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ANTI-UROLITHIASIS EVALUATION OF SIDDHA FORMULATION KARPOORA SILASATHU PARPAM IN WISTAR RATS

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ABSTRACT

Kidney stone disease has become a rising problem and the third prevalent disorder affecting the urinary tract with high recurrence. This disorder involves a complex of events, such as crystal nucleation, supersaturation, growth, aggregation, retention within renal tubules and migration to the renal papillary surfaces. Surgery, lithotripsy, and local calculus disruption using a high-power laser are used to treat calculi. However, these procedures are expensive and recurrence is quite common. Moreover, traditional remedies are known to contain multiple constituents, acting through multiple pathways such as antioxidant, analgesic, diuretic, pH neutralizing, etc. Siddha system of medicines gains paramount importance in treating urolithiasis by its versatile combination of therapeutic agents, one such formulation claimed for treating kidney stone is Karpoora silasathu parpam (KSSP). Still now there is no literature evidencing the efficacy of this formulation. Hence the main aim of the present study is to evaluate the anti-urolithiasis potential of KSSP in ethylene glycol (EG) induced urolithiasis in rats. Result of the study indicates increased urinary crystal with high threshold of urinary calcium and phosphate were observed in rats belongs to ethylene glycol treatment which has shown the severity of onset of urolithiasis induced by EG in the experimental animals. There was significant decrease in the serum BUN, Creatinine and Uric acid level observed in KSSP treatment group (200 & 400 mg/kg) rats which reveals the anti-urolithiasis potential of the formulation. It was concluded from the results of the present investigation that administration of KSSP reduced and prevented the growth of urinary stones. Therefore, KSSP might helpful to prevent the early stages of stone development in patients with urolithiasis.

KEY WORDS: ***Urolithiasis, Siddha, Karpoora silasathu parpam, Ethylene glycol, Urinary stones, Anti-urolithiasis***

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1. Introduction

Urolithiasis or urinary stone, the presence of one or more calculi in the urinary tract, is a serious, debilitating problem worldwide, affecting approximately 12% of the population. It is the third most prevalent disease of the urinary system that affects about 11% population in India [1]. The highest risks of developing urolithiasis have been reported in Asian countries with lifetime recurrence rates of up to 50% [2]. Due to the very high incidences of recurrence and detrimental effects on renal functions, kidney stones have seriously jeopardized the public health and affected the quality of life [3]. Further, it increased the risk of developing chronic kidney disorders and is also associated with different cardiovascular diseases [4].Among several types of renal stones such as oxalate, phosphate, struvite, cysteine, etc., the most common (80%) stones are composed of calcium oxalate (CaOx) [5].

In India, renal stone have become serious concern due to its prevalence in majority of the population every year [6]. Renal stone formation is highly unpredictable with complex etiology. Various endogenous and exogenous factors and multivariate pathogenesis are involved in renal stone formation. Some endogenous factors like improper metabolism of calcium, oxalic acid, phosphorus and uric acid. Nitrogenous waste products like urea also contribute to renal stone formation. People's food habits, dehydration, hot climate, hard water usage are involved in exogenous factors [7]. Supersaturation of urine with components like calcium, oxalate and phosphate which initiate renal stone formation which is followed by nucleation, crystal growth and crystal aggregation process. Many stone inhibitors are available in urine like magnesium, citrate which make the soluble complex with calcium ions and reduces the supersaturation level of CaOx ions, but its inhibition capacity varies person to person [8].

The modern techniques available in the management of urinary calculi depend on the size and location of calculi, the degree of obstruction, kidney function and associated functions [9]. Open surgical procedures for treating urinary stones are currently not used and were replaced by modern techniques, including extracorporeal lithotripsy or ureteroscopy. Despite the availability of these minimally invasive techniques, with dietary modifications the recurrence rate is still expected to be nearly 50% [10]. Therefore, alternative or complementary medicines with minimal side-effects might be useful.

Since the ancient time of human history herbs and mineral have been used to treat various ailments, for the reason that the herbals have been generally considered to be safe and nontoxic as compared to synthetic medicine. In Indian traditional system of medicine, different parts of plants were being used and their pharmacological properties have also proved [11]. Siddha system of medicines gains paramount importance in treating urolithiasis by its versatile combination of therapeutic agents, one such formulation claimed for treating kidney stone is Karpoora silasathu parpam (KSSP). Still now there is no literature evidencing the efficacy of this formulation. Hence the main aim of the present study is to evaluate the anti-urolithiasis potential of KSSP in ethylene glycol (EG) induced urolithiasis in rats.

2. Materials and Methods

2.1. Experimental Animals

Healthy adult wistar albino 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 handling unit. A 12 light / dark cycle were maintained .Room temperature was maintained between 22 ± 2o C and relative humidity 50–65%. They were provided with food (Sai feeds, Bangalore, India) and water ad libitum. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of science and technology, Chennai, Tamil Nadu, India (IAEC no: SU/CLATR/IAEC/XV/164/2020).

2.2. Animal grouping and Methodology [12,13]

The animals were grouped into four groups of 6 animals each. Group I (Control group) -received normal saline, Group II – Urolithiatic control received EG (0.75% w/v, p.o.) in drinking water for 28 days ad libitum (Day1 and Day 28). Group III - Received EG (0.75% w/v, p.o.) in drinking water and treated with 200mg/kg of KSSP for the period of 28 days. Group IV Received EG (0.75% w/v, p.o.) in drinking water and treated with 400mg/kg of KSSP for the period of 28 days.

2.3. Sample Collection [14,15]

At the end of the study, before sacrifice, the animals were fasted for overnight with free access to water. Animals were sacrificed with excess anesthesia. Blood samples were collected from retro orbital sinus puncture and stored in EDTA (ethylenediamine –tetra acetate) test tubes for Hematological analysis and in clot activator coated test tubes for serum biochemical analysis. Kidney sample were harvested and carefully investigated for gross lesions. The organ (kidney) were preserved in 10% formalin for histopathological assessment.

2.4. Urine Sample Analysis

Urine samples (24 h) will be collected on the 28th day by keeping the animals in an individual metabolic cage. The animal had free access to drinking water during urine collection period.

2.5. Parameters

The parameters such as serum magnesium, calcium, Phosphate, uric acid and urine biochemistry such as BUN, pH, uric acid and Creatinine was estimated.

2.6. Histopathological Analysis [16]

Sample obtained from the study were immersed in 10% formalin for 24 h-48h for histopathological examination. After standard processing, the cut tissue was embedded in paraffin (Leica TP1020 tissue processor) and cut into 5 µm thick sections in a rotary microtome (Leica RM2255 - Fully Automated Rotary Microtome). The sections were stained with haematoxylin-eosin (Merck). Histological measurement and photographs were taken with Olympus CX31, Trinocular Biological Microscope (magnification 10x & 40 x).

2.7. Statistical Method

The statistical analysis was carried by one-way analysis of variance ANOVA (GRAPH PAD PRISM 5 computer program). Results are expressed as ±SEM. The data were statistically analyzed by ONE WAY ANOVA followed by Dunnett's multiple comparison test. Probability P values < 0.05 were considered as significant.

3. Results

3.1. Effect of KSSP on Urine output, pH and Kidney weigh of EG Induced Urolithiatic rats

It was observed from the result of the present investigation that there was a significant increase in the volume of urine output as well as pH and on average weight of kidney in rats treated with (EG) (0.75%), whereas treatment with KSSP to group III and IV rats reveals measurable decrease in urine output, pH and on average weight of kidney at both the dose level. Results were tabulated in Table 1.

Table 1: Effect of MAC on Urine output, pH and Kidney weigh of EG Induced Urolithiatic rats

Group	Treatment and Dose	Urine Out put	pH	Average weight of Kidney in gms
I	Normal Saline	8.08 ± 0.39	6.72 ± 0.37	1.38 ± 0.04
II	Ethylene glycol (EG)-0.75%, p.o.	13.65 ± 0.60 *	8.3 ±0.29	2.80 ± 0.10
III	EG + 200 mg/kg of KSSP	11.33 ± 0.49 *	6.63 ± 0.24	2.47 ± 0.16
IV	EG + 400 mg/kg of KSSP	10.17 ± 0.79 *	6.13 ± 0.36	2.13 ± 0.09

Values represent mean ± SEM of 6 experimental animals.
* P< 0.05; ** P< 0.01; *** P < 0.001.

3.2. Effect of KSSP on Serum biochemistry of EG Induced urolithiatic rats

It was observed from the datas of the present investigation that there was a significant increase in blood urea nitrogen (BUN), creatinine and uric acid level of rats treated with (EG) (0.75%). Treatment with KSSP to group III and IV rats reveals significant decrease in urine serum biomarkers such as blood urea nitrogen (BUN), creatinine and uric acid. As shown in Table 2.

Table 2: Effect of KSSP on Serum biochemistry of EG Induced urolithiatic rats

Group	Treatment and Dose	Blood urea nitrogen (BUN) (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
I	Normal Saline	15.33±1.02	0.50±0.06	4.70 ±0.32
II	Ethylene glycol (EG) (0.75% w/v, p.o.)	36.67 ± 1.96*	2.28± 0.16*	9.58± 0.28*
III	EG + 200 mg/kg of KSSP	29.83 ±1.58*	1.22 ±0.20*	7.52± 0.42*
IV	EG + 400 mg/kg of KAAP	24.67 ± 1.38*	0.95 ±0.11*	5.58 ±0.34*

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001.

3.3. Effect of KSSP on Urine Biochemistry of EG Induced Urolithiatic rats

It was observed from the results of the present investigation clearly that there was a significant

increase in urine calcium and phosphate level in rats treated with (EG) (0.75%). Treatment with KSSP to group III and IV rats reveals significant decrease in urine calcium and phosphate level at both the dose level of 200 and 400mg/kg. Similarly marginal decrease in the level of urine magnesium were observed in EG treatment group and the same were restored back to the normal in KSSP treatment groups. As shown in Table 3.

Table 3: Effect of MAC on Urine Biochemistry of EG Induced Urolithiatic rats

Group	Treatment and Dose	Magnesium (mg/24hr)	Calcium (mg/24hr)	Phosphate (mg/24hr)
I	Normal Saline	5.13 ± 0.25	6.18 ± 0.12	40.57 ± 2.5
II	Ethylene glycol (EG) 0.75%, p.o.)	1.37 ± 0.06*	28 ± 1.62*	87.67 ± 1.87*
III	EG + 200 mg/kg of KSSP	3.80 ± 0.18*	21.63 ± 0.65*	58 ± 2.62*
IV	EG + 400 mg/kg of KSSP	4.02 ± 0.34*	17.72 ± 1.14*	48.50 ± 2.41*

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001.

3.4. Effect of KSSP in controlling size of CaOX crystals in urine sample of rats

It was observed from the datas obtained from the study that there was a significant increase size and density of the CaOX crystals in urine sample of rats treated with (EG: 0.75%). Treatment with KSSP reveals significant decrease in size and number of CaOX crystals in urine sample of rats belongs to group III and IV. As shown in Figure 1.

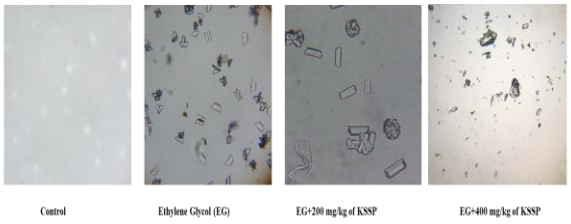

Figure 1 : Size of CaOX crystals in urine sample of rats

3.4. Effect of MAC on Histology of EG Induced urolithiatic rats

Histopathology of kidney sample retrieved from control group reveals normal lumen of vessels and bowman's space. Appearance of proximal and distal convolutes tubules was normal with no evidence of atrophy as observed in sample belongs to group I. Vascular, degenerative with very mild inflammatory changes were been observed along with swollen tubular basement membrane, further increased deposition of crystal deposition in renal tubule were observed. Significantly reduced level of crystal deposition was observed in sample belongs to KSSP 200mg/kg and 400 mg/kg treated rats. Accumulation of calcium oxalate deposits inside the tubules was much controlled in treatment group when compare to EG alone treated group. As shown in Figure 2.

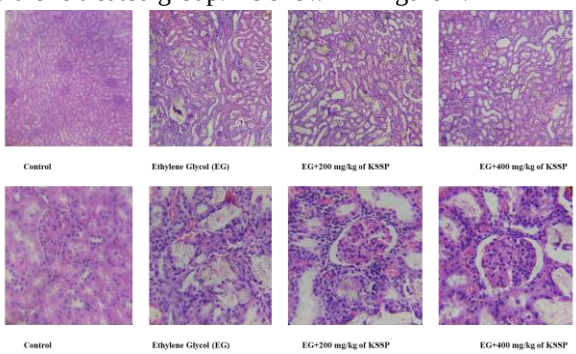

Figure 2: Effect of KSSP on Histopathology of Rat Kidney (H&E) Staining

4.Discussion

Calcium oxalate stone development is a multifactorial process involving various etiological factors. Studies have shown that calcium oxalate crystal deposition leads to the cellular injury mediated by lipid peroxidation through free oxygen radical generation. Studies revealed that these cellular injuries favor the events of calcium oxalate retention in renal tubules which is significant for further stone development [17]. Recent clinical data are also supporting this finding that formation of urinary stones leads to the oxidative stress in patients [18].

Oral administration of 0.75% ethylene glycol (EG) over a period in rats developed kidney stones (or urolithiasis) mainly composed of calcium oxalate (CaOx). The pathophysiological mechanisms responsible for the alterations elicited in this model could be related to an increase in the urinary oxalate (Ox) concentration. Indeed, EG is easily absorbed along the intestine and metabolized in the liver to Ox, leading to hyperoxaluria [19,20].

An increase in urinary phosphorus excretion was observed in ethylene glycol induced urolithic rats. Increased excretion of phosphorus has been reported in stone formers [21]. Increased urinary phosphorus excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition [22].It was

observed from the results of the present investigation clearly that there was a significant increase in urine calcium and phosphate level in rats treated with (EG) (0.75%). Treatment with KSSP to group III and IV rats reveals significant decrease in urine calcium and phosphate level at both the dose level of 200 and 400mg/kg. Similarly marginal decrease in the level of urine magnesium were observed in EG treatment group and the same were restored back to the normal in KSSP treatment groups.

It has been reported that, level of inorganic phosphate and uric acid has been increased in stone formers and toxin agents induced by urolithiatic rats. Increased urinary phosphate level along with oxalate provides an appropriate environment for formation of calcium phosphate crystals, which further induces calcium oxalate deposition in renal [23]. Uric acid which interferes with calcium oxalate solubility, induces the nucleation of calcium oxalate and reduces the inhibitory activity of glycosaminoglycans [24]. It was observed from the datas of the present investigation that there was a significant increase in blood urea nitrogen (BUN), creatinine and uric acid level of rats treated with (EG) (0.75%). Treatment with KSSP to group III and IV rats reveals significant decrease in urine serum biomarkers such as blood urea nitrogen (BUN), creatinine and uric acid.

Hyperoxaluria and hypercalciuria are considered as the major risk factor in pathogenesis of kidney stone formation. Continuing hypercalciuria promotes the nucleation and subsequent precipitation of CaOx crystals from the urine. Some studies have suggested that disorders of renal tubular calcium reabsorption are the major cause of hypercalciuria. However, hyperoxaluria is more important risk factor in the pathophysiology of urolithiasis than hypercalciuria [25]. Because hyperoxaluria is associated with the production of free radicals and related oxidative damage of renal epithelium which provide a nidus for crystal attachment and ultimately causes crystal aggregation and retention in renal tubules [26]. It was observed from the datas obtained from the study that there was a significant increase size and density of the CaOX crystals in urine sample of rats treated with (EG: 0.75%). Treatment with KSSP reveals significant decrease in size and number of CaOX crystals in urine sample of rats belongs to group III and IV.

Hyperoxaluria is one of the major risk factor in the pathogenesis of kidney stone formation [27], as it cause oxidative stress and damages the renal epithelial cells thereby providing a nidus for crystals attachment and ultimately cause crystal aggregation retention and deposition in the kidney [28]. Therefore, decrease in oxalate may explain its decrease in oxidative stress and renal crystal deposition. Histopathology of kidney sample retrieved from control group reveals normal lumen of vessels and bowman's space. Appearance of proximal and distal convolutes tubules was normal with no evidence of atrophy as observed in sample belongs to group I. Vascular, degenerative with very mild inflammatory changes were been observed along with swollen tubular basement membrane, further increased deposition of crystal deposition in renal tubule were observed. Significantly reduced level of crystal deposition was observed in sample belongs to KSSP 200mg/kg and 400 mg/kg treated rats. Accumulation of calcium oxalate deposits inside the tubules was much controlled in treatment group when compare to EG alone treated group.

5. Conclusion

Kidney stone disease is a multi-factorial disorder resulting from the combined influence of epidemiological, biochemical and genetic risk factors. Interference with crystal growth and aggregation therefore seems a possible therapeutic strategy for the prevention of recurrent stone disease. It was concluded from the results of the present investigation that administration of Karpoora silasathu parpam reduced and prevented the growth of urinary stones. Therefore, Karpoora silasathu parpam might helpful to prevent the early stages of stone development in patients with urolithiasis.

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6. References

1. Leye A, Jaeger P, Robertson W, Unwin R. Renal stone disease. Medicine. 2007;35:415–9.
2. Lopez M, Hoppe B. History, epidemiology and regional diversities of urolithiasis. Pediatr Nephrol. 2010;25:49–59.

3. Gao S, Chen W, Peng Z, Li N, Su L, Lv D, et al. Urinary metabonomics elucidate the therapeutic mechanism of Orthosiphon stamineus in mouse crystal-induced kidney injury. J Ethnopharmacol. 2015;166:323–32.
4. Ando R, Nagaya T, Suzuki S, Takahashi H, Kawai M, Okada A, et al. Kidney stone formation is positively associated with conventional risk factors for coronary heart disease in Japanese men. J Urol. 2013;189:1340–6.
5. Ghelani H, Chapala M, Jadav P. Diuretic and antiurolithiatic activities of an ethanolic extract of Acorus calamus L. rhizome in experimental animal models. J Tradit Complement Med. 2016;6:431–6.
6. Sohgaura A., Bigoniya P. A review on epidemiology and etiology of renal stone. Am. J. Drug Discov. Dev. 2017;7:54–62.
7. Smyslova O.A., Markaryan A.A., Evdokimova O.V., Glazkova I.U., Yaroshenko M.A. Characteristics of the new comprehensive herbal medicine for the treatment and prevention of urolithiasis. Biol. Med. 2015;7:4.
8. Rathod N.R., Biswas D., Chitme H.R., Ratna S., Muchandi I.S., Chandra R. Anti-urolithiatic effects of Punica granatum in male rats. J. Ethnopharmacol. 2012;140:234–238.
9. Aboumarzouk O.M., Kata S.G., Keeley F.X., McClinton S., Nabi G. Extracorporeal shock wave lithotripsy (ESWL) versus ureteroscopic management for ureteric calculi. Cochrane Database of Syst Rev. 2012;5:1–46.
10. Knoll T. Stone disease. Eur Urol Suppl. 2007;6:717–722.
11. Amer N. The histological changes on liver and spleen of mice treated with alcoholic and aquatic extract of Cupressus sempervirens. Al-Anbar Medical Journal. 2012;10(2):51–57.
12. Selvam R, Kalaiselvi P, Govindaraj A, Murugan VB, Kumar AS. Effect of A. lanatai leaf extract and Vediuppu Chunnam on the urinary risk factors of calcium oxalate urolithiasis during experimental Hyperoxaluria. Pharmacol Res. 2001;43:89–93.
13. Shukkur MF, Abdul SE, Devarajan A, Ramasamy S, Sethumadhvan S, Nachiappa GR. Credential of Spiruina diet on stability and flux related properties on the biomineralization process during oxalate mediated renal calcification in rats. Clin Nutr 2005;24:932-42.
14. Parasuraman S, Raveendran R, Kesavan R. Blood sample collection in small laboratory animals. J Pharmacol Pharmacother. 2010;1:87–93.
15. Verley H. Practical Clinical Biochemistry. New Delhi: CBS Publishers; 2003.
16. Suvarna, S.K., C.Layton and J.D. Bancroft. Bancroft's theory and practice of histological techniques. 7th edn, Churchill Livingstone, London.2013.
17. Dal Moro F, Mancini M, Tavolini IM, de Marco V, Bassi P. Cellular and molecular gateways to urolithiasis: a new insight. Urologia Internationalis. 2005;74(3):193–197.
18. Huang HS, Ma MC, Chen CF, Chen J. Lipid peroxidation and its correlations with urinary levels of oxalate, citric acid, and osteopontin in patients with renal calcium oxalate stones. Urology. 2003;62(6):1123–1128.
19. Ravindra D.H., Sunil S.J. Effect of hydro-alcoholic extract of Vernonia cinerea Less against ethylene glycol-induced urolithiasis in rats. Indian J. Pharmacol. 2016;48:434–440.
20. Scheid C.R., Cao L.C., Honeyman T., Jonassen J.A. How elevated oxalate can promote kidney stone disease: Changes at the surface and in the cytosol of renal cells that promote crystal adherence and growth. Front. Biosci. 2004;9:797–808.
21. Soundararajan P., Mahesh R., Ramesh T., Begum V.H. Effect of Aerva lanata on calcium oxalate urolithiasis in rats. Indian J. Exp. Biol. 2006;44:981–986.
22. Karadi R.V., Gadge N.B., Alagawadi K.R., Savadi R.V. Effects of Moringa oleifera Lam. root-wood on ethylene glycol induced urolithiasis in rats. J. Ethnopharmacol. 2006;105:306–311.
23. Karadi R.V., Gadge N.B., Alagawadi K.R., Savadi R.V. Effect of Moringa oleifera Lam. root-wood on ethylene glycol induced urolithiasis in rats. J. Ethnopharmacol. 2006;105:306–311.
24. Selvam R., Kalaiselvi P., Govindaraj A., Bala Murugan V., Sathish Kumar A.S. Effect of A. lanata leaf extract and vediuppu chunnam on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria. Pharmacol. Res. 2001;43:89–93.

25. Li X, Liang Q, Sun Y, Diao L, Qin Z, Wang W, et al. Potential mechanisms responsible for the antinephrolithic effects of an aqueous extract of Fructus aurantii. Evid Based Complement Alternat Med. 2015;2015:491409.
26. Hirose M, Yasui T, Okada A, Hamamoto S, Shimizu H, Itoh Y, et al. Renal tubular epithelial cell injury and oxidative stress induce calcium oxalate crystal formation in mouse kidney. Int J Urol. 2010;17:83–92.
27. Tisselius HG. Solution chemistry of supersaturation. In: Coe FL, Favus MJ, Pak CYC, Parks JH, Preminger GM, editors. Kidney stones: Medical and surgical management. Lippincott-Raven; Philadelphia, PA: 1996. pp. 33–64
28. Aihara K, Byer KJ, Khan SR. Calcium phosphate-induced renal epithelial injury and stone formation: involvement of reactive oxygen species. Kidney Int. 2003;64:1283–1291.