Open Access Journal

Indian Journal of Medical Research and Pharmaceutical Sciences

December 2016;3(12) ISSN: ISSN: 2349-5340

DOI: 10.5281/zenodo.197062 Impact Factor: 3.052

EVALUATION OF ANTI-UROLITHIATIC POTENTIAL OF NOVEL SIDDHA FORMULATION SEENAKARAPARPAM ON ETHYLENE GLYCOL-INDUCED UROLITHIASIS IN WISTAR ALBINO RATS

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Keywords:

Seenakaraparpam, urolithiasis, ethylene glycol, anti-urolithiatic activity, biochemical estimation.

Abstract

Urinary calculi are the third prevalent disorder of the urinary system. Approximately 80% of these calculi are composed of calcium oxalate and calcium phosphate. In India, urolithiasis constitutes one of the commonest afflictions requiring surgical intervention and by conservative estimates there are about 5–7 million patients suffering from urinary calculus disease in India. It is not only the higher prevalent disease condition which requires early attention, but rather the more problematic disorder with, higher rate of recurrence after surgical removal. The main aim of the present study is to investigate the anti-urolithiatic activity of *Seenakaraparpam* (SKP) on ethylene glycol-induced urolithiasis in wistar albino rats. All the experimental animals except normal control received ethylene glycol (0.75%) in drinking water for a period of 28 days for induction of urolithiasis. Rat belongs to treatment group co-administered with SKP at the dose of 100,200 and 300 mg/kg b.wt, p.o from 1st to 28st day, Animals belongs to standard group received cystone 500 mg/kg, p.o. Parameters such as volume and urinary biochemical parameters were measured after 14 & 28 days, Blood was collected on the 28st day for biochemical estimation. Treatment with SKP significantly reduced the excretion of oxalate and Ca and phosphate, protein, uric acid and creatinine in compared to lithiatic control group.The results of the study also shows that serum parameters such as calcium, uric acid, creatinine, oxalate, phosphate levels were increased significantly in animals belongs to lithiatic control. Treatment with SKP at the dose of 100, 200 and 300mg/kg and standard drug cystone at the dose of 500 mg/kg shown significant decrease in serum calcium, uric acid, creatinine, oxalate, phosphate levels. The level of magnesium was restored near to normal in treatment group animals. In conclusion formulations like SKP may be considered as a potential lead for the clinical management of urolithiasis

Introduction

Urinary stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. In addition, the incidence of kidney stones has been increased in western societies in the last five decades, in association with economic development. Most calculi in the urinary system arise from a common component of urine, e.g. calcium oxalate (CaOx), representing up to 80% of analyzed stones [1].Currently, open renal surgery for nephrolithiasis is unusual and used only rarely since the introduction of extracorporeal shockwave lithotripsy (ESWL), which has revolutionized urological practice and almost become the standard procedure for eliminating kidney stones. However, in addition to the traumatic effects of shock waves, persistent residual stone fragments and the possibility of infection, suggest that ESWL may cause acute renal injury, a decrease in renal function and an increase in stone recurrence [2,3].

Kidney stone formation is a complex process and it results as a cascade of events, including crystal nucleation, growth and aggregation, crystal retention within the renal tubules [4]. Usually kidney stones are yellow or brown

December 2016;3(12) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.197062 Impact Factor: 3.052

color with a smooth or gagged structure. Some common type of kidney stones are calcium oxalate, calcium phosphate, struvite, uric acid and cysteine, among of which calcium stones are the most common form of kidney stones in both humans and rats [5]. Urolithiasis, also called calculi or uroliths, is a condition which involves the process of stone formation in the kidney. Renal stones are a universal cause of blood in the urine and pain in the abdomen, with a reported incidence about 12% in the general population [6]

A number of vegetable drugs have been used in India and elsewhere which claim efficient cure of urinary stones [7]. In the indigenous system of medicine, the *SeenakaraParpam* (SKP) is reported to be useful in the treatment of urinary stones. However, so far no systematic study has been reported with respect to the anti-urolithiatic property of SKP. Hence the main aim of the present study is to evaluate the anti-urolithiatic property of SKP using ethylene glycol induced hyperoxaluria model in rats

Materials and methods

Evaluation of Anti-urolithiatic activity by Ethylene glycol induced urolithiasis in rats

Animals

Healthy adult albino wistar rats weighing between 200-220 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100 % fresh air by air conditioning. A 12h light/dark cycle was maintained. Room temperature was maintained between 22±2° C and relative humidity 40–65%. They were provided with food and water *ad libitum*. All the animals were acclimatized to the laboratory about 7 days prior to experimentation

Experimental Protocol

Ethylene glycol induced hyperoxaluria method was used to assess the anti-urolithiatic activity in albino wistar rats. Animals were divided into 6 groups of 6 animals each. All the experimental animals except normal control received ethylene glycol (0.75%) in drinking water for a period of 28 days and a single dose of sodium oxalate injection (35 mg/kg ,i.p) on 14th day for induction of urolithiasis. Rat belongs to treatment group co-administered with SP at the dose of 100,200 and 300 mg/kg b.wt, p.o from 1st to 28st day, Animals belongs to standard group received cystone 500 mg/kg, p.o.

Grouping

Group I: Control group rats received normal saline
Group II: Administered with ethylene glycol (0.75%) + Sodium oxalate injection (35mg/kg ,i.p)
Group III: Administered with ethylene glycol and treated with SKP at 100 mg/kg, p.o
Group IV: Administered with ethylene glycol and treated with SKP at 200 mg/kg, p.o
Group V: Administered with ethylene glycol and treated with SKP at 300 mg/kg, p.o
Group VI: Administered with ethylene glycol and treated with cystone 500 mg/kg, p.o
24 hour urine samples will be collected on 14th and 24thday by housing rats at individual metabolic cages using sodium azide as preservative .Parameters such as volume, specific gravity and pH of the urine will be noted immediately after urine sample collection and stored at -80^{o} C, for further electrolyte estimation. Blood will be collected from retro-orbital plexus of each rat, on day 28 and subjected to electrolytes estimation.

Collection and analysis of urine

All animals were kept in individual metabolic cages and 24 h urine samples were collected on 14th, and 28th day of calculi induction treatment. The volume and calcium content of urine were measured. Calcium in urine was estimated using kit by “COBAS MIRA PLUS” auto analyzer. Urine was analyzed for oxalate [8], magnesium [9], phosphate [10], uric acid [11], citrate [12] and total protein [13].

Serum analysis

The blood was collected from the retro-orbital sinus under anesthetic condition and serum was separated by centrifugation at 10,000g for 10 min and analyzed for creatinine and uric acid. The creatinine kit (Reckon Diagnostics Pvt. Ltd., India) and uric acid diagnostic kit (Span Diagnostics Ltd., India) were used to estimate serum

Indian Journal of Medical Research and Pharmaceutical Sciences
December 2016;3(12) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.197062 Impact Factor: 3.052

creatinine and uric acid levels respectively. Estimation of calcium, oxalate, inorganic phosphorus and magnesium carried out using standard methods.

Statistical analysis

The results were expressed as mean ± standard error mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Newmannkeul's multiple range tests and $p < 0.05$ was considered significant.

Results

Effect of SeenakaraParpam on Urine volume output on Day 0, 14 and 28th Day

The result of 24hrs urine collection on 0th, 14th and 28th day shows that there is a significant decrease in the urine volume excretion in lithiatic control group treated with ethylene glycol. Treatment with SKP at the dose of 100mg/kg, 200mg/kg, 300mg/kg to group 3 to 5 and cystone 500mg/kg to group 6 shown significant (P<0.01) increase in urine volume rate on 14th and 28th day of the study period.

Table 1: Effect of SKP on urinary output in urolithiasis induced rats.

Days	GP1	GP2	GP3	GP4	GP5	GP6
0	7.60± 0.40	7.45± 0.42	7.80± 0.70	7.55± 0.75	8.50± 0.95	8.25± 0.90
14	7.80± 0.60	6.90± 0.35**	8.35± 0.90**	9.05± 1.30**	10.30± 1.50**	11.25± 1.60**
28	8.30± 0.70	6.30± 0.25**	8.60± 1.20**	10.20± 1.35**	11.50± 1.65**	11.90± 1.75**

- Values are expressed as mean ± SEM
- Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.

Effect of *SeenakaraParpam* on Urinary Parameters on Day 14th and 28th Day

The result of 24hrs urine collection on 14th& 28th day shows that there is a significant ($P < 0.001$) increase in the level of oxalate in the animals belongs to group II treated with ethylene glycol alone. Treatment with SKP at the dose of 100mg/kg, 200mg/kg, 300mg/kg to group 3 to 5 and cystone 500mg/kg to group 6 shown significant (P<0.01) reduction in the level of oxalate excretion in animals belongs to treatment group. The results are shown in the table no 2 and 3.

Table 2 :Effect of SKP on Urinary Biochemical Parameters on 14th Day of Induction

GP	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
GP_1	70.90± 2.90	4.25± 0.50	5.70± 0.52	8.22± 0.65	0.84± 0.10	17.80± 1.43	35.90± 2.80
GP_2	155.20 ± 5.25**	1.05 ± 0.12**	25.15± 1.94**	16.56 ± 1.62**	1.59 ± 0.12**	30.65 ± 3.12**	76.60 ± 4.26**

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GP3	88.30 ± 3.90 **	2.55 ± 0.25**	19.45 ± 2.10**	10.56 ± 0.94**	0.98 ± 0.09**	24.22 ± 2.46**	45.55 ± 3.73**
GP4	83.70 ± 3.60**	2.80 ± 0.35**	13.90 ± 0.82**	12.20 ± 0.85**	0.89 ± 0.11**	22.30 ± 2.29**	40.80 ± 3.15**
GP5	85.50± 3.65**	2.60 ± 0.40**	15.75 ± 0.63**	9.90 ± 0.80**	0.87 ± 0.09**	23.10 ± 1.89**	36.30 ± 2.28**
GP6	80.25± 2.95**	3.30 ± 0.60**	17.75 ± 0.45**	8.90 ± 0.80**	0.81 ± 0.10**	20.20 ± 1.90**	35.30 ± 2.28**

- Values are expressed as mean ± SEM
- Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.

Table 3 : Effect of SKP on Urinary Biochemical Parameters on 28th Day of Induction

GP	Protein (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
GP1	74.90 ±3.86	4.22 ±0.48	7.10 ±0.72	3.40 ±0.52	0.90 ±0.28	18.40 ±1.40	31.50 ±2.44
GP2	163.40 ±8.30**(a)	1.85 ±0.51**(a)	21.55 ±1.50**(a)	13.50 ±1.42**(a)	1.56 ±0.54**(a)	50.30 ±4.55**(a)	80.56 ±4.76**(a)
GP3	90.15 ±5.58**(b)	2.05 ±0.37**(b)	14.55 ±0.55**(b)	8.50 ±0.56**(b)	1.12 ±0.15**(b)	26.58 ±2.60**(b)	47.72 ±3.34**(b)
GP4	87.46 ±5.36**(b)	3.96 ±0.55**(b)	12.46 ±0.70**(b)	8.25 ±0.16**(b)	1.24 ±0.50**(b)	23.50 ±2.50**(b)	44.45 ±3.16**(b)
GP5	83.22 ±4.55**(b)	3.65 ±0.5**(b)	11.75 ±0.45**(b)	7.95± 0.50**(b)	1.55 ±0.00**(b)	21.22 ±2.09**(b)	41.10 ±2.70**(b)
GP6	84.55 ±4.87**(b)	3.52 ±0.50*(b)	11.15 ±0.05**(b)	7.12± 0.30**(b)	1.24 ±0.50**(b)	20.72 ±2.10**(b)	40.70 ±2.20**(b)

- Values are expressed as mean ± SEM
- Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.

The urinary calcium excretion was 5.70±0.52mg/dl/24hr and 7.10±0.72mg/dl/24hr on day 14th& 28th respectively of control group rats .It increased significantly to 25.15± 1.94mg/dl/24hr and 21.55± 1.50mg/dl/24hr ($P < 0.01$) on day 14th and 28th day in animals belongs to group II.

The level of calcium excretion was significantly reduced in animals treated treatment with SKP at a dose of 100 200 and 300mg/kg and cystone herbal tablet at a dose of 500mg/kg (Group III to VI). The results are shown in the table no: 2 and 3.

Urinary phosphate, protein, uric acid and creatinine excretion rate were gradually increased in group II animals on the 14th& 28th day. However treatment with SKP and standard drug cystone to group III to VI shown considerable decrease in the level of phosphate, protein, uric acid and creatinine in the urine sample collected for analysis.

Magnesium excretion rate of control group animals was estimated as 4.25±0.50 mg/dl/24hr, 4.22±0.48 mg/dl/24hr on 14th& 28th day. Treatment with ethylene glycol to group II animals shows gradual decrease in magnesium level to 1.05±0.12 mg/dl/24hr on 14th day and 1.85± 0.51 mg/dl/24hr on 28th day. Treatment with SKP and cystone shows

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December 2016;3(12) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.197062 Impact Factor: 3.052

significant increase in magnesium level of 2.55±0.25 mg/dl/24hr, 2.05±0.37mg/dl/24hr in group III , 2.80±0.35mg/dl/24hr,3.96± 0.55 mg/dl/24hr in group IV, 2.60± 0.40 mg/dl/24hr, 3.65± 0.50 mg/dl/24hr in group V, 3.30±0.60 mg/dl/24hr, 3.52±0.50 mg/dl/24hr on group VI ($P< 0.01$) respectively on 14th day & 28th day.

Effect of SeenakaraParpam on Serum Parameters on 28 days treatment.
The result obtained from the study shows that the calcium, uric acid, creatinine, oxalate, phosphate levels were increased significantly in lithiatic control groups. Treatment with SKP at the dose of 100,200 and 300mg/kg to group III to V and cystone 500mg/kg to group VI shown significant decrease in the level of calcium, uric acid, creatinine, oxalate and phosphate. On contrary the magnesium levels were decreased significantly in lithiatic control animals **.** After treatment with SKP at a dose of 100mg/kg, 200mg/kg and 300mg/kg to group III to V and cystone at the dose of 500mg/kg to group VI restored the magnesium level near to normal level. The results were tabulated in table no 4.

Table 4 :Effect of SKP on Serum Parameters on 28th Day

GP	Magnesium (mg/dl)	Calcium (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
GP_1	4.90 ±0.56	10.50 ±1.52	3.25 ±0.20	0.46 ±0.23	6.5 ±0.67	12.86 ±1.53
GP_2	1.98 ±0.45$^{**(a)}$	17.40 ±2.44$^{**(a)}$	9.72 ±1.20$^{**(a)}$	1.21 ±0.53$^{**(a)}$	13.50 ±1.65$^{**(a)}$	29.21 ±3.45$^{**(a)}$
GP_3	3.78 ±0.56$^{**(b)}$	12.75 ±1.78$^{**(b)}$	4.25 ±0.52$^{**(b)}$	0.68 ±0.99$^{**(b)}$	9.48 ±0.88$^{**(b)}$	24.10 ±2.85$^{**(b)}$
GP_4	3.97 ±0.22$^{**(b)}$	12.82 ±1.50$^{*(b)}$	4.10 ±0.40$^{**(b)}$	0.73 ±0.01$^{**(b)}$	8.82 ±1.78$^{**(b)}$	22.85 ±2.75$^{**(b)}$
GP_5	3.86 ±0.55$^{**(b)}$	11.08 ±1.42$^{**(b)}$	3.76 ±0.42$^{**(b)}$	0.59 ±0.05$^{**(b)}$	8.85 ±0.75$^{**(b)}$	18.23 ±1.55$^{**(b)}$
GP_6	4.10 ±0.55$^{**(b)}$	10.01 ±1.42$^{**(b)}$	3.01 ±0.42$^{**(b)}$	0.52 ±0.05$^{**(b)}$	7.04 ±0.15$^{**(b)}$	17.23 ±1.75$^{**(b)}$

- Values are expressed as mean ± SEM

Values were found out by using ONE WAY ANOVA Followed by Newman keul's multiple range tests.

Discussion

Once kidney stone develops, the recurrence rate is estimated to be 14% at 1 year, 35% at 5 years, and 52% at 10 years. The incident in general population is about 1 in 1000 adults per year. The cause of urolithiasis is still unknown but probably positive family history, overweight, obesity, or increased BMI. Some other causes include low urine volume <1500 mL/day, high dietary animal protein intake, increased urine excretion of calcium oxalate, uric acid and cystine. Urinary tract structural abnormalities leading to stasis of urine flow [14].

Increased urinary calcium is a factor favoring the nucleation and precipitation of calcium, oxalate or phosphate from urine and consequent crystal growth. It has been reported that hyperabsorption of calcium is due to defective renal tubular reabsorption [15]. Stone formation in ethylene glycol-fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate [16].

Siddha preparations are 'parpam', 'chenduram', 'chunnam', 'mezhugu'. These are metallic and mineral preparations by transmutation processes of converting the metal to ashes and waxy constituency which exerts only therapeutic properties and devoid of any metallic traces. These are also the byproducts noticed during Siddhars Alchemical practices.

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Siddha systems of medicine have several medicines/formulations towards the clinical management of urolithiasis which ensures the safety and efficacious treatment,that are time tested and used so far. Hence, the present study aimed at evaluating the safety and efficacy of Seenakaraparpam, a Siddha formulation indicated for the management of urolithiasis in order to validate its traditional claim.In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans[17] and also earlier studies have shown that the amount of stone deposition in female rats was significantly less [18].

Urinary supersaturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidence in previous studies indicated that in response to 28 day period of ethylene glycol (1% v/v) administration, young male albino rats form renal calculi composed mainly of calcium oxalate [19].The biochemical mechanisms for this process are related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate. Similar results have been obtained when rats were treated with ethylene glycol and ammonium oxalate. Therefore, this model was used to evaluate the antilithiatic effect of SKP at a dose of 100,200 and 300mg/kg and cystone herbal tablet at a dose of 500mg/kg against urolithiasis [20,21].

Nearly 8% of kidney stones are composed of calcium salts, and the most common metabolic abnormality associated with kidney stones in humans is excessive urinary calcium excretion (idiopathic hypercalciuria [IH]). About 40–45% of patients with IH have a family history of nephrolithiasis [22]. The worldwide incidence of urolithiasis is quite high, and in spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi. However, surgery is the last resort; persistent endeavors have been made to overcome this painful disease [23].

In the present study oxalate and calcium excretion progressively increased in calculi- induced animals belongs to group II, since it is accepted that hyperoxaluria, is a far more risk factor in the pathogenesis of renal stones than hypercalciuria [24], and the changes in urinary oxalate levels are relatively much more important than those of calcium [25]. Increased urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate (or) apatite (calcium phosphate) from urine and subsequent crystal growth [26]. However treatment with SKP at a dose of 100, 200 and 300mg/kg and cystone tablet at the dose of 500mg/kg significantly lowered the levels of oxalate as well as calcium excretion. An increase in urinary phosphate is observed in calculi induced rats belongs to disease control group. Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which is epitaxially induces calcium oxalate deposition [27]. Treatment with SKP at the dose of 100, 200 and 300mg/kg and cystone herbal tablet at a dose of 500mg/kg restored phosphate level, thus reducing the risk of stone formation.

Medicinal ingredients in Siddha Vaidya are classified into three main groups: Thavaram (medicines derived from plants), Jangamam (those derived from animals), and Thatu (those derived from earth and organic toxins). Thavaram includes the thousands of whole plants and plant products [28].The National Siddha Formulary of India lists more than 10000 well practiced Siddha formulations described in Gunavagadam (Siddha pharmacology) [29].For a medicine to be effective, the inorganic substances have to be brought to their atomic form. Siddhars developed the knowledge of bringing inorganic substances into atomic and ionic form which is easily absorbed by the system, when ground with herbal juices and put on the fire. The specialized

The increases in urinary uric acid excretion were observed in urolithiatic rats. Increased excretion of uric acid has been reported in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of glycosaminoglycans. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation. Treatment with SKP at the dose of 100,200 and 300mg/kg and cystone herbal tablet at a dose of 500mg/kg lowered the excretion of uric acid and reduces the risk of stone formation.

Supersaturation, a step in the pathogenesis of nephrolithiasis, occurs when substances that make up the stone are found in the high concentration in urine, when urine volume decreases, and when urinary concentration of chemicals that

December 2016;3(12) ISSN: ISSN: 2349-5340
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inhibit stone formation decreases. Inhibitors of crystallization include citrate, magnesium, phosphate; nephrocalcinetc [30,31].Low urinary magnesium content is a common feature in stone formers [32]. A similar condition was observed in group II rats. Treatment with SKP at a dose of 100,200 and 300mg/kg and cystone herbal tablet at a dose of 500mg/kg elevated the urinary magnesium level, and thus, reduced the propensity to crystallize, thereby creating an ambience unfavourable for precipitation.

Increased excretion of proteins has been noted in hyperoxaluric rats and stone formers [33].A high urinary colloidal concentration favors crystal growth [34]. Such a condition was observed with ethylene glycol treated rats, in this study. Administration of SKP at the dose of 100,200 and 300mg/kg and cystone herbal tablet at a dose of 500mg/kg reduced the urinary protein excretion in the treated group rats, and hence minimizes the conditions favourable for crystal growth.
In urolithiasis, the Glomeruli Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinary system. Due to this, the waste products, particularly nitrogenous substances such as creatinine and uric acid get accumulated [35].Also increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi- producing diet (CPD) [36]. Elevated oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with poly unsaturated fatty acids in the cell membrane [37]. In urolithiatic rats there is a marked renal degeneration was observed as indicated by the elevated serum levels of creatinine and uric acid. However, the prophylactic treatment SKP at a dose of 100, 200 and 300mg/kg and cystone herbal tablet at a dose of 500mg/kg causes diuresis and hastens the process of dissolving the preformed stones and prevention of new stone formation in the urinary system. Increase in calcium and oxalate levels in the renal tissue of EG-treated rats were observed. Prophylactic treatment with SKP at the dose of 100,200 and 300mg/kg and cystone 500mg/kg suppresses this increase in intracellular calcium. It was concluded from the result of the present investigation that the siddha formulations like Seenakaraparpam may be considered as a potential lead for the clinical management of urolithiasis.

Acknowledgements

I wish to acknowledge and thank the TheTamilnaduDr.M.G.R Medical university, Chennai, The National Institute of Siddha, Chennai, KM College of Pharmacy Madurai for their support for this research work.

References

1. Ravindra VK, Navneet BG, Alagawadi KR, Rudraprabhu VS. Effect of Moringaoleifera Lam. root - wood on ethylene glycol induced urolithiasis in rats. J Ethnopharmacol 2006;105:306-4.
2. Vyas BA, Vyas RB, Joshi SV, Santani DD. Antiurolithiatic activity of whole-plant hydroalcoholic extract of Pergulariadaemia in rats. J Young Pharm 2011;3:36-40.
3. Tania AV, Cristina DD, Ana PS, Maria TR, Antonio JL, Caden S. Evaluation of the antiurolithiatic activity of the extract of Costusspiralis Roscoe in rats. J Ethnopharmacol 1999;66:193-8.
4. Coe F and Parks J. Pathophysiology of kidney stones and stratergies for treatment. HospPract., 23: 145-168.
5. Sunitha J, Asha S and Taju G. 2012. Protective effect of spirulina on ethylene glycol induced urolithiasis in rats. Inter Res J Pharmacy 1983, 3: 444-448.
6. Kumar V, Abbas AK, Fausto N, Robbins SLandCotran RS. Robbins and Cotran pathologic basis of disease.2015: 1012.
7. Atmani F, Slimani Y, Mimouni M, Hacht B. Prophylaxis of calcium oxalate stones by Herniariahirsuta on experimentally induced nephrolithiasis in rats. British Journal of Urology International 2003,92: 137–140.
8. Robertson WG., Renal stones in the tropics. SeminNephrol 2003, 23:77- 87.
9. Chell ARM. Urolithiasis historical, comparative and pathophysiological aspects: A review. Journal of the Royal Society of Medicine 1989, 82: 669-671.
10. MartinoMarangella, Corrado Vitale, Michele Petrarulo, Michele Bruno. Renal stones: from metabolic to physiochemical abnormalities. How Useful are inhibitors?.Journal of Nephrology 2000, 13: 51- 60.
11. Ross Morton A, Eduard A, Iliescu, James, Wilson WL. Nephrology: Investigation and treatment of recurrent kidney stones. CMAJ 2002; 2:166.
12. King JS. Etiology factors involved in urolithiasis.A review of recent Research.The Journal of Urology

December 2016;3(12) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.197062 Impact Factor: 3.052

1967; 97:587- 591.
13. Vermeulen CW. Experiments on causation of urinary calculi. In, Essays in Experimental Biology. University of Chicago Press, Chicago 1962: 253-269.
14. Prasad KVSRG, Bharathi K, Srinivasan KK. Evaluation of Musa Parasidica Linn Cultivar "Puttubale" stems juice for antilithiatic activity in albino rats. Indian Journal Physiology and Pharmacology 1993, 37:337-341.
15. Huang HS, Ma MC, Chen J, Chen CF. Changes in the oxidant- antioxidant balance in the kidney of rats with nephrolithiasis induced by ethylene glycol. Journal of Urology 2002, 167:2584 -2593.
16. Adhirai M, Selvam R. Vitamin E pretreatment prevents cyclosporine A-induced crystal deposition in hyperoxaluric rats. Nephron 1997, 75:77-81.
17. Muthu KA, Selvam R. Effect of depletion of reduced glutathione and its supplementation by glutathione monoester on renal oxalate retention in hyperoxaluria. Journal of Nutrition and Biochemistry 1997, 8:445-450.
18. Coef L, Favus MJ, Pak CYC, Parks JH. Solution Chemistry of Supersaturation, Kidney Stones: In, Medical and Surgical Management, (Tisselius, H.G., ed.) Preminger G.M. Lippincott Reven, Philadelphia, 1996:33.
19. Lemann JJ, Worcestor EM, Gray RW.Hypercalciuria and stones. American Journal of Kidney Diseases 1991, 26:105-110.
20. Roger K, Low MD, Stoller ML . Uric acid nephrolithiasis. Urologic Clinics of North America 1997, 24:135-148.
21. Ryall RL, Harnet RM, Marshall VR. The effect of urine pyrophosphate, citrate, magnesium and glycosaminoglycans on the growth and aggregation of calcium oxalate crystals invitro. Clin. Chem. Acta 1991, 112:349-356.
22. Grases F, Genestar C, Conte A, March P, Costa BA. Inhibitory effect of pyrophosphate, citrate, magnesium and chondriotin sulfate in calcium oxalate urolithiasis. British Journal of Urology 1989, 64:235-237.
23. Khan SR. Animal models of kidney stone formation: An analysis. World Journal of Urology 1989, 64:236-243.
24. Groyer PK, Resnick M. Evidence for the presence of abnormal proteins in the urine of recurrent stone formers. Journal of Urology 1995, 153:1716-1721.
25. Finch AM, Kasidass GP, Rose GA. Urine composition in normal subjects after oral ingestion of oxalate rich foods. Clinical Science, 1981, 60:411-418.
26. Ghodkar PB. Chemical Tests in Kidney Disease. In, Text book of Medical Laboratory Technology, 1st ed, Bhalani Publishing House, Mumbai, 1994: 118-132.
27. Sumathi R, Jayanthi S, Kalpana DV, Varalakshmi P. Effect of DL–alpha- Lipoic acid on tissue lipid peroxidation and antioxidant systems in normal and glycolate treated rats. Pharmacological Research 1993; 27:1-10.
28. Saravanan N, Senthil D, Varalakshmi P. Effect of L-cysteine on lipid peroxidation in experimental urolithiatic rats. Pharmacological Research, 1995; 32:165-169.
29. Ernster L, Nordenbrand K. Oxidation and Phosphorylation. In, Methods in Enzymology, (Ronald, W.E., Maynard, E.P. ed.) Academic Press, New York, 1967; 10:574 – 580.
30. Karadi RV, Palkar MB, Gaviraj EN, Gadge NB, Mannur VS, Alagawadi KR. Antiurolithiatic property of Moringaoleifera root bark. Pharmaceutical Biology 2008; 46:861-865.
31. Varatharrajan S, Veena CK, Varalakshmi P. Antiurolithiatic effect of lupeollinoleate in experimental hyperoxaluria. Journal of Natural Products 2008; 71:1509-1512.
32. Soundararajan P, Mahesh R, RameshT, Hazeena VB. Effect of Aervalanata on calcium oxalate urolithiasis in rats. Indian Journal of Experimental Biology 2006; 44:981-986.
33. Mousa-Al-Reza H, Alireza K, Zahra H, Mohammadreza P. Ethanolic extract of Nigella sativa L seeds on ethylene glycol- induced kidney calculi in rats. Urology Journal 2007; 4:86-90.
34. Arafat OM, Tham SY, Sadikun A, Zhari I, Haughton PJ, Asmawi MZ. Studies on diuretic and hypouricemic effects of orthosiphonstamineus methanol extracts in rats. Journal of Ethnopharmacology 2008; 118:354-360.
35. Grases F, costa-Bauza.Potentiometric study of the nucleation of calcium oxalate in the presence of several

December 2016;3(12) ISSN: ISSN: 2349-5340
DOI: 10.5281/zenodo.197062 Impact Factor: 3.052

additives.Clinical chemistry and enzymology communication 1991; 3:319-328.
36. Grases F, costa-Bauza A, March JG,MasarovaL.Glycosaminoglycans, uric acid and calcium oxalate urolithiasis. Urological research 1991;19:375-380.
37. Volak J, Stodola J, SeveraF.PlantasMedicinales ,Artia Ed., Praque, Czechoslovakia,1983.