



## ANTIUROLITHIATIC EFFECT OF *AERVA LANATA LINN* EXTRACT ON ETHYLENE GLYCOL INDUCED URINARY CALCULI MODEL IN RATS

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### ABSTRACT

To evaluate the potential of Aqueous Extract of *Aerva lanata Linn extract* for the treatment of urolithiasis. The aqueous extract of *Aerva lanata (L)* was evaluated for antiurolithiatic activity in male albino Wistar rats. Ethylene glycol (0.75%) in drinking water was fed to all the groups (Groups II-V) except normal control (Group I) for 28 days to induce urolithiasis. Groups II, served as positive control (hyperurolithiatic), Groups III and IV served as curative regimen and received Extract (500 and 1000mg/kg body weight) from 15<sup>th</sup> day till 28<sup>th</sup> day once daily by oral route and Group V standard (cystone 750 mg/kg), respectively. Oxalate, calcium and phosphate were monitored in the urine and kidney. Serum BUN, creatinine, and uric acid were also recorded. The aqueous extract of *Aerva lanata (L)* were safe orally and exhibited no gross behavioral changes in the rats. In hypercalculi animals, the oxalate, calcium, and phosphate excretion grossly increased. However, the increased deposition of stone forming constituents in the kidneys of calculogenic rats were significantly ( $P < 0.001$ ) lowered by treatment with extract. These results confirm that *Aerva lanata (L)* possess potent antiurolithiatic activity. The results obtained suggested that the *Aerva lanata (L)* extract has a potent antiurolithiatic agent.

**Keywords:** *Aerva lanata (L)*, Cystone, calcium, hyperoxalurea, oxalate and phosphate.

### INTRODUCTION

Urolithiasis, the formation of urinary stones, is one of the oldest known diseases. Archaeological findings give profound evidence that humans have suffered from kidney and bladder stones for centuries, even examinations of Egyptian mummies have revealed kidney and bladder stones. The plant *Aerva lanata (L)* is said to be diuretic and demulcent. Its diuretic action is said to be very effective in the treatment of urethral discharges and gonorrhoea and is of value in cases of lithiasis and as an anthelmintic. The whole plant or parts of it is used as a diuretic herbal drink, tea, porridge, extract or as a decoction with other herbs<sup>1</sup>. The plant extract has also been reported to possess anti-inflammatory<sup>2</sup>, antimalarial, antivenin, analgesic and sedative activities<sup>3</sup>. Also it is used to treat urinary calculi, hematemesis, bronchitis, nasal bleeding, cough, scorpion stings, fractures, spermatorrhoea, to clear uterus after delivery and to prevent lactation. In folklore practice hot water extract of *Aerva lanata (L)* has been reported to be useful in Diabetes Mellitus<sup>4</sup>. Recent study has been reported on the aqueous extract having strong potential Antioxidant activity<sup>5</sup>. The purpose of the study was to evaluate the effect of aqueous extract of *Aerva lanata (L)*.

### MATERIALS AND METHODS

#### Drugs and chemicals

All of the drugs and biochemicals were purchased from Sigma Chemical Company Inc., St Louis, MO, USA, and the chemicals used were of analytical grade.

#### Plant material

Aerial parts of *Aerva lanata (L)* was collected from Coimbatore in the month of January and identified authenticated by Dr.G.V.S. Murthy, Scientist 'F' & Head of Office, Botanical Survey of India, Coimbatore. The aerial parts of the plant were shade dried and powdered. They were extracted with distilled water by cold maceration process.

Phytochemical studies on *Aerva lanata (L)* revealed that it contains flavonoid glycosides, aervitrin, aervolanine, aervoside, amyirin betulin, campesterol, canthin-6-one, 10-hydroxy-canthin-6-one, carboline-1 propionic acid, chrysin,  $\beta$ -ecdysone, daucosterol, hentriacontane, narcissin,  $\beta$ -sitosterol, syringic acid, feruloyl tyramine and vanillic acid<sup>6</sup>. Preliminary phytochemical analysis indicated that the leaf extract of *Aerva lanata (L)* contain sterols, glycoside, flavonoids, carbohydrates and tannins. A trace of alkaloid has also been detected<sup>7</sup>.

#### Animals

For acute toxicity studies, Wistar albino mice of either sex weighing between 25-30g and for the antiurolithiatic study albino rats of 150-200g were selected. The animals were acclimatized to standard laboratory conditions (temperature:  $25 \pm 2^\circ\text{C}$ ) and maintained on 12-h light and 12-h dark cycle and were provided with regular rat food (Sai meera foods Pvt Ltd., Bangalore, India) and drinking water *ad libitum*. The animal care and experimental protocols were in accordance with Institutional Animal Ethical Committee (IAEC).



### Preparation of stock solution

One gram of aqueous extract of *Aerva lanata* (L) was accurately weighed and was further dissolved in 2% CMC with distilled water so as to prepare 100mg/ml concentration at room temperature for oral administration by gastric intubation method.

### Acute toxicity studies

The acute oral toxicity study was carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD-425). One-tenth and one-fifth of the maximum tolerable doses were taken as therapeutic doses for further study.

### Ethylene glycol induced urolithiasis model

Ethylene glycol (0.75%) induced hyperoxaluria method was used to assess the antiurolithiatic activity in albino rats.<sup>8</sup> Animals were divided into five groups containing six animals in each group. Group I served as normal control and received regular rat food and drinking water *ad libitum*. Ethylene glycol (0.75%) in drinking water was fed to Groups II-V for induction of calculi till 14<sup>th</sup> day. Groups III and Group IV served as curative regimen, received aqueous extract of *Aerva lanata* (L) (500mg/kg and 1000mg/kg body weight) from 15<sup>th</sup> day till 28<sup>th</sup> day by oral route. Group V received standard antiurolithiatic drug, Cystone<sup>9</sup> (750mg/kg body weight) from 15<sup>th</sup> day till 28<sup>th</sup> day.

### Collection and analysis of urine

All animals were placed on metabolic cages and urine samples of 24h were collected on 28<sup>th</sup> day. Animals were

allowed for free access to drinking water and the urine were collected. A drop of concentrated hydrochloric acid had been added to the urine samples before being stored at 4°C. Urine samples were analyzed for contents like calcium<sup>10</sup>, phosphate<sup>11</sup> and oxalate<sup>12</sup>. The other estimations were performed using standard methodology.

### Serum Analysis

The blood samples were collected by the retro-orbital puncture, under anesthetic conditions after the experimental period. Creatinine, urea nitrogen, and uric acid<sup>13</sup> were separated from serum by centrifugation and analyzed.

### Kidney homogenate analysis

Kidneys and liver from animals were isolated from the abdomen and the isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. The sample of about 100 mg of the kidney were dried and boiled in 10 mL of 1 N hydrochloric acid for 30 min and homogenized. The homogenates were centrifuged and the supernatant was separated<sup>14</sup>. The kidney homogenate were analyzed for the contents like calcium, phosphate and oxalate and tabulated.

### Statistical analysis

Results were expressed as mean  $\pm$  S.E.M. Differences among data were determined using one-way ANOVA followed by Dunnet 'T' test (INSTAT software for Windows, Version-V-3). P<0.05 were considered significant.

**Table 1: Effect of Extract on Urinary Parameters in Normal and Urolithiatic Rats**

Parameter (Unit)	Group I, Normal Control	Group II, Calculi-induced control	Group III, Calculi-induced+ Extract (500mg/kg)	Group IV, Calculi-induced+ Extract (1000mg/kg)	Group V, Cystone-treated
Urine (mg/dl)					
Oxalate	0.36 $\pm$ 0.02	3.63 $\pm$ 0.10 <sup>w,φ</sup>	2.09 $\pm$ 0.08 <sup>w,φ,Φ</sup>	1.23 $\pm$ 0.06 <sup>w,φ,Φ</sup>	0.52 $\pm$ 0.03 <sup>w,φ</sup>
Calcium	1.26 $\pm$ 0.06	4.50 $\pm$ 0.11 <sup>w,φ</sup>	3.03 $\pm$ 0.12 <sup>w,φ,Φ</sup>	1.56 $\pm$ 0.10 <sup>w,φ,λ</sup>	1.49 $\pm$ 0.05 <sup>w,φ</sup>
Phosphate	3.66 $\pm$ 0.03	7.28 $\pm$ 0.05 <sup>w,φ</sup>	5.17 $\pm$ 0.04 <sup>w,φ,Φ</sup>	3.77 $\pm$ 0.05 <sup>w,φ</sup>	3.80 $\pm$ 0.08 <sup>w,φ</sup>

Values of urine parameters are assessed in 24 h urine sample. All values are expressed as mean  $\pm$  S.D. for six animals in each group. <sup>a</sup>Comparisons made between Group I Vs all other groups. <sup>b</sup>Comparisons made between Group II Vs all treatment group. <sup>c</sup>Comparisons made between Group V Vs all other groups. Statistically significant at P < 0.05; P < 0.01, P<0.001; <sup>NS</sup>P>0.05. <sup>∞</sup>-0.05, <sup>#</sup>- 0.01,<sup>ψ</sup>-0.001-<sup>a</sup>compared to normal; <sup>b</sup>φ-0.05, <sup>γ</sup>-0.01, <sup>φ</sup>-0.001- compared to Control; <sup>c</sup>λ-0.05, <sup>δ</sup>-0.01, <sup>Φ</sup>-0.001- compared to standard; NS- Not significant

**Table 2: Effect of Extract on kidney Parameters in Normal and Urolithiatic Rats**

Parameter (Unit)	Group I, Normal Control	Group II, Calculi-induced control	Group III, Calculi-induced+ Extract (500mg/kg)	Group IV, Calculi-induced+ Extract (1000mg/kg)	Group V, Cystone-treated
Kidney (mg/g)					
Oxalate	1.40 $\pm$ 0.05	5.72 $\pm$ 0.06 <sup>w,φ</sup>	2.61 $\pm$ 0.03 <sup>w,φ,Φ</sup>	1.77 $\pm$ 0.04 <sup>w,φ</sup>	1.60 $\pm$ 0.07 <sup>w,φ</sup>
Calcium	3.22 $\pm$ 0.03	4.78 $\pm$ 0.15 <sup>w,φ</sup>	4.27 $\pm$ 0.10 <sup>w,φ,Φ</sup>	3.98 $\pm$ 0.08 <sup>w,φ,λ</sup>	3.41 $\pm$ 0.06 <sup>w,φ</sup>
Phosphate	2.34 $\pm$ 0.02	3.73 $\pm$ 0.09 <sup>w,φ</sup>	3.36 $\pm$ 0.07 <sup>w,φ,Φ</sup>	3.07 $\pm$ 0.04 <sup>w,φ,Φ</sup>	2.51 $\pm$ 0.06 <sup>w,φ</sup>

All values are expressed as mean  $\pm$  S.D. for six animals in each group. <sup>a</sup>Comparisons made between Group I Vs all other groups. <sup>b</sup>Comparisons made between Group II Vs all treatment group. <sup>c</sup>Comparisons made between Group V Vs all other groups. Statistically significant at P < 0.05; P < 0.01, P<0.001; <sup>NS</sup>P>0.05. <sup>∞</sup>-0.05, <sup>#</sup>- 0.01,<sup>ψ</sup>-0.001-<sup>a</sup>compared to normal; <sup>b</sup>φ-0.05, <sup>γ</sup>-0.01, <sup>φ</sup>-0.001- compared to Control; <sup>c</sup>λ-0.05, <sup>δ</sup>-0.01, <sup>Φ</sup>-0.001- compared to standard; NS- Not significant



**Table 3:** Effect of Extract on Serum Parameters in Normal and Urolithiatic Rats

Parameter (Unit)	Group I, Normal Control	Group II, Calculi-induced control	Group III, Calculi-induced+ Extract (500mg/kg)	Group IV, Calculi-induced+ Extract (1000mg/kg)	Group V, Cystone-treated
Serum (mg/dl)					
BUN	37.60±0.14	49.96±0.47 <sup>w,Φ</sup>	45.35±0.32 <sup>w,φ,Φ</sup>	42.17±0.27 <sup>w,φ</sup>	39.29±0.47 <sup>w,φ</sup>
Creatinine	0.74 ± 0.01	0.93 ± 0.02 <sup>w,φ</sup>	1.63±0.04 <sup>w,φ,Φ</sup>	1.25±0.03 <sup>w,φ,Φ</sup>	0.80 ± 0.01 <sup>w,γ</sup>
Uric acid	1.48 ± 0.06	3.63 ± 0.10 <sup>w,φ</sup>	2.25±0.09 <sup>w,φ,Φ</sup>	1.79±0.07 <sup>w,φ</sup>	1.70 ± 0.03 <sup>w,φ</sup>

All values are expressed as mean ± S.D. for six animals in each group. <sup>a</sup>Comparisons made between Group I Vs all other groups. <sup>b</sup>Comparisons made between Group II Vs all treatment group. <sup>c</sup>Comparisons made between Group V Vs all other groups. Statistically significant at P < 0.05; P < 0.01, P < 0.001; <sup>NS</sup>P > 0.05. ∞-0.05, #- 0.01, <sup>w</sup>-0.001-<sup>a</sup>compared to normal; <sup>b</sup>φ-0.05, γ-0.01, φ-0.001- compared to Control; <sup>c</sup>λ-0.05, δ-0.01, Φ-0.001- compared to standard; NS- Not significant

## RESULTS

Acute toxicity studies observed that animals tolerated a maximum dose of 5000 mg/kg b.w. with no mortality and noticeable behavioral changes in all groups. Therefore, 1/10<sup>th</sup> of the maximum tolerated dose 500 and 1/5<sup>th</sup>, 1000 mg/kg b.w. were chosen for further studies.

Increased oxalate, calcium, and phosphate excretion were observed after the chronic administration of 0.75% (v/v) ethylene glycol thereby resulting in hyperoxaluria [Table 1], [Group II]. Extract of *Aerva lanata* (L) significantly (P < 0.001) lowered the elevated levels of oxalate, calcium and phosphate in urine and kidney [Table 1 and 2], [Group III and IV]. The deposition of the crystalline components in the renal tissue, namely oxalate, phosphate, and calcium, were increased in the stone forming rats (Group II).

The Extract of *Aerva lanata* (L) treatment significantly (P < 0.001) reduced the renal contents of stone forming constituents in Groups III and IV. The serum uric acid, creatinine and BUN increased significantly in the calculi-induced animals (Group II), [Table 3] compared to control, while serum creatinine elevated in Group II, thus indicating marked renal damage. However, *Aerva lanata* (L) extract treatment in (Group III) significantly (P < 0.001) lowered the elevated serum levels of creatinine, uric acid, and BUN.

Administration of ethylene glycol 0.75% (v/v) aqueous solution to Wistar rats resulted in hyperoxaluria. The oxalate, calcium and phosphate excretion increased in calculi-induced animals. However, *Aerva lanata* (L) extract treatment lowered the elevated levels of oxalate, calcium and phosphate in urine and kidney compared to cystone-treated animals. The *Aerva lanata* (L) extract treatment significantly lowered the renal contents of these stone forming constituents.

Anti-urolithiatic studies of plant extract of *Aerva lanata* (L) were given in Tables 1, 2, and 3 for urinary, kidney and serum parameters respectively.

## DISCUSSION

The urinary system of male rats resembles to that of humans and hence in the present study, male rats were selected to induce urolithiasis. Urinary super saturation with respect to stone-forming constituents were considered to be one of the causative factors in calculogenesis<sup>14</sup>. Evidence in previous studies indicated

that in response to 14 days period of ethylene glycol (0.75%, v/v) administration, young albino rats form renal calculi composed mainly of calcium oxalate<sup>15</sup>. The biochemical mechanisms for this process were related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed animals was caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate. Similar results were obtained when rats were treated with ethylene glycol and ammonium oxalate<sup>16 & 17</sup>.

The serum creatinine was elevated in Group II, indicating marked renal damage, while serum uric acid and BUN were remarkably increased in calculi-induced animals as compared to control group. The *Aerva lanata* (L) extract treatment significantly (P < 0.01) lowered the elevated serum levels of creatinine, BUN and uric acid. The urine analysis data of control group and treated group did not reveal any major abnormalities except bilirubin and urobilinogen.

Hyperoxaluria induced by chronic administration of 0.75% (v/v) ethylene glycol increased oxalate, calcium and phosphate excretion [Group II]. Extract of *Aerva lanata* (L) significantly (P < 0.001) lowered the elevated levels of oxalate, calcium and phosphate in urine and kidney parameters. The deposition of the crystalline components in the renal tissue, namely oxalate, phosphate, and calcium, were increased in the stone forming rats (Group II)<sup>18</sup>. The extract of *Aerva lanata* (L) treatment significantly (P < 0.001) reduced the renal content of stone forming constituents in both regimens (Groups III and IV). However, *Aerva lanata* (L) extract treatment in (Group III) significantly (P < 0.001) lowered the elevated serum levels of creatinine, uric acid, and BUN.

Oxalate, calcium and phosphate excretion were grossly increased in calculi-induced animals<sup>8</sup>. However, supplementation with extract significantly lowered the elevated levels of oxalate, calcium and phosphate in urine and kidney as compared to cystone-treated animals<sup>19</sup>. The *Aerva lanata* (L) extract treatment significantly reduced the renal content of these stone forming constituents.

In this study, the excretion of oxalate and calcium progressively increased in calculi-induced animals. It is accepted that hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria<sup>20</sup>, the changes in urinary oxalate levels are



relatively much more important than those of calcium<sup>21</sup>. Increased urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate from urine and subsequent crystal growth<sup>22</sup>. However, extract lower the levels of oxalate as well as calcium excretion.

Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition<sup>23</sup>. Treatment of *Aerva lanata* (L) extract restored the phosphate level, thus reducing the risk of stone formation.

In urolithiasis, the glomerular filtration rate had been decreased due to the obstruction to the outflow of urine by stones in urinary system and hence particularly nitrogenous substances such as urea, creatinine and uric acid get accumulated in blood<sup>24</sup>. In calculi-induced rats, marked renal damage was seen as indicated by the elevated serum levels of creatinine and uric acid and BUN. However, the Diuretic activity of extract of *Aerva lanata* (L) was studied and the study indicated that the extract at a dose of 800 mg/kg acted as a diuretic, with respect to control. *Aerva lanata* (L) aqueous suspension (2 g/kg body wt/dose/day for 28 days) to CaO<sub>2</sub> urolithic rats had reduced the oxalate-synthesizing enzymes, and diminished the markers of crystal deposition in the kidney. The results of the study confirmed that *Aerva lanata* (L) can be used as a curative agent for urolithiasis<sup>2,25</sup>. The significant lowering of serum levels of accumulated waste products is attributed to the enhanced GFR and the anti-lipid peroxidative property<sup>26,27</sup> of *Aerva lanata* (L) extract. Histopathological examination did not reveal any abnormality in the kidney of drug treated animals.

### CONCLUSION

In conclusion, the data presented indicated that the administration of aqueous extract of *Aerva lanata* (L) to rats fed with ethylene glycol induced urolithiasis, reduced the growth of urinary stones. Further studies are required to understand the mechanism underlying this effect and lowering urinary concentrations of stone forming constituents.

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