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Evaluation of the Lithotriptic Activity of the Siddha Herbo-Mineral Formulation Aya Veera Maathirai in Ethylene Glycol-Induced Urolithiatic Albino Rats

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ABSTRACT

Introduction: The kidneys are among the most vital organs in the human body, regulating fluid balance, electrolytes, and waste excretion. Urolithiasis, the third most common renal disorder of renal system. The Siddha system includes several formulations for *Kalladaippu* (kidney stones), though many lack scientific validation. *Aya Veera Maathirai* (AVM), a traditional herbo-mineral preparation for urinary calculi, has not been systematically evaluated for lithotriptic activity. **Objective:** To evaluate the lithotriptic potential of AVM in an ethylene glycol induced calcium oxalate urolithiasis model in female Wistar albino rats. **Materials and Methods:** Urolithiasis was induced with 1% ethylene glycol for 28 days. Thirty female rats were divided into five groups, normal control, lithiatic control, standard drug (Cystone 100 mg/kg), AVM 25 mg/kg, and AVM 50 mg/kg. Treatments were administered orally for 15 days. Urine and serum biochemical parameters were measured, and kidney tissues were examined histopathologically. **Results:** Ethylene glycol induced significant alterations in urinary and serum markers, and tubular injury. AVM treatment restored these parameters in a dose dependent manner, with the 50 mg/kg dose achieving effects comparable to Cystone. Histopathological analysis showed minimal tubular degeneration, reduced mesangial hypercellularity, and complete absence of crystal deposition in the high dose (50 mg/kg) AVM group. **Conclusion:** AVM exhibits significant lithotriptic activity, effectively, normalizing biochemical markers and preventing calcium deposition, supporting its potential in urolithiasis management.

Key words: *Aya Veera Maathirai*, Siddha medicine, urolithiasis, lithotriptic activity, *Kalladaippu*

INTRODUCTION

Urolithiasis, also known as nephrolithiasis or kidney stone disease, is a common disorder of the urinary system characterized by the formation of crystalline aggregates due to the precipitation of urinary solutes within the kidneys, bladder, or urinary tract.(1) When calculi migrate from the renal pelvis into the ureter, bladder, or urethra, the condition is referred to as urolithiasis (derived from the Greek words ouron meaning urine and lithos meaning stone). (2,3) Clinically, urolithiasis presents with severe abdominal or flank pain radiating toward the groin, hematuria, urinary tract infections, and may progress to renal dysfunction if left untreated. (4)

Urolithiasis is ranked as the third most prevalent renal disorder worldwide, following urinary tract infections and prostate diseases.(3,4) Epidemiological studies indicate a rising global prevalence, affecting approximately 13% of the population at some point in their lifetime,(5) with significant geographical variations 7-13% in North America, 5-9% in Europe, and 1-5% in Asia(6). The disease is more prevalent between the ages of 20 and 40 years and occurs more frequently in males than females, with male-to-female ratios increasing over time (e.g., from 1.86:1 in 1997 to 2.7:1 in 2006 in Germany). Overall prevalence rates show 5.5% in males and 4.0% in females, (5,7) largely attributed to the stone promoting effects of testosterone and the inhibitory effects of estrogen(8).

The recurrence rate of urolithiasis is notably high, with studies reporting relapse rates of 50% within 5–10 years and up to 75% within 20 years after the first episode.(1) Some datasets suggest that more than 50% of patients experience recurrence within five

years, while recurrence rates range from 70–81% in males and 47–60% in females [3]. Recurrent urolithiasis contributes significantly to long-term morbidity, including arterial hypertension, septicemia, and a twofold increased risk of acute or chronic kidney disease. Moreover, urolithiasis accounts for more hospitalizations than other urological disorders such as benign prostatic hyperplasia, prostate cancer, and bladder cancer [5].

Regarding stone composition, calcium oxalate stones predominate, accounting for approximately 60–90% of cases, either alone or in combination with calcium phosphate. Other stone types include calcium phosphate (10–20%), struvite (9–14%), uric acid (5–10%), cystine (1–5%), and mixed stones (approximately 4%).[5] Renal calculi consist predominantly (about 80%) of calcium oxalate and calcium phosphate, with nearly half being idiopathic calcium oxalate stones [9]. Risk factors for stone formation and recurrence include younger age at onset, male gender, multiple prior stone episodes, family history, elevated body mass index (BMI), dietary habits, climatic conditions, and comorbidities such as hypertension and obesity [1].

The management of urolithiasis depends on stone size, location, and composition. Stones smaller than 5 mm may pass spontaneously. However, larger stones or those that fail to pass require interventional procedures such as Extracorporeal Shock Wave Lithotripsy (ESWL), Ureteroscopy (URS), or Percutaneous Nephro-lithotomy (PNL).[7] Although effective, these procedures are expensive and often associated with complications, recurrence, and adverse effects. Current pharmacological therapies aim at altering urinary chemistry but are not always free from side effects, highlighting the need for safer and cost effective alternatives [1].

In Siddha literature, urolithiasis is described as *Kalladaippu,* specifically *Vatha Kalladaippu*, and is classified under *Neerinai Arukkal Noigal* (obstructive uropathies), as documented in classical texts such as *Yugi Vaidhya Sindhamani* [9,10]. Several classical Siddha formulations possess diuretic, anti-inflammatory, and lithotriptic properties and have traditionally been used to dissolve or expel urinary calculi and to reduce recurrence. However, many of these formulations remain scientifically unexplored with respect to their pharmacological activities.

Contemporary medical management of nephrolithiasis is often costly and associated with adverse effects.[11] In contrast, traditional systems of medicine rely on remedies derived from *Jeevam* or *Sangamam* (animal products), *Thathu* (inorganic substances), and *Mooligai*/*Thavaram* (herbal products).[12] Among these, *Aya Veera Maathirai* (AVM) is a classical herbo-mineral formulation documented in *Kannusamiyam Ennum Vaithiya Segaram*. It is traditionally indicated for hydrocele, *Vatha Pitha Soolai* (rheumatic disorders), *Kudaichal* (neuralgic pain), and *Kalladaippu* (renal calculi) [13].

Although described in Siddha literature, the lithotriptic potential of *Aya Veera Maathirai* has not been scientifically evaluated. Hence, the present study assesses its lithotriptic activity using an ethylene glycol-induced urolithiasis model in Wistar albino rats.

MATERIALS AND METHODS

Selection of the Drug

The herbo-mineral formulation *Aya Veera Maathirai* was selected from the *Siddha* literature *Kannusamium Ennum Vaiththiya Segaram* (Page number 47, 48).

Drug Recognition and Authentication

The collected raw materials were identified and authenticated by a qualified expert from the Faculty of Medicinal Botany (**Authentication Certificate No: NISMB8282025**) and the Department of Gunapadam, National Institute of Siddha, Chennai-47. (**Authentication Certificate No: Gun/Aut/37/24).**

Preparation of *Aya Veera Maathirai*

Purified iron powder (*Aya podi*) 70 grams and Hydragyrum perchloride (*Veeram*) 35 grams are ground with *vediuppu thravagam* for 3 days, then dried and fried until black smoke vanishes. After getting a good shade, powder it and put it in a moist free, air tight container. One component of this completed medicine, then each one part of *Zingiber officinale* (*Chukku*), *Piper longum (Thippili), Piper nigrum (Milagu), Emblica officinalis (Nelli vattral), Terminalia chebula (Kadukkai), Piper cubeba (Vaal milagu)* and two part of *Croton tiglium* (*Nervaalam*) were added, ground with lemon juice for 2 hours, formed into tablets and then allowed to dry in the shade.

LITHOTRIPTIC ACTIVITY

Selection and Maintenance of Experimental Animals

Healthy female Wistar albino rats (150–200 g) were procured from Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Madhavaram. Animals were housed in well-ventilated polypropylene cages (3–4 per cage) under controlled environmental conditions (Temperature 25 ± 2°C, 12-hour light/dark cycle, relative humidity 30–70%). They were provided with a standard pellet diet and water ad libitum. Animals were acclimatized for 7 days prior to the experimental study.

All experimental procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), National Institute of Siddha. **Approval No: NIS/IAEC-27/R07/13122024/E9**.

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 FOR LITHOTRIPTIC ACTIVITY

Table no 1: Experimental design for Lithotriptic activity

S.no	Group	Treatment Details	No. of Animals
1	**Group I** (Normal control)	Received normal saline (vehicle control) and standard diet at a dose of 10 ml/kg orally for 28 days.	6(F)
2	**Group II** (Lithiatic group)	Received 1% ethylene glycol in drinking water to induce urolithiasis for 28 days.	6(F)
3	**Group III** (Standard drug - Cystone)	Received 1% ethylene glycol in drinking water for 28 days followed by Cystone treatment at a dose of 100 mg/kg orally for 15 days.	6(F)
4.	**Group IV** (AVM- 25 mg/kg)	Received 1% ethylene glycol in drinking water for 28 days followed by AVM treatment at a dose of 25 mg/kg orally for 15 days.	6(F)
5.	**Group V** (AVM- 50 mg/kg)	Received 1% ethylene glycol in drinking water for 28 days followed by AVM treatment at a dose of 50 mg/kg orally for 15 days.	6(F)

In this experiment, a total of 30 female rats were used and divided into five groups of six animals each. Group I served as the curative control, while Groups II were rendered lithiatic using 1% ethylene glycol. Group III received the standard antiurolithiatic drug Cystone (100 mg/kg), whereas Group IV and Group V were administered AVM at dose levels of 25 mg/kg and 50 mg/kg, respectively. All treatments were given orally according to the prescribed dosing schedule. At the end of the treatment period, the animals were placed in metabolic cages to collect 24-hour urine samples for biochemical evaluation.

Collection and analysis of urine samples

All animals were housed individually in metabolic cages, and 24-hour urine samples were collected on the 28th day of the experiment. Animals were allowed free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the collected urine samples, which were then stored at 4 °C until analysis. Urine was analyzed for Calcium, phosphorus and Sodium content.[11]

Serum analysis

At the end of the experimental period, blood samples were collected from the retro-orbital vein under anesthesia. Animals were subsequently euthanized by Thiopental sodium injection. Serum was separated by centrifugation at 10,000 × g for 10 minutes and analyzed for urea, creatinine, uric acid, and Blood Urea Nitrogen (BUN). [11]

Histopathological examination

Kidneys were excised from animals of each group and washed with normal saline. The right kidney was fixed in 10% formalin, processed by routine histological methods, sectioned, and stained with hematoxylin and eosin (H&E) for microscopic examination.[14]

STATISTICAL ANALYSIS

Statistical Analysis Data were expressed as mean ± SD (n = 6) and analyzed using one-way ANOVA followed by Dennett's post hoc test. Statistical significance was set at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

Test Results

Blood Analysis

Table no 2: Lithotriptic Activity of AVM

Group	Urea	Creatinine	Uric Acid	BUN
Group I (Normal Control)	49.33 ± 5.39	1.38 ± 0.27	4.88 ± 0.48	23.02 ± 2.51
Group II (Ethylene Glycol Control)	41.67 ± 12.75	1.58 ± 0.18	2.13 ± 1.64	17.45 ± 3.41
Group III (Standard–Cystone)	47.5 ± 3.94	1.4 ± 0.32	1.52 ± 1.17	22.13 ± 1.85
Group IV (AVM-25 mg/kg)	40.33 ± 3.33	1.33 ± 0.19	1.55 ± 1.12	18.82 ± 1.54
Group V (AVM-50 mg/kg)	42.83 ± 4.45	1.72 ± 0.2	5.7 ± 7.35	16.63 ± 8.17

Mean (±) S.D; P<0.05 - significant, when compared to Control Group

Urine Analysis

Table no 3: Lithotriptic Activity in AVM

Group	Calcium	Phosphorous	Sodium
Group I (Normal Control)	50.67 ± 8.89	73.67 ± 13.88	156 ± 36.05
Group II (Ethylene Glycol Control)	48.17 ± 9.75	89.67 ± 6.41	**99.33 ± 26.73***
Group III (Standard–Cystone)	51.5 ± 2.51	**51.17 ± 20.87***	**61.17 ± 16.74***
Group IV (AVM-25 mg/kg)	43.17 ± 5.85	86.5 ± 5.5	**83.67 ± 15.97***
Group V (AVM-50 mg/kg)	51.17 ± 3.66	88.5 ± 2.66	**110.33 ± 30***

Mean (±) S.D; *P<0.05 - significant, when compared to Control Group

Histopathology Results (Kidney)

Group I: Treated with regular food and drinking water

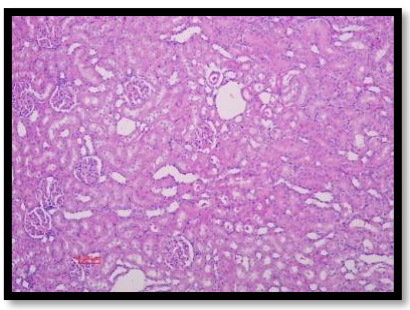


Fig no: 1 A

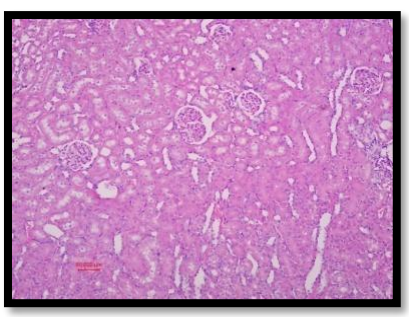


Fig no: 1 B

Group II: Treated with Ethelene glycol with drinking water.

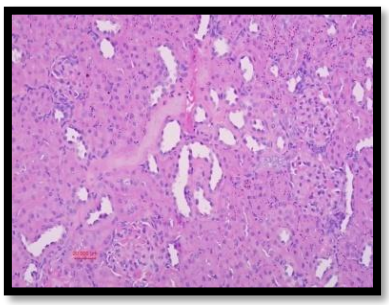


Fig no: 2A

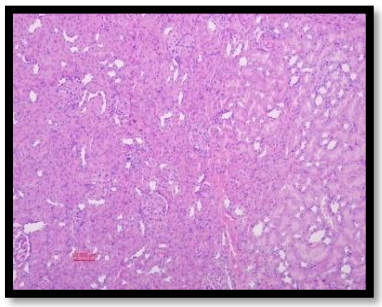


Fig no: 2 B

Group III : Treated with standard Received 1% ethylene glycol in drinking water for 28 days followed by Cystone treatment at a dose of 100 mg/kg orally for 15 days.

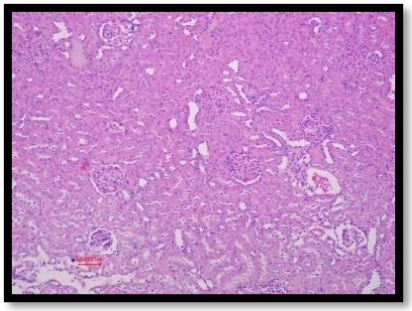


Fig no : 3 A

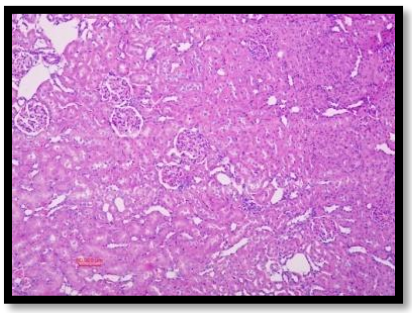


Fig no:3 B

Group IV (AVM- 25 mg/kg) : Received 1% ethylene glycol in drinking water for 28 days followed by AVM treatment at a dose of 25 mg/kg orally for 15 days.

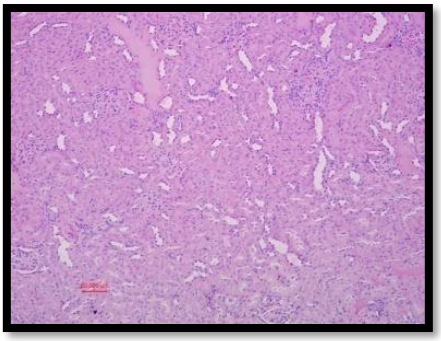


Fig no: 4 A

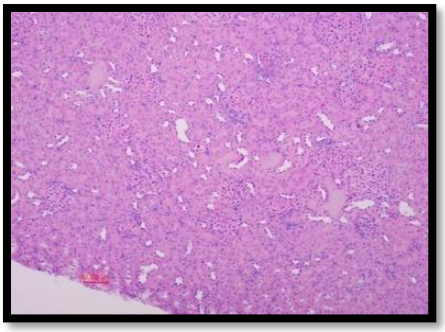


Fig no: 4 B

Group V (AVM- 50 mg/kg): Received 1% ethylene glycol in drinking water for 28 days followed by AVM treatment at a dose of 50 mg/kg orally for 15 days.

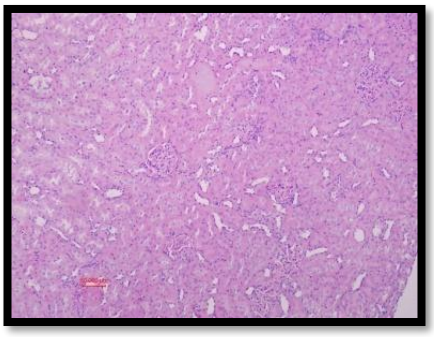


Fig no:5 A

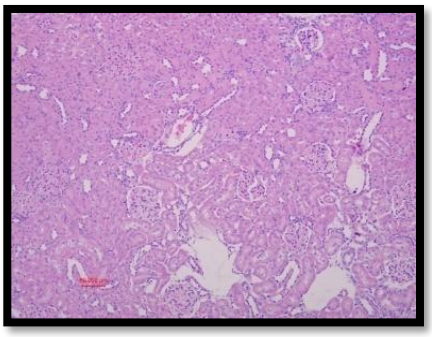


Fig no: 5 B

Result

The effect of ethylene glycol induced urolithiasis and subsequent treatments on serum and urinary biochemical parameters is presented in Tables 2 and 3, and Charts 1–7. Administration of ethylene glycol produced marked alterations in renal function markers in the lithiatic control group compared with the normal control animals. Changes in serum urea, creatinine, uric acid, and blood urea nitrogen (BUN) levels indicated impairment of renal function following stone induction.

Treatment with the standard drug Cystone (100 mg/kg) markedly modulated serum biochemical parameters, restoring urea, creatinine, and BUN levels toward normal control values. Similarly, administration of *Aya Veera Maathirai* (AVM) at doses of 25 and 50 mg/kg resulted in improvement of serum renal biomarkers when compared with the lithiatic control group. AVM at 25 mg/kg showed a reduction in serum creatinine and BUN levels, while the higher dose of AVM (50 mg/kg) demonstrated comparable modulation of renal parameters, indicating a protective effect against ethylene glycol–induced renal dysfunction.

Urinary biochemical analysis revealed significant alterations in calcium, phosphorus, and sodium excretion in ethylene glycol–treated rats. The lithiatic control group exhibited significant changes in urinary electrolyte levels, particularly sodium, when compared with the normal control group ($P < 0.05$). Treatment with Cystone significantly reduced urinary phosphorus and sodium levels compared with controls ($P < 0.05$). AVM treatment influenced urinary electrolyte excretion in a dose-dependent manner; the 25 mg/kg dose reduced urinary calcium levels, while the 50 mg/kg dose maintained calcium levels comparable to the normal control group. Significant variations in urinary sodium levels were observed in all treated groups compared with controls ($P < 0.05$).

Histopathological examination of kidney sections supported the biochemical findings. Normal control animals showed intact renal architecture without evidence of crystal deposition. In contrast, kidneys from the lithiatic control group exhibited pathological alterations, including tubular damage and crystal deposition. Treatment with Cystone markedly reduced crystal formation and preserved renal histoarchitecture. AVM treatment demonstrated dose-dependent protection, with the 25 mg/kg group showing reduced crystal deposition and the 50 mg/kg group exhibiting near-normal renal architecture with absence of visible crystal deposits.

Overall, AVM effectively modulated ethylene glycol induced biochemical and renal pathological changes, with enhanced protection observed at the higher dose.

Discussion

Aya Veera Maathirai (AVM) herbo-mineral formulation has been traditionally described in Siddha literature for the management of urinary calculi. However, scientific evidence substantiating its lithotriptic efficacy remains limited. Therefore, the present study was undertaken to evaluate its lithotriptic potential against ethylene glycol induced urolithiasis in Wistar albino rats.

The ethylene glycol induced urolithiasis model is a well established method for replicating calcium stone formation and associated renal dysfunction and has been widely used in lithotriptic studies. Ethylene glycol disrupts oxalate metabolism, leading to hyperoxaluria, calcium oxalate crystal deposition, and renal tubular injury through oxidative stress and depletion of antioxidant defenses. In the present study, ethylene glycol administration caused significant alterations in serum urea, creatinine, uric acid, and blood urea nitrogen levels, indicating impaired renal function in lithiatic control group.

Treatment with the standard drug Cystone (100 mg/kg) effectively corrected the ethylene glycol–induced biochemical disturbances, restoring serum renal parameters toward values observed in the normal control group. Similarly, animals treated with AVM at 25

mg/kg and 50 mg/kg demonstrated significant improvement in serum urea, creatinine, and BUN levels compared with the lithiatic control group, indicating attenuation of EG-induced renal injury.

Likewise, rats treated with AVM at doses of 25 mg/kg and 50 mg/kg showed significant improvements in serum urea, creatinine, and blood urea nitrogen levels compared with the lithiatic control group, indicating mitigation of EG induced renal injury. Notably, the higher dose of AVM (50 mg/kg) produced greater normalization of renal biomarkers, suggesting a dose dependent protective effect. The diuretic and anti-inflammatory properties of its herbal constituents *Zingiber officinale, Piper longum, Terminalia chebula, and Phyllanthus emblica* also contribute to these effects.

Urinary biochemical parameters are key determinants of stone pathogenesis, as supersaturation of calcium and phosphate facilitates crystal nucleation and growth. Ethylene glycol administration significantly disrupted urinary electrolyte excretion, notably sodium and phosphorus, thereby enhancing urinary supersaturation and crystal aggregation. In contrast, Cystone treatment markedly reduced urinary phosphorus and sodium levels, supporting its established antiurolithiatic efficacy.

AVM modulated urinary electrolyte excretion in a dose dependent manner. The 25 mg/kg dose significantly reduced urinary calcium, indicating inhibition of calcium oxalate supersaturation and crystal formation, while the 50 mg/kg dose maintained calcium levels comparable to normal controls, suggesting stabilization of mineral homeostasis. Altered urinary sodium excretion may further reduce crystal retention. Collectively, these findings indicate that AVM interferes with multiple stages of stone formation, including crystal nucleation and aggregation.

Histopathological findings further substantiated the renoprotective effect of AVM. The ethylene glycol–treated group showed moderate tubular dilation, epithelial degeneration, and pronounced mesangial hypercellularity, indicative of crystal-induced renal injury. In contrast, Cystone-treated rats exhibited only mild tubular alterations with reduced cast formation. AVM produced dose-dependent renal protection; the 25 mg/kg dose showed mild tubular degeneration with focal dilation, whereas the 50 mg/kg dose demonstrated minimal tubular changes, markedly reduced mesangial hypercellularity, and absence of crystal deposition, indicating effective prevention of calcium oxalate accumulation.

Conclusion

Aya Veera Maathirai demonstrated that clear lithotriptic effect in ethylene glycol induced urolithiasis. It corrected key biochemical disturbances, improved renal function, and showed dose dependent protection against calcium oxalate deposition, with the 50 mg/kg dose offering the greatest benefit. These results confirm *Aya Veera Maathirai* potential as an effective formulation for managing urolithiasis and support further studies to establish its clinical value.

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