WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8. Issue 1. 1036-1044 Research Article ISSN 2278 – 4357

IN-VITRO EVALUATION OF ANTI-UROLITHIASIS POTENTIAL OF NOVEL SIDDHA FORMULATION PANCHA LAVANA PARPAM USING STRUVITE CRYSTAL GROWTH INHIBITION ASSAY

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Article Received on
31 October 2018,

Revised on 21 Nov. 2018,
Accepted on 11 Dec. 2018,

DOI: 10.20959/wjpps20191-12916

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ABSTRACT

Urolithiasis is a clinical condition characterized by formation of stones in the urinary tract that causes variable degrees of pain, bleeding, and leads to secondary infection The worldwide occurrence of urolithiasis is increasing and calcium oxalate (CaOx) is the primary constituent of the majority of stones formed in the urinary system of patients reported with urolithiasis. Research focus on drug development towards urolithiasis majorly relies on prevention of crystal aggregates which may tend to become stone later. Crystal growth occurs after supersaturation and then primary nucleus of kidney stone was formed. Crystal aggregation is affected by viscous bindings, such as external crystalline compounds with multiple binding sites. Siddha system of medicine had range of formulation in particular with parpam that can tend to be having higher penetration and also possess significant activity in preventing crystal aggregation. Pancha lavana parpam (PLP) is a novel siddha made of salts and indigenous herbal juices.

Present investigation aimed at evaluating the anti-urolithiasis potential of the formulation PLP by struvite crystal growth inhibition assay at two dose level of 0.5% and 1%. The potency of the formulation PLP was critically evaluated by comparing the crystal size of drug treated medium with that of the control gel medium. Average size of the crystal was higher in the control medium with the length of 1.92 ± 0.35 cm and the size of the crystal was significantly decreased in medium contains 0.5% and 1 % of test drug PLP with the average length of 1.24 ± 0.11and 0.96 ± 0.14cm respectively. From the result of the present study it was clealry evident that the siddha formulation PLP has significant anti-urolithiasis property in the tested Invitro medium and may correlated clinically with proper preclinical studies.

KEYWORDS: Siddha system, Urolithiasis, Pancha lavana parpam, Crystal growth, Kidney.

1. INTRODUCTION

Kidney stone disease is a crystal concretion formed usually within the kidneys. It is an increasing urological disorder of human health, affecting about 12% of the world population. It has been associated with an increased risk of end-stage renal failure. The etiology of kidney stone is multifactorial. The most common type of kidney stone is calcium oxalate formed at Randall's plaque on the renal papillary surfaces. Globally, kidney stone disease prevalence and recurrence rates are increasing,[1] with limited options of effective drugs.

Urolithiasis affects about 12% of the world population at some stage in their lifetime.[2] It affects all ages, sexes, and races[3] but occurs more frequently in men than in women within the age of 20–49 years.[4] If patients do not apply metaphylaxis, 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.[5] However, lifetime recurrence rate is higher in males, although the incidence of nephrolithiasis is growing among females.[6] Therefore, prophylactic management is of great importance to manage urolithiasis.

In the present scenario there are no perfect drugs of choice available for the management of urolithiasis, some modern therapies includes thiazide diuretics and citrates are been used as a preventive therapies. 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.[7] In this regard, traditional herbal medicine can be a potent source of new anti-

urolithiatic remedies, because it is shown that their extracts and compounds have biological activities. A number of plants have been used which claim to treat the kidney stone.[8]

Siddha medicine is one of the most ancient medical systems of India. Siddha is the mother medicine of ancient Tamils/Dravidians of peninsular South India. The word Siddha means established truth. The persons who were associated with establishing such a Siddha school of thought were known as Siddhars. They recorded their mystic findings in medicine, yoga, and astrology in Tamil. Fundamental Principles of Siddha include theories of Five Elements (Aimpootham), and Three Forces/Faults (Mukkuttram). The Eight Methods of Examination (Envakai Thervukal) is used to determine diagnosis, etiology, treatment and prognosis. Siddha has safe herbal and herbo mineral treatment for several disease. Parpam is ne unique formulation of siddha comprises of herbs and other salts the fineness nature of this preparation made it very unique versatile in crossing biological barrier and offers extensive binding with the target thereby usage of such formulation may provide extended benefits at even lower therapeutic dose level when it comes clinically. The main aim of the present investigation is to evaluate the possible anti-urolithiasis property of the siddha formulation PLP by struvite crystal growth inhibition assay.

2. MATERIALS AND METHODS

2.1. Preparation of Pancha Lavana Parpam

2.1.1. Ingredients

- Pancha Lavanam (Five salts) - 100gms
- Acalypha Juice (Kuppai meni saru) - Quantity Sufficient
- Square spurge latex (Sadhura kalli saru) - Quantity Sufficient
- Daemia juice (Uththamani saru) - Quantity Sufficient
- Mudar Leaf Juice (Erunkkan elai saru) - Quantity Sufficient

The above raw materials and herbal ingredients were identified and authenticated by respective authority before usage of the same for the PLP formulation.

2.1.2. Formulation of PLP[9]

Grind a mixture of the salts with acalypha Juice for 2 days and calcine with dung cake. Grind the resulting product with square spurge latex for 2 days followed by calcine with dung cake. Resulting mixture were grounded with daemia juice for 2 days followed by calcine with dung

cake and the resultant product grounded with mudar leaf juice for 2 days with calcine with dung cake. Finally the finely powder product was then calx to obtain an ash coloured parpam.

2.1.3. Test Drug concentration

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

2.2. Struvite Crystal growth inhibition Assay[10]

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% 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 serves 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 growth of crystal were carried out for five consecutive days.

3. RESULT

3.1. Effect of the formulation PLP on Size variation of Struvite crystals in Gel medium

Average size of the crystal was higher in the control medium with the length of 1.92 ± 0.35 and similarly the average size of the crystal was significantly decreased in medium contains 0.5% of test drug PLP with the average length of 1.24 ± 0.11 cm. Average size of the crystal was even much reduced in medium contains 1 % of test drug PLP with the Avg length of 0.96 ± 0.14 cm. As shown in the figure 1A to 1C and table 1.

Microscopic observation of crystal belongs to control medium reveals the presence of large aggregate whereas treatment with 0.5% of the test formulation PLP reveals significant decrease in the aggregates resulting in projection of individual crystals similarly treatment with 1% of the test formulation PLP shown that fragmented crystals reveals 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.

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

S. No.	Medium	Average Length of the Crystals
1	Control Gel medium	1.92 ± 0.35
2	Gel medium + 0.5 PLP	1.24 ± 0.11
3	Gel medium +1% PLP	0.96 ± 0.14

Data are given as Mean ± SD (n=3).

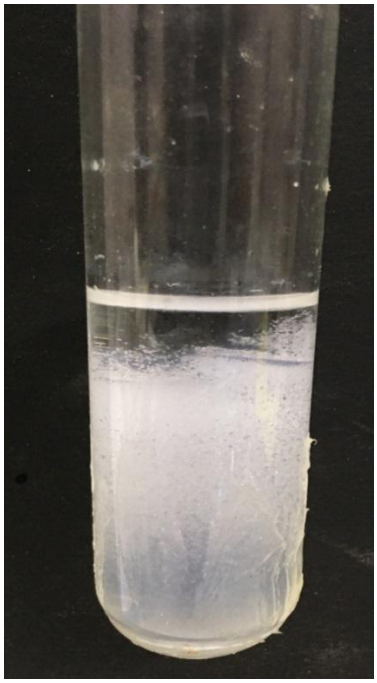

Figure 1 A: Control medium.

Figure 1 B: Medium + 0.5% *PLP*.

Figure 1 C: Medium +1% *PLP*.

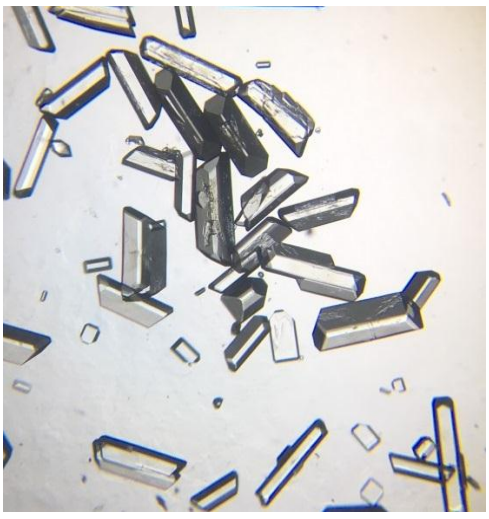

Figure 2A: Crytals in Control Gel medium.

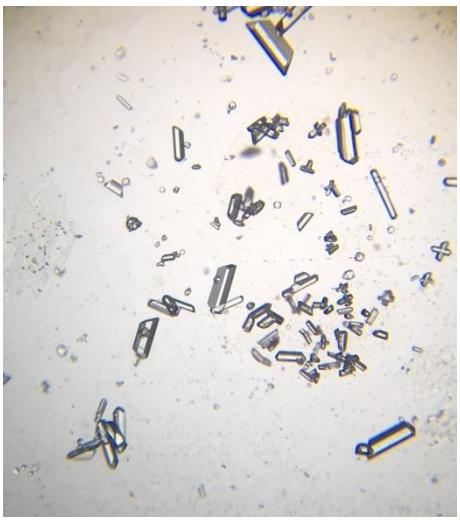

Figure 2B: Crytals in Gel medium with 0.5 % of *PLP*.

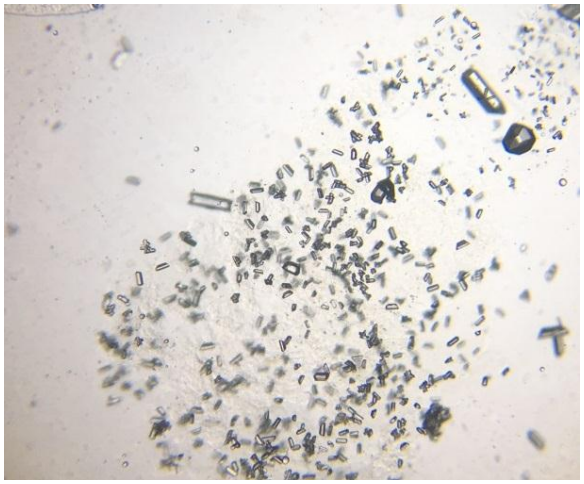

Figure 2 C: Crytals in Gel medium with 1 % of *PLP*.

4. DISCUSSION

Clinical therapy for kidney stone prevention depends upon addressing the cause of stone formation. Generally, to prevent the first episodes of kidney stone formation or its secondary episodes, proper management of diet and the use of medications are required. Primary prevention of kidney stone disease via dietary intervention is low-cost public health initiative with massive societal implications. Thus, nutritional management is the best preventive strategy against urolithiasis.[11] Kidney stones are the third most common urinary tract problems, after urinary tract infections and prostate diseases. Most people with kidney stones suffer from severe colic pains that are not relieved by conventional pain killers and may require narcotic analgesics. In addition to pain, urinary tract obstruction, urinary tract infection, hydronephrosis and severe bleeding may occur and in some cases, surgery is required to remove or break stones.[12]

It has been estimated that 80% of the world's population relies on traditional medicine to treat their diseases.[13] Medicinal plants have a long history of use and are globally safer than synthetic drugs.[14] They are a reliable source for drug discovery.[15] Today, researchers have focused on the drug discovery from medicinal plants.[16] It has been estimated that at least one third of all medicinal product have plant origin.[17] Medicinal plants are regarded as an acceptable, cheap, easily available and safe source of active compounds for pharmaceutical.[18] The therapeutic effects of medicinal plants on kidney and urinary tract disorders have been variously studied and their efficacy has been demonstrated.[19] From the results if the present investigation it was observed that the average size of the crystal was higher in the control medium with the length of 1.92 ± 0.35 and similarly the average size of

the crystal was significantly decreased in medium contains 0.5% of test drug PLP with the average length of 1.24 ± 0.11 cm. Average size of the crystal was even much reduced in medium contains 1 % of test drug PLP with the Avg length of 0.96 ± 0.14 cm.

Synthetic drugs are not only expensive and insufficient for the treatment of diseases but also have deception and side effects. The kind of this situation stresses need to search a novel drug for treating such disease.[20] Siddha system of traditional medicine has numerous novel formulations that can act by multiple mechanism and also in the present study microscopic observation of crystal belongs to control medium reveals the presence of large aggregate whereas treatment with 0.5% of the test formulation PLP reveals significant decrease in the aggregates resulting in projection of individual crystals similarly treatment with 1% of the test formulation PLP shown that fragmented crystals reveals the inhibition potential of the trial drug when compared to that of the control medium crystals.

5. CONCLUSION

Stone formation in the kidney is one of the oldest and the most widespread diseases known to man. It is a common chronic disorder affecting 10-15% population worldwide. Urinary oxalate is directly related to diet, but the majority of oxalate is produced via metabolism of glyoxylic acid. The need of the hour is to develop an effective, safe and standardized traditional preparation for the management of urolithiasis. From the data's obtained from the present study it was observed that the formulation PLP has significantly decreases the size of the crystal and also arrest the growth which was evident microscopically. Hence it was concluded that anti-urolithiatic potential of the siddha formulation Pancha lavana parpam may be due to biologically active component present in the formulation further, research and studies needed to isolate the active principles and investigate them in order to identify a promising compound responsible for this action.

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