Research Article

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AN *IN VITRO* EVALUATION OF ANTI-UROLITHIASIS POTENTIAL OF SIDDHA FORMULATION SAGALANOI CHOORANAM USING STRUVITE CRYSTAL GROWTH INHIBITION ASSAY

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ABSTRACT

Renal calculus is a rising concern, with a prevalence of 12% across the world and 12% in India, which is around 2 million people affected by Urolithiasis. The rise in the ratio has been correlated to genetic predisposition, working in high temperatures, high caffeine intake, lack of physical activity, and groundwater consumption. Yet the major risk factor is a diet that consists of excess animal proteins, which reduces urinary PH and citrate excretion, leading to the formation of Renal calculi. The main aim of this article is to evaluate the anti-urolithiatic potential of a polyherbal Siddha formulation Sagalanoi chooranam obtained from the classical Siddha text Brahmamuni Karukidai Soothiram 380. From the results, it was concluded that the drug has a high potential as an alternative for the management of renal calculus in Siddha. The ingredients efficiently manage renal calculus due to their potent anti-urolithiasis activity, diuretic and anti-inflammatory activity as cited in modern research articles and by mitigating azhal and Vata humour as mentioned in classical Siddha texts. The effect of Sagalanoi chooranam on struvite crystal growth inhibition assay through a significant reduction in the crystal size further validates the drug's efficiency in managing Renal calculus. In such a scenario, the Siddha drug Sagalanoi chooranam can be used as an individual drug or alongside Biomedicine in the management of Renal calculi.

Keywords: Kalladaippu, Sagalanoi chooranam, Siddha, Renal calculus

INTRODUCTION

Renal calculus or Urolithiasis is one of the most common forms of renal disease characterized by pain from the loin to the groin, dysuria, oliguria, yellow-colored urination, hematuria, nausea, and vomiting. It occurs more frequently in men than in women. It is the most painful and prevalent urological disorder of the urinary system. Urine contains a variety of waste products that are dissolved in it. If there is excess waste in a limited amount of liquid, crystals can develop. These crystals have the ability to attract other substances and combine to create a solid mass that may continue to grow unless it is expelled from the body through urination. Typically, these substances are expelled from the body through urine. For most individuals, adequate hydration helps to flush out these substances or other components in urine to prevent the formation of stones.[1] The chemicals responsible for stone formation include calcium, oxalate, urate, cystine, xanthine, and phosphate. [2] In Siddha medicine, Renal calculi is compared to Kalladaippu. According to Siddhar Yugi, as mentioned in his 'Yugi Vaithiya Chinthamani,' he deals with Kalladaippu under 'Kalladaippu Roga Nithanam'. He has documented the knowledge of this disease along with Siddhars Theran and Agasthiyar[3]. Polyherbal Siddha formulation Sagalanoi chooranam is analysed for its anti-urolithiasis activity, which helps treat renal calculus. Sagalanoi chooranam contains herbs such as *Anethum graveolens* Linn (Sathakuppai) [4], *Cuminum cyminum* Linn (Seeragam) [5], *Nigella sativa* Linn (Karunjeeragam) [6], *Saccharum officinarum* Linn[7,8], *Coriandrum sativum* Linn[9] (Kothumalli) have potent diuretic action, anti-bacterial and antispasmodic action that is essential in treating Urolithiasis. Although steroids, Ibuprofen, Alpha-blockers, and Calcium channel blockers have been suggested for Urolithiasis in modern medicine, they mainly aim at reducing the clinical symptoms and inflammation while the stone remains unaffected. Even with invasive procedures such as percutaneous nephrolithotomy and procedures such as extracorporeal shock wave lithotripsy and laparoscopic ureterolithotomy, Renal calculi is a major challenge in biomedicine due to its high recurrence rate and high-cost demands. In recent trends, people have been inclined to accept scientific and traditional medicine. Their choices are subjective, personal, and based on their cultural, economic, and social influences.

MATERIALS AND METHODS

Ingredient	Botanical Name	Quantity
Seeragam	*Cuminum cyminum* Linn	35 gm
Athimathuram	*Glycyrrhiza glabra* Linn	35 gm
Mathanakamapoo	*Cycas circinalis* Linn	35 gm
Karunjeeragam	*Nigella sativa* Linn	35 gm
Sanna lavangam	*Syzygium aromaticum* Linn	35 gm
Sathakuppai	*Anethum graveolens* Linn	35 gm
Kothumalli	*Coriandrum sativum* Linn	210 gm
Sugar	*Saccharum officinarum* Linn	210 gm
Seeni karkandu		420 gm

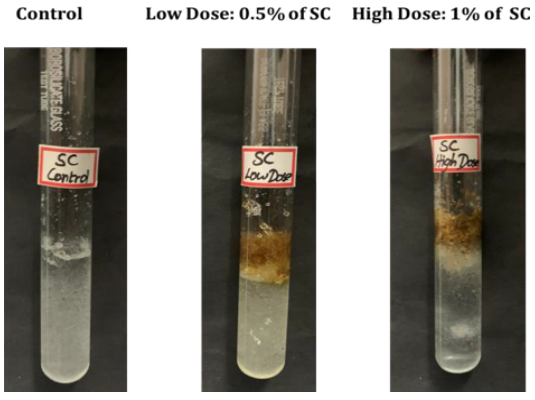
The ingredients were purchased from a local country shop and purified individually according to Brahmamuni Karukidai Soothiram 380 [10] and powdered together and mixed with the required quantity of powdered sugar and were given to analysis at Nobel Research Solutions, No 51 Selva Vinayagar Koil Street, Perambur, Chennai, India.

Test drug concentration

The test drug was prepared in two different concentrations of 0.5% and 1%, then dispersed in a 1.0 M magnesium acetate solution. An aqueous solution containing 0.5M ammonium dihydrogen phosphate was mixed with a sodium metasilicate solution with a specific gravity of 1.05 in the appropriate amount using a magnetic stirrer to achieve a pH value of 7.0. The pH of the reaction was monitored using a pH probe meter. The resulting gel solution of 10 mL was then transferred into test tubes measuring 140 mm in length and 25 mm in diameter. After the gelation occurred, 5 mL of supernatant solutions containing 0.5% and 1% concentration of the test drug in 1.0 M magnesium acetate were gently poured onto the set gels in the test tubes to assess the growth inhibition of Struvite crystals. As a control for crystal growth, 5 ml of 1.0 M magnesium acetate without the test drug was added as supernatant to control tubes. All procedures took place in a sterile environment in a laminar flow hood to prevent microbial contamination. Before use, all test tubes and glassware were sterilized at 120 °C for 15 minutes using an autoclave. Once the supernatant solution was poured, the test tubes were sealed with airtight stoppers. The experiment was conducted at room temperature, and crystal growth was observed over five days.[11,12]

RESULTS AND OBSERVATIONS

Growth Pattern of crystal in control and drug-added medium

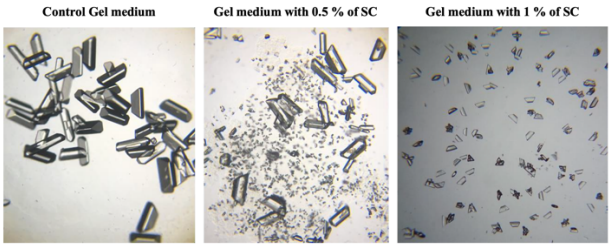


1. Struvite crystals growth in the control Gel medium
2. Struvite crystals growth in Gel medium with 0.5% of Sagalanoi chooranam
3. Struvite crystals growth in Gel medium with 1% of Sagalanoi chooranam

Size variation of Struvite crystals

A - Control Gel medium with size variations in struvite crystals
B- Variations in size of Struvite crystals in Gel medium with 0.5 % of test drug
C- Variation in size of Struvite crystals in Gel medium with 1 % of test drug

Microscopic view of Struvite crystals size after fragmentation



Report on the Average Length of the Crystal in a different medium

Medium	Average Length of the Crystals in cm
Control Gel medium	2.76 ± 0.32
Gel medium + 0.5 % SC	1.4 ± 0.1
Gel medium + 1 % SC	0.72 ± 0.16

DISCUSSION

The control medium showed a higher average crystal size, measuring 2.76 ± 0.32 in length. When the medium contained 0.5% of the test drug Sagalanoi chooranam, the crystal size decreased significantly to an average length of 1.4 ± 0.1 cm. Similarly, in the medium containing 1% of the test drug Sagalanoi chooranam, the size of crystal aggregates was measured at 0.72 ± 0.16 cm.

The ingredients of the Siddha drug Sagalanoi chooranam indicated for Kalladaippu contain drugs that have anti-urolithic and diuretic activity. According to Siddha's sastric texts, the drugs have a diuretic activity that helps dissolve renal calculus.[13] The

ingredients' sweet taste and cold potency aid in mitigating the aggravated Vata and Pitta humour, which is the leading cause of Kalladaippu according to Siddha texts. Further, the ingredients have phytochemicals that can resolve associated symptoms and features of calculus, such as inflammation, bacterial growth, nausea, and vomiting. This research article has validated the anti-urolithiatic activity of the drug. Hence, it can be a perfect treatment choice for renal calculus.

CONCLUSION

The ingredients of Sagalanoi chooranam contain alkaloids that are highly useful in managing Renal calculus. Scientific evidence has validated the diuretic, anti-inflammatory, nephroprotective and anti-bacterial potential of the herbs used in the drug. The effectiveness of Sagalanoi chooranam as a potent lithotriptic and diuretic drug was validated through a significant reduction in the size of the struvite crystals. The study showed substantial variations in the size of the crystals between the gel medium in the control group and the gel medium with 0.5% and 1% of Sagalanoi chooranam. There is a considerable decrease in the struvite crystal size in microscopic view after fragmentations and in the various gel mediums. An enhanced dissolution rate and fragmentation of grown struvite crystals indicate the promising anti-urolithiasis property of Sagalanoi chooranam in the tested medium. Clinical trials can validate the potential of the drug.

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