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Original Article

Exploration of Anti-Urolithiasis Potential of Traditional Siddha Formulations Amukkara Chooranam and Karisalai Karpam Chooranam by Struvite Crystal Growth Inhibition Assay

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ABSTRACT

Background: Urolithiasis is a medical condition characterized by formation of stone which comprises of calcium oxalate, magnesium ammonium phosphate and uric acid. Reoccurrence becomes the primary hindering factor in providing relief for urolithiasis; hence there is a need of alternate therapeutic strategy that may effectively combat and halt the formation and nucleation of the crystals. **Objective:** The main aim of the present investigation is to explore the anti-urolithiasis potential of the two versatile siddha formulations Amukkara Chooranam (AKC) and Karisalai karpam chooranam (KKC) using diffusion gel growth technique. **Materials and Methods:** Silica hydrogel matrix was divided in to three groups which were control, AKC and KKC treated groups. Test drugs were 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 2.12 ± 0.22 cm, whereas the crystal size was significantly decreased in medium contains 0.5% and 1% of AKC with the size of 1.4 ± 0.15 and 1.14 ± 0.18 cm. Similar type of findings were observed in medium consist of 0.5% and 1 % KKC with the size of 1.52 ± 0.13 and 1.08 ± 0.17 cm. **Conclusion:** Results clearly indicates that both the siddha formulations 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.

Key words: Lithotripsy, Traditional medicines, Polyherbal, Crystals, Amukkara Chooranam, Karisalai karpam chooranam.

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INTRODUCTION

Urinary stone troubles the mankind since 4000 B.C. Development of newer therapeutic lead towards management of stone formation may requires extensive knowledge on understanding the mechanism of stones formation and aggregation. Deposition of stone attracts the immunogenic response resulting in inflammation and declined physiological activity.[1-5] 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. Prevalence rate are more in men rather than in women population. In case of patients with improper prophylaxis the relapsing rate of secondary stone formations is estimated to be 10-23% per year, 50% in 5-10 years and 75% in 20 years of the patient.[6] This alarming demography indicates that the metabolic disorder like urolithiasis requires drugs that can acts by multiple mechanisms in both prevention and reoccurrence as well.[7,8]

As reported by WHO majority of the people around the globe nearly 80% relies on herbal supplement for their essential health care needs.[9] As per the demography published in the year 2013 around 13,000 herbs have been screened for their potential medicinal applications. Siddha system of medicine majorly relies on ancient traditional preparations for treating several infectious and non-communicable diseases. As per the vedic literature it has been provoked that this method of treatment has emerged from southern region of India and progressed though out the world. Contribution of herbs towards siddha formulation is considerably innumerable as its playing a very vital role in healing, rejuvenation and mode of action of the drugs.

Karisalai Karpam is a traditional siddha preparation consists of seven herbs which includes *Eclipta prostrata, Wedelia calendulacea, Indigofera tinctoria, Sphaeranthus indicus, Centella asiatica, Acalypha indica, Coldenia procumbens*. According to folklore claim this formulation has been widely used for treating liver disorders such as jaundice, enlargement of liver and spleen, hepatospleenomegaly, anemia and is also beneficial in skin diseases.[10] *Amukkara Chooranam* is a polyherbal siddha formulation blended with combination of some unique herbs such as *Eugenia cryophyllus*, *Mesua ferrea* auct, *Elettaria cardamomum, Piper nigrum*, *Piper longum* Linn, *Zingiber officinalis* and *Withania somnifera*. This formulation has been extensively used for clinical ailment against gastric disorders, rheumatic pain, leucorrhoea, anaemia, tuberculosis, insomnia and sexual insufficiency.[11] Still now there is no proper

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literature evidence available on proving the anti-urolithiasis potential of these two siddha formulations. Hence this prompted us to pursue the present investigation on evaluating the anti-urolithiasis potential of these traditional formulations using struvite crystal growth inhibition assay.

MATERIALS AND METHODS

Procurement of test drugs

Siddha formulations Amukkara Chooranam and Karisalai Karpam Chooranam were procured from The Indian Medical Practitioners Co-operative Pharmacy and Stores from Thiruvanmiyur, Chennai, Tamil Nadu 600041. Samples was archived at centre for laboratory animal technology and research, Sathyabama institute of science and technology, Chennai 600119, Tamil Nadu, India.

Test drug concentration

Both the formulations Amukkara Chooranam and Karisalai Karpam Chooranam 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. As shown in Figure 1.

Crystal growth inhibition assay

Method adopted as per the procedure described by Chauhan *et al.*[12] Entire procedure were carried out in aseptic medium using sterilized tubes and glass wares. The gel medium consist 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 AKC and KKC in 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 was carried by one way analysis of variance ANOVA (GRAPH PAD PRISM 5 computer program). Results are expressed as ±SD. 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.

RESULTS

Effect of AKC on size variation of struvite crystals

From the datas obtained from the stuvite crystal growth assya it was observed that the average size of the crystal in control medium was found to be 2.12 ± 0.22 cm which was comparatively higher when compared to that of the AKC treated medium. There was significant decrease in the average size of the crystal was observed in medium contains 0.5% and 1% of AKC with the average length of 1.4 ± 0.15 and 1.14 ± 0.18 cm. Results were illustrated in the Figure 2 and data's represented in Table 1.

Effect of KKC on size variation of struvite crystals

It was observed that there was significant decrease in the average size of the crytals belongs to the medium contains 0.5% and 1% of KKC with the size range of 1.52 ± 0.13 and 1.08 ± 0.17 cm respectively. Results were illustrated in the Figure 3 and data's 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 AKC and KKC reveals significant decrease in the size resulting in projection of individual crystals. As shown in the Figures 4-8.

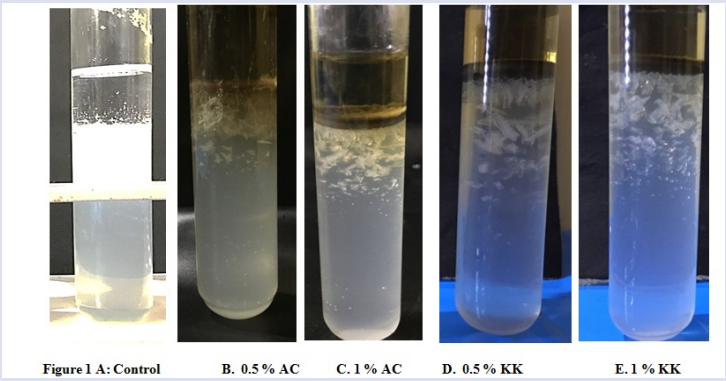

Figure 1: Crystal growth in control and drug treated medium.

Table 1: Effect of AKC and KKC on average length of the crystals.

S.No	Medium	Length of the Crystals in (Cm)
1	Control Gel medium	2.12 ± 0.22
2	Gel medium + 0.5% *AKC*	1.4 ± 0.15
3	Gel medium + 1% *AKC*	1.14 ± 0.18
4	Gel medium + 0.5% *KKC*	1.52 ± 0.13
5	Gel medium + 1% *KKC*	1.08 ± 0.17

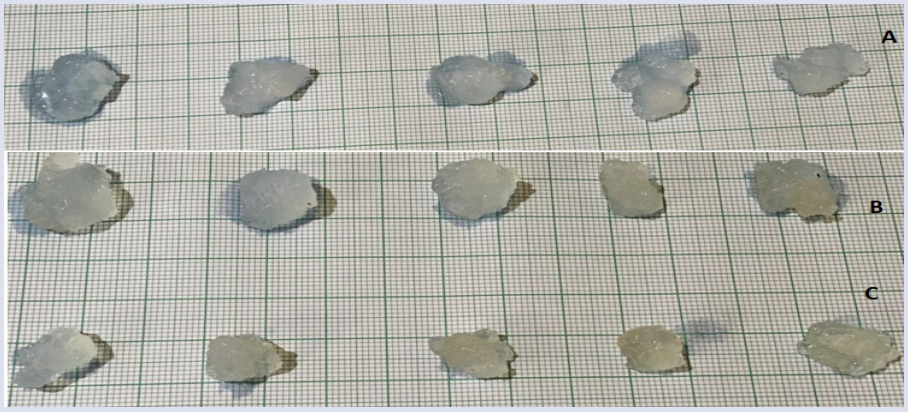

Figure 2: Size variation of struvite crystals in control and AKC treated medium.

A - Size variation of Struvite crystals in Control Gel medium
B- Size variation of Struvite crystals in Gel medium with 0.5% of *AKC*
C- Size variation of Struvite crystals in Gel medium with 1% of *AKC*

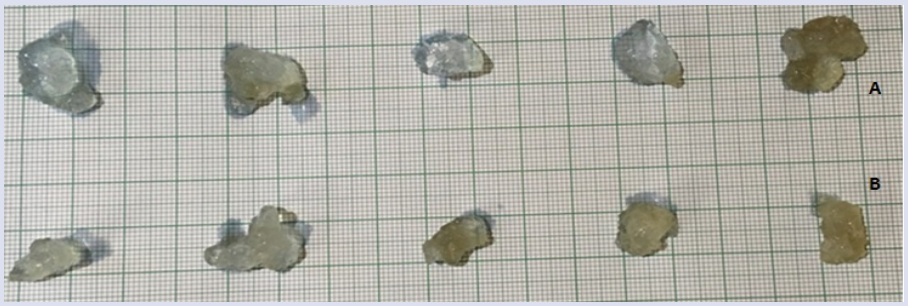

Figure 3: Size variation of struvite crystals KKC treated medium.

A- Size variation of Struvite crystals in Gel medium with 0.5% of *KKC*
B- Size variation of Struvite crystals in Gel medium with 1% of *KKC*

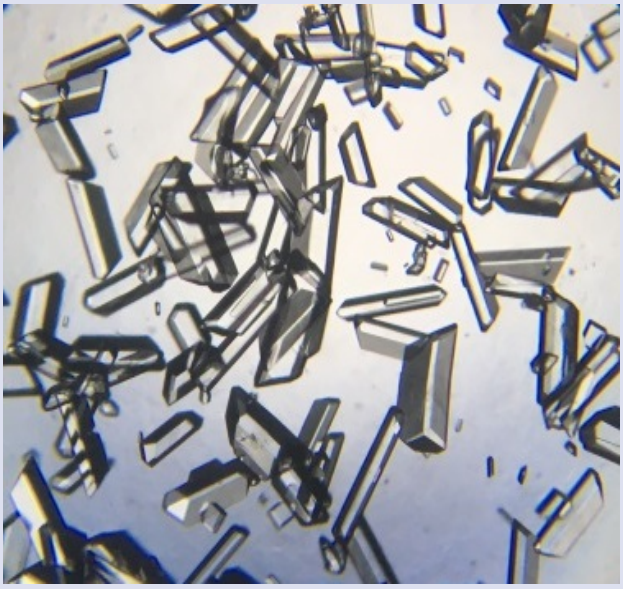

Figure 4: Microscopic observation of crystal in control medium.

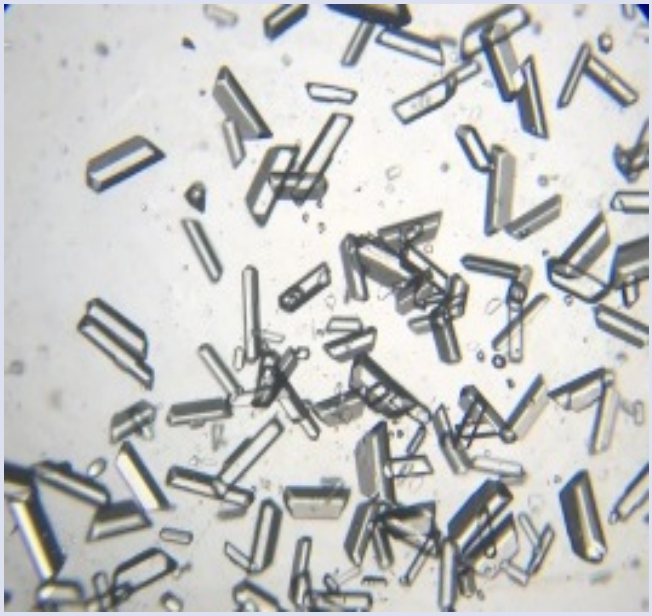

Figure 5: Microscopic observation of crystal in 0.5% AKC treated medium.

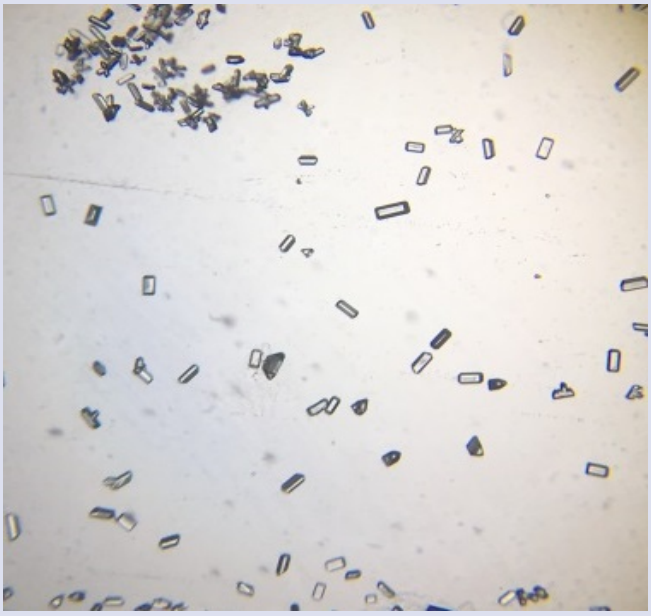

Figure 6: Microscopic observation of crystal in 1% AKC treated medium.

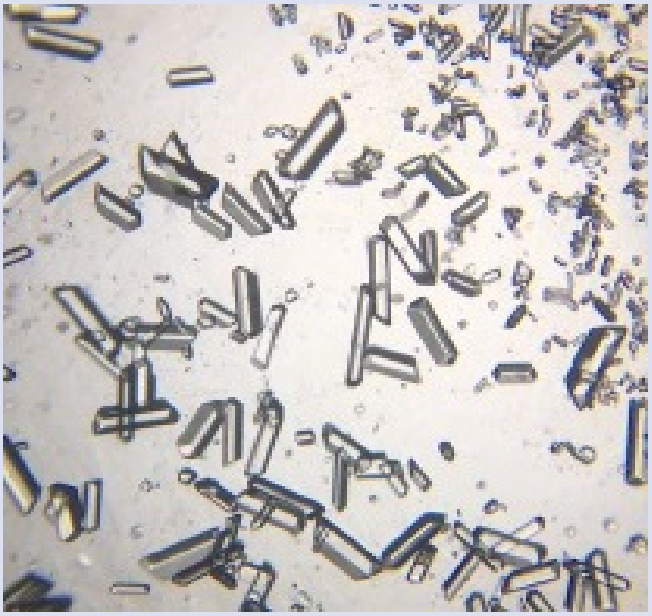

Figure 7: Microscopic observation of crystal in 0.5% KKC treated medium.

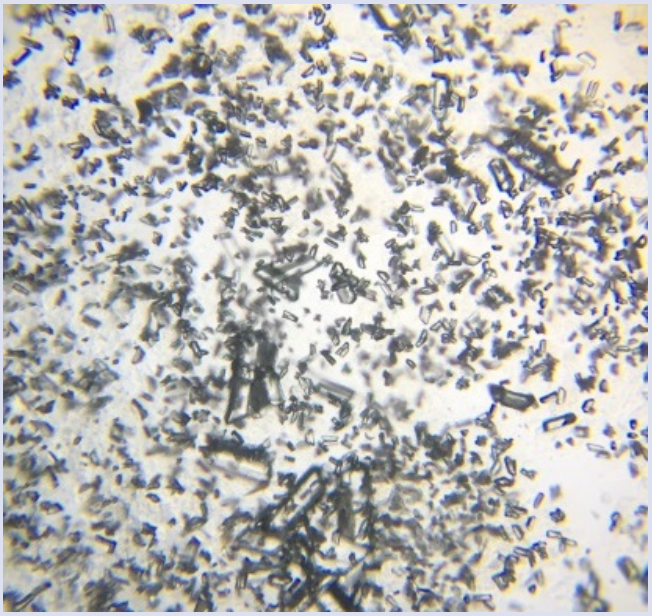

Figure 8: Microscopic observation of crystal in 1% KKC treated medium.

DISCUSSION

It was evident through several research findings that increased uric acid, sodium and calcium level is the core triggering factor in crystal formation. pH, saturation, nucleation, infections are the other contributing factor in worsening the disease condition. Inflammation in kidney has significant impact on filtration physiology which further stimulates crystal retention and formation of struvite crystals.[13,14] Renal oxidative stress triggers the episodes of lipid peroxidation which damages some of the functional unit of the kidney such as glomerulus, tubules and collecting duct hence drug that quenches the free radicals could be a better therapeutic substitute for managing this medical condition.[15]

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 %.[16,17]Allopurinol is an approved drug of choice currently used for reducing the uric acid level.[18] But upon sustained usage this uricosuric agent may offers tremendous side effects like allergy, rashes, itching including altered liver function. Altered dietary regimen and lifestyle modification may bring out 50% reduction in the relapsing rate.[19,20]

Researchers are constantly striving hard towards identification of lead for treating urolithiasis either from the synthetic or from herbal origin. Complex formation with ions that tend to promotes crystal growth is the most expected mechanism of the newer therapeutics to become successful against kidney stone.[21] Siddha formulations mainly act by impeding the super saturation of urine and also aids in excretion of excess salt and uric acid thereby it effectively maintains the normal hemostasis and reverse the physiology. *In-vitro* assays provides reliable results from which the drugs shall be screened for their promising activity. In the present investigation the efficacy of the siddha formulations were screened by comparing the crystal size of the control and drug treated matrix medium. The average size of the crystals in the control medium was found to be 2.12 ± 0.22 cm, whereas the crystal size was significantly reduced in medium contains 0.5% and 1% of AKC with the size of 1.4 ± 0.15 and 1.14 ± 0.18 cm. Similar type of findings were observed in medium consist of 0.5% and 1% KKC with the size of 1.52 ± 0.13 and 1.08 ± 0.17 cm.

CONCLUSION

From the results it was concluded that both the siddha formulations exerted extensive antilithogenic property in the tested medium. The mode of action may be due to the presence of potential bioactive phytocomponents that may tend to interact with crystal forming ions which hinders the formation and aggregation of crystals. This investigation provided an evidence-based data which could be utilized for prompting the usage of *Amukkara Chooranam and Karisalai Karpam Chooranam* towards management of urolithiasis in near future.

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CONFLICTS OF INTEREST

The author declares that there is no conflicts of interest.

ABBREVIATIONS

AKC: Amukkara Chooranam; KKC: Karisalai Karpam Chooranam; BC: Before Christ; WHO: The World Health Organization; ANOVA: Analysis of Variance

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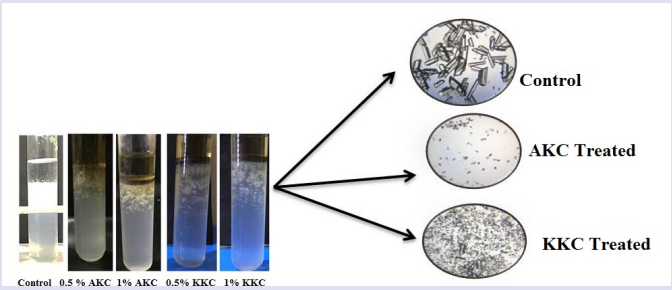
GRAPHICAL ABSTRACT



SUMMARY

Present investigation aimed at exploring the anti-urolithiasis potential of the two versatile siddha formulations Amukkara chooranam (AKC) and Karisalai karpam chooranam (KKC) using diffusion gel growth technique. Silica hydrogel matrix was divided in to three groups which were control, AKC and KKC treated groups. Test drugs were 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. The average size of the crystals in the control medium was found to be 2.12 ± 0.22 cm, whereas the crystal size was significantly decreased in medium contains 0.5% and 1% of AKC with the size of 1.4 ± 0.15 and 1.14 ± 0.18 cm. Similar type of findings were observed in medium consist of 0.5% and 1% KKC with the size of 1.52 ± 0.13 and 1.08 ± 0.17 cm. Results clearly indicates that both the siddha formulations 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.

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