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Original Article

Evaluation of Antiurolithiatic Potential of Methanolic Stem Extract of *Spermacocce articularis* L.f.: An *In vitro* and *In vivo* Approach

S Gopika, MK Nisha*, E Gaayathiri Devi, A Raja Rajeswari, R Vasandhlakshmi

ABSTRACT

Context: Polygenic urolithiasis has a complicated etiology and even more varied therapeutic outcomes. *Spermacoce articularis* L.f. has been used historically for stone treatments in several traditional medical systems. **Aim:** The current study aimed to investigate the *in vitro* and *in vivo* anti-urolithiatic potential of *Spermacoce articularis* Stem Extract (SASE). **Methods:** *In vitro* antiurolithiatic potential on the CaOx crystallization was evaluated using nucleation and aggregation assays. *In vivo*, activity was assessed on renal calculi-induced Wistar rats by polyethylene glycol (0.75%) in drinking water for 14 days. SASE and cystone with two experimental doses (250 and 500 mg/kg, p.o.) were dispensed for ten days. Various biochemical parameters were assessed in the kidneys' serum, urine, and histological sections. In addition, SASE inhibited CaOx crystallization by reducing the density of crystals, triggering the breakdown of CaOx crystals, and hindering their growth. Cystone demonstrated comparable outcomes. **Results:** Upon treatment with SASE, urinary, serum, kidney homogenates, and antioxidants were significantly improved ($p<0.05$) to normal levels. The histopathology of the kidney section showed no damaged cells of SASE treated and Cystone treated compared with that of control animals. **Conclusion:** This research validates the traditional idea and suggests that SASE is advantageous in preventing the growth of urinary stones.
Keywords: Calcium oxalate, Polyethylene glycol, *Spermacoce articularis*, Urolithiasis, *In vivo.*

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INTRODUCTION

Urolithiasis or urinary stone formation in the urinary system is an extremely painful condition that has plagued people since ancient times.This multifactorial disorder is mainly caused by the abnormality in metabolism and urinary tract, urinary infection is a combination of genetic, metabolic, and epidemiological risk factors[1].Low consumption of fruits, vegetables, and fluids, as well as high intake of animal proteins, salt, and sweetened beverages, have a significant impact on urolithiasis incidence.An abnormal rise in the excretion of compounds such as calcium, cystine, oxalate, phosphate, uric acid, urate, and xanthine, or a decrease in urinary level causes stone formation.[2] The primary inhibitors of stone formation in the urinary tract are magnesium and citrate, and stone formation results from low or absent concentrations of these inhibitors in the urine.[3]

According to reports, calcium oxalate and calcium phosphate makeup 80% of renal stones, with struvite-containing magnesium ammonium phosphate accounting for 10%.[4]Urolithiasis has been managed with a variety of treatment approaches and their combinations, including diet, diuretics, probiotic therapy, expulsion therapy, and chelating agents.[5]Current-day medical expenses are either higher or accompanied by adverse effects. The healthcare system may incur significant expenses and face severe problems when intervention treatments are utilized for therapy. Hence, in many countries, including India, phytotherapeutic agents are widely used as complementary and alternative medicines for the treatment of urolithiasis. The majority of Ayurvedic treatments were derived from plants and have been proven effective.These plants are a cheap source of drugs, widely available, affordable, and thought to be quite safe with few to no adverse effects. In Ayurveda, *S. articularis* L.f. which belongs to the family, Rubiaceae, is known as "Madanaghanti" and in Tamil "Nathaisuri"possesses various pharmacological properties, that cure inflammation of gums, and spleen complaints, used as an astringent in hemorrhoids and gallstones, demulcent in diarrhea and dysentery, and decoction of herb in the treatment of headaches.[6] Although *S. articularis* is used traditionally there is no report on the antiurolithiatic potential. Thus, the present objective was to investigate and validate the Antiurolithiatic property of *S. articularis* extract against calcium oxalate crystallization and PEG-induced kidney calculi rat model.

MATERIALS AND METHODS

Plant material and extraction

The fresh stem of *Spermacoce articularis* L.f. was collected from Attappady, Kerala. The plant samples were identified and authenticated taxonomically by the Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India, with Voucher No: BSI/SRC/5/232022/Tech. The fresh stem was cleaned, chopped, and shade-dried for about ten days at room temperature and powdered. In the Soxhlet apparatus, the refined stem samples (10g), were packed tightly in Whatman No. 1 filter paper for extraction with methanol solvent (100ml). The methanol-extracted fractions were concentrated to a crude using a rotary evaporator under reduced pressure at 37°C and the extract was used for *in vitro* and *in vivo* studies.

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Evaluation of *In-vitro* antiurolithiatic activity

Nucleation assay

The effect of stem extract on calcium oxalate (CaOx) crystal development was examined using nucleation assay.Buffer containing Tris-HCl of 0.05 mol/l and sodium chloride (NaCl) of 0.15 mol/l was prepared and maintained at pH 6.5. 5 mmol/l of calcium chloride ($CaCl_2$) and 7.5 mmol/l of sodium oxalate ($Na_2C_2O_4$) solution was prepared using the buffer. Different concentrations (100, 200, 300, 400, 500, and 600 µl) of SASE were mixed with 3 ml calcium chloride and sodium oxalate solution each and were kept in incubation at 37°C for 30 min. The absorbance (OD) of the mixture was observed (620 nm). The percentage inhibition (%) of nucleation by SASE was obtained using the formula and compared to the standard cystone.[7]

$$\% \text{ Inhibition} = (1 - OD_{Sample} / OD_{Control}) \times 100$$

Aggregation assay

Dissolution of the aggregated CaOx crystal by the stem extract was analyzed using the standard procedure[8], with a minor modification. Calcium chloride and sodium oxalate were combined to get CaOx monohydrate crystals at 50 mmol/l. Compensated the solution in a water bath at 60°C for 1 hour, and cooled to 37°C all night. Extracted the crystals using centrifugation and evaporated at 37°C. CaOx crystals were employed at a final concentration of 0.8mg/ml, buffered with 0.05mol/l Tris and 0.15mol/l NaCl at pH 6.5. Experiments were carried out at 37°C in the presence or absence of a sample. The inhibitory percentage of aggregation (Ir) was then estimated by calculating the turbidity of the sample to that acquired in the control using the formula

Ir = (1- Sample turbidity /Control turbidity) x 100

Microscopic evaluation

After which, a few drops of the mixture from different concentrations were placed in different slides and the reduction of crystal size and morphology of CaOx crystals formed with the presence and absence of *S.articularis* stem methanol extract was determined using a trinocular microscope at 1000 x magnification.

Evaluation of *in vivo* anti-urolithiatic activity

Experimental Animals

Thirty healthy adult male albino rats of Wistar strain (*Rattus norvegicus*) weighing 150–200 g were acquired from the animal house of Biogene lab, Bangalore. Rats were housed under controlled environmental conditions (Temperature 28±2°C; 12:12 h light: dark cycle; 50±10% humidity)in plastic cages with filter tops. All rats were fed with commercial rat pellets (SAI Animal Feed Ltd, Bangalore) and were given water ad libitum. Before experimental work, the rats were acclimated in the animal house for 10 days.

The experimental protocol was approved ethically for the *in vivo* study by the Institutional Review Board (IRB) of Avinashilingam Institute for Home Science and Higher Education for Women with the study reference (AIW: IAEC.2023:09).

Acute toxicity assay

The acute oral toxicity study was carried out as per Organization for Economic Co-operation and Development (OECD) Guidelines 425. One animal is utilised in a step by step process. The stem methanol extract was administered at the dose level of 2000 mg/kg and observed mortality after 24 hr. One-tenth of the median lethal dose (LD_{50})was taken as an effective dose.[9]

Ethylene *glycol-induced urolithiasis in Wistar albino rats*

Ethylene glycol 0.75% (0.75 ml of ethylene glycol in 100 ml of drinking water) to rats for 28 days for the production of calcium oxalate stone in rats. Such an induced urolithiasis model was used to study the antiurolithiatic activity in male Wistar albino rats. The adult male Wistar albino rats were divided into 5 groups, from Group I to Group V, each with 6 rats,Group 1 was reserved as Normal control, whereas all other groups were induced with urolithiasis (stone).Group II served as lithiatic control and received vehicle 1% tween 80.Group III to V received a daily oral solution of 0.75% ethylene glycol till day 14 days and each group were treated with standard cystone (100 mg/kg), SASE low dose (PEG+L.D 250 mg/kg) and SASE high dose (PEG+H.D 500 mg/kg) respectively from 15 to 28 days. Group IV and V were served as curative treatment groups.

Collection and analysis of urine, blood and serum

After all the treatment, all rats were encased in individual metabolic cages. During urine collection, the animals had no restriction to drinking water. On the 28th-day treatment, the pH of the urine samples (24 hours) was determined using a digital pH meter shortly after the collection, anda drop of strong hydrochloric acid was added before storing the collected urine at 4°C. It was then analyzed for calcium[10], phosphate[11], oxalate[12], urea[13], uric acid[14] and citrate[15] were performed by using standard methods. The hematological parameters like RBC, WBC and Hb percentage were estimated.

Serum samples were obtained by the retro-orbital collection of blood from the eyes of the rats using 5% isoflurane on day 28.A cervical dislocation procedure was used to slaughter rats for histopathological examination. Separated the serum from the blood using a cooling centrifuge at 10,000 rpm for 10 minutes to separate the serum from the blood, and the samples were labelled. The serum was stored in a refrigerator and measured for creatinine and uric acid levels[16].

Measurement of Body and Kidney Weight

The body weight was measured for each group of rat at the beginning and end of the treatment using a weighing balance, and the changes were recorded.All the rats were sacrificed, and at the end of the study, actual weight of the dissected kidney was measured.

Determination of antioxidant enzymes and lipid peroxidation

Preparation of tissue homogenate

SASE post-treated liver of the sacrificed rats was isolated, cleansed with normal saline (0.9%), and stored for 12 h for *in vivo* antioxidant studies.10% homogenate of the separated liver was achieved using a motor-driven Teflon-coated homogenizer and 0.1 M Tris-HCl buffer (pH 7.4) and centrifuged at 10,000 rpm for 10 minutes at 5˚C. The collected supernatant was used for further *in vivo* experiments. Antioxidant enzymes, viz. Superoxide dismutase (SOD)[17], Catalase (CAT)[18], Glutathione peroxidize (GPX)[19], Reduced glutathione (GSH)[20] and Lipid peroxidation (LPO)[21]liver tissues of all the tested rats were determined. Protein estimation was carried out using Folin-Ciocalteau and Biuret reaction combination[22]. The results of the antioxidants SOD, and CAT were expressed as U/mg protein, and GPX, GSH, and LPO were expressed as µmol/mg protein, µg/mg protein, and nmol/mg protein respectively.

Histopathological analysis of kidneys

On day 29 rats were anesthetized, sacrificed through an incision on their abdomen, and both kidneys were removed. A cold solution of normal saline was used to rinse the extraneous tissue of isolated kidneys

to remove fat deposits, then it was fixed in a neutral formalin (pH 7.4) solution for 10%. Then, the kidney tissue portion was fixed in 10% neutral formalin(pH 7.4) solution. One of the two kidneys that were isolated was immersed in paraffin films using a traditional method, then sliced into 5 µm thin sections by a rotary vertical microtome. A hematoxylin and eosin solution was used to stain the sections to indicate changes in the histopathological analysis and crystal deposition.[16] Hematological parameters like hemoglobin content, total erythrocyte count,and leukocyte count were analyzed in the blood.[23]

Statistical analysis

The results of biochemical paraeters are expressed as mean ± SEM. One-way analysis of variance was used to determine the significance of the urine and blood serum analysis, followed by Dunnett's test. A significance level of P< 0.05 was established for statistical significant.

RESULTS AND DISCUSSION

Evaluation of *In vitro* antiurolithiatic activity

Nucleation assay

The effect of inhibition on the nucleation of calcium oxalate crystals using the extract was determined by a spectrophotometric assay.[24]The inclusion of SASE reduced the turbidity of the solution by disintegrating the oxalate crystallization after 30 minutes of incubation. An increase in the percentage of calcium oxalate crystallization'inhibition was observed with an increase in the content of plant extracts. All the SASE concentrations showed a significant reduction (P<0.01) in the nucleation of CaOx crystals when compared with cystone. The SASE at 600 µg/ml showed the highest inhibition of 67.16±0.002% compared to cystone 65.22 ± 0.003% at 600 µg/ml presented in (Figure 1). The images displayed in (Figure 2A-H)observed under the light microscope exposed a paramount of crystal formation in the negative control, followed by a significant reduction in the sizeand number of crystalsat different concentrations (100 µg/ml-600 µg/ml) of SASE indicating dose-dependent inhibitory effect in par with the standard cystone at 600 µg/ml. It is observed that in the light microscopic image of A (the control) without SASE, the number of crystals was the highest. Figure 2C-H represents the plant extract showing maximum inhibition effect at different concentrations (100 µg/ml-600 µg/ml) when compared with Figure 2B of cystone at 600 µg/ml. This indicated a dose-dependent manner of crystals formed after 30 min of incubation. In the crystal growth experiment shown in the nucleation assay, at the constant time against different concentrations, the presence and absence of SASE determined the rate at which the crystals grow. Nucleation is the spontaneous crystallization of dissolved materials in a supersaturated solution due to a thermodynamically driven phase change. Significant inhibition of CaOx crystal nucleation was seen in the presence of the extract when compared to the presence of cystone. Reduction in the size of the crystals formed in the presence of the extract further exhibited the extract's growth inhibitory capabilities. This indicated that the extract in the CaOx crystallization assay has anti-crystallization activity. The extract may have anti-crystallization properties due to its capacity to form complexes with free calcium and oxalate ions, thereby preventing the formation of CaOx complexes. CaOx crystals should be reduced in size because they tend to spontaneously dissolve in urine.[25,26]

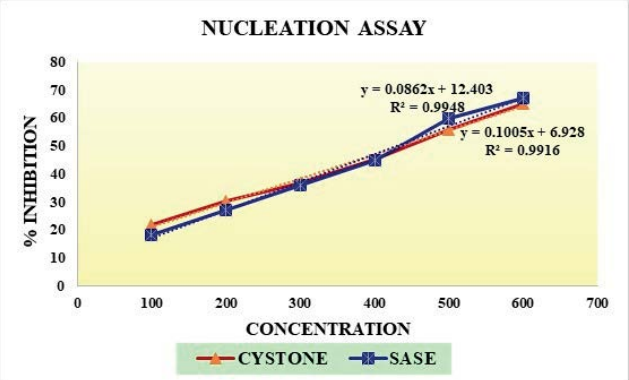

Figure 1: *In vitro* Antiurolithiatic Activity of SASE extract using Nucleation assay. Values are expressed as mean ± S.D.

Aggregation assay

At 600 µg/ml (88.4 ± 0.153%), SASE was found to have a more effective inhibitory effect on the aggregation of CaOx crystals than cystone (77.09 ± 0.007%). A concentration-dependent increase of SASE causes a reduction in CaOx crystal size and increases the inhibition percentage (Figure 3). In the images displayed in (Figure 4A-H)an increase in crystal growth was observed under a light microscope in the negative control, followed by a significant reduction in crystal size and number as SASE concentrations increased (100 µg/ml -600 µg/ml, suggesting a dose-dependent inhibitory effect comparable to cystone at 600µg/ml.

Same as the nucleation assay, in (Figure 4A-H) it is observed that in the light microscopic images of A (the control) without SASE, the number of crystals was the maximum. Figure 4C-H represent the plant extract showing maximum inhibition effect at various concentrations (100 µg/ml-600 µg/ml) when compared with Figure 4 B of cystone at 600 µg/ml. Incubation for 30 minutes resulted in crystals forming in a dose-dependent manner. A constant time against various concentrations of methanol stem extract determined the rate of crystal growth in the aggregation assay experiment. This study also exhibited strong inhibitory effect of SASE on the crystallization of CaOx. These results were in agreement with the previous reports studied in *Spermacoce articularis*[27]*Boldoa purpurascens*[28] *Ocimum bacilicum* seed extracts[29]*Daucus carota.*[30]Urinary supersaturation, which results from alterations in urine chemistry such as hyperoxaluria and hypercalciuria, crystallizes, aggregates, and eventually forms stones.[31]

In vivo Antiurolithiatic Activity

Acute toxicity study

The oral administration of SASE up to a dosage of 2000 mg/kg showed no signs of toxicity in rats, and for 14 days there was no death. Therefore, for the anti-urolithiatic investigation in the present study, the therapeutic dosage was determined to be 250 mg/kg and 500 mg/kg body weight.

Effect of SASE on the urine, blood and serum parameters

On par with the normal control group (Group I), the administration of PEG in the rats with induced urolithiasis (Group II) led to increased renal excretion of phosphate on the 28th day, along with hypercalciuria and hyperoxaluria. Urinary calcium levels (***p<0.05; 13.8±0.551), oxalate (***p<0.05;12±0.374), and phosphorus (p<0.05;7.21±0.242) were improbably elevated in animals induced with stones. However, the levels of calcium (nsp<0.05; 7.76±0.32), oxalate (**p<0.05; 6.67±0.759), and phosphorus (**p<0.05; 5.05 ±0.101) in the animals in the standard drug cystone-treated group were significantly lower. When compared to the disease control group, treatment with SASE (250 mg/kg) did not significantly lower the elevated levels of calcium (***p>0.05; 10.20±0.199) or oxalate (***p<0.05; 8.02±0.208) or phosphorus (p<0.05; 5.47±0.294). However, when SASE (500 mg/kg, p.o) was administered, the levels of calcium, oxalate, and phosphorus (*p<0.05; 8.69 ± 0.214, nsp<0.05; 5.57±0.395, p<0.05 4.37±0.144) in urine were reduced. Urine BUN, creatinine, and uric acid levels

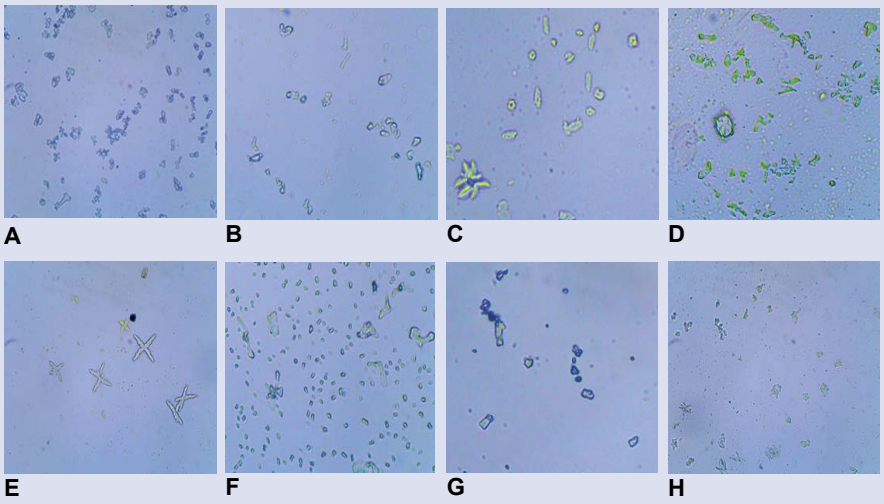

Figure 2: Nucleation Assay - Micrograph of *S. articularis* L.f. in the presence and absence of stem methanol extract. A-Control, B-cystone at 600 µg/ml, C to H represents *S. articularis* L.f. stem methanol extract at different (100, 200, 300, 400, 500, and 600 µg/ml) concentrations.

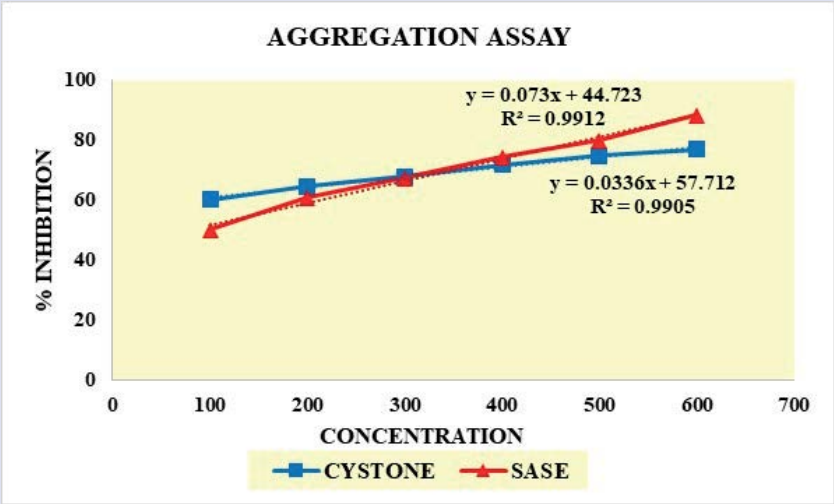

Figure 3: *In vitro* Antiurolithiatic Activity of SASE extract using Aggregation assay. Values are expressed as the mean ± S.D.

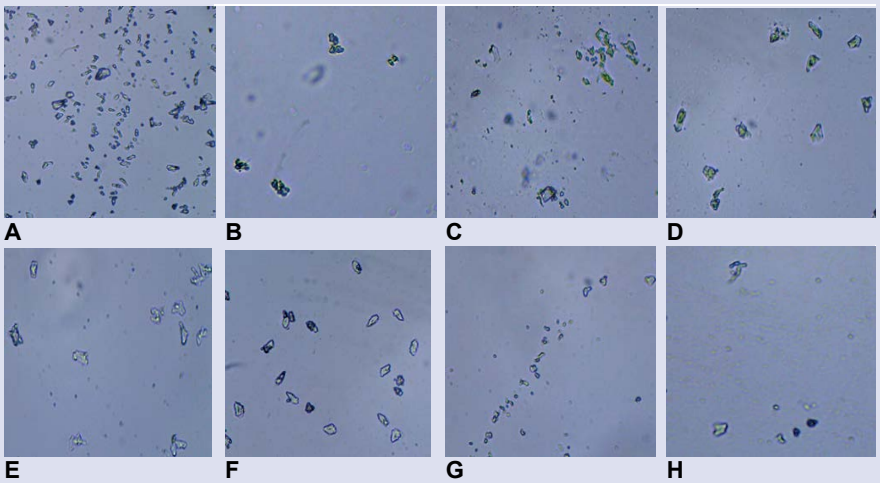

Figure 4: Aggregation Assay - Micrograph of *S. articularis* L.f. in the presence and absence of stem methanol extract. A-Control, B-cystone at 600 µg/ml, C to H represents *S. articularis* L.f. stem methanol extract at different (100, 200, 300, 400, 500 and 600 µg/ml) concentrations.

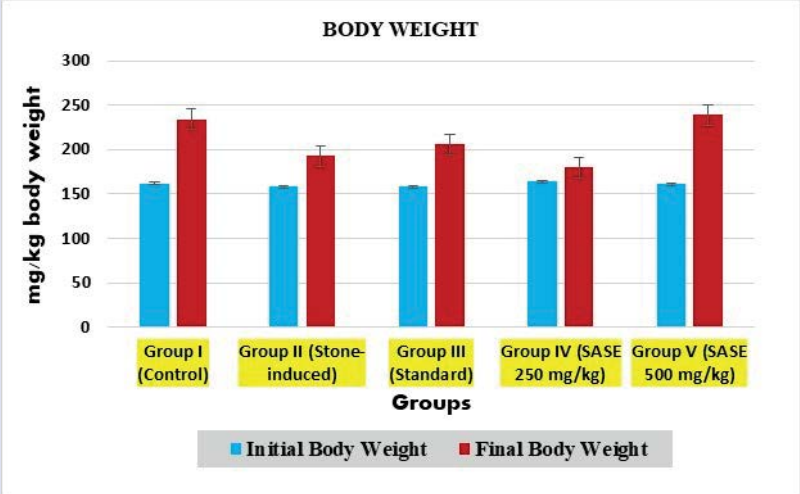

Figure 5: Body weight of control and treatment groups. Values are expressed as the mean ± S.D. Statistical significance (p) was calculated by one-way ANOVA followed by Dunnett's. ns- not significant **P< 0.05 calculated by comparing the treated group with the control group. n = 6 in each group.

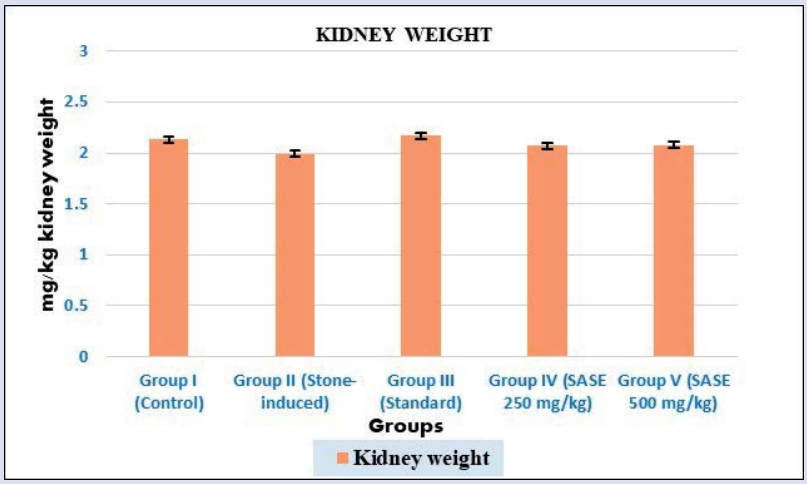

Figure 6: Kidney weight of control and treatment groups. Values are expressed as the mean ± S.D. Statistical significance (p) was calculated by one-way ANOVA followed by Dunnett's. ns- not significant **P< 0.05 calculated by comparing the treated group with the control group. n = 6 in each group.

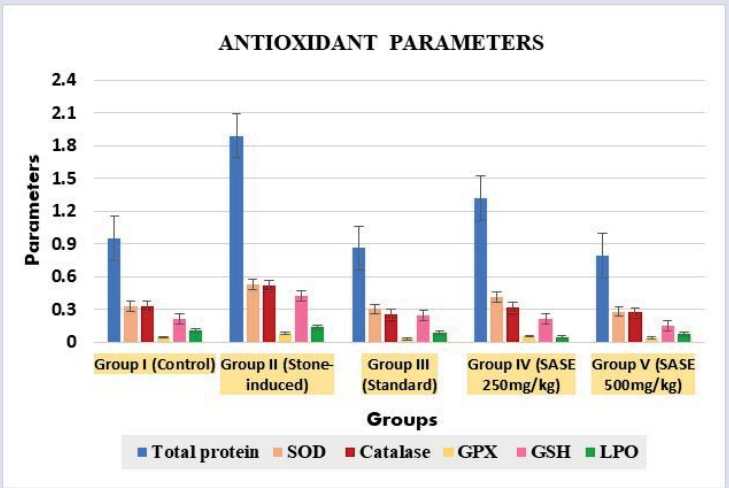

Figure 7: Effect of SASE on Antioxidant Parameters. Values are expressed as the mean ± S.D. Statistical significance (p) was calculated by one-way ANOVA followed by Dunnett's. ns- not significant *P<0.05, **P< 0.05, ***P<0.05 calculated by comparing treated group with control group. n = 6 in each group.

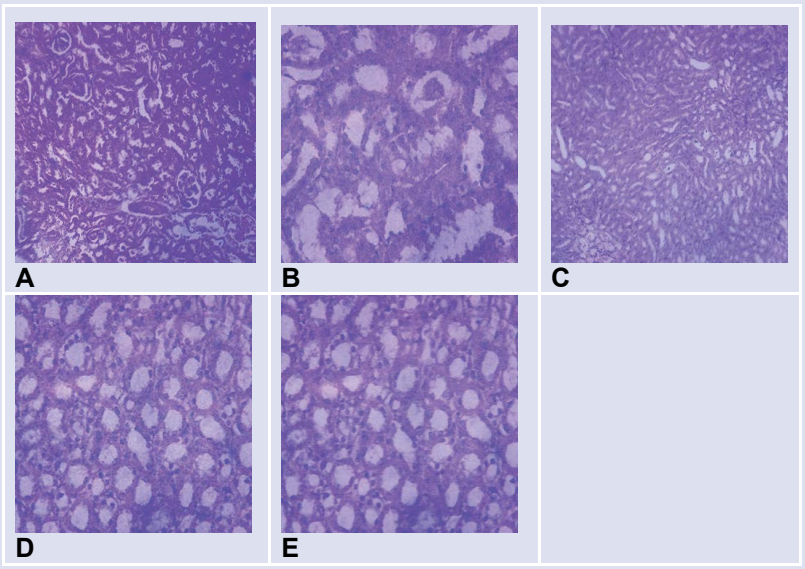

Figure 8: Histopathological analysis of kidney at 400X: A - Group I (Control), B - Group II Poly Ethylene glycol (1%) (Stone Induced), C - Group III cystone (Standard), D - Group IV Methanol stem extract 250 mg/kg body weight and E - Group V Methanol stem extract 500 mg/kg body weight.

Table 1: Effect of *S. articularis* stem methanol extract (SASE) on different parameters of urine analysis in Urolithiasis Male Wistar Rats. Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns- not significant, *P<0.05, **P< 0.05, ***P<0.05 calculated by comparing treated group with control group.

©	Urine Analysis (mg/dl)							
	Bun- Blood Urea Nitrogen	Creatinine	Uric acid	Oxalate	Citrate	Calcium	Magnesium	Phosphorus
Group I (Control)	18.4±1.1	1.2±0.346	0.733±0.0882	4.12±0.32	22.6±0.722	7.1±0.453	3.1±0.121	5.15±0.364
Group II (Stone-induced)	103±7.4***	7.87±0.677***	5.47±2.12*	12±0.374***	8.89±0.248***	13.8±0.551***	1.93±0.0924 ns	7.21±0.242
Group III (Standard)	36.5±6.96ns	2.47±0.467ns	2±0.208ns	6.67±0.759**	16.80±0.517***	7.76±0.32 ns	2.78±0.323	5.05±0.101
Group IV (SASE 250mg/kg)	73.3±4.11***	5.47±0.406***	2.57±0.674ns	8.02±0.208***	11.60±0.703***	10.20±0.199***	2.32±0.0577 ns	5.47±0.294
Group V (SASE 500mg/kg)	43.9±3.54*	2.47±0.348ns	1.9±0.436ns	5.57±0.395 ns	17.60±0.419***	8.69±0.214*	2.83±0.026 ns	4.37±0.144

Table 2: Effect of *S.rticularis* stem methanol extract (SASE) on different parameters of serum and haematology in Urolithiasis Male Wistar Rats. Values are expressed as the mean ± S.D. Statistical significance (p) calculated by one way ANOVA followed by dunnett's. ns- not significant *P<0.05, **P< 0.05, ***P<0.05 calculated by comparing treated group with control group.

Group	Serum (mg/dl)			Haematological parameters		
	Bun- Blood Urea Nitrogen	Creatinine	Uric acid	RBC ($X10^6$/µl)	WBC ($X10^3$/µl)	HB (g/dl)
Group I (Control)	54.3±3.48	0.867±0.176	3.1±0.173	6.07±0.172	11.3±0.876	15.6±0.458
Group II (Stone-induced)	153±7.26***	3.6±0.404***	11.7±0.586***	5.45±0.319ns	11.6±0.994	13.7±1.3
Group III (Standard)	58.3±10.4ns	1.07±0.12ns	3±0.361ns	6.16±0.274ns	13.4±0.762	13.2±0.907
Group IV (SASE 250mg/kg)	98.3±11.3*	1.73±0.521ns	7.3±0.608**	4.86±0.0437*	10±0.984	12.6±0.546
Group V (SASE 500mg/kg)	61±12.1ns	1.03±0.393ns	4.93±0.913ns	5.85±0.331ns	12.2±1.27	14.2±0.649

were significantly elevated in the rats given ethylene glycol treatment when compared to Group I and restored by SASE treatment in Group IV & V which was similar to Group III. Comparing group II to the control group (group I), urinary citrate and magnesium excretion was reduced after the administration of ethylene glycol. The levels of citrate and magnesium were nearly returned to normal after supplementing with SASE (250 and 500 mg/kg), which also markedly increased (P < 0.05) this parameter (Table 1). The concentration of BUN (153±7.26), creatinine (3.6±0.404), and uric acid (11.7±0.586) in the serum was highly increased (***p<0.05) in the stone induced group, indicating renal damage (Table 2). However, group V treated with SASE 500 mg/kg significantly reduced the concentrations of BUN, creatinine, and uric acid in contrast to group III and IV.

The addition of ammonium chloride accelerated the stone formation in the rat model of ethylene glycol-induced urolithiasis. There were lower levels of calcium, phosphate, and oxalate in urine excretion in extract-treated groups. Calcium phosphate crystals are formed when urine phosphate and oxalate levels are elevated, which creates the ideal condition for stone formation.[32] Urine that has a higher calcium content is more likely to precipitate and nucleate CaOx, which leads to the formation of crystals. Calcium excretion in urine and increased calcium deposition in the kidney are due to either increased absorption from the intestine or defective renal tubular reabsorption; patients with renal calcium stones were found to have hyper absorptive calcium.[33] Magnesium levels are low in rats that form stones. The production of calcium oxalate crystals in the urine is known to be inhibited by magnesium, because magnesium binds free oxalate and makes it more soluble, thus preventing calcium oxalate crystals from forming in urine. There have been reports that magnesium-containing diet interventions may reduce the supersaturation of lithogenic salts in the urine.[32]Citrate, a significant urolithiasis inhibitor, prevents calcium oxalate and phosphate from precipitating and aggregating by forming a soluble complex with calcium. This investigation supported our results where the administration of SASE and cystone resulted in an increase in citrate concentrate, which may have decreased calcium oxalate crystallization.[34] As compared to normal, the lithiatic group experiences a decrease in renal function due to elevated urine and serum creatinine, BUN, and uric acid levels. All groups showed increased hemoglobin, leukocytes and erythrocyte counts compared to stone-induced groups athematological analysis,after 28 days Table 2. A significant increase was observed to be 14.2±0.649, 12.2±1.27, and 5.85±0.331 respectively in Group V (500 mg/kg) when compared to the stone induced group. Similar result was found in the extract of *Pedalium murex*[23] with erythrocyte count of 6.21±1.02, leucocytes count of 605± 37.3 and haemoglobin amount of 14.1±1.23.

Measurement of Body and Kidney Weight

The mean body and kidney weight of the five experimental groups i.e., control (Group I), stone induced-PEG (Group II), PEG+STD (Group III), SASE-treated PEG+L.D 250 mg/kg (Group IV), PEG+H.D 500 mg/kg (Group V), were recorded practically the same for all the groups before the commencement of treatment (day 1) and the changes observed after 28 days are summarized in (Figure 5 & 6).It showed that animals in cystone and SASE-treated 500 mg/kg had a significant gain ($p<0.05$) in mean body weight than the stone-induced group after 28 days of treatment which coincides with the previous reports.[35,36,37]The results of wet kidney weight were significantly similar in the treated groups concerning normal control cystone-treated group (Group III), and plant extract-treated groups(Group IV and Group V). An increase in kidney weight in the PEG-treated animals (Group II) observed in the present study indicated stone formation. Compared to groups treated with extract, the disease control group's kidney weight was greater as a result of CaOx crystal precipitation.[38]Our results were in par with the previous findings of. [39,40]The cystone and plant extract treated groups exhibited antiurolithiatic activity, which may have prevented stone accumulation, therefore providing pain relief for the animals. This might be the cause of the considerable rise in body weight that results from increasing food consumption. Similar result was exhibited by *S. hispida* methanol extract (100 mg/kg), the rats body weight in normal group was found to be increasing (192.9±4.595) ($P< 0.05$) in contrast to cisplatin treated group (179.3±6.103) ($P <0.05$)[37] and hydroalcoholic extract of *Copaifera langsdorffii* leaf exhibited significant antiurolithiatic activity with the increase in body weight in plant extract-treated groups when compared to control groups.[41]

Effect of extract on antioxidants and lipid peroxidation activity in the experimental rats

The total protein level was significantly ($P<0.05$) increased to 1.89 ± 0.552 mg/dl in the Ethylene glycol-treated group Figure 7. This increased total protein level was considerably decreased after treating withcystone and with SASE based on extract concentration. The 250 and 500 mg/kg extract concentrations of SASE were observed equipotent($P<0.05$) to the standard cystone. For *in vivo* antioxidant activity, Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPS), and Reduced glutathione (GHS) were found to have decreased ($P<0.05$) and to have inclined ($p<0.05$) in the PEG-treated lithiasis rats (Group II) in contrast to the normal control rats (Group I). Antioxidants levelswere ($p<0.05$) increased and LPO was ($p<0.05$) reduced in the SASE(250 mg/kg) treated rats (Group III),SASE (500 mg/kg) treated rats (Group IV), and the standard cystone treated rats (Group V) when correlated with PEG treated lithiatic rats (Group II). In the experimental models, the 500 mg/kg SASE treatment decreased lipid peroxidation and enhanced antioxidant activities.

Increased LPO and GSH levels in the control group of our study indicated that hyperoxaluria promoted extensive ROS production. The extract has a dose-dependent antioxidant effect, as evidenced by a significant drop in the antioxidant levels of GSH, LPO, and catalase in the SASE-treated Groups IV and V. Renal epithelial cells are toxically exposed to oxalate, which causes lipid peroxidation (LPO) mediated by free radicals. The antioxidant response of SASE was in a dose-dependent manner similar to previous reports of *Musa paradisiaca* pseudo stem.[42]

Histopathological studies

Large spaces, increased tubular dilatation, and abnormal calcium oxalate crystal deposition were observed in the urolithiatic rats exposed to PEG. Less crystal deposition and dilation were seen in the treatment groups, and Group III's cystone treatment showed notable improvements, as shown in Figure 8C. SASE treatment Figure 8D &E with varying doses i.e. (Group IV and V), suggestively lowered the calcium oxalate crystals deposition, renal tubules dilation and interstitial inflammation compared to Group-II. The presence of rosette-like, polycrystalline CaOx crystals indicated that the particles have adhered to and retained in the renal tubules. On the other hand, SASE treatment significantly decreased the CaOx crystal deposits that was showed in urine microscopy and kidney histology.In the normal group, kidney tissues were examined microscopically and no cellular necrosis or inflammatory infiltration was visible in the interstitial space of the kidney tubules. Several inflammatory infiltrations were observed in the renal cortex and renal tubular epithelium of rats injected with PEG. Alternatively, SASE treatment reduced the large range of infiltrates. A dose of SASE treatment was shown to protect the renal tubular necrosis score (Group IV and V) compared to the PEG treated Group II.In the present study supplementation with SASE and cystone, restored oxalate and calcium levels in urine in both curative as well as preventive regimens as compared to untreated animals. Test findings revealed that SASE and standard cystone markedly reduced the stone size and crystalluria which exhibits considerable antiurolithiatic activity. PEG-treated rats for 28 days excreted abundant and aggregated CaOx crystals stones in their urine. According to the results, the SASE reduced the aggregation and thereby prevented the formation of stones, or broke down the calcium particles into subtle constituents that were not visible. Through excreting a smaller amount of debris from the kidney and decreasing the possibility of them being deposited in the urinary tract, this effect may help prevent the formation of urinary stones.[43,44]

CONCLUSION

Although advances in medical research have been made in recent decades, urolithiasis recurrence remains a severe issue for many

patients, prompting them to look for treatment alternatives, such as herbal therapies. The present results of this study, clearly demonstrated that *S. articularis* stem extract has the *in vitro* antiurolithiatic potential against CaOx stones. Furthermore, the extract was administered to reduce the progression of the disease in rats in urolithiasis model. Therefore, more research should be performed using SASE to identify the constituents exhibiting anti-urolithiatic activity in order to discover potential lead molecules for future studies into drug formulation.

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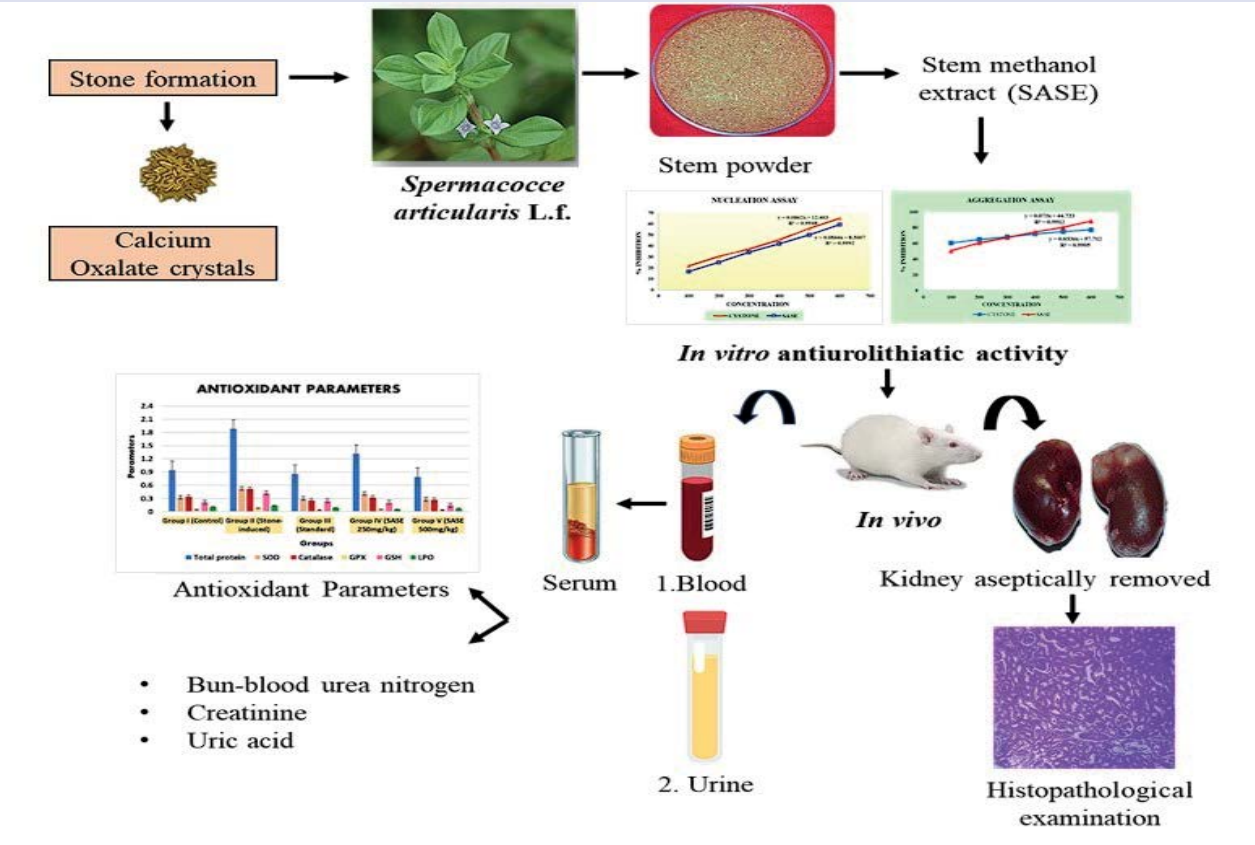
GRAPHICAL ABSTRACT



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