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IN-VITRO EVALUATION OF ANTI-UROLITHIASIS POTENTIAL OF KARUVANGA CHENDOORUM BY STRUVITE CRYSTAL GROWTH INHIBITION ASSAY

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ABSTRACT

Urolithiasis is a kind of metabolic disorder were individual's biological system is unable to maintain the calcium hemostasis which was characterized by the formation of stone in the kidney, bladder or urethra. Still, now there is no proper treatment available for clinical management of this condition and there are no proper drugs available in modern medicine that can correct the altered physiology of hemostasis. Siddha system of medicine comprises of natural remedy with therapeutic effects that can dissipate and remove even the larger stones would eliminate the need for a surgery and the risks associated with it. Karuvanga chendoorum (KC) is one such novel Siddha formulation presently used for the treatment of Pramiyam(syphilis), Perumpadu (leucorrhea) and Neerizhivu (diabetic). The main aim of the present investigation is to carry out the anti-urolithiasis activity of the formulation KC by struvite crystal growth inhibition assay at two

dose level of 0.5% and 1%. The efficacy of the KC was evaluated by comparing the crystal size of treated medium with that of the control. The average size of the crystal was higher in the control medium with the length of 1.44 ± 0.08 cm and the size of the crystal was significantly decreased in medium contains 0.5% and 1% of test drug KC with the average length of 0.98 ± 0.13 and 0.58 ± 0.17 cm. From the result of the study, it was concluded that the test drug KC has Promising anti-urolithiasis property in the tested medium.

KEYWORDS: Urolithiasis, Siddha system, Karuvanga chendoorum, Struvite crystal, Kidney, Calcium hemostasis.

INTRODUCTION

Urolithiasis is a clinical condition characterized by the presence of calculi in the kidney and other part of the urinary tract, including the ureters and bladder. Nearly about 80% of these calculi are chemically composed of calcium oxalate and phosphate.[1] The relapse rate of urolithiasis without any precaution or preventive treatment is approximately 10% per year.[2] Epidemiological studies revealed that the urolithiasis is more prone to men (12%) than women (6%) and is more predominant with increasing ages between 20 and 40 in both men and women.[3] Urolithiasis is a multifaceted process which includes crystal nucleation, aggregation, and growth of insoluble particles.[4] It is assumed that when the urine becomes saturated with insoluble materials as a result of the excessive rate of excretions which leads to the formation of crystals and aggregates to form a stone[5], urolithiasis needs both preventive and curative therapy because of having a higher rate of reoccurrences of kidney stone.

Kidney stone formation is a complex process and it results in a cascade of events, including crystal nucleation, growth, and aggregation, crystal retention within the renal tubules.[6] Usually, kidney stones are yellow or brown color with a smooth or gagged structure. Some common type of kidney stones is 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.[7] Urolithiasis, also called calculi, 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.[8]

The 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.[9] 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.[10,11]

Kalladaippu noi, also called as Achmari is characterized by sudden retardation in the urine output. The onset of pain in the tip of the genitalia, burning sensation over the urethral orifice occurs while urination. Further, the symptoms also emerge by radiating pain on either side of the vertebral column.

Karuvanga chendoorum is a novel Siddha formulation made upon a unique combination of lead (Karuvangam), Rasam (Mercury) and Aavarai panchangam (Whole plant of *Cassia auriculata*) hopefully in Siddha system of medicine these preparations belongs to the category of the herbal mineral formulation. KC is used for the treatment of various ailment since years together, currently, it has been used by the Siddha physicians for the treatment of Pramiyam (syphilis), Perumpadu (leucorrhea), Moothira kiricharangal (Urethral meatal stenosis), Kalladippu (renal calculi) and Neerizhivu (diabetic).[12]

Siddha systems of medicine have several formulations towards the clinical management of urolithiasis which ensures the safety and efficacious treatment, that are time tested and used so far. Hence, the main aim of the present investigation is to evaluate the anti-urolithiasis activity of the formulation KC by struvite crystal growth inhibition assay.

MATERIALS AND METHODS

Preparation of Karuvanga chendoorum

Ingredients

- Lead(Karuvangam)
- Rasam(Mercury)
- Aavarai panchangam (Whole plant of Cassia auriculata)

The above mineral and herbal ingredients were identified and authenticated by geochemist and botanist.

Purification

140 gm Lead (Karuvangam) was placed in an iron vessel; it was heated up to melt. Followed by this melted Karuvangam was poured 7 times in the following medicinal liquids such as Lemon juice (Elumechai charu), Gingelly oil (Nalenai), Cow's urine (Komiyam), Horse gram decoction (Kollu kudineer) and Vinegar (Kaadi), then purified karuvangam was washed and dried. 40 gm mercury is triturated with brick powder and turmeric powder for one hour respectively and washed with water. Then the mercury is boiled with the juice of Acalypha indica until it is detoxified.[13]

Formulation

The purified 125 gm of Karuvangam was kept in the iron vessel the ingredient was heated up to melting in nature, at the time 35 gm of purified mercury was added to make a metallic compound and then Aavarai panchangam added and fried slowly for 12 hours. The above medicinal substances were fried another 12 hours up to light yellow color powder (Murungai poo niram) by the process of frying. Finally, the above chendoorum was sieved in the cloth and made in fine Karuvanga chendoorum (KC). The chendoorum was kept in air tight container.

Adjuvant: Honey, Ghee.

Dose: Panavetai (488 mg).

Test Drug concentration

Test drug was prepared at two different concentrations of 0.5 and 1% dispersed in 1.0 M magnesium acetate solution.

METHODOLOGY

An aqueous solution of 0.5M Ammonium dihydrogen phosphate was admixed with the sodium metasilicate solution of specific gravity 1.05 in the appropriate amount using magnetic stirrer so that the pH value 7.0.pH of the reaction was ensured by using pH probe meter. The gel solution of 10 mL was transferred into the test tubes of 140 mm length and 25 mm diameter. After the gelation took place, 5 mL of supernatant solutions of 0.5 and 1% concentration of test drug in 1.0 M magnesium acetate were gently poured on the set gels in test tubes to enumerate the growth inhibition of Struvite crystals. About 5 ml of 1.0 M magnesium acetate without test drug were added as supernatant to control tubes which serve as crystal control group. All the procedures were done in the aseptic medium in laminar flow

hood to avoid microbial contaminations. All test tubes and other glassware were autoclaved at 120°C for 15 min. After pouring supernatant solution, the test tubes were capped with airtight stopples. The experiment was conducted at the room temperature. Study on the growth of crystal was carried out for five consecutive days.[14]

RESULT

Effect of KC on Size variation of Struvite crystals in Gel medium

The average size of the crystal was higher in the control medium with the length of 1.44 ± 0.08 and similarly the average size of the crystal was significantly decreased in medium contains 0.5% of test drug KC with the average length of 0.98 ± 0.13 cm. The average size of the crystal was even much reduced in medium contains 1 % of test drug KC with the Avg length of 0.58 ± 0.17 cm. As shown in the figure 1A to 1 C and Table 1.

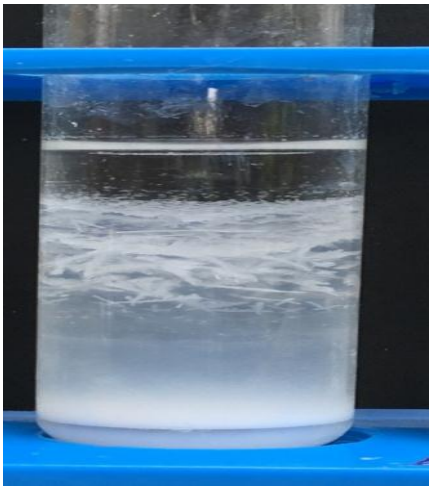

Figure 1 A: Growth of Struvite crystals in control Gel medium.

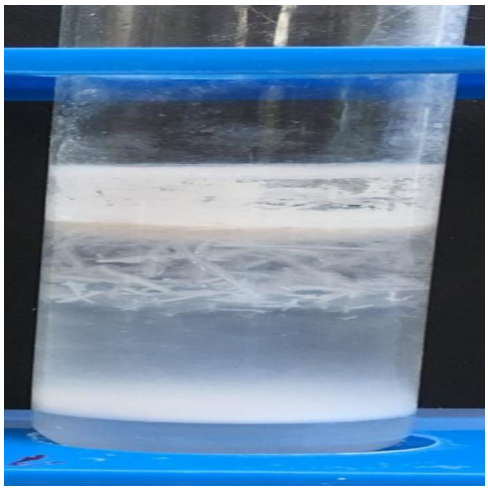

Figure 1 B: Growth of Struvite crystals in Gel medium with 0.5% of *KC*.

Figure 1 C: Growth of Struvite crystals in Gel medium with 1% of *KC.*

Table 1: Report on Average Length of the Crystal in different medium.

S.No	Medium	Average Length of the Crystals
1	Control Gel medium	1.44 ± 0.08
2	Gel medium + 0.5 KC	0.98 ± 0.13
3	Gel medium +1% KC	0.58 ± 0.17

Data are given as Mean ± SD (n=3).

Microscopic observation of crystal belongs to control medium reveals the presence of large aggregate whereas treatment with 0.5% of the test formulation KC reveals significant decrease in the aggregates resulting in projection of individual crystals similarly treatment with 1% of the test formulation KC shown that fragmented crystals reveal the inhibition potential of the trial drug when compared to that of the control medium crystals. As shown in the figure 2A to 2 C.

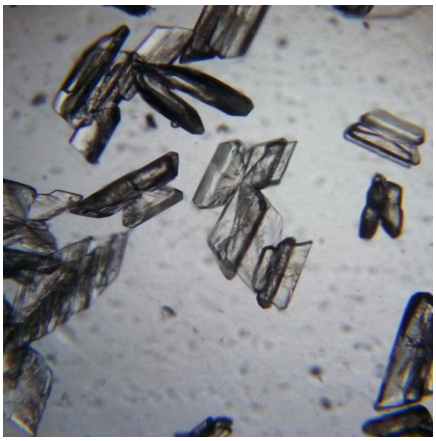

Figure 2 A: Microscopic view of Struvite crystals in Control Gel medium.

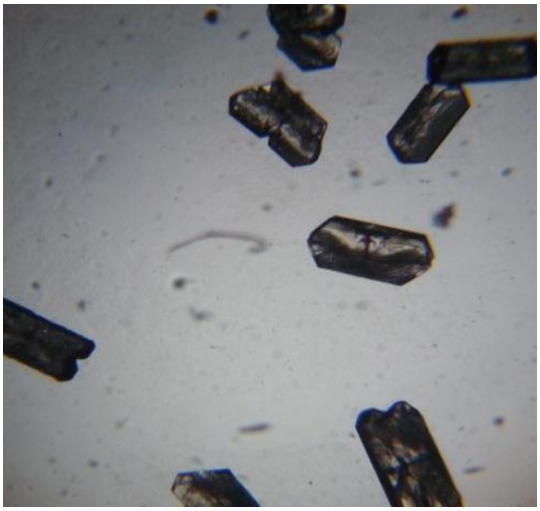

Figure 2 B: Microscopic view of Struvite crystals in Gel medium with 0.5% of *KC.*

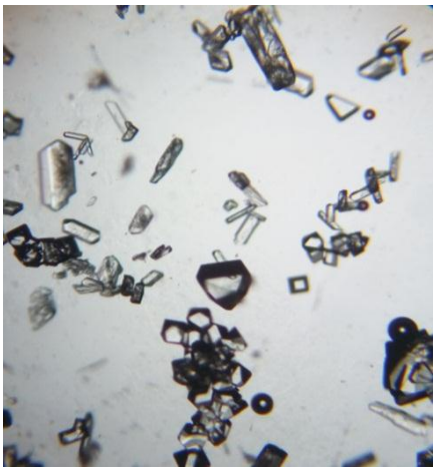

Figure 2 C: Microscopic view of Struvite crystals in Gel medium with 1% of *KC.*

DISCUSSION

In the present scenario, there are no perfect drugs of choice available for the management of urolithiasis, some modern therapies include thiazide diuretics and citrates are been used as a preventive therapy. Sophisticated surgical procedure available for treatment includes extracorporeal shock wave lithotripsy (ESWL), and percutaneous nephrolithotomy (PCNL) are being used for the management of stones. Moreover, these are less convincing and cause side effects such as hemorrhage, hypertension, tubular necrosis, and subsequently fibrosis of the kidney.[15]

Urinary obstruction caused due to crystal deposition greatly reduces the volume of urine excretion. This obstruction causes hypertension and associated issue and presently most of the physicians recommend the usage of diuretics. Greater advantages of using diuretics are it

increases the urine output and reduces hypertension. But the major concern preventing the usage of diuretics is the development of hypokalemia, hyperglycemia, hyponatremia, hyperlipidemia and metabolic imbalance.[16]

Numerous Siddha formulation act as a potent diuretics are no emerged among the public awareness the advantages of using Siddha formulations are cost effective, high potency, prolong the duration of action, multiple mechanisms with no side effects. Now there is a tremendous hike in the usages of Siddha medicines among the public for various ailments due to its endless advantage and its non-toxic nature at long-term usage.[17]

The average size of the crystal was higher in the control medium with the length of 1.44 cm and similarly the average size of the crystal was significantly decreased in medium contains 0.5% of test drug KC with the average length of 0.98 cm. The average size of the crystal was even much reduced in medium contains 1 % of test drug KC with the Avg length of 0.58cm.

In urolithiasis, the stones in the urinary tract obstruct urine outflow resulting in the decreased glomerular filtration rate. This leads to the deposition of waste products in the blood, mainly nitrogenous substances such as urea and creatinine.[18]

The formation of the most calcium containing calculi is more complex and surprisingly not yet understood completely. Current evidence suggests that both free and fixed stone formation is possible. The long accepted simple explanation of the formation of calcium oxalate (CaOx) stones is supersaturation.[19] Deviating from the hypothesis new insights suggest a primary plaque formation in the interstitial space of the renal papilla. This plaque acts as support commonly referred as nidus made of either calcium phosphate crystals or organic matrix derived from damaged membranes. This membrane damage is mainly because of various apoptotic pathways as ROS, oxidative stress, altered pH and co-morbid conditions damaging the kidney.

Siddha system of medicine attains greater importance in recent days as most of the novel Siddha preparation considered to be a valuable lead for the treatment of various infectious, non-infectious and other metabolic disorders in mankind. Gaining popularity mandate the drug to be standardized to compete in the global market.[20]

Microscopic observation of crystal belongs to control medium reveals the presence of large aggregate whereas treatment with 0.5% of the test formulation KC reveals significant

decrease in the aggregates resulting in projection of individual crystals similarly treatment with 1% of the test formulation KC shown that fragmented crystals reveal the inhibition potential of the trial drug when compared to that of the control medium crystals.

CONCLUSION

From the results of the present investigation, it was clear that the formulation KC reveals promising anti-urolithiasis property in the tested medium. Further, the molecular mechanism underlying the crystal growth inhibition has to be properly documented through standard in-vivo and molecular biology techniques.

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