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Evaluation Of Anti-Urolithiasis Potential of Siddha Formulation Mahaboopathiparpam Using Struvite Crystal Growth Inhibition Assay

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ABSTRACT

Abstract: Urolithiasis is a medical term used to describe the formation of stones, known as calculi, within the urinary tract. It can vary in severity from asymptomatic small stones to large stones causing significant pain, obstruction of urine flow and potential complications such as urinary tract infections or kidney damage. Preventing the recurrence of urolithiasis poses a significant challenge, emphasizing the necessity for innovative therapeutic approaches that can effectively inhibit the formation and initial stages of crystal development.

Objective: The main aim of the present investigation is to explore the anti-urolithiasis potential of siddha formulation Mahaboopathiparpam using diffusion gel growth technique.

Materials and Methods: Silica hydrogel matrix was divided in to three groups which were control and two MBP treated groups. Test drug was screened at two dose levels of 0.5% and 1%. The efficiency of the formulations was screened by comparing the crystal size of the control and treatment medium.

Results: The average size of the crystals in the control medium was found to be 1.4 ± 0.23 cm, whereas the crystal size was significantly decreased in medium contains 0.5% and 1% of MBP with the size of 1.16 ± 0.15 and 0.74 ± 0.21 cm. **Conclusion:** Results clearly indicates that the siddha formulation offers maximum percentage inhibition on the crystal growth in the tested medium, this efficacy may be due to presence of versatile phytocomponents present in the formulations.

Keywords: *Anti-urolithiasis, Siddha formulation, Phytocomponents, Crystals, Mahaboopathiparpam*

1. INTRODUCTION

Nephrolithiasis or urolithiasis or ureterolithiasis, i.e. the formation of urinary calculi, usually known as renal stone or kidney stone is a serious, debilitating problem in all societies throughout the world.(1)The people with kidney stone in the US as 12% of males and 6% of females and in India also has 12% get affected but it is approximately 12% of the world population get renal stone disease, with a reappearance rate of 70–81% of males and 47–60% of females.(2)The primary components found in most urinary calculi are oxalates and phosphates. Within the phosphates category, substances such as struvite (also known as ammonium magnesium phosphate hexahydrate) and newberyite (magnesium hydrogen phosphate trihydrate) have been identified as constituents of urinary stones.Urolithiasis has become a global issue due to its higher prevalence in both developed and developing countries. Nearly 12% of the world populations are at potential risk of some stages of

urolithiasis.(3) Struvite stones are among the most difficult and dangerous problems in stone disease because of the potential of life threatening complications from infection. Worldwide, struvite compose 30% of all kidney stones in humans.(4) An elevated urinary pH reduces the solubility of magnesium ammonium phosphate and favors precipitation of Struvite crystals.(5) Struvite crystallization is related to the urinary tract infections by microorganism producing ureases (6).They are mainly the microorganisms from species of proteus which are isolated in the cases of 70% of the so called infectious stones (7).The symptoms of urolithiasis can vary depending on the size and location of the stones. Small stones may pass through the urinary tract unnoticed, while larger stones can cause severe pain, often described as a sharp, stabbing sensation in the back or side of the abdomen. Other symptoms may include blood in the urine, difficulty urinating, and frequent urination.Traditional medication is an essential source of potentially valuable compounds for the development of potential therapeutic agents.(8) In the traditional Indian system of medicine, many herbal medicines have been recommended for the treatment of urinary stone problem and some of them have been experimentally evaluated (9). Urolithiasis is synonymously called “Kalladaippu Noi” in Siddha texts. Symptoms of kalladaippu include colicky pain radiating from the costal arch obliquely to the lower abdomen, loin to groin and testes, burning micturition, fever, nausea, or vomiting. In the Siddha system, many herbal and herbo-mineral formulations are mentioned in the texts for kalladaippu diseases. Mahaboopathiparpam is taken into study from the Siddha literature, “Sikithcharathna deepam” , (Author: S.Kannusamy Pillai) a traditional siddha preparation consists of:

1.Pavazha parpam (1 part)

2.Vengaraparpam (2 parts)

3.Maankombu parpam (3 parts)

4.Aamaiootu parpam (4 parts)

5.Muthusirpi parpam (5 parts)

6.Karudakal parpam (6 parts)

7.Padikaara parpam (7 parts)

8.Sangu parpam (6 parts)

And is indicated for Neeradaipu, Kalladaipu, Sadhaiaddaipu, Neererichal, Neerkattu, Marbunoi, Shayam, Eelai, Megakangai

2. MATERIALS AND METHODS:

Procurement of test drugs:

Siddha formulationMahaboopathiparpam was procured from Sai Siddha Medicals, Tambaram, Sanatorium, Chennai, Tamil Nadu 600047.

Test drug concentration:

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

Single diffusion gel growth technique:

Struvite crystal growth assay is a novel in-vitro technique for preliminary screening of drugs for evaluating its antilithogenic property. The growth of struvite was simulated in the laboratory condition by allowing the crystals to grow in a controlled silica hydro gel medium. In the gel growth assay, the gel medium reciprocate the three dimensional platform which supports the crystals to attain its maximum growth without exerting major opposing forces. This relative in-vitro condition provides high structural perfection. This relative in-vitro condition provides high structural perfection. As shown in Figure 1.

Crystal growth inhibition assay:

Method adopted as per the procedure described by Chauhan et al.(10)Entire procedure were carried out in aseptic medium using sterilized tubes and glass wares. The gel medium consists of ammonium dihydrogen phosphate and sodium metasilicate of was transferred into the test tubes and allow the gelation to proceed until uniform matrix will be formed. Soon after gelation, 5 mL of supernatant solutions of 0.5 and 1% concentration of Mahaboopathiparpamin 1.0 M magnesium acetate were gently added through side wall of the tubes. Magnesium acetate alone without test drugs serves as crystal control group. Setup was then monitored for around 5 days under room temperature for enumerating the growth pattern of the crystal in control and traded medium.

Data and sample analysis:

The statistical analysis utilized one-way analysis of variance (ANOVA) using the GRAPH PAD PRISM 5 software. Results are presented as mean ± standard deviation (SD). Subsequently, Dunnett’s multiple comparison test was employed for statistical analysis. Significance was determined by probability (P) values < 0.05.

3. RESULTS:

Effect of MBP on size variation of struvite crystals:

From the data obtained from the struvite crystal growth assay it was observed that the average size of the crystal in control medium was found to be 1.4 ± 0.23 cm which was comparatively higher when compared to that of the MBP treated medium. There was significant decrease in the average size of the crystal, was observed in medium contains 0.5% and 1% of MBP with the average length of 1.16 ± 0.15 and 0.74 ± 0.21 cm. Results were illustrated in the Figure 2 and the data is represented in Table 1.

Microscopic observation on size variation of struvite crystals in control and drug treated medium:

Microscopic observation of crystal belongs to control medium reveals the presence of large aggregate whereas treatment with 0.5% and 1% of the MBP reveals significant decrease in the size resulting in projection of individual crystals. As shown in the Figure3-5.

A: Control **B:0.5% of MBP** **C:1% of MBP**

Figure 1: Growth Pattern of crystal in control and drug added medium

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

S.NO	Medium	Average Length of the Crystals in cm
1	Control Gel medium	1.4 ± 0.23
2	Gel medium + 0.5 % MBP	1.16 ± 0.15
3	Gel medium + 1 % MBP	0.74 ± 0.21

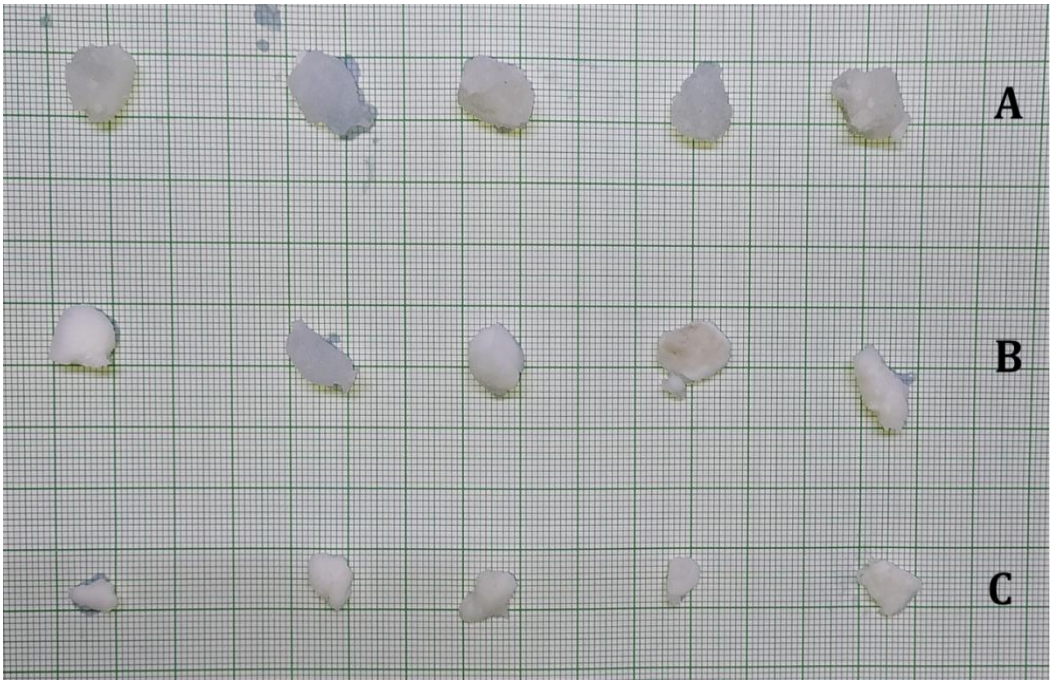

Figure 2: Size variation of Struvite crystals

A - Size variation of Struvite crystals in Control Gel medium

B- Size variation of Struvite crystals in Gel medium with 0.5 % of MBP

C- Size variation of Struvite crystals in Gel medium with 1 % of MBP

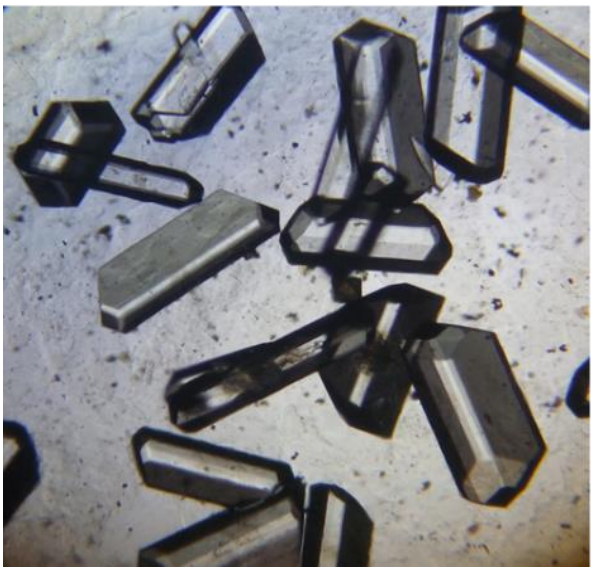

Figure 3: Microscopic observation of crystal in Control Gel medium

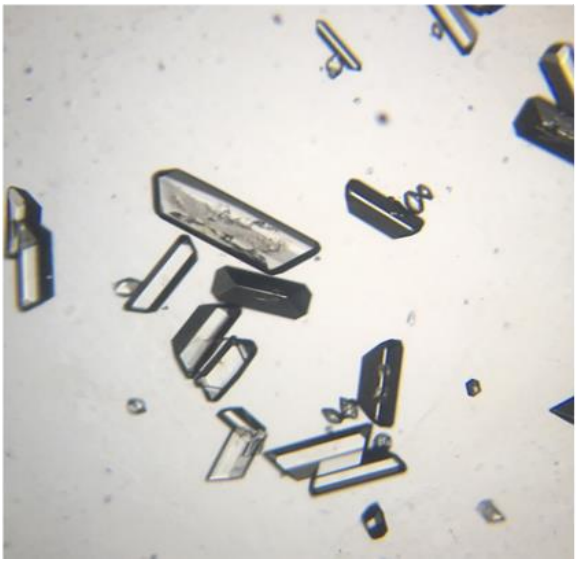

Figure 4: Microscopic observation of crystal in Gel medium with 0.5 % of MBP

Figure 5: Microscopic observation of crystal in Gel medium with 1 % of MBP

4. DISCUSSION:

Studies have highlighted that elevated levels of uric acid, sodium, and calcium play a central role in the formation of crystals. Factors such as pH balance, saturation levels, nucleation, and infections also contribute to the exacerbation of the condition. Inflammation in the kidneys significantly affects their filtration function, leading to increased retention of crystals and the formation of struvite crystals.(11,12) Renal oxidative stress initiates episodes of lipid peroxidation, resulting in damage to functional units of the kidney like glomeruli, tubules, and collecting ducts. Therefore, medications that neutralize free radicals could offer a promising therapeutic approach for managing this medical condition. (13)

In earlier days clinical management of kidney stones requires surgical treatment where as in recent times there are several modern sophisticated methods available for the same which inclusive of percutaneous nephrolithotomy and shockwave lithotripsy. Propensity of reoccurrence is the most common pathetic scenario in both of these invasive procedures which is about 50 %. (14,15)

Changes in diet and lifestyle can lead to a 50% decrease in the recurrence rate. Scientists are continuously working on synthetic sources or herbal remedies to identify potential treatments for urolithiasis.

Siddha formulations primarily work by preventing urine from becoming overly concentrated and assisting in the elimination of surplus salt and uric acid, thereby restoring normal physiological balance. In-vitro tests offer dependable outcomes for screening drugs with potential activity. In the current study, the effectiveness of Siddha formulations was evaluated by

comparing crystal sizes in the control group with those in the group treated with the medication.The average size of the crystals in the control medium was found to be 1.4 ± 0.23 cm, whereas the crystal size was significantly reduced in medium contains 0.5% and 1% of MBP with the size of 1.16 ± 0.15 and 0.74 ± 0.21 cm.

5. CONCLUSION:

From the results it was concluded that the siddha formulation, Mahaboopathiparpam exerted extensive antilithogenic property in the tested medium. The mechanism of action could result from the presence of bioactive phytochemicals, which might interact with ions involved in crystal formation, thus impeding the process of crystal formation and aggregation.This investigation provided an evidence-based data which could be utilized for prompting the usage of Mahaboopathiparpam towards management of urolithiasis.

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CONFLICTS OF INTREST:

The author declares that there is no conflicts of interest.

ABBREVIATIONS:

MBP-Mahaboopathiparpam ; ANOVA- Analysis of variance.

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