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ORIGINAL ARTICLE

Antiurolithiatic Effect of *Sirupeelai Samoola Kudineer*: A Polyherbal Siddha Decoction on Ethylene Glycol-induced Renal Calculus in Experimental Rats

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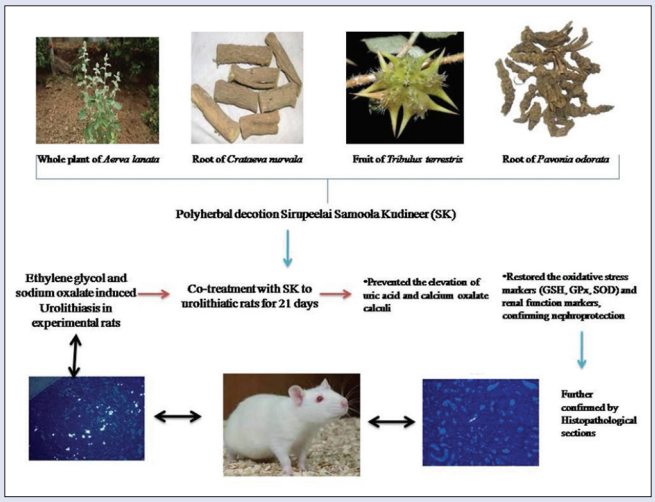
ABSTRACT

Background: *Sirupeelai Samoola Kudineer* (SK), a polyherbal decoction containing four medicinal plants has been used in Siddha system of medicine, practiced in Southern parts of India for the management of urolithiasis. **Objective:** The present study is carried out to scientifically validate the traditional claim and to study the mechanism of action of the drug. **Materials and Methods:** In the present study, anti-urolithiatic effect of SK was evaluated in Sprague-Dawley rats using ethylene glycol through drinking water and intraperitoneal injection of sodium oxalate. Renal damage was confirmed by the increased production of thiobarbituric acid reactive substance (TBARS). **Results:** Co-treatment with SK to urolithiatic rats for 21 days significantly prevented the elevation of renal and urinary stone biomarkers in plasma and renal tissue thereby preventing renal damage and the formation of renal calculi. Administration of SK at all doses and cystone restored the antioxidant (glutathione) levels by preventing the elevation of TBARS in the kidney tissue, which was further confirmed by histological sections. **Conclusions:** SK treatment promotes diuresis which leads to flushing of the renal stones and maintains the alkaline environment in the urinary system which probably mediates the antilithiatic activity. SK provides structural and functional protection to the kidneys by enhancing its physiological function against stone formation and validates its clinical use.

Key words: Ethylene glycol, oxidative stress, Siddha drug *Sirupeelai Samoola Kudineer*, sodium oxalate, urolithiasis

SUMMARY

- SK exhibited antilithiatic and diuretic potential in ethylene glycol and sodium oxalate induced urolithiasis in rats
- Elevated urinary stone markers (Calcium, oxalate, uric acid, magnesium and phosphates) in plasma and renal tubular enzymes (LDH, GGT, ALP, AST ALT) in urolithiatic rats were reversed by SK treatment
- SK administration significantly reduced the level of renal stress markers like Urea, Creatinine, LPO and elevated SOD, GPx, GSH levels aiding in nephroprotection
- SK also provides structural and functional protection against ethylene glycol-induced renal calculus in rats as evidenced by histopathological studies.



Abbreviations used: SK: Sirupeelai Samoola Kudineer; TBARS: ThioBarbituric Acid Reactive Substances; SOD: SuperOxide Dismutase; GPx: Gluthathione peroxidase; GSH- Glutathione; LPO: Lipid peroxidation as measured as TBARS; AST: Aspartate AminoTransferase; ALT: Alanine Amino transferase; GGT: Gamma Glutamyl Transferase; LDH: Lactate Dehydrogenase.

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INTRODUCTION

Urinary stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout the lifespan of a patient. Urolithiasis known as *Kal Adaippu* in Siddha system of medicine is a common disorder found in humans of all ages due to changing lifestyle and dietary choices, leading to increased incidence and prevalence around the globe.[1] It is a multi-factorial process caused due to an imbalance between the promoters and the inhibitors of renal calculi and finally may cause kidney failure also. About 75% of renal stones are composed of calcium oxalate crystals.[2] Current modern medications include α-1-blockers and Calcium channel blockers and technologies such as percutaneous irrigation chemolysis and Extracorporeal Shock Wave Lithotripsy provide effective treatment. However, adverse drug reactions such as hemorrhage, hematuria, tubular necrosis, and subsequent fibrosis of the kidney are identified.[3]

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Traditional medicines, mainly Chinese medicines and Indian medicines are becoming more and more popular as alternative and supplementary remedies over recent years because of its low cost and nontoxic nature.[4] For centuries, a large number of herbal and herbo-mineral preparations have been used in traditional system of medicine and elsewhere which claim an efficient cure for urinary stones.[5] These preparations are gaining importance in controlling hyperoxaluria and subsequent stone formation because of its minimal side effects. *Sirupeelai Samoola Kudineer* (SK), a decoction prepared by combining four herbs namely the whole plant of *Aerva lanata*, root of *Crataeva nurvala*, fruit of *Tribulus terrestris,* and root of *Pavonia odorata* is used therapeutically to treat urolithiasis in Siddha system of Medicine practiced in South India.[6] Earlier whole plant of *A. lanata*, root of *C. nurvala*, fruit of *T. terrestris,* and root of *P. odorata* in the formulation of SK have been reported for their various biological properties such as antioxidant, diuretic and antilithiatic properties.[7-10] Even though the individual herbs have both scientific and traditional claim for their diuretic, antioxidant and calculus restraining property, there is no proper scientific data available for the antilithiatic property of SK formulation. Several models for inducing urolithiasis are available off late, to evaluate the drug response but they have some disadvantages such as hematuria, and mortality.[3,9] In the present study, a modified induction methodology was adopted to induce urolithiasis using ethylene glycol and sodium oxalate to produce renal stone efficiently with lesser mortality. Therefore, in the present study, the antilithiatic potential of SK was scientifically evaluated against experimentally induced urolithiasis in rats using ethylene glycol and sodium oxalate and thereby also checks the attenuation of nephrotoxicity induced by ethylene glycol.

MATERIALS AND METHODS

Chemicals

Diagnostic kits were purchased from Accurex Biomedical Pvt. Ltd., Mumbai. Cystone tablet of Himalaya Drug Co., Bangalore was purchased commercially. All other chemicals used were of analytical grade purchased from SRL Mumbai.

Experimental animals

Male Sprague-Dawley rats (250–280 g) were obtained from Sri Venkateswara Enterprises, Bangalore. Animals were maintained at 25°C ± 2°C in 12-h dark/12-h light cycles, with both standard pelleted diet and water *ad libitum* in accordance to the CPSCEA guidelines. The experiments were carried after necessary clearances from Institutional Animal Ethics Committee of Sri Ramachandra University, Chennai, India. (Approval No: IAEC-XII/SRU/85/2008).

Test drug–*Sirupeelai Samoola Kudineer*

A sufficient amount of dry raw materials of *A. lanata* (whole plant), *C. nurvala* (root), *T. terrestris* (fruit) and *P. odorata* (root) were obtained commercially from the local market in Chennai, Tamilnadu and authenticated by Prof. V. Jayaraman, Director, Plant Anatomy Research Centre, Tambaram. SK was prepared as per the methodology in Siddha texts.[6,11] It is a decoction prepared by boiling the raw materials (each 5 g) with 8 parts of water (160 ml) and reducing to 1/8th (20 ml of decoction with a yield of 35 mg/ml). As part of the standardisation procedure, the quality of the plant material used were checked for any contaminants including toxic elements and is reported[11,12] Fresh decoction was prepared daily for oral administration.

The dose of the SK was selected based on the proposed human therapeutic dose indicated (60–120 ml) in Siddha formulary[6,13] and practiced currently. The calculation was done on the basis of body surface area of human to rat for the conversion of human dose to rat dose.[14] Earlier, we had screened the toxicity of SK; the results suggested that 4.5 ml/kg exhibited the No Observed Adverse Effect Level and was found to be safer.[11] Hence, for the present diuretic activity and antiurolithiatic study, 4.5 ml/kg was taken as mid dose and from that one lower dose (3.0 ml/kg) and one higher dose (6.0 ml/kg) was chosen to be tested.

Evaluation of diuretic activity of *Sirupeelai Samoola Kudineer*

The diuretic activity in rats was tested using standard protocols.[15-17] The animals were starved and also deprived of food for 18 h before the experiment and were divided into five groups of animals containing six each ($n = 6$). Normal vehicle-treated rats (water) saved as Group I, whereas Group II animals were given a standard diuretic drug Furosemide[18] (30 mg/kg b. w) in order to identify if the test drug exhibited a similar mode of action, while SK was treated at graded doses to Group III, IV, V rats.

All the groups received normal saline orally at 60 ml/kg body weight. Forty-five minutes later, the first group received only water second group received Furosemide (30 mg/kg body wt.) and the third, fourth, and fifth group received test drug SK (UF 2) at doses of 4.5, 9.0, and 18.0 ml/kg body wt., respectively. Immediately after administration of the drug, the rats (one/cage) were placed in metabolic cages, specially designed to separate urine and fecal matter and observed at room temperature of 25°C ± 0.5°C.[19] During this period of the experiment, no food but only water was made available to the animals. The total volume of urine excreted by the animals was collected in a metabolic cage and measured at 1 h intervals, collected and measured up to 5 h after the test drug administration to relate to the urine output. Other observations such as color, pH, specific gravity, protein, ketone bodies, bilirubin, blood cells, nitrites, sugars and urobilinogen were determined using URISCAN 9 SG Strip, YD Diagnostics, Korea. Further, the concentration of Na^+ and K^+ which relates to the electrolyte balance and Ca^{2+} were analyzed using flame photometer.[19,20]

Experimental design

Thirty-six rats were divided into six groups (n = 6/group). Normal rats (Group I) received water for 21 days. All the experimental animals except normal control received ethylene glycol (0.75%) in drinking water for 21 days and a single dose of sodium oxalate injection (35 mg/kg, i.p.) on 14th day for urolithiasis induction. Urolithiatic rats (Group II) served as positive control. Urolithiatic rats (Groups III, IV and V) co-treated with SK at a dose of 3, 4.5, and 6 ml/kg b.wt, p.o./day, respectively, Cystone co-treated rats (Group VI) at 500 mg/kg b.wt, p.o./day served as a standard drug-treated group. 24 h urine samples were collected by housing rats in individual metabolic cages on the 7th, 14th, and 21st day using sodium azide as preservative. Specific gravity, pH and volume, of the urine were noted immediately after urine specimen collection and stored at −80°C. At the end of the experimental period, on the 21st day blood was collected through retro-orbital puncture for further analysis followed by sacrifice of the animals and isolation of vital organs as per CPSCEA guidelines.

Biochemical assays

Plasma was obtained from the blood withdrawn in a tube containing 0.2 ml of 11% trisodium citrate, after centrifugation and stored at −80°C for further analysis. The isolated kidney tissue was washed in saline, weighed and homogenised using phosphate buffer (pH-6.9). The supernatant obtained was used for the array of biochemical parameters. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), and lactate dehydrogenase (LDH), were analyzed in plasma and kidney tissue followed by urea, creatinine, uric acid and total protein estimation in

plasma, urine and kidney tissue using Accurex kits, Accurex Biomedical Pvt. Ltd., Mumbai. Calcium, oxalate, inorganic phosphorus, and magnesium in plasma, urine and kidney were estimated using standard methods[21] Oxidative stress markers such as thiobarbituric acid reactive substance (TBARS), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx) were measured using standard methods in the kidney tissue.[22]

Histopathological examination

The kidney tissue obtained from all experimental groups (n = 4/group) were washed immediately with saline and then fixed in 10% buffered neutral formalin solution. After fixation, the tissue was processed and embedded in paraffin. Tissues (3–5 µm thick) were sectioned and stained with hematoxylin and eosin. These sections were then examined under a high power light microscope for the histoarchitectural changes and photomicrographs were taken for documentation. Bright white oxalate crystals were identified and counted using a polarizing microscope (Nikon Eclipse LV.100).

Statistical analysis

All the results were expressed in mean ± standard error of mean. The data were statistically analyzed by one-way ANOVA followed by least significant difference using SPSS 10 version (IBM software, NewYork City, USA). $P < 0.05$ and < 0.01 were considered as statistically significant.

RESULTS

Diuretic activity of *Sirupeelai Samoola Kudineer*

The total urine output of the treated and control groups are given in Figure 1. Water intake was normal during treatment. The total urine output of the rats administered with Furosemide (30 mg/kg) and SK formulation were found to be elevated. The rats which received Furosemide (30 mg/kg) excreted nearly two and half fold volume of urine as compared to the normal control rats (saline treated). The rats which received low (4.5 ml/kg), mid (9.0 ml/kg), and high dose (18/0 ml/kg) SK excreted nearly two-fold, two-fold, and one and half fold volume of urine respectively as compared to the control treated (saline treated). The color of the urine of rats in test drug and furosemide-treated groups appeared almost identical to that of the control group. Specific gravity (1–1.025 range) and protein (10 mg/dl) was found to be within normal limits. Qualitatively ketone bodies, nitrites, bilirubin, blood cells, sugars, and urobilinogen were not found in all drug-treated groups. pH of the urine was found to be slightly acidic in furosemide-treated rats as compared to the normal control saline-treated rats. The excretion of sodium and potassium was found to be increased and the Na^+/K^+ ratio was found to be decreased in all test drug-treated rats and in furosemide-treated rats compared to the control rats and was found to be significant in furosemide ($P < 0.05$), mid ($P < 0.01$), and high doses ($P < 0.001$). However, there was no change in calcium levels. All the results were comparable with control, and the significant changes are shown in Table 1.

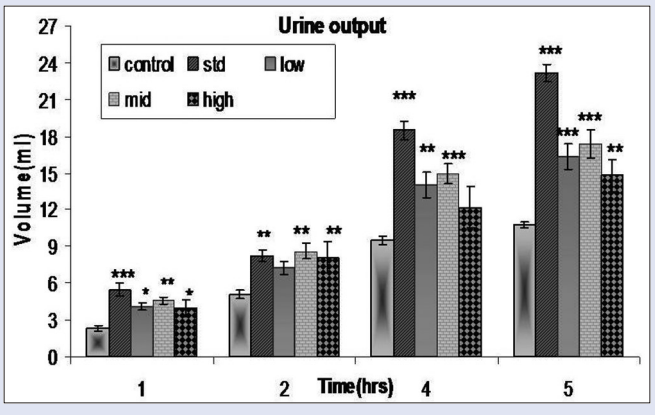

Figure 1: Total urine output of the treated and control groups

Antiurolithiatic activity of *Sirupeelai Samoola Kudineer*

Based on the findings of the preliminary standardization of renal stone induction in pilot study using ethylene glycol and sodium oxalate, the antiurolithiatic activity of SK was tested by identifying the changes in urinary stone markers, renal markers, stress markers, and histopathological findings which are discussed below.

Changes in urinary stone markers

The stone markers mainly calcium and oxalate was significantly increased to about 4 folds in plasma, 3 folds in urine, and 4 folds in kidney tissues of urolithiatic animals when compared with normal controls [Tables 2 and 3]. Treatment with SK and cystone showed 60% and 69%, reduction in urinary calcium levels and 66.6% and 52% reduction in plasma calcium levels respectively. Whereas, calcium level in the kidney tissues were reduced to 68.2% and 51.14% in SK- and cystone-treated rats. Urinary oxalate level was found to be reduced on SK and cystone treatment (60.01% and 58%) respectively while they showed reduction in oxalate level of 55% and 44% in plasma and 41.21% and 45% in the kidney tissues, respectively. Contradictorily, the excretion of inorganic phosphorus, uric acid and magnesium in the urine and their levels in plasma and kidney were found to be decreased in urolithiatic rats [Tables 2 and 3]. The inorganic phosphorus in urine, plasma, and kidney was significantly reversed in all the test drugs treated rats while the magnesium levels were significantly reversed in urine and kidney but not up to the mark in plasma.

Changes in renal markers

Markers in renal functions such as urea, creatinine, and protein were augmented in urine, plasma and kidney tissues [Tables 2 and 3] of urolithiatic rats when compared to normal control rats. As calcium and oxalate, these levels were also considerably decreased by SK at all doses and cystone co-treatment. Similarly, the activities of marker enzymes such as AST, ALT, ALP, LDH, and GGT were found to be elevated significantly ($P < 0.05$) in both kidney and plasma of Group II rats when compared to normal Group I rats and the results are shown in Table 4. Simultaneous administration of SK and cystone maintained these levels to near normalcy compared to the urolithiatic rats.

Changes in renal stress markers

In the present study, stress marker lipid peroxide level (TBARS) was found to be increased significantly in kidney tissues of urolithiatic groups in comparison to the normal group. TBARS level in kidney tissue were significantly maintained to normal by all SK treated and standard administered groups when compared to induction group. Concurrently, renal SOD, GPx, reduced GSH level were significantly decreased in induction group whereas it is restored to normal by all SK treated rats and it was significant in high dose (SK)-treated groups. The results are shown in Table 5.

Histopathological findings

Histopathological sections of normal rats showed normal renal architecture under light microscopy is shown in Figure 2a. Patchy renal

Table 1: Effect of *Sirupeelai Samoola Kudineer* formulation on urine parameters

Treatment	pH	Sodium (mEq/L)	Potassium (mEq/L)	Na/K ratio	Calcium (mEq/L)
Control (normal vehicle treated)	6.49±0.18	302.33±37.66	77.33±14.07	4.68±0.89	3.07±0.19
Standard furosemide (30 mg/kg)	5.90±0.23*	628.67±76.94***	270.67±74.29**	3.01±0.55*	3.00±0.66
Low dose (4.5 ml/kg p.o)	6.54±0.11	426.67±44.87	123.33±8.79	3.43±0.12	2.72±0.28
Mid dose (9 ml/kg p.o)	6.21±0.11	401.03±18.98	162.00±9.95	2.54±0.24**	3.65±0.15
High dose (18 ml/kg p.o)	6.63±0.31	319.00±30.53	182.67±30.26	1.95±0.31***	1.86±0.18*

Data were expressed in mean±SEM (n=6). P values were evaluated using one-way ANOVA followed by LSD. Standard furosemide versus control and test drugs versus control (*P<0.05, ** P<0.01 and ***P<0.001). LSD: Least significant difference; SEM: Standard error mean

Table 2: Effect of *Sirupeelai Samoola Kudineer* on stone and renal markers in urine and plasma

Sample	Experimental groups	Stone markers					Renal markers		
		Calcium	Oxalate	Uric acid	Magnesium	Inorganic phosphorus	Urea	Creatinine	Total protein
		mg/ml			µg/ml		mg/ml		
Urine	Normal control	0.28±0.02	9.86±0.16	2.40±0.28	0.40±0.05	3.96±0.12	23.81±1.29	0.55±0.02	10.00±4.47
	Urolithiatic rats	0.73±0.04^{++}	23.58±2.78^{++}	0.45±0.08	0.15±0.02^{++}	1.00++±0.26^{++}	47.15±2.68^{++}	1.55±0.1^{++}	70.00±18.89^{++}
	Induction + SK (3.0 ml/kg)	0.29±0.05**	11.60±1.42**	1.18±0.07	0.32±0.09	2.90±0.46**	29.16±2.18**	0.69±0.07**	25.00±15.00*
	Induction + SK (4.5 ml/kg)	0.26±0.02**	11.46±3.20**	1.24±0.23	0.40±0.12*	3.98±0.45**	25.78±4.00**	0.68±0.10**	28.33±14.70*
	Induction + SK (6 ml/kg)	0.24±0.02**	9.18±1.58**	1.61±0.26	0.42±0.04*	4.21±0.06**	25.78±3.13**	0.68±0.12**	13.33±3.33**
	Induction + cystone (500 mg/kg)	0.34±0.05**	7.55±1.56**	2.14±0.11	0.40±0.07*	3.07±0.35**	22.18±1.45**	0.57±0.05**	28.33±14.70*
					mg/dl				g/dl
Plasma	Normal control	2.55±0.59	2.08±0.22	8.23±1.96	17.15±3.25	12.68±0.96	23.45±0.99	1.25±0.26	5.49±0.42
	Urolithiatic rats	9.20±1.25^{++}	9.24±1.18^{++}	1.22±0.24^{++}	7.22±1.61^{++}	6.78±0.91^{++}	59.15±5.42^{++}	3.20±0.63++	11.70±1.65^{++}
	Induction + SK (3.0 ml/kg)	5.62±0.98*	4.65±0.32**	2.88±0.74**	8.84±1.41	10.13±0.91*	43.83±5.67	1.70±0.29**	6.38±0.33^{++}
	Induction + SK (4.5 ml/kg)	4.44±1.91**	6.22±0.72**	3.21±0.60**	9.31±1.62	10.03±0.84	39.96±3.20**	1.64±0.46**	5.66±0.36^{++}
	Induction + SK (6 ml/kg)	3.72±0.93**	4.15±0.53**	3.12±0.22**	10.38±1.29	9.86±0.84	34.14±3.97**	1.19±0.08**	5.47±0.38^{++}
	Induction + cystone (500 mg/kg)	4.13±0.86**	5.87±0.21**	2.90±0.16**	7.27±1.10	10.05±1.43	40.02±4.42	1.62±0.28**	6.34±0.97^{++}

Data were expressed in mean±SEM (n=6). P values were evaluated using LSD multi comparison test. Normal control versus urolithiasis induction (^{++}P<0.01), urolithiasis induction versus induction + SK-treated group and cystone-treated group (*P<0.05, **P<0.01). SK: *Sirupeelai Samoola Kudineer*; SEM: Standard error of mean; LSD: Least significant difference

Table 3: Effect of *Sirupeelai Samoola Kudineer* on renal and stone markers in kidney tissues of experimental rats

Experimental groups	Stone markers (mg/g tissue)					Renal markers (mg/g tissue)	
	Calcium	Oxalate	Uric acid	Magnesium	Inorganic phosphorus	Urea	Creatinine
Normal control	0.28±0.05	0.09±0.02	2.46±0.29	1.72±0.04	2.05±0.07	2.87±0.20	0.82±0.10
Urolithiatic rats	1.25±0.04^{++}	0.36±0.03^{++}	1.58±0.20^{++}	0.39±0.11^{++}	1.08±0.25^{++}	14.46±1.92^{++}	2.19±0.10^{++}
Induction + SK (3.0 ml/kg)	0.45±0.06**	0.24±0.03**	1.74±0.08	1.50±0.18**	1.71±0.36	6.89±0.47**	0.84±0.16**
Induction + SK (4.5 ml/kg)	0.41±0.06**	0.26±0.02**	1.79±0.26	1.68±0.24**	2.02±0.35**	3.67±0.66**	0.52±0.09**
Induction + SK (6 ml/kg)	0.47±0.09**	0.26±0.02**	1.76±0.33	1.55±0.26**	1.27±0.22	3.78±0.37**	1.08±0.05**
Induction + cystone (500 mg/kg)	0.67±0.11**	0.21±0.02**	1.62±0.13	1.45±0.19**	1.79±0.16*	3.53±0.45**	0.93±0.09**

Data were expressed in mean±SEM (n=6). P values were evaluated using LSD multi comparison test. Normal control versus urolithiasis induction (^{++}P<0.01), urolithiasis induction versus induction + SK-treated group and cystone-treated group (*P<0.05, **P<0.01). SK: *Sirupeelai Samoola Kudineer*; SEM: Standard error of mean; LSD: Least significant difference

tubular damage with congestion of blood vessels, severe glomeruli inflammation, and mild interstitial nephritis were seen in urolithiatic group [Figure 2b]. In SK co-treated group (3 ml/kg), there were few micro calculi with mild inflammation, mild interstitial nephritis, and patchy tubular damage (not shown). Likewise, in the SK co-treated animals (4.5 ml/kg, Group IV), less number of calculi with blood vessel congestion, inflammation, and mild tubular damage were seen (not shown). However, in SK (6.0 ml/kg) co-treated group, mild tubular

Table 4: Effect of *Sirupeelai Samoola Kudineer* on renal tubular enzymes in plasma and kidney tissues

Sample	Experimental groups	AST	ALT	ALP	GGT	LDH
Plasma (U/L)	Normal control	44.58±3.51	31.59±1.15	131.50±9.26	7.14±0.7	228.52±9.45
	Urolithiatic rats	139.58±18.94^{++}	51.78±4.73^{++}	398.92±32.39^{++}	14.83±3.57^{+}	530.47±17.81^{++}
	Induction + SK (3.0 ml/kg)	75.09±2.82**	22.03±1.43**	190.47±23.05**	10.72±2.56	246.75±44.95**
	Induction + SK (4.5 ml/kg)	63.65±3.15**	26.01±2.91**	186.83±16.94**	8.33±1.80*	307.29±29.63**
	Induction + SK (6 ml/kg)	82.28±12.84**	26.77±0.78**	184.04±40.30**	6.50±0.76**	287.65±33.76**
	Induction + cystone (500 mg/kg)	92.01±11.37**	29.70±1.26	224.38±22.11**	10.33±0.97	295.18±21.96**
Kidney (U/mg protein)	Normal control	3.87±0.52	17.15±1.67	20.23±1.32	17.01±1.28	4.38±0.48
	Urolithiatic rats	9.51±1.17^{++}	52.81±8.48	63.14±9.37^{++}	122.08±19.99^{++}	19.67±0.95^{++}
	Induction + SK (3.0 ml/kg)	5.87±0.86*	57.60±13.38	50.29±6.10**	48.10±11.45**	9.08±1.39*
	Induction + SK (4.5 ml/kg)	9.19±1.32	64.10±16.87	49.42±4.52**	67.30±14.04**	10.40±2.20
	Induction + SK (6 ml/kg)	10.41±2.17	70.43±2.73	74.66±5.13**	44.27±2.07**	12.21±4.74
	Induction + cystone (500 mg/kg)	7.38±0.90	100.89±22.17*	84.36±7.84**	72.77±7.92**	13.26±1.57

Data were expressed in mean±SEM (n=6). P values were evaluated using LSD multi comparison test. Normal control versus urolithiasis Induction (^{+}P<0.05, ^{++}P<0.01), urolithiasis induction versus induction + SK-treated group and cystone-treated group (*P<0.05, **P<0.01). SK: *Sirupeelai Samoola Kudineer*; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma glutamyltransferase; LDH: Lactate dehydrogenase; LSD: Least significant difference; SEM: Standard of error mean

Table 5: Effect of *Sirupeelai Samoola Kudineer* on renal stress markers in normal and urolithiatic rats

Experimental groups	LPO (nm/g tissue)	SOD (units/mg protein)	GPx (µcg/mt/mg protein)	GSH (µm/g tissue)
Normal control	104.58±10.06	60.67±8.92	78.17±9.41	5.94±0.35
Urolithiatic rats	143.00±3.08^{++}	27.15±1.27^{++}	42.63±4.57^{++}	3.87±0.15^{++}
Induction + SK (3.0 ml/kg)	102.59±3.73**	52.67±2.83**	66.40±10.58*	5.35±0.11**
Induction + SK (4.5 ml/kg)	98.05±3.80**	64.80±2.24**	73.76±15.77**	6.35±0.29**
Induction + SK (6 ml/kg)	115.78±4.01**	40.87±1.28*	59.32±11.10	4.71±0.07*
Induction + cystone (500 mg/kg)	109.24±7.01**	48.38±5.34**	46.78±5.10	5.14±0.35**

Data were expressed in mean±SEM (n=6). P values were evaluated using LSD multi comparison test. Normal control versus urolithiasis induction (^{++}P<0.01), urolithiasis induction versus induction + SK-treated group and cystone-treated group (*P<0.05, **P<0.01). SK: *Sirupeelai Samoola Kudineer*; SEM: Standard Error Mean; GSH: Glutathione; LPO: Lipid peroxidation; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; LSD: Least significant difference

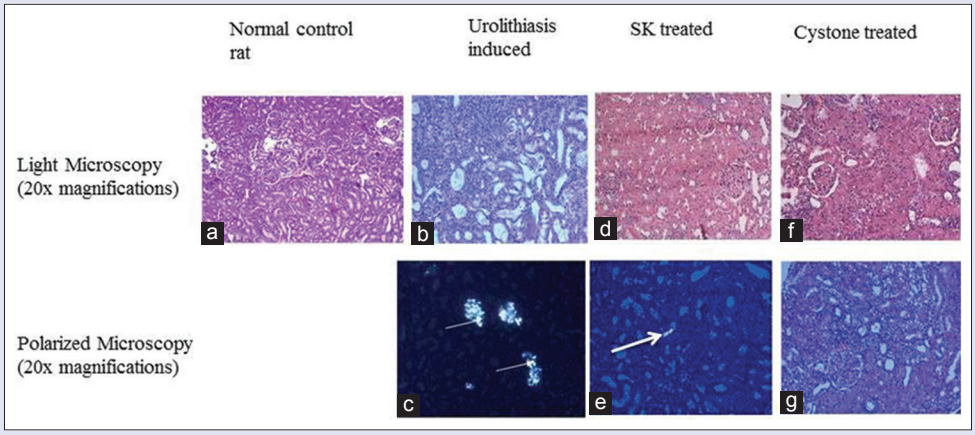

Figure 2: Histological Comparisons of Kidney Tissue of Normal and Induction Rats. (a) Light microscopy (×20) of histological section of kidney tissue of Normal Control rat; (b) light microscopy (×20) of histological section of kidney tissue of Urolithiasis induced rat; (c) polarized microscopy (×20) of histological section of kidney tissue of Urolithiasis induced rat. Histological examinations of kidney tissues of *Sirupeelai Samoola Kudineer* treated rat. (d) Light microscopy (×20) of histological section of kidney tissue of *Sirupeelai Samoola Kudineer* treated rat (6.0 ml/kg); (e) polarized microscopy (×20) of histological section of kidney tissue of *Sirupeelai Samoola Kudineer* treated rat (6.0 ml/kg). Histological examination of kidney tissues of cystone administered rat. (f) Light microscopy (×20) of histological section of kidney tissue of cystone-treated rat (500 mg/kg); (g) polarized microscopy (×20) of histological section of kidney tissue of cystone-treated rat (500 mg/kg)

damage with congestion of blood vessels was observed [Figure 2d]. In the cystone co-treated group, there was a calculus with mild inflammation and acute tubular necrosis [Figure 2f]. Under polarized microscopic examination, Group II rats exhibited many number of polarizing bright white oxalate crystals [Figure 2c] whereas SK and cystone co-treated rats reveal considerably lesser number of oxalate crystals [Figure 2e and 2g].

DISCUSSION

Sirupeelai Samoola Kudineer is being traditionally used as diuretic and antiurolithiatic agentin the management of urolithiasis since antiquity and is still practiced by many Siddha practioners. The findings of the present study scientifically validates the antilithiatic potential of SK mediated by its diuretic potential and lowering of stone forming constituents in the

urine as compared to the standard drug. The nephroprotective action is also discussed below as evidenced by the attenuation of oxidative stress in the kidney tissues. Kidneys are the targets for ethylene glycol toxicities where it gets oxidized to oxalic acid leading to hyperoxaluria, decreases the glomerular filtration rate (GFR) and finally obstructs the urine outflow which leads to the accumulation of nitrogenous waste products in the blood.[21,23] Either ethylene glycol or sodium oxalate was frequently used to induce urolithiasis in the experimental animals mainly resulting in hyperoxaluria thereby leading to increased deposition of calcium oxalate crystals in the renal system.[2,24] In the present study, a model of inducing experimental renal calculi was standardized by administering ethylene glycol and low dose of sodium oxalate (35 mg/kg). This was confirmed by the substantial presence of bright white crystals viewed under a polarizing microscope. These deposited oxalates mediate oxidative stress by enhancing lipid peroxidation and induces renal damage against oxalate mediated nephronal impairment[11]

Hyperoxaluria is reported to be a more significant risk factor in the pathogenesis of urolithiasis which induces renal membrane damage resulting in calcium, oxalate, and protein leakages into the blood stream. The stone markers such as calcium, oxalate, inorganic phosphorus, magnesium, and uric acid play a vital role in the formation of stones.[25,26] Diuresis reduces the risk of stone formation by reducing the saturation product of calcium oxalate. In the present study, the urinary output is increased in both drug-treated groups which implies the diuretic effect of the test drug thereby increasing urine output and reducing the possibility of stone formation. Ethylene glycol/sodium oxalate-induced rats (Group II) showed marked elevation in urinary stone markers such as calcium, oxalate, and protein and lowered the magnesium, uric acid, and inorganic phosphorus levels and also maintained slight acidity in urine implying successful induction. Increased urinary calcium and oxalate concentration might favor the nucleation and precipitation of calcium oxalate from urine causing supersaturation of urinary colloids which when trapped acts as a nidus, leading to subsequent crystal growth.[27,28] These urinary stone markers excreted in urine and elevated in plasma and kidney during ethylene glycol/sodium oxalate administration was reversed by SK at all doses and cystone treatment. Magnesium and inorganic phosphorus are the potent inhibitors of stone formation, their elevation in drug treatment might reduce the supersaturation of calcium oxalate followed by decreased growth and nucleation rates of calcium oxalate crystals.[27,29,30] Restoration of magnesium excretion by reduced excretion of oxalate and calcium by the drug decreases the supersaturation of the urine with respect to calcium oxalate thereby decreasing the risk of stone formation.[29] Similar results were obtained when *A. lanata* and lupeol a flavanoid from *C. nurvala* were used as an antilithic agent against induction.[9,29,31] Due to the obstruction of the outflow of urine by stones in the urinary system, the GFR gets reduced which leads to the accumulation of waste products, particularly nitrogenous substances in the blood stream.[23] In the present study, the calculi-induced rats (Group II) showed a significant elevation in urea, creatinine, uric acid of plasma, kidney, and urine which confirms the renal damage. Similarly, treatment with SK and cystone significantly lowered the accumulation of these waste products in plasma as well as in the kidney and their elimination in urine thereby attributing the maintenance of the glomerular function.

The liver is considered to be the major site of oxalate synthesis in the rat and role of the two enzymes-glyoxylate oxidase and LDH-have been well recognized. Ethylene glycol/sodium oxalate induction induces hepatic and renal cellular damage thereby altering the cytosolic enzymes levels (GOT, GPT, ALP, GGT, and LDH) in the liver and kidney.[32] Most of the urinary enzymes originating in the kidneys are localized to specific regions and cellular components of the nephron.[33] Studies pertaining to these enzymes will thereby showing the pathological status of the kidney. In the present study, renal injury was also evidenced by the increased activities of AST, ALT, LDH (renal tubular cytoplasmic enzyme), GGT (brush border membrane luminal surface enzyme), and ALP (brush border membrane enzyme). This might be due to the leakage of these enzymes into the general circulation from the collateral circulation.[31,34] Administration of cystone and SK at all doses prevented the leakage of these marker enzymes and maintained its level in tissues in comparison to lithiatic rats.

Defined as an impaired balance between free radical production and antioxidant capacity resulting in accumulation of oxidative products, oxidative stress is a well-recognized mechanism playing important roles in several human diseases. Oxidative stress may be amplified by a concomitant decrease in cellular enzymatic (SOD, GPx) and nonenzymatic antioxidants (reduced GSH, nitrite) that may favor the propagation of oxidative alterations from intra- to extra-cellular spaces and from confined to distant sites, thus realizing a systemic oxidative stress state.[35] In the present study, renal damage was confirmed by the increased production of TBARS with a concomitant decrease in the levels of antioxidants in the kidney tissue of urolithiatic rats. Administration of SK at all doses and cystone restored the antioxidant levels by preventing the elevation of TBARS in kidney. These might be due to the antioxidant potential of the individual herbal ingredients such as *T. terrestris*, *A. lanata,* and *C. nurvala*.[36,37] In addition, histopathological observation shows significant changes like patchy renal tubular damage, congestion of blood vessels, severe glomerular inflammation with amplified crystals in rats administered with ethylene glycol and sodium oxalate. These observations along with changes in renal and stone markers confirmed the severity of induction. Co-treatment with SK (6.0 ml/kg) and cystone (500 mg/kg) withstands the severity of induction and reduces the formation of oxalate crystals in renal tissue as evidenced by histopathological examination also. These results and observations suggest that SK would be a drug of choice as a nephroprotective agent probably due to the nephroprotective effects exhibited by the individual ingredients *A. lanata*, *C. nurvala*, *T. terrestris,* and *P. odorata* present in the formulation.[8,9,31]

CONCLUSIONS

The results scientifically validate that the administration of SK and cystone against urolithiasis by preventing the elevation of marker enzymes, and urinary risk factors and proves its nephron protective and lithotrophic potential. The mechanism underlying this effect is unknown but is apparently related to increased diuresis and lowering of urinary stone forming constituents and attenuation of oxidative stress probably due to the phytoconstituents such as polyphenols, flavonoids, and tannins in the plant source. Thus, SK provides structural and functional protection along with antilithiatic activity against ethylene glycol and sodium oxalate-induced renal calculus in experimental animals and scientifically proves the use of SK in the treatment of urolithiasis in Siddha system of Medicine one of the oldest systems of Indian medicine.

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Conflicts of interest

There are no conflicts of interest.

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