at ###P<0.001, ##P<0.01, #P<0.05 compared with normal control. ***P<0.001, **P<0.01, *P<0.05 compared with EG group.

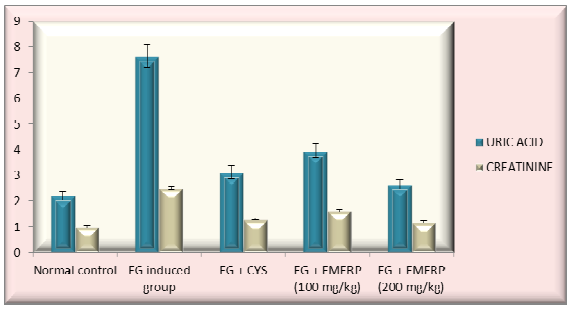

Fig 4: Effect of EeAs on uric acid and creatinine

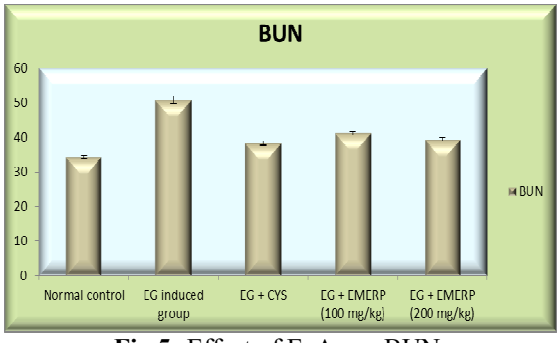

Fig 5: Effect of EeAs on BUN

All values are expressed as mean ± SEM (n = 6). Significance at ###P<0.001 compared with normal control. ***P<0.001 compared with model control.

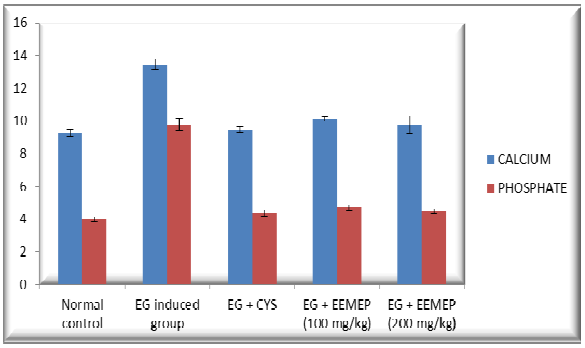

Fig 6: Effect of EeAs on calcium and phosphate

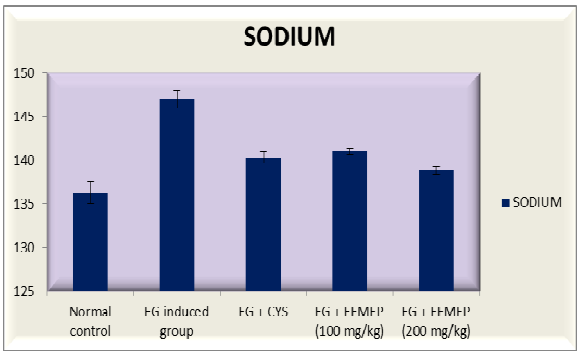

Fig 7: Effect of EeAs on Sodium

All values are expressed as mean ± SEM (n = 6). Significance at ###P<0.001 compared with normal control. ***P<0.001, **P<0.01 compared with model control.

Histopathology of Kidney:

Histopathological examination of kidney section of normal control animals showed intact nephron structure. But in model control group there was significant damage to the kidney cells. Rupture of bowmen capsule and tubules as well as infiltration of cells were observed with ethylene glycol treated animals. This damage was significantly prevented by four weeks treatment of all test drugs and standard drug. Ethylene glycol induced damage was also reversed by two weeks treatment of *Alphonsea sclerocarpa.*

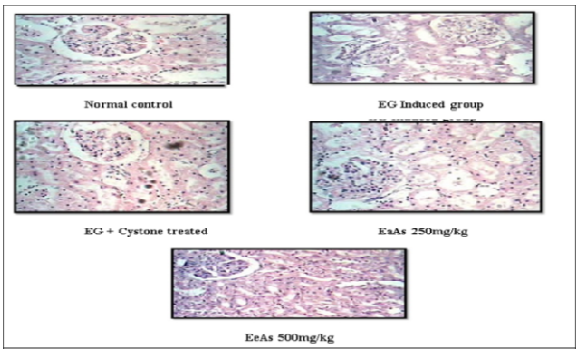

Fig 8: H&E staining of rat kidney

4. Conclusion

In the present investigation the medicinal plant *Alphonsea sclerocarpa* was explored for its phytochemical nature and antiurolithiatic properties. The conclusion drawn from this investigation can be summarized as: Preliminary phytochemical screenings of both the drugs were carried out for the detection of different plant constituents. In ethylene glycol treated animal model, *Alphonsea sclerocarpa* (500 mg/kg, p.o.) showed significant results when compared with standard drug - Cystone. It was also found to have significant prevention and reverse alteration in the kidney function parameters (Uric acid, BUN and Creatinine), when compared with cystone.*In-vivo* treatment of ethanolic extract of *Alphonsea sclerocarpa* root Extract shows significant anti urolithiatic activity in ethylene glycol induced urolithiasisin rats.In histopathological examinations of kidney also shows reduction in renal damagewith the rootplant *Alphonsea sclerocarpa* root Extract has shown promising antiurolithiatic activity and support folklore claims of these plants as antiurolithiatic. The mechanism of action of the plant for antiurolithiatic is apparently related to increased diuresis and lowering of urinary concentrations of stone forming constituents, though it should be confirmed by the extensive exploratory studies. Finally, future research is recommended on these seeds for authentication in human beings.

Table1: Ash analysis of *A.sclerocarpa*

Sl. No.	Evaluation parameters	*A. sclerocarpa* root Yield (% W/W)
1	Total ash	5.804 ± 0.321
2	Water- soluble ash	1.042 ± 0.062
3	Acid insoluble ash	1.553 ± 0.078
4	Foreign organic matter	0.064 ± 0.321
5	Moisture content	10.802 ± 0.728

Table 2: Phytochemical constituents of *A. sclerocarpa* extract

S. No	Phytochemicals	Ethanolic extract
1	Alkaloids	+++
2	Saponins	+
3	Tannins	+
4	Steroids	+

5	Anthocyanines	+
6	Flavonoids	+
7	Anthraquinones	-
8	Reducing sugar	-
9	Coumarin	-
10	Proteins	+

Table 3: Effect of ethanolic extracts of *Alphonsea sclerocarpa* on various physical parameters in ethylene glycol induced renal stone

Groups	Urine Volume	Urine pH
Normal control	18.17 ± 0.872	6.52± 0.092
EG induced group	16.50 ± 0.763	8.08± 0.071###
EG + CYS	21.50 ± 0.806**	7.09 ± 0.110***
EG + EeAs (250 mg/kg)	22.17 ± 0.872 **	7.42 ± 0.066***
EG + EeAs (500 mg/kg)	19.67 ± 0.760	6.91 ± 0.070***

Table 4: Effect of ethanolic extracts of *Alphonsea sclerocarpa* on various physical parameters in ethylene glycol induced renal stone

Groups	Uric Acid	BUN	Creatinine
Normal control	2.21 ± 0.165	34.37 ± 0.425	1.01 ± 0.048
EG induced group	7.63 ± 0.451###	50.76 ± 1.043###	2.51 ± 0.060
EG + CYS	3.12 ± 0.266***	38.36 ± 0.595***	1.30 ± 0.040***
EG + EeAs (250 mg/kg)	3.96 ± 0.266***	41.18 ± 0.515***	1.62 ± 0.045***
EG +EeAs (500 mg/kg)	2.65 ± 0.173***	39.35 ± 0.438***	1.19 ± 0.045***

Table 5: Effect of ethanolic extracts of *Alphonsea sclerocarpa* on various Calcium, Inorganic Phosphate and Sodium in ethylene glycol induced renal stone

Groups	Calcium	Phosphate	Sodium
Normal control	9.25 ± 0.213	3.99 ± 0.128	136.3 ± 1.282
EG induced group	13.46 ± 0.316 ###	9.74 ± 0.361 ###	147.0 ± 1.033 ###
EG + CYS	9.47 ± 0.182***	4.35 ± 0.187***	140.3 ± 0.666***
EG + EeAs (250 mg/kg)	10.11 ± 0.147***	4.67 ± 0.183***	141.0 ± 0.365***
EG + EeAs (500 mg/kg)	9.78 ± 0.53***	4.47 ± 0.143***	138.8 ± 0.477***

5. References

[1] Moe, OW (2006). "Kidney stones: pathophysiology and medical management". The Lancet 367 (9507): 333–44.

[2] Johri, N; Cooper B, Robertson W, Choong S, Rickards D, Unwin R (2010). "An update and practical guide to renal stone management". Nephron Clinical Practice116 (3): c159–71

[3] Reilly Jr. RF, Chapter 13: Nephrolithiasis, pp. 192–207 in Reilly Jr. and Perazella (2005)

[4] De Mais, Daniel. ASCP Quick Compendium of Clinical Pathology, 2nd Ed. ASCP Press, Chicago, 2009

[5] Soundararajan P, Mahesh R, Ramesh T, Hazeena Begum V. Effect of Aerva Lanata on calcium oxalate urolithiasis in rats. Indian journal of experimental biology, 44, 2006, 981-986. 2.

[6] Rana Gopal Singh, Sanjeev Kumar Behura, Rakesh Kumar. Litholytic Property of Kulattha (Dolichous Biflorus) vs Potassium Citrate in Renal Calculus Disease: A Comparative Study. JAPI, 58, 2010, 287. 3.

[7] Kalpana Devi V, Baskar R,Varalakshmi P. Biochemical effects in normal and stone forming rats treated with the ripe kernel juice of Plantain (Musa Paradisiaca). Ancient Science of Life, 3 & 4, 1993, 451 – 461. 4.

[8] Bahuguna YM, Rawat MSM, Juya V, Gnanarajan G. Antilithiatic effect of grains of Eleusine Coracana. Saudi Pharmaceutical Journal, 17, 2009, 182. 5.

[9] Surendra K pareta, Kartik Chandra Patra, Ranjit Harwansh. In-vitro calcium oxalate crystallization inhibition by Achyranthes indica Linn.Hydroalcoholic extract: An approach to antilithiasis. International Journal of Pharma and Bio Sciences, 432. 6.

[10] Kalpana Devi V, Baskar R,Varalakshmi P. Biochemical effects in normal and stone forming rats treated with the ripe kernel juice of Plantain (Musa Paradisiaca). Ancient Science of Life, 3 & 4, 1993, 451 – 461.

[11] C.Turk (chairman),T.K.c.(2011).guidelines on urolithiasis.update march 2011,1-103.

[12] Reilly Jr. RF, Chapter 13: Nephrolithiasis, pp. 192–207 in Reilly Jr. and Perazella (2005)

[13] Coe, FL; Evan, A; Worcester, E (2005). "Kidney stone disease". The Journal of Clinical Hoppe, B;

Langman, CB (2003). "A United States survey on diagnosis, treatment, and outcome of primary hyperoxaluria". Pediatric Nephrology 18 (10): 986–91. Investigation 115 (10): 2598-2608

[14] Hoppe, B; Langman, CB (2003). "A United States survey on diagnosis, treatment, and outcome of primary hyperoxaluria". Pediatric Nephrology 18 (10): 986–91.

[15] National Endocrine and Metabolic Diseases Information Service (2006). "Hyperparathyroidism (NIH Publication No. 6–3425)".

[16] National Endocrine and Metabolic Diseases Information Service (2008). "Renal Tubular Acidosis (NIH Publication No. 09–4696)"

[17] De Mais, Daniel. ASCP Quick Compendium of Clinical Pathology, 2nd Ed. ASCP Press, Chicago, 2009.

[18] Clinical Pathology of Ethylene Glycol Toxicosis. Archived from the original on 2 May 2012. Retrieved 2012-0

[19] Carl A. Osborne, D. P. (n.d.). Minnesota Urolith Center, University of Minnesota. Monitoring CaOx Urolith Prevention , 1-2.