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Original Article

Evaluation of *Aerva Lanata* Flower Extract for Its Antilithiatic Potential *In Vitro* and *In Vivo*

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Abstract

Despite considerable progress in Medical therapy there is no satisfactory drug to treat kidney stones therefore. This study is aimed to look for an alternative treatment by using *Aerva lanata.* The effect of aqueous extract of dried flower of *Aerva lanata* against ethylene glycol. Induced renal calculi in albino wistar rats and cystone tablets in popular medicine for kidney stone patients. This two extracts comparative study, a renal calculus was induced in rats by ingesting 0.75% ethylene glycol in drinking water for 28 days and was concentrated on estimation of calcium, oxalate, phosphate, magnesium excretion in the urine. This elevated urinary calcium with high urinary oxalate might lead to calcium oxalate stone formation, following administration of extracts significantly lower urinary calcium and oxalate were observed to compared with the cystone. This enabled us to conclude that the extract is antilithiatic activity.

Keywords: Antilithiatic, *Aerva lanata*, cystone, ethylene glycol.

1. Introduction

Kidney stones are one of the most common disorders of the urinary tract. Each year people make almost 3 million visits to health care providers and more than half a million people go to emergency rooms for kidney stone problems (9). A kidney stone also known as a renal calculus which is a solid concretion or crystal aggregation formed in the kidney from dietary minerals in the urine. The existence of the kidney stones was first recorded thousands of years ago. In 1901,a kidney stone was discovered in pelvis of an ancient Egyptian mummy and was dated to 4,800Bc.(3). Stone formation is more common in males than females as females tend to deadlines after the menopause, which may explain the equal incidence for stone formation in older people of both sexes. High body mass index also associated with increased risk of stone formation. Not only in humans but animals and birds also suffer from the urinary stone problem. The occurrence in some areas is so disquieting that they are known as "stone belts" (2).Many medicinal plants have been used since ages to treat urinary stones though the rationale behind their use is not well established through systematic and pharmacological studies, except for some composite herbal drugs and plant medicines are which great demand both in developed as well as in developing countries for the health care because of their wide biological and medicinal activities, higher safety margin and costs(8). Weeds are comprised of the more aggressive, troublesome and undesirable elements of the world's vegetation. *Aerva lanata* was grown in waste places as weed and used as an important medicinal plant for a long period of time *Areva lanata* commonly called in Tamil sirru-pulay or poolaipoo and in English stone breaking plants. It is biennial weed, native of south Asia, Saudi Arabia, Tropical Africa and South Africa, India, Srilanka etc. It belongs to the family Amaranthacea Genus *Aerva* and species *A.Lanata* since not much documented evidences are available regarding its therapeutic benefits for lithiasis. The aim of this research effort is to evaluate the antilithiatic potential of *Aerva lanata*. Antilithiatic potential of the flower extracts encompassing its preventive effect on calcium oxalate crystallization was studied in various in vitro assays and in vivo using the male wistar rats.

2. Materials and Methods

Collection of Plant Material

The flowers of *Aerva lanata* were collected from Tirunelveli. The plant was identified by the botanical survey of India, Tamilnadu Agricultural University, Coimbatore. The flowers of the plant were powdered and passed through the course sieve (0.2 mm).A series of extraction was carried out for the plant material. About 5g of the powdered sample was taken in a thimble and placed in a soxhlet apparatus and was extracted by hot percolation method using 200ml of different solvents with increasing polarity namely petroleum ether, benzene, chloroform, ethyl acetate, methanol,ethanol and aqueous. The extraction was carried out until the plant material become colourless. Then the extract was collected and evaporated in boiling water both at 60^0C. The residue was mashed and stored in an air tight container in a refrigerator.

Preparation of Aqueous Extract

Aqueous Extract of the plant sample also prepared as follows. To about 1g of the powdered sample, added l00 ml of distilled water and kept in a water bath at 60^0C for 2 hrs. Filtered and centrifuged thrice and the supernatant was collected evaporated in a flash evaporator and stored in an air tight container in the refrigerator.

Preparation of Cystone Extract

The tablet of cystone was powdered and from this about 35mg of the powdered sample was dissolved in 900µl of distilled water and added 100 µl of Tween 80. The extract was stored in an air tight container in the refrigerator.

Concentration of Extract

The plant extract obtained using various solvents were made up to a final concentration of 35µg/µl was utilized for various assays.

Selection of Animals for *In Vivo* Studies

Healthy male wister rats were received from animal breeding centre, Agriculture university of Thrissur, Kerala. Male wistar rats weighted between 150 -200 g were chosen for this study. The animals were acclimatized in polypropylene cages and maintained at 27 ± 2^0C, under 12 hr light/dark cycles. They were provided with rat chow and drinking water. The animal care and experimental protocols were in accordance with Institutional Animal ethical committe (IAEC) and the registration No.623/02/b/ CPSCEA.

Ethylene Glycol Induced Lithiasis

Ethylene glycol is a metabolic precursor of oxalate. Regular administration of ethylene glycol caused hyperoxaluria in ethylene glycol feed animals and causes increased renal retention and excretion of oxalate, calcium and phosphate. Ethylene glycol induced hyperoxaluric rats were used to assess the antilithiatic activity in male wistar rats, animals were divided into five groups containing four animals in each group.

Treatment Groups

Control - Animals were feed with regular water and food for 28 days.

Lithiatic Control - Animals were feed with 0.75 % ethylene glycol in drinking water up to 28 days.

Extract control - Treatment with the aqueous extract of *Aerva lanata* (3.2mg/kg body weight) orally daily up to 28 days.

Preventive Regimen - Animals were feed with 0.75% ethylene glycol in drinking water and the aqueous extract of *Aerva lanata* (3.0mg/kg body weight) orally up to 28 days.

Curative Regimen - Animals were feed with 0.75% ethylene glycol in drinking water and the aqueous extract of *Aerva lanata* (3.2mg/ kg body weight) from 15^{th} to 28^{th} days.

Preventive Regimen - Animals were feed with 0.75% ethylene glycol in drinking water and the cystone extract (3.2 mg/kg body weight) orally up to 28 days.

Curative Regimen - Animals were feed with 0.75% ethylene glycol in drinking water for 1 - 14 days and followed by the cystone extract (3.2 mg/kg body weight) from 15^{th} to 28^{th} days.

Assessment of antilithiatic activity

Collection and analysis of urine

All animals were kept in metabolic cages separately and urine sample of all groups were collected from the period of treatment on 0^{th}, $7^{th,}$ 14th, $21^{st,}$ and 28^{th} days. Animals had free access to drinking water and food was given for every 4^{th} hour and left over night, during the urine collection period one or two drops of toluene were added to the urine before stored in refrigerator. Calcium (5) oxalate, (7) phosphate (6), uric acid (2) and creatinine (1) levels in the urine were analyzed.

Serum Analysis

On 28^{th} day the blood was collected from the retro - orbital under anesthetic condition and the animals were sacrificed by cervical decapitation serum was separated and further analyzed for estimation of creatinine by the methods as described for the analysis of urine.

Statistical Analysis

The analysis was performed using faster's LSD method (version 3.1). Statistical significance was determined by one way analysis of variance (ANOVA), and also the two factorial statistics in WASP was also used. The result obtained for various *in vitro* and *in vivo* biochemical assays performed in the present study.

3. Results and Discussion

Nucleation Assay

The extent to which the nucleation of calcium oxalate crystals were inhibited by the flower extracts of *Aerva lanata* was determined and presented in figure 1. The results of the assays performed indicate that all calcium oxalate monohydrate crystals were hexagonal in control samples.When the *Aerva lanata* flower extract at varying concentrations (100,200,400,600,800 and 1000 µg/ml) were added. The calcium oxalate monohydrate crystals lost their crystalline nature and also disaggregated in to smaller particles.

Growth Assay

The aggregated clusters of calcium oxalate particles grow further. The extent to which the growth of calcium oxalate crystals were inhibited by the presence of the flower extracts of *Aerva lanata* was determined and presented in figure 2.Among the various solvent extracts analyzed, aqueous extract at its higher concentration (3200 µg) possessed a significant($P<0.001$) inhibitory effect against

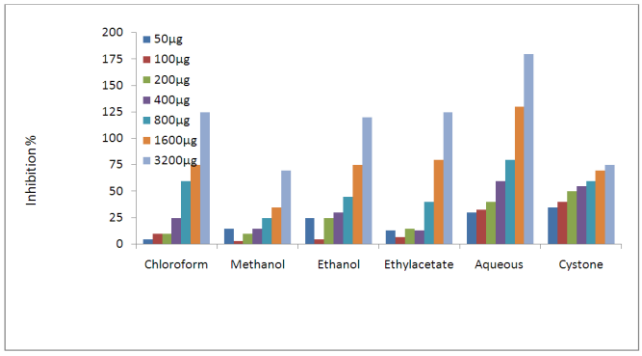

Fig 1: Effects of *Aerva lanata* extract nucleation of calcium oxalate crystals (after 60 mn)

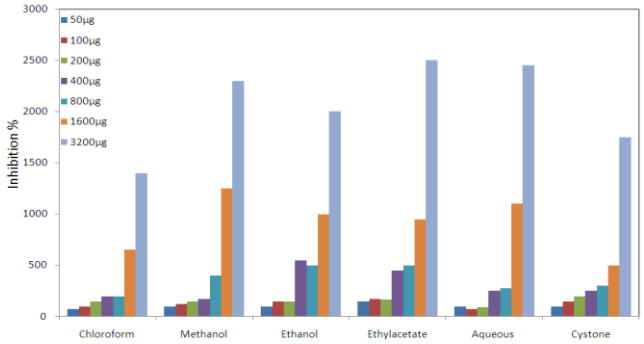

Fig 2: Effect of *Aerva lanata* extracts on calcium oxalate crystal growth

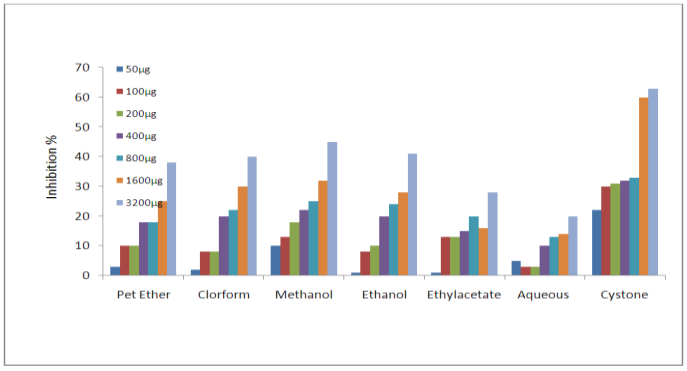

Fig 3: Effect of *Aerva lanata* extract on calcium oxalate crystal aggregation

the crystal nucleation, aggregation and growth process. All other extracts also exhibited a considerable inhibitory action.

Aggregation Assay

It was found that calcium oxalate monohydrate crystal aggregation was steadily decreased by the increased concentration of flower extract. Microscopic view of calcium oxalate crystals showed that the crystal size was bigger and aggregated in the control. The size of the crystal was considerably reduced and very well dispersed by the presence of the flower *Aerva lanata*.

Preventive and curative groups excretion of urinary calcium oxalate phosphorus, uric acid and creatinine level was reduced significant ($P<0.001$) when compared with ethylene glycol treated group.

Table 1: Levels of Calcium Excreted in the Urine Samples of Lithiasis Induced Rat

TREATMENT GROUPS	Days				
	0	7	14	21	28
Group I	0.82 ± 0.01	0.75 ± 0.02	0.78 ± 0.08	0.80 ± 0.08	0.80 ± 0.08
Group II	0.79 ± 0.01^{a}	3.31 ±0.01^{ab}	5.13 ± 0.02^{a}	5.87 ± 0.02^{a}	6.76 ± 0.02^{a}
Group II	0.74 ± 0.02	0.75 ±0.01^{ab}	0.81 ± 0.01	0.84 ±0.01^{ab}	0.07 ± 0.02^{a}
Group IV	0.79 ± 0.01	1.62 ± 0.02^{a}	1.51 ±0.01^{ab}	1.26 ± 0.01^{a}	1.20 ±0.02^{ab}
Group V	0.87 ±0.01^{ab}	2.98 ±0.01^{ab}	5.85 ± 0.02^{a}	3.2 ± 0.02^{a}	2.98 ±0.02^{ab}
Group VI	0.87 ± 0.01	2.13 ± 0.02a	1.33 ±0.02^{ab}	1.18 ± 0.01^{a}	0.86 ± 0.01^{a}
Group VII	0.83 ± 0.01	3.11 ±0.02^{ab}	5.90±0.02^{abc}	2.65 ± 0.01^{a}	1.06±0.01^{abc}
CD(0.050)	0.051	0.058	0.062	0.050	0.058

Table 2: Levels of Oxalate Excreted in the Urine Samples of Lithiasis Induced Rats

TREATMENT GROUPS	Days				
	0	7	14	21	28
Group I	1.49 ± 0.01	1.40 ±0.012	1.39 ± 0.01	1.41 ± 0.01	1.45 ± 0.02
Group II	1.83 ± 0.02	1.89 ±0.01^{ab}	1.91 ± 0.03	1.93 ± 0.01	1.95 ±0.03
Group II	1.8 ± 0.01^{ab}	1.82 ±0.01^{ab}	1.82±0.03^{a}	1.83 ± 0.03^{a}	1.80 ± 0.02^{a}
Group IV	1.45 ±0.01^{ab}	1.53±0.03^{abc}	1.63±0.03^{abc}	1.61 ±0.03^{ab}	1.58 ±0.01^{ab}
Group V	1.6 ± 0.01^{ab}	1.73 ± 0.01^{a}	1.86±0.01^{abc}	1.63±0.01^{abc}	1.31 ±0.01^{ab}
Group VI	1.71± 0.02^{a}	1.81± 0.03^{a}	2.51±0.02^{ab}	2.29±0.02^{a}	1.35±0.01^{ab}
Group VII	1.8±0.01^{a}	1.96±0.02^{a}	2.02±0.01^{a}	1.85±0.03^{ab}	1.70±0.02^{ab}
CD(0.050)	0.055	0.059	0.062	0.055	0.057

Table 3: Levels of Phosphorus Excreted in the Urine Samples of Lithiasis Induced Rat

TREATMENT GROUPS	Days				
	0	7	14	21	28
Group I	3.61±0.01	3.58±0.01	3.59±0.02	3.60± 0.02	3.61±0.01
Group II	3.64±0.02[a]	4.89±0.01[a]	5.33±0.01	6.72±0.02	7.33±0.02
Group III	3.63±0.01[ab]	3.64±0.01[ab]	3.63±0.01[ab]	3.64±0.01[ab]	3.65±0.01[ab]
Group IV	3.65±0.02[a]	3.64±0.02[ab]	3.73±0.02[ab]	3.66±0.01[ab]	3.61±0.02[ab]
Group V	3.47±0.01[abc]	4.22±0.02[abc]	4.87±0.01[abc]	4.47±0.02[abc]	3.11±0.01[abc]
Group VI	3.33±0.01[c]	3053±0.01[abc]	3.54±0.01[abc]	3.53±0.01[abc]	3.47±0.01[abc]
Group VII	3.61±0.01[ab]	3.98±0.02[ab]	4.60±0.01[ab]	4.02±0.01[ab]	3.21±0.01[ab]
CD(0.050)	0.052	..042	0.062	0.049	0.054

Table 4: Levels of Uric acid Excreted in the Urine Samples of Lithiasis Induced Rat

TREATMENT GROUPS	Days				
	0	7	14	21	28
Group I	1.15±2.17	1.16±3.47	1.12±1.24	1.09±0.36	1.12±0.09
Group II	1.14±1.12[bc]	1.17±0.96	1.25±2.87[ab]	1.26.43[a]	1.29±1.59[a]
Group III	1.19±0.04[C]	1.20±0.18[a]	1.21±0.47[abc]	1.20±0.59[ab]	1.20±0.36[a]
Group IV	1.16±3.07[a]	1.18±4.40[ab]	1.26±2.09[ab]	1.22±0.59[ab]	1.17±0.07[ab]
Group V	1.10±1.16[a]	1.13±1.15[abc]	1.19±0.81[ab]	1.14±1.08[ab]	1.11±0.40[ab]
Group VI	1.11±1.16[abc]	1.16±3.88[a]	1.23±0.97[ab]	1.17±0.63[abc]	1.14±0.40[ab]
Group VII	1.17±2.76[ab]	1.20±7.06[a]	1.26±0.91[ab]	1.22±0.28[abc]	1.19±0.56[ab]
CD(0.050)	5.489	0.363	0.567	1.882	2.093

Table 5: Levels of Creatinine Excreted in the Urine Samples of Lithiasis Induced Rat

TREATMENT GROUPS	Days				
	0	7	14	21	28
Group I	0.12 ± 0.02	0.13±0.01	0.09±0.00	0.12±0.00	0.09±0.00
Group II	0.08±0.00[a]	0.14±0.02[a]	0.15±0.01	0.21±0.01	0.23±0.01
Group III	0.06±0.00[b]	0.07±0.01	0.14±0.01[b]	0.16±0.00[ab]	0.18±0.01[b]
Group IV	0.07±0.00[b]	0.09±0.00	0.11±0.00[ab]	0.14±0.01[ab]	0.14±0.00
Group V	0.09±0.03[abc]	0.16±0.01[ab]	0.17±0.01[bc]	0.13±0.00[ab]	0.10±0.00[a]
Group VI	0.10±0.01[ab]	0.16±0.01[ab]	0.18±0.00[ab]	0.15±0.00[abc]	0.15±0.01[ac]
Group VII	0.07±0.01[b]	0.10±0.01[ab]	0.16±0.00[ab]	0.14±0.00[ab]	0.10±0.01[a]
Group VIII	0.12±0.02[ab]	0.17±0.[01ab]	0.19±0.00[ad]	0.17±0.01[a]	0.16±0.01[a]
CD(0.050)	0.024	0.048	0.044	0.052	0.033

The values are mean ± SD for 4 animals in each group

a – Statistically significant (P<0.0001) compared to control

b - Statistically significant (P<0.0001) compared to lithiatic control (respective treatment days)

c –Statistically Significant (P<0.0001) compared to aqueous extract (respective treatment days)

Conclusion

In our study the urinary output was markedly decreased in lithiatic control rats on 28 days. In plant extract and standard treated rats the urinary volumes were increased when compared to that lithiatic group. This suggested that extract following ethylene glycol administration the excretion of calcium, oxalate, phosphate and protein were found to be increased in lithiatic group while in standard. Curative and preventive groups were significantly decreased (P<0.01) the magnesium level was decreased in lithiatic group while in standard and extract treated groups were increased.

Significantly (P<0.01). The creatinine levels of extract treated rats restored to normal limits and the creatinine clearance was also found to be improved. These effects could conclude that the *Aerva lanata* aqueous flower extracts shows better antiurolithiatic activity than cystone.

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