AYUSHDHARA
ISSN: 2393-9583 (P)/ 2393-9591 (O)
An International Journal of Research in AYUSH and Allied Systems

Research Article

EVALUATION OF ANTI-UROLITHIATIC ACTIVITY OF SIDDHA HERBAL FORMULATION *KALLADAIPPUKU KUDINEER* ON 0.75% ETHYLENE GLYCOL INDUCED UROLITHIASIS MODEL

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Article info

Article History:
Received: 11-01-2026
Accepted: 18-02-2026
Published: 15-03-2026

KEYWORDS:
Kalladaippuku Kudineer, Siddha medicine, Anti-Urolithiatic activity, Ethylene glycol induced model.

ABSTRACT

The Siddha system of medicine employs a wide range of herbal and mineral formulations for the management of Urolithiasis. In the Siddha system of medicine, urolithiasis is correlated with *Kalladaippu*. This study aimed to investigate the anti-urolithiatic activity of *Kalladaippuku Kudineer* using an experimental animal model**.** Wister albino rats of number 30 were randomly divided into five groups (n=6 per group) and used for the study. Urolithiasis was induced in all groups, except the normal control, by administering 0.75% ethylene glycol in the drinking water. Group I served as the normal control, Group II as the disease control, Group III as the standard control, while Groups IV and V received *Kalladaippuku Kudineer* (KK) at low and high doses of 200 and 400mg/kg body weight, respectively. After 28 days of treatment, serum biochemical parameters including blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine were estimated. Histopathological examination of kidney tissue was performed to assess any treatment-related or toxicological changes. Data are expressed as mean ± standard error of the mean (SEM) and were analyzed using one-way analysis of variance (ANOVA). A p-value < 0.05 was considered statistically significant. In the present study, treatment with *Kalladaippuku Kudineer* (KK) demonstrated a significant dose-dependent anti-urolithiatic effect at doses of 200 and 400mg/kg body weight in experimental animals. Administration of the low dose of KK (200mg/kg b.w.) markedly reduced serum and urinary abnormalities. In contrast, the high dose of KK (400 mg/kg b.w.) produced notable improvements in renal biochemical parameters, including blood urea nitrogen (BUN), creatinine, oxalate, and calcium levels, when compared with the standard control group (Group III).

INTRODUCTION

Urolithiasis is a pathological condition characterized by the formation and retention of calculi within the urinary tract, including the kidneys, ureters, bladder, and urethra. In India, the prevalence of Urolithiasis is estimated to be approximately 12%, with a higher incidence reported among males, particularly in the age group of 35–60 years. Traditional systems of medicine continue to contribute substantially to healthcare, particularly in the management of chronic disorders. The Siddha system of medicine, one of the oldest traditional medical systems, was developed by Siddhas-ancient scholars known for their extensive knowledge of medicine, alchemy, yoga, and spirituality. Siddha medicine is founded on the principles of the five-elemental theory and emphasizes preventive, promotive, and curative approaches to health. Currently, the management of Urolithiasis includes pharmacological therapy and surgical interventions such as extracorporeal shock wave lithotripsy (ESWL), which remains a widely adopted treatment modality. However, ESWL is associated with certain limitations, including the risk of renal tissue injury, impaired renal function, and recurrence of calculi. These concerns have led to

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	https://doi.org/10.47070/ayushdhara.v13i1.2494

increased interest in alternative therapeutic approaches with improved safety profiles. Medicinal plants have long been recognized as valuable sources of novel therapeutic agents. Several plant-based formulations, including *Phyllanthus niruri, Aerva lanata,* and *Crataeva magna,* have demonstrated inhibitory effects on calcium oxalate crystallization and reduced recurrence of urinary calculi in experimental and clinical studies. In the classical Siddha literature named "*Agasthiyar Vaithiya Kaaviyam*–1500", the formulation *Kalladaippuku Kudineer* is indicated for the management of *Kalladaippu*, a condition clinically comparable to urolithiasis. Despite its traditional use, systematic scientific evaluation of its antilithiatic efficacy remains limited. The standardization of KK was done in Regional Research Institute of Unani Medicine, Chennai. The quantitative test for preliminary phytochemical screening of *Kalladaippuku Kudineer* shows the presence of carbohydrates, favonoids, quinones and carboxylic acid. The toxicological evaluation of KK was also completed in Madras veterinary college, Vepery, Chennai. Therefore, the present study was undertaken to evaluate the anti-urolithiatic potential of *Kalladaippuku Kudineer* using an experimental animal model.

MATERIAL AND METHODS

Kalladaippuku Kudineer is a herbal formulation consisting of:

1. *Nathaichoori - Spermacoce hispida*
2. *Naaipagal- Momordica dioica*
3. *Karunjeeraga - Nigella sativa*

Animal Selection

Healthy Wistar albino rats weighing between 150 and 200g were selected and are housed in polypropylene cages under standard laboratory conditions, maintained at a temperature of 23±2 °C with a relative humidity of 55±5% and a 12-h light/dark cycle. The animals were provided with standard pelleted rat feed and water ad libitum. The animal care and Experimental protocols were in accordance with Institutional Animal Ethical Committee (IAEC) -proposal No: AKCP/ IAEC/ 20/2025-2026, constituted under CPCSEA. Prior to the commencement of the experiment, all animals were acclimatized for a period of 15 days.

Chemicals and Drugs

Ethylene glycol (0.75% v/v) was procured from S. D. Fine Chemicals, Bangalore, India. The standard drug, Cystone, was purchased from Himalaya Herbal Healthcare, Bangalore, India. All other chemicals and solvents used in the study were of analytical grade. Demineralized water was used throughout the experimental procedures.

Drug Administration

The test drug KK and the standard drug Cystone were administered orally using a stainless-steel oral gavage tube. The required doses of KK were freshly prepared as mentioned in the text book prior to administration.

Acute Toxicity Study

The acute oral toxicity study was carried out in Madras Veterinary College, Vepery, Chennai, as per the OECD guidelines. No emerging signs, toxicity and mortality was observed during the study. One-tenth of the median lethal dose (LD50) was taken as an effective dose.

Anti-Urolithiatic Activity of KK

Urolithiasis was induced by administering ethylene glycol at a final concentration of 0.75% (v/v) in the drinking water. After acclimatization, the animals were randomly divided into five groups (Group I–V), each consisting of six rats and the test drug KK was given from day 15 to day 28.

Group	Treatment	Dose
Group I	Normal control	Standard feed & drinking water
Group II (Disease control)	Ethylene glycol	0.75%v/v in drinking water
Group III (Standard control)	Ethylene glycol + cystone	0.75%v/v + 750mg/kg
Group I V	Ethylene Glycol+ KK single dose	0.75%v/v + 200mg/kg
Group V	Ethylene Glycol + KK double dose	0.75%v/v + 400mg/kg

Serum Analysis

At the end of day 28, the blood was collected from the retro-orbital sinus under anaesthetic condition and serum was separated by centrifugation at 10,000g for 10 min and analyzed for Creatinine, uric acid, BUN and other biochemical evaluation.

Urine Analysis

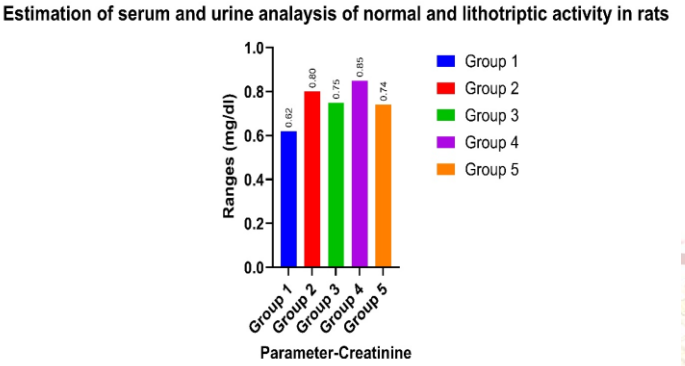
All animals were kept in individual metabolic cages and 24 hr urine samples were collected on 14th, and 28th day. Urine samples were analyzed for calcium, oxalate, magnesium, phosphate, and pH. Following sample collection, the animals were sacrificed, and both kidneys were excised for histopathological examination to assess the presence and extent of renal calculi formation.

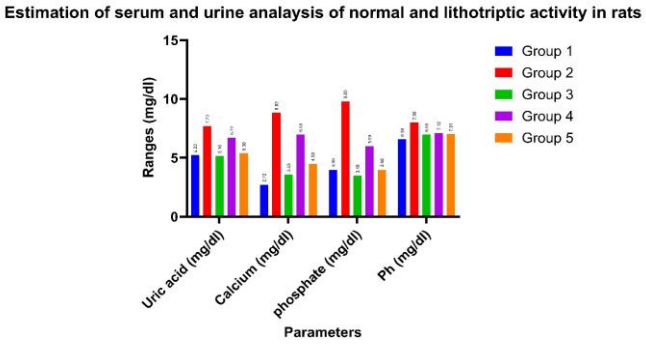
Statistical Analysis

The urinary and blood parameters data were recorded, calculated and expressed as Mean±SEM. The results were analysed using one-way ANOVA followed by Dunnett's multiple Comparison test. P values <0.05 were considered as statistically significant.

Table 1: Estimation of Urine Analysis of normal and Lithotriptic activity in Rats

Group	Treatment	Uric acid (mg/dl)	Oxalate (mg/dl)	Calcium (mg/dl)	Phosphate (mg/dl)	pH (mg/dl)
Group 1	Control	5.23±0.3	0.39±0.2	2.72±0.3	3.98±0.65	6.58±0.9
Group 2	Ethylene Glycol	7.70±0.32	2.98 ±0.98	8.83±0.8	9.80 ±0.56	7.99 ±0.56
Group 3	STD (Cystone) + Ethylene Glycol	5.16±0.2	0.38±0.2	3.58±1.1	3.48±1.4	6.98 ±1.3
Group 4	Ethylene Glycol + KK single dose	6.72 ±1.1	2.98 ±0.9	6.98±1.1	5.99 ±0.98	7.12 ±0.9
Group 5	Ethylene Glycol + KK Double Dose	5.38±0.6	1.15 ±0.2	4.50±1.3	3.98±1.1	7.01 ±1.4

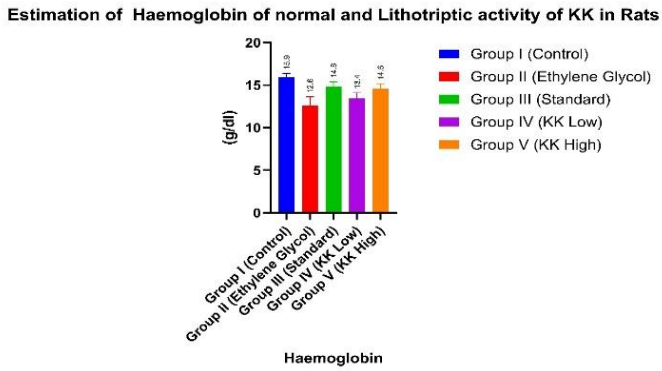


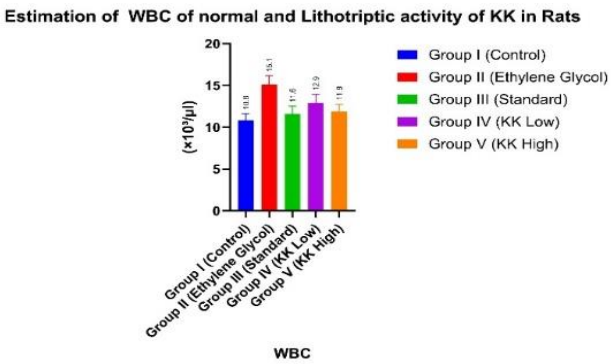


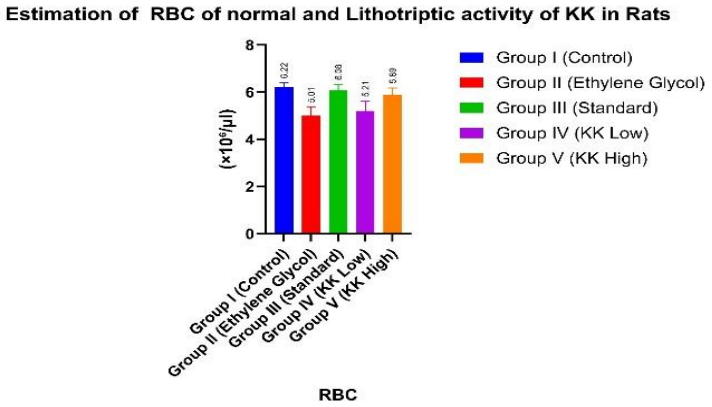
All values were expressed as mean ± SEM, n = 6, * P < 0.05, **P < 0.01, ***P < 0.001 as compared to the disease control group. Results were done by one-way ANOVA followed by Dunnett's test.

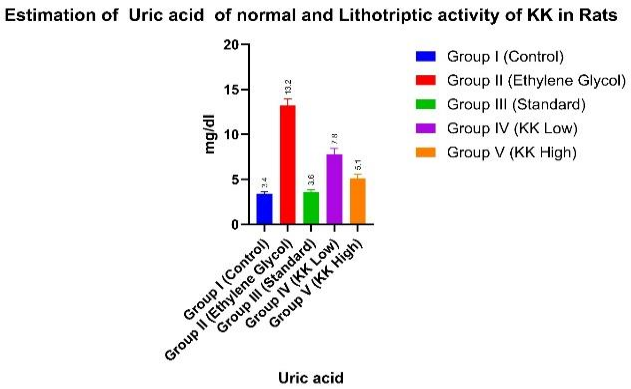
Table 2: Estimation of Serum Analysis of normal and Lithotriptic activity of KK in Rats

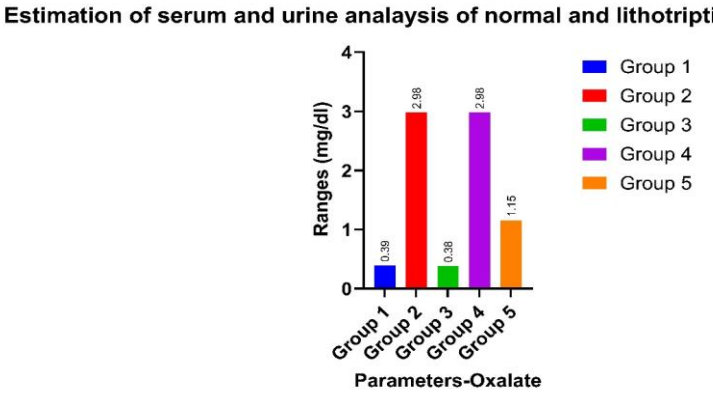
Group	BUN (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	RBC ($\times 10^6$/µl)	WBC ($\times 10^3$/µl)	HB (g/dl)
Group I (Control)	17.21 ± 1.54	0.62±0.21	3.4 ± 0.19	6.22 ± 0.18	10.8 ± 0.84	15.9 ± 0.47
Group II (Ethylene Glycol)	36.55 ± 1.2	0.80 ±0.54	13.2 ± 0.74***	5.01 ± 0.36*	15.1 ± 1.08	12.6 ± 1.02*
Group III (Standard)	18.70 ± 1.33	0.75±0.32	3.6 ± 0.28	6.08 ± 0.25	11.6 ± 0.91	14.8 ± 0.63
Group IV (KK Low)	30.17 ± 1.45	0.85 ±0.4	7.8 ± 0.62**	5.21 ± 0.41*	12.9 ± 1.02*	13.4 ± 0.71*
Group V (KK High)	25.18 ± 1.56	0.74±0.3	5.1 ± 0.47	5.89 ± 0.29	11.9 ± 0.88	14.6 ± 0.58





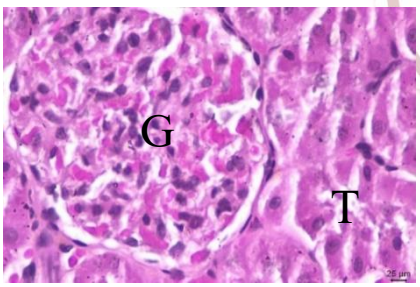
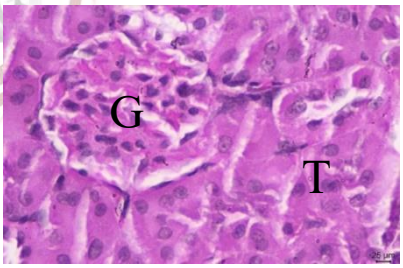
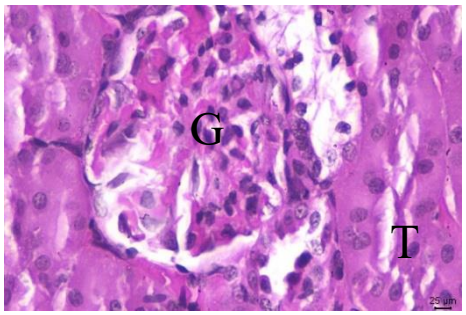
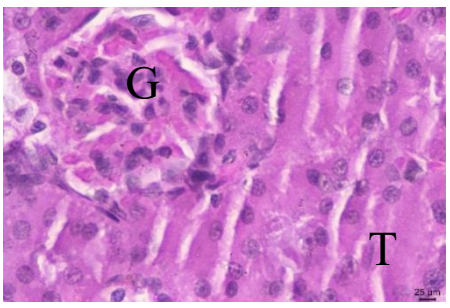


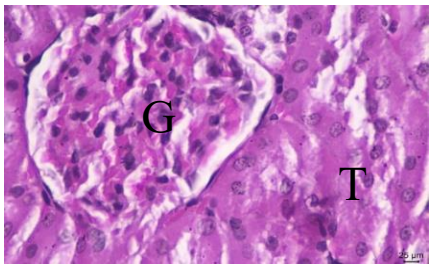




All values were expressed as mean ± SEM, n = 6, * P < 0.05, **P < 0.01, ***P < 0.001 as compared to the disease control group. Results were done by one-way ANOVA followed by Dunnett's test.

Histopathology Report

 Kidney from control group showing normal glomeruli (G) and tubules (T) with no significant pathological changes. H&E.	 Kidney section from control group –II served as lithiatic control and received 0.75% ethylene glycol & vehicle 1% tween showing Degeneration, tubular, multifocal, moderate. H&E.
 Kidney section from group-III served as standard control received 0.75% ethylene glycol & standard cystone (750 mg/kg), Showing Degeneration, tubular, multifocal, minimal.H&E.	 Kidney section from Group IV served as treatment control received 0.75% ethylene glycol &KK low dose - received p.o.) Showing Degeneration, tubular, multifocal, mild.H&E.



Kidney section from Group V served as treatment control received 0.75% ethylene glycol & KK high dose – received p.o.) showing Degeneration, tubular, multifocal, Minimal.

DISCUSSION

Urolithiasis is a multifactorial disorder characterized by urinary supersaturation of lithogenic solutes, oxidative stress–mediated renal injury, and subsequent crystal nucleation, aggregation, and retention within renal tubules. In the present investigation, ethylene glycol (0.75% v/v) administration successfully induced experimental urolithiasis in Wistar albino rats, a well-validated and reproducible model that closely resembles human calcium oxalate stone formation**.** Rats in the disease control group (Group II) exhibited significant elevations in serum blood urea nitrogen (BUN), creatinine, and uric acid levels, indicating compromised renal function secondary to crystal deposition and tubular obstruction. Concurrently, urinary oxalate, calcium, phosphate, and pH levels were markedly increased, reflecting enhanced supersaturation of lithogenic ions and a greater predisposition to calcium oxalate crystallization. These metabolic derangements are well-recognized contributors to stone formation and progression, as elevated urinary oxalate and calcium concentrations promote crystal nucleation, while phosphate and altered urinary pH facilitate crystal growth and aggregation. Histopathological evaluation further substantiated these biochemical findings, revealing multifocal crystal deposition and mild tubular epithelial degeneration in the disease control group. Tubular epithelial injury plays a critical role in stone pathogenesis by exposing underlying adhesion molecules, thereby facilitating crystal attachment, aggregation, and retention within the renal tubules. Administration of the standard anti-Urolithiatic formulation Cystone (Group III) significantly ameliorated the altered biochemical parameters and reduced urinary lithogenic constituents. Histological examination demonstrated minimal tubular damage, confirming its established therapeutic efficacy in preventing crystal formation and preserving renal architecture. Treatment with *Kalladaippuku Kudineer* (KK) exhibited a dose-dependent protective effect. The low-dose KK group (200 mg/kg, Group IV) showed partial normalization of serum and urinary biochemical parameters, accompanied by mild tubular degeneration on histological assessment. In contrast, the high-dose KK group (400 mg/kg, Group V) demonstrated significant improvement in renal function markers, including reductions in serum BUN, creatinine, and uric acid levels, as well as decreased urinary oxalate, calcium, and phosphate concentrations. Additionally, urinary pH values approached normal physiological levels, indicating reduced crystallization potential. Histopathological findings in the high-dose group revealed minimal tubular degeneration, comparable to that observed in the standard drug-treated group. Collectively, these findings indicate that KK possesses significant anti-Urolithiatic activity, likely mediated through reduction of urinary supersaturation, inhibition of crystal nucleation and aggregation, and preservation of renal tubular epithelial integrity. The observed nephroprotective effects further underscore its therapeutic potential in the management of urolithiasis. However, further studies exploring its molecular mechanisms and clinical translation are warranted.

CONCLUSION

The present study on *Kalladaippuku Kudineer* possess significant anti-Urolithiatic activity by inhibiting the crystal nucleation and aggregation**,** reduction of urinary supersaturation, significant improvement in renal function markers, including reductions in serum BUN, creatinine, and uric acid levels, as well as decreased urinary oxalate, calcium, and phosphate concentrations at a dose of 200 and 400 mg/kg. It can be concluded that *Kalladaippuku Kudineer* demonstrated significant anti-Urolithiatic activity comparable to the standard drug.

Acknowledgement

The author expresses sincere gratitude to Mr. M.Santhanakumar, Associate professor, HOD, Department of pharmacology, Arulmigu kalasalingam college of pharmacy for rendering the necessary facilities to carry out this research and the author also extends heartfelt thanks to the faculty of Department of PG Pothu Maruthuvam for providing support.

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Cite this article as:
S. Swathi, K. Sudhamathi Pushparaj, R. Menaka, U. Chitra. Evaluation of Anti-Urolithiatic Activity of Siddha Herbal Formulation Kalladaippuku Kudineer on 0.75% Ethylene Glycol Induced Urolithiasis Model. AYUSHDHARA, 2026;13(1):7-12.
https://doi.org/10.47070/ayushdhara.v13i1.2494
Source of support: Nil, Conflict of interest: None Declared

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