

DETECTION OF ANTI-UROLITHIASIS ACTIVITY IN MALLIKAI CHOORNAM USING STRUVITE CRYSTAL GROWTH INHIBITION ASSAY

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Article Received on
30 March 2018,

Revised on 20 April 2018,
Accepted on 10 May 2018,

DOI: 10.20959/wjpr201810-12371

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ABSTRACT

Urolithiasis is one among the three prevalent disorders in the urinary system. 10 -15% of urinary calculi are formed due to the presence of urease producing bacteria more prevalent in women. The present study was aimed to grow struvite crystals in vitro using single diffusion gel growth technique and to understand the effect of Mallikai Choornam on its growth. Test drug was prepared at two different concentrations of 0.5 and 1% dispersed in 1.0M magnesium acetate solution were gently poured on the set gel in the test tubes to enumerate the growth inhibition of struvite crystals. In the result of the study, it was concluded that the test drug Mallikai Choornam has shown inhibitory

activity on struvite crystal growth.

KEYWORDS: Urolithiasis, struvite, crystal growth, Mallikai choornam.

INTRODUCTION

A large number of people (upto 20% of the population world wide) are suffering from urinary stone problem.^[1] Struvite (Magnesium ammonium phosphate- $MgNH_4 PO_4 \cdot 6H_2O$) is the predominant crystalline component. Struvite crystallization is related to urinary tract infection by microorganism producing urease.^[2] They are mainly the microorganism from the species of proteus. These studies indicate that herbal preparation could inhibit the growth of the crystals, forming the kidney stones. As opposed to the treatment using antibiotics, these herbal preparation is claimed to cause no bacterial resistance and other adverse effects.

However only a few studies have been conducted on the effects of herbs on the crystal growth of struvite, as one of the main components of renal calculi.^[3,4] The ingredient of the Mallikai Choornam are well known for their pharmacological activities mainly as antibacterial, antifungal, antioxidant, anti-inflammatory etc.. keeping in view of this, such the present work of anti-urolithiasis activity in Mallikai Choornam using struvite crystal growth inhibition assay (in vitro) has been aimed.

MATERIAL AND METHODS

Collection of raw drugs

Raw drugs were purchased from country medical shop at Chennai, these drugs were authenticated by Medicinal Botany department, NIS Chennai(No:NISMB2732017/02/2017). Medicine were prepared at Gunapadam laboratory, NIS,Chennai. Project Id: NRS/AS/0033/02/2017

Preparation of mallikai choornam^[5]

Ingredients

Thaniya(Corriandram sativam.Linn) -315grams(9palam)
Paranki chakkai(Smilax china.linn) -35grams(1palam)
Athimathuram(Glyciriza glabra.Linn) -35grams(1palam)
Karunjeeragam(Nigella sativa.Linn) -35grams(1palam)
Jeeragam(Cumminum ciminum.Linn) -35grams(1palam)
Sajara(Shorae robusta.Gaertn)^[6] -35grams(1palam)
Ealam(Eletteria cardamomum.Marton) -35grams(1palam)
Sannalavanga pattai(Cinnamomum zylanicum.Presl) -35grams(1palam)
Kirambu(Syzygium aromaticum.Linn) -35grams(1palam)
Vithai illa thirachai(Vitis vinifera.Linn) -35grams(1palam)

Purification of raw drugs

- Paranki chakkai-Powered and steamed with cow's milk and then dried.
- Athimathuram-washed with clean water,skin pealed and made into small pieces.
- Karunjeegam-Cleaned and kept in sunlight then fry it to golden colour.
- Sajara – Boil with tender coconut
- Jeeragam Ealam, Sanna lavanga pattai, Kirambu, Vithai illa thiratchai – Clean and kept in sunlight

- Thaniya – Tie coriantrum sativum seeds using a cloth and boil it by dipping in lemon juice, then dry in sunlight.^[7]

Method of preparation

Coriander seeds are dried in sunlight, the remaining raw drug are fried to till they reach golden colour and powdered. Mix all the powder. The prepared drug will be stored in a clean and dry tight glass container.

Anti-urolithiatic activity

Objective

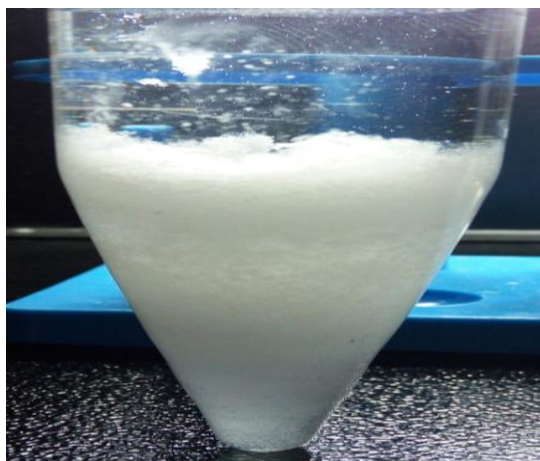
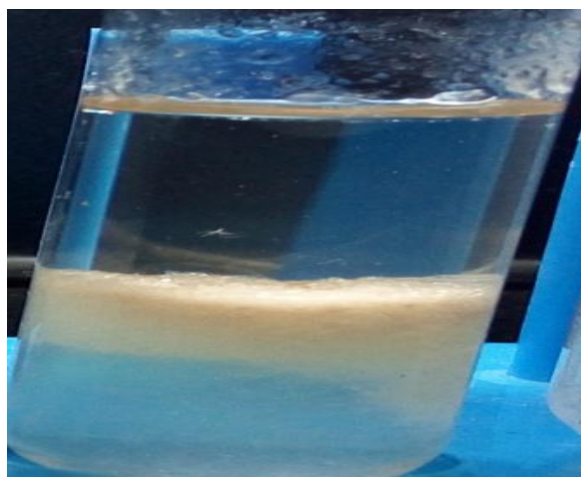
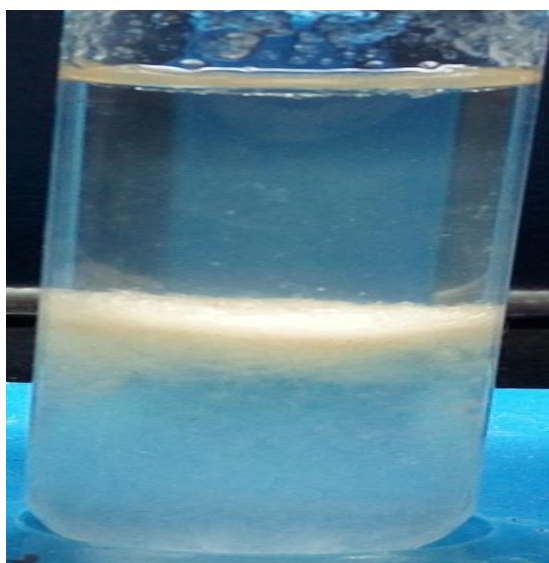
The single diffusion gel growth technique was adopted to evaluate anti-urolithiatic[8] potential of the study drug Mallikai Choornam.

Test Drug concentration

Test drug was prepared at two different concentration 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 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% conc 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 serves as crystal control group. All the procedures was 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 growth of crystal were carried out for five consecutive days.

Growth Pattern of crystal in control and drug added medium**Growth of Struvite crystals in control Gel medium****Growth of Struvite crystals in Gel medium with 0.5 of *Mallikai Choornam*****Growth of Struvite crystals in Gel medium with 1% of *Mallikai Choornam***

Size variation of Struvite crystals



A

B

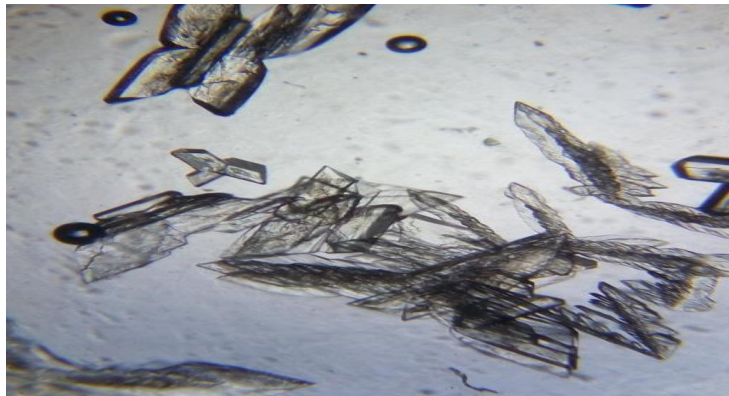
C

A - Size variation of Struvite crystals in Control Gel medium

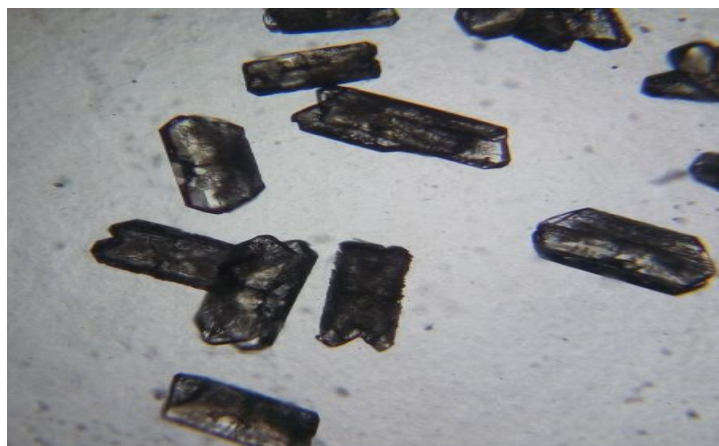
B- Size variation of Struvite crystals in Gel medium with 0.5 % of *Mallikai Choornam*

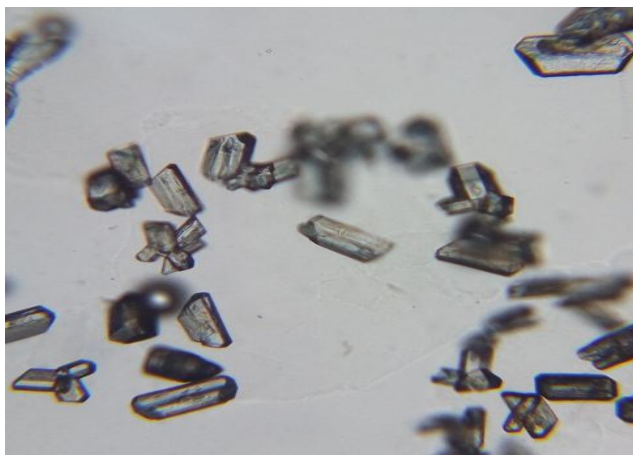
C- Size variation of Struvite crystals in Gel medium with 1 % of *Mallikai Choornam*

Microscopic view of Struvite crystals size after fragmentation.



Control Gel medium

Gel medium with 0.5 % of *Mallikai Choornam*



Gel medium with 1 % of *Mallikai Choornam*

RESULTS AND DISCUSSION

The present study investigated the anti-urolithiasis activity of poly herbal preparation using struvite crystal growth inhibition assay. This results were tabulated.

Report on Average Length of the Crystal in different medium

| S. No. | Medium | Average Length of the Crystals in cm |
|--------|------------------------------|--------------------------------------|
| 1 | Control Gel medium | |
| | Mean | 1.14 |
| | Std. Deviation | 0.1517 |
| | Std. Error | 0.06782 |
| 2 | Gel medium + 0.5 % MC | |
| | Mean | 0.66 |
| | Std. Deviation | 0.1673 |
| | Std. Error | 0.07483 |
| 3 | Gel medium +1% MC | |
| | Mean | 0.46 |
| | Std. Deviation | 0.1342 |
| | Std. Error | 0.06 |

Control Medium

Average size of the crystal was higher in the control medium with the Avg length of 1.14 cm.

Gel medium + 0.5 % MC

Average size of the crystal was significantly decreased in medium contains 0.5% of test drug MC with the Avg length of 0.66 cm.

Gel medium +1% MC

Average size of the crystal was much reduced in medium contains 1 % of test drug MC with the Avg length of 0.46 cm.

From the result of the study it was concluded that the test drug Mallikai Choornam has anti-urolithiasis activity in the tested medium.

CONCLUSION

In view of the antibiotic resistance associated with the use of commercially available antibiotics especially in immune compromised patients and childrens. It is concluded that the natural products from medicinal plants, one of the alternative source of combating infections in human beings which may also be of cost effective, efficacious and easily available in treating infectious calculi.^[9]

REFERENCES

1. Nicki R. Colledge, Bricm R. Walker, Devidson's principles and practice of Medicine, 21st edition, 2010.
2. B. Bindu, Studies on the effect of phyllanthus emblica extract on the growth of urinary type struvite crystals in vitro, clinical phytoscience, 8 aug 2015.
3. Stefanus Muryanto, inhibition of struvite crystal growth in the presence of herbal extract orthosiphon aristatus BL. MIQ, 2016.
4. Jolanta Prywer, Effect of curcumin against proteus mirabilis during crystallization of struvite from artificial urine, Hindawi publishing corporation, 2012; 7.
5. C.Kannusami pillai, Chikitcha Rathna Deepam, edition; 2007; 122.
6. Lr. Clonol, K.R. Krithikar & Majoe B.D. Basu, The Indian Medicinal plants, Edition, 1: 290.
7. Devaasirvatham Samuvel, Marunthu Sei Iyalum Kalaiyum; 29, 30, 11.
8. Chauhan, C.K., K.C. Joseph, B.B. Parekh and M.J. Joshi, Growth and characterization of Struvite crystals, Ind. J. Pure Appl. Phys., 2008; 46: 507-512.
9. S.Kalpana, Inhibition of calcium oxalate crystallization in vitro by extract of banana cultivar monthan, International journal of pharmacy and pharmaceutical science, 2013; 5(4).