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Effect of *Aerva lanata* on calcium oxalate urolithiasis in rats

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Calcium oxalate (CaOx) stone was induced in rats using 0.75% of ethylene glycol in drinking water for 28 days. Ethylene glycol treated rats showed significant increase in the activities of oxalate synthesizing enzymes such as glycolic acid oxidase (GAO) in liver and lactate dehydrogenase (LDH) in liver and kidney. CaOx crystal deposition, as indicated by increased excretion of stone-forming constituents in urine, such as calcium, oxalate, uric acid, phosphorus and protein and decreased concentration of inhibitors, such as citrate and magnesium was observed in ethylene glycol induced urolithic rats. Histopathological studies also confirmed the deposition of CaOx crystals. Administration of *Aerva lanata* aqueous suspension (2g/kg body wt/dose/day for 28 days) to CaOx urolithic rats had reduced the oxalate synthesizing enzymes, diminished the markers of crystal deposition in the kidney. The results of the present study confirmed that *A. lanata* can be used as an curative agent for urolithiasis.

Keywords: *Aerva lanata*, Oxalate synthesizing enzymes, Stone forming constituents

Kidney stone disease is a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 70-81% in males, and 47-60% in females[1]. The majority of stones, up to 80%, are composed mainly of calcium oxalate (CaOx)[2]. Many remedies have been employed during ages to treat urinary stones. Most of the remedies were taken from plants and proved to be useful, though the rationale behind their use is not well established except for a few plants and some proprietary composite herbal drugs and they are reported to be effective with no side effects[3].

Aerva lanata is a plant from the family Amaranthaceae locally known as sirupoolai in Tamil. *A. lanata* is endowed with chemical components such as flavonoids, alkaloids, steroids, polysaccharides, tannins, saponins, etc[4-6]. The plant has been documented earlier for its therapeutic effects in controlling kidney disorders[7], diuretic[8], anti-inflammatory[8], anti-diabetic[9], anti-tumor[10], and antimicrobial[11].

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The present study evaluated for the possible therapeutic potential of aerial parts of *A. lanata* aqueous suspension in experimentally induced calcium oxalate urolithic rats.

Materials and Methods

Plant material—Fresh *A. lanata* aerial parts were collected during the months of September to December. The aerial parts were dried thoroughly under shade and powdered finely. The powder was suspended in distilled water as aqueous suspension and used for the study.

Animals—Male albino rats of Wistar strains weighing approximately 140-150 g were used. All animal experiments and maintenance were carried out according to the ethical guidelines suggested by the Institutional Animal Ethics Committee. Animals were housed in polypropylene cages under controlled conditions of 12 h light/dark cycle at 27°±2°C. All the rats received standard pellet diet (Amrut rat feed, Pune) and water *ad libitum*.

Stone induction—Kidney stones were induced by 0.75% of ethylene glycol in drinking water for 28 days *ad libitum*. After 28 days induction, the animals were used for the study.

Experimental design—In the experiment a total of 24 rats (12 urolithic rats, 12 normal rats) were used. The rats were divided into 4 groups of 6 rats each. Group I (Gr I)-Normal control rats; Group II (Gr II)-

Normal rats given *A. lanata* aqueous suspension (ALAS; 2 g/kg body wt/dose/day for 28 days) using an intragastric tube; Group III (Gr III)-Urolithic rats; and Group IV (Gr IV)-Urolithic rats given ALAS (2 g/kg body wt/dose/day for 28 days) using an intragastric tube.

At the end of 28 days, the animals were housed in metabolic cages and collected urine after 24 h. After urine collection, the animals were mild anaesthetized and sacrificed by cervical decapitation. Liver and kidney were quickly dissected into ice-cold saline. They were trimmed free of connective tissue and minced. A 10% of homogenate was prepared in tris-HCl buffer *p*H 7.4.

Biochemical assays—The total homogenate was used for assaying oxalate synthesizing enzymes like glycolic acid oxidase (GAO[12]; only in liver), lactate dehydrogenase (LDH)[13] and oxalate[14] level were assayed both in liver and kidney. The urine collected after 24 h was subjected to analyze calcium[15], oxalate[14], uric acid[16], phosphorus[17], citrate[18], magnesium[19] and protein[20].

Histological assays—The tissue pieces taken from the kidney of the rats were fixed by neutral buffered formalin (10%) and subsequently embedded in paraffin. The sections (5 μm thick) were stained by haematoxylin and eosin to study the histopathological changes and calcium oxalate crystal deposition.

Statistical analysis—The results were presented as the mean SD. One-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparison. Values of $P<0.05$ was considered to be significant. SPSS 7.5 version was used for this analysis.

Results

Liver and kidney act as the main sites of endogenous oxalate synthesis. The activities of oxalate synthesizing enzymes were assayed in the control and experimental groups and are presented in Table 1. The ethylene glycol induced urolithic rats exhibited significant increase in liver GAO activity when compared to that of the normal control rats. The LDH activity and oxalate level were monitored in liver and kidney. These were significantly raised in the urolithic rats when compared with that of the normal control rats. Treatment with ALAS reduced the activities of GAO, LDH and oxalate levels to near normal control. GAO and LDH activities and oxalate level were not altered in ALAS administered drug control rats as compared with normal control rats.

Calcium and oxalate excretion were significantly increased while citrate and magnesium excretion were significantly decreased in 24 h urine of ethylene glycol induced urolithic rats when compared with the normal control rats. ALAS therapy reverted the above changes to near normal. Significant changes were not observed in ALAS supplemented drug control rats as compared with normal control rats (Table 2).

Phosphorus, uric acid and protein excretion were significantly elevated in 24 h urine of urolithic rats as compared to normal control rats. ALAS therapy reduced the phosphorus, uric acid and protein to near normal. ALAS supplemented drug control rats did not show any change as compared to normal control (Table 3).

The histopathological observations of kidney showed normal structure and architectural intactness without any apparent damages in control (Fig. 1A)

Table 1—Effect of ALAS on GAO, LDH and oxalate in urolithic rats
[Values are mean ± SD for 6 rats in each group]

Parameters	Group I	Group II	Group III	Group IV
Liver				
Glycolic acid oxidase	1.68 ± 0.08	1.58 ± 0.06	2.27 ± 0.08[a]***	1.89 ± 0.07[b]***
Lactate dehydrogenase	1.52 ± 0.10	1.56 ± 0.12	4.07 ± 0.17[a]***	1.47 ± 0.13[b]***
Oxalate	1.27 ± 0.07	1.19 ± 0.07	1.86 ± 0.08[a]***	1.32 ± 0.06[b]***
Kidney				
Lactate dehydrogenase	2.73 ± 0.17	2.64 ± 0.13	4.92 ± 0.18[a]***	3.02 ± 0.10[b]***
Oxalate	0.62 ± 0.06	0.57 ± 0.04	1.78 ± 0.07[a]***	0.75 ± 0.05[b]***

GAO, nmol of glyoxylate formed/mg protein; LDH, U/mg protein; Oxalate, mg/g tissue. Superscript letters represent P <0.05 (Tukey's test). [a]As compared with group I, [b]As compared with group III. *P <0.05, **P <0.01, ***P <0.001.

Table 2—Effect of ALAS on urinary calcium, oxalate, citrate and magnesium in urolithic rats
[Values are mean ± SD for 6 rats in each group]

Biochemical assay	Group I	Group II	Group III	Group IV
Calcium	0.64±0.03	0.64±0.04	2.14±0.03[a]***	0.76 ± 0.05[b]***
Oxalate	0.40±0.02	0.37±0.02	2.93±0.05[a]***	0.97 ± 0.04[b]***
Citrate	1.94±0.13	1.96±0.04	0.83±0.05[a]***	1.88 ± 0.06[b]***
Magnesium	1.04±0.06	1.08±0.04	0.68±0.03[a]***	0.99 ± 0.04[b]***

Calcium, oxalate, citrate, magnesium, mg/24 h urine. Superscript letters represent P <0.05 (Tukey's test).
[a]As compared with group I, [b]As compared with group III.
*P <0.05, **P <0.01, ***P <0.001.

Table 3—Effect of ALAS on urinary uric acid, phosphorus and protein in urolithic rats

[Values are mean ± SD for 6 rats in each group]

Biochemical assay	Group I	Group II	Group III	Group IV
Uric acid	1.06±0.04	1.03±0.05	1.37±0.03[a]***	1.13±0.05[b]***
Phosphorus	5.42±0.26	5.34±0.23	7.21±0.28[a]***	5.72±0.29[b]***
Protein	2.14±0.17	2.14±0.14	3.13±0.07[a]***	2.23±0.13[b]***

Uric acid, phosphorus, protein, mg/24 h urine. Superscript letters represent P <0.05 (Tukey's test).
[a]As compared with group I, [b]As compared with group III.
*P <0.05, **P <0.01, ***P <0.001.

and ALAS supplemented drug control rats (Fig. 1B). Kidney of ethylene glycol induced urolithic rats showed polymorphic irregular crystals accumulated in the tubules that caused dilation of the proximal tubules and led to dialation and tubularrehxis of the tubules along with interstitial inflammation (Fig. 1C, 1D). ALAS dissolved the CaOx stone and restored the renal structure in ethylene glycol induced urolithic rats (Fig. 1E).

Discussion

Ethylene glycol disturbs oxalate metabolism by way of increase the substrate availability that increases the activity of oxalate synthesizing enzymes in the rats. GAO and LDH catalyses the coupling of oxidation and reduction of glyoxylate results in the formation of glycolate and oxalate[21]. In the present study, significantly increased activities of GAO in liver and LDH in liver and kidney of ethylene glycol induced urolithic rats that may be due to substrate mediated induction of the enzymes. A similar increase was also observed in glyoxylate, pyridoxine deficient diet and glycolate-administered rats[22-24]. Administration of ALAS brought about a significant reduction in GAO activity in liver and LDH activity in liver and kidney of urolithic rats.

Increased activity of GAO and LDH confirmed their direct link to endogenous oxalate deposition in ethylene glycol induced urolithiasis. Administration of ALAS reduced the oxalate level in liver and kidney of urolithic rats.

The study of the urinary chemistry with respect to the stone-forming minerals will provide a good indication of the risk of stone formation. In the present study, observed hypercalciuria in ethylene glycol induced urolithic rats might be a factor favouring the nucleation and precipitation of calcium oxalate of apatite (calcium phosphate) from urine and subsequent crystal growth[25]. Treatment with ALAS reduced the level of calcium excretion in ethylene glycol induced urolithic rats.

Hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria. In the present study, urinary oxalate was increased in ethylene glycol induced urolithic rats. It has been reported that oxalate plays an important role in stone formation and has about 15-fold greater effect than urinary calcium[26]. The reduction in oxalate excretion was observed on ALAS treatment. This indicates that its pentacyclic triterpenes mainly butelin act as inhibiting some steps of oxalate synthesis from glycolic acid.

In the present study, decreased level of urinary citrate was observed in ethylene glycol induced urolithic rats. Hypocitraturia is the major metabolic abnormality in patients with renal stones[27]. Investigations of citrate metabolism in stone formers[28] have shown that tubular citrate re-absorption is the main mechanism regulating urinary citrate excretion. ALAS administration brought the urinary citrate excretion to near normal in ethylene glycol induced urolithic rats.

Urinary magnesium was significantly diminished in ethylene glycol induced urolithic rats. Magnesium complexes with oxalate, thus reducing calcium oxalate supersaturation in urine and, as a consequence growth and nucleation rate of calcium oxalate crystals[29,30]. Low levels of magnesium are also encountered in stone formers as well as stone forming rats[31]. ALAS treatment restored the magnesium excretion and thus could reduce the growth of calcium oxalate crystals in ethylene glycol induced urolithic rats.

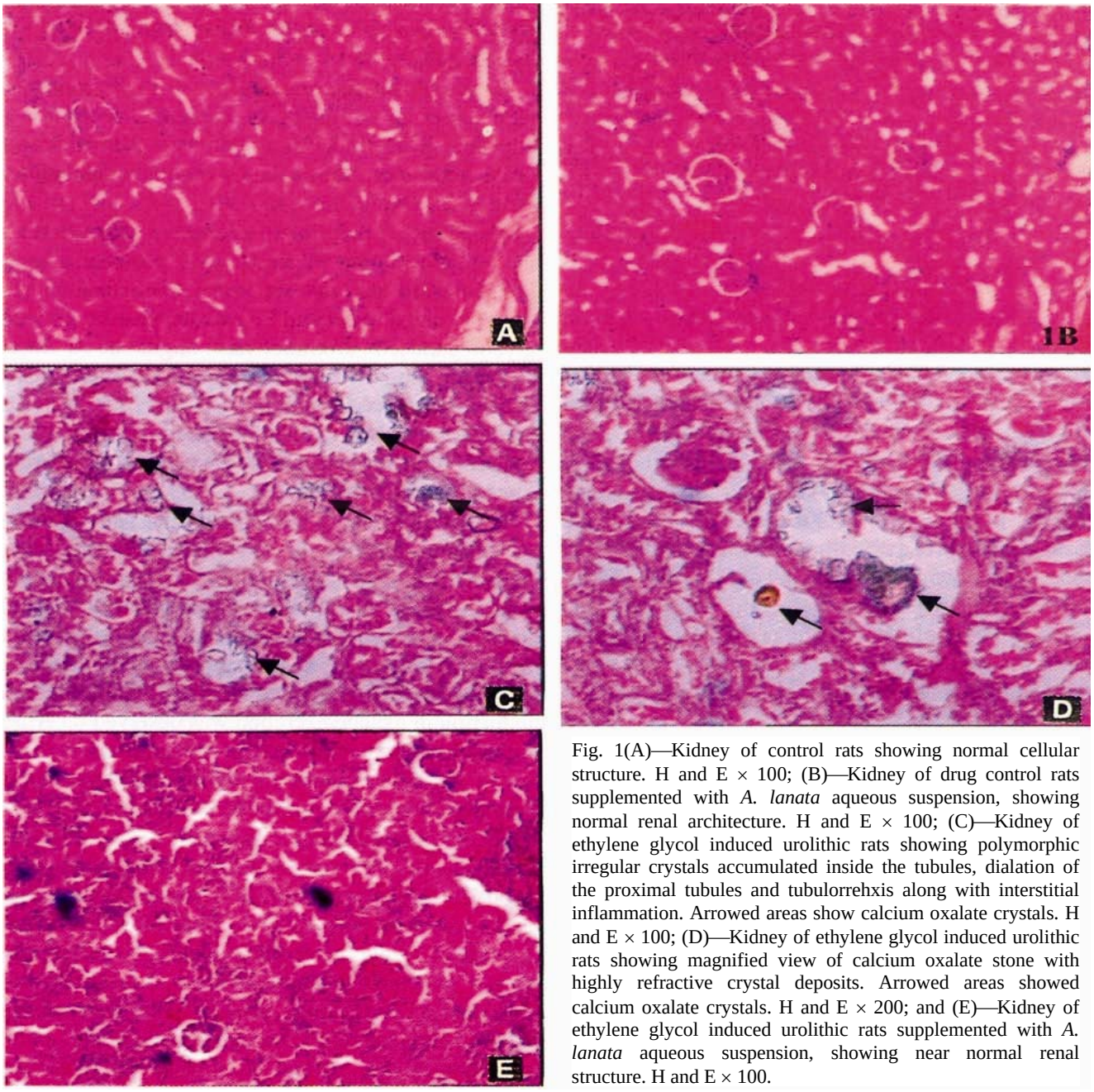

Fig. 1(A)—Kidney of control rats showing normal cellular structure. H and E × 100; (B)—Kidney of drug control rats supplemented with *A. lanata* aqueous suspension, showing normal renal architecture. H and E × 100; (C)—Kidney of ethylene glycol induced urolithic rats showing polymorphic irregular crystals accumulated inside the tubules, dialation of the proximal tubules and tubulorrehxis along with interstitial inflammation. Arrowed areas show calcium oxalate crystals. H and E × 100; (D)—Kidney of ethylene glycol induced urolithic rats showing magnified view of calcium oxalate stone with highly refractive crystal deposits. Arrowed areas showed calcium oxalate crystals. H and E × 200; and (E)—Kidney of ethylene glycol induced urolithic rats supplemented with *A. lanata* aqueous suspension, showing near normal renal structure. H and E × 100.

Uric acid is known to promote calcium oxalate crystal growth[32]. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation[33]. In the present study, higher concentration of urinary uric acid was observed in ethylene glycol induced urolithic rats. ALAS treatment restored the uric acid level to normal thus reducing the risk of stone formation.

A gradual increase in urinary phosphorus excretion was observed in ethylene glycol induced urolithic rats. Increased excretion of phosphorus has been reported in stone formers[34] and hyperoxaluric rats[35,36]. Increased urinary phosphorus 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[37]. On ALAS treatment, the phosphorus level was maintained

to normal and reducing the risk of stone formation.

The present observation showed proteinuria in ethylene glycol induced urolithic rats. Proteinuria reflects proximal tubular dysfunction[38]. Administration of ALAS had profound effects on minimizing the excretion of protein and thus might have prevented the nidus formation for crystal nucleation.

Microscopic examination of kidney sections derived from ethylene glycol induced urolithic rats showed polymorphic irregular crystals deposits inside the tubules which causes dialation of the proximal tubules along with interstitial inflammation this might be attributed to oxalate. Atmani *et al*.[39] have also shown that crystal deposits are intensely birefringent, polycrystalline, and arranges in rosette characteristic of calcium oxalate crystals. The presence of such deposits is an evidence of adhesion and retention of pentides within renal tubules. Ethylene glycol induced urolithic rats treated with ALAS had increased the solubility of CaOx crystal deposits and restored the normal renal architecture. This recovery effect may be due to flavonoids such as kaemepferol-3-rhamnoside and kaempferol-3-rhamnogalactoside, triterpenes such as betulin and tannins etc. The present study concludes that ALAS is more effective and this indigenous medicinal plant can be used successfully as an attractive alternative treatment for urolithiasis.

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