



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2): 1859-1862
Received: 03-01-2019
Accepted: 06-02-2019

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Design, development and standardization of novel polyherbal syrup against renal calculi

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Abstract

Renal calculi are the most common urinary disorder of urinary tract and its incidence is high throughout the world. Traditional practitioners prescribe the whole plants of *Tribulus terrestris* (Zygophyllaceae) and *Aerva lanata* (Amaranthaceae) for the treatment of renal calculi. This study was aimed to design, develop and to characterize the novel polyherbal syrup using decoction of the whole plants of *Aerva lanata* and *Tribulus terrestris* and to evaluate its efficacy against renal calculi by *in vitro* nucleation assay method. The formulated polyherbal syrup significantly inhibited nucleation of calcium oxalate crystals and the microscopical examination showed significant decrease in the number of calcium oxalate crystals for the standard drug cystone and formulated syrup. The result provides the scientific validation for the traditional uses of the herbal plants and the formulated novel polyherbal syrup might be an effective therapy for the management of urolithiasis.

Keywords: Polyherbal syrup, *Aerva lanata*, *Tribulus terrestris*, urolithiasis, renal calculi

Introduction

Renal calculi are the most common urinary disorder of urinary tract and its incidence is high across globe. It affects 10-12% of the population in industrialized countries and the recurrence rate is 50%. In renal calculi the modern medication for urolithiasis includes surgical techniques like extracorporeal shock wave lithotripsy, intracorporeal lithotripsy, urethroscopy with lithotripsy and for the management of urolithiasis non-steroidal anti-inflammatory drugs are being used [1]. Approximately 75% of renal stone are calcium oxalate crystals and the formation are due to its supersaturation and crystallization followed by nucleation, growth and aggregation [2].

In siddha system for the management of renal calculi, whole plant of *Aerva lanata* and *Tribulus terrestris* is boiled in 1500ml of water and the decoction is concentrated to 500ml. The concentrated decoction is consumed early morning in empty stomach [3]. Hence there is no scientific validation for its efficacy against renal calculi, the study is designed to formulate the decoction into novel polyherbal syrup and an attempt was made to prove its efficacy against renal calculi using *in vitro* nucleation assay method.

Materials and Methods**Materials**

Calcium chloride, sodium oxalate, sodium chloride, Hydrochloric acid were procured from Merck, Mumbai, India.

Plant Collection

Whole plants of *Aerva lanata* and *Tribulus terrestris* were collected from Vazhudavur, Pondicherry, India. The plants were identified and authenticated in the French Institute of Pondicherry, Pondicherry, India (Accession No. 27057, 28055). The voucher specimen is deposited at Department of Pharmacognosy, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health sciences, Pondicherry, India.

Formulation of Polyherbal syrup**Preparation of Decoction [4].**

250 gms of coarsely powdered whole plants of *Aerva lanata* (125gms) and *Tribulus terrestris* (125gms) were mixed with 4000ml of water and boiled until the total volume become one fourth of the initial volume. The decoction is filtered and the filtrate was taken to prepare the polyherbal syrup.

Preparation of Simple syrup as per USP [5].

Sucrose 666.7 Gms is weighed and added to 300ml of distilled water and heated until the sucrose is completely dissolved. Final volume is Made upto 1000ml with distilled water.

Preparation of Polyherbal syrup [4].

One part of the prepared decoction is mixed with five parts of simple syrup USP (1:5 ratio). Methyl paraben and peppermint oil is added to the above mixture quantity sufficient to.

Characterization of Polyherbal syrup [6].

Physical appearance, PH, Specific gravity, Refractive index, Viscosity is carried out as per standard guideline given in IP.

***In-vitro* Urolithiatic activity by Nucleation assay [7,8].**

The effect of the novel polyherbal syrup formulated on Calcium oxalate crystal nucleation is determined by nucleation assay method. Calcium chloride 5mmol/l solution and sodium oxalate 5mmol/l solution are prepared in Tris buffer PH 6.5. Dilutions of the formulation and standard drug Cystone (446mg) is prepared in 100, 200, 300, 400, 500 and 600 microgram/ml solution. One millilitre of each

concentration of formulation and cystone individually is mixed with 3ml of calcium chloride solution followed by the addition of 3ml sodium oxalate solution. The final mixture was incubated for 30 mins at 37 °C. The optical density of the mixtures is then measured at 620nm wavelength. The percentage inhibition of nucleation by standard drug and test sample is calculated using the following formula

$$\% \text{ Inhibition} = \frac{\text{OD control} - \text{OD test}}{\text{OD control}} \times 100$$

The assay was done in triplicates and the average is calculated for the accuracy of the results.

Microscopic examination of calcium oxalate crystals [9,10].

A drop of turbid solution of each test samples and standard drug mixture after incubation is examined microscopically for qualitative analysis using compound microscope under 10x magnification.

Results

Fig 1: Habitat of *Aerva lanata*



Fig 2: Habitat of *Tribulus terrestris*

Table 1: Physical appearance of polyherbal formulation

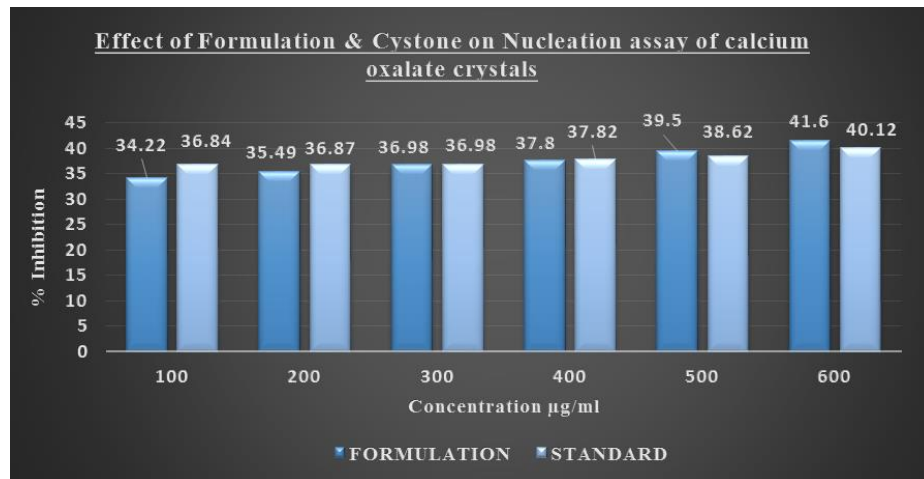
S.No	Parameter	Value
1	Colour	Yellowish brown
2	Odour	Peppermint odour
3	Taste	Sweet
4	Nature	Viscous Syrup

Table 2: Characterization of formulated polyherbal syrup

S.No	Parameter	Value
1	PH	6.98
2	Specific gravity	0.97 gm/ml
3	Viscosity	0.087 Poise
4	Refractive index	1.443

Table 3: Effect of polyherbal syrup and cystone on Nucleation of Calcium oxalate crystals

S. No	Conc. (µg/ml)	% Inhibition (Average of triplicates)	
		Formulation	Cystone
1.	100	34.22	36.84
2.	200	35.49	36.87
3.	300	36.98	37.98
4.	400	37.80	37.82
5.	500	39.50	38.62
6.	600	41.60	40.12



Graph 1: Graphical representation of % inhibition of formulation and cystone on Nucleation assay

Qualitative Microscopical examination of effect of Formulation and standard on Nucleation of calcium oxalate crystals (10x magnification)

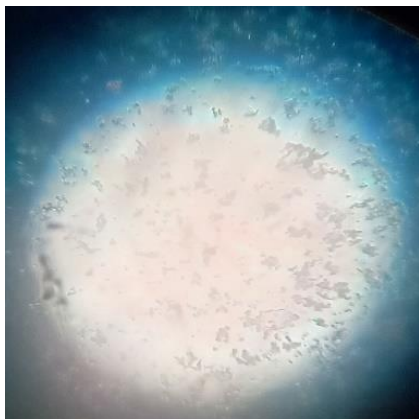


Fig 3: Control – Increased number of CaOx crystals



Fig 4: Cystone 600µg/ml – Decreased number of CaOx crystals

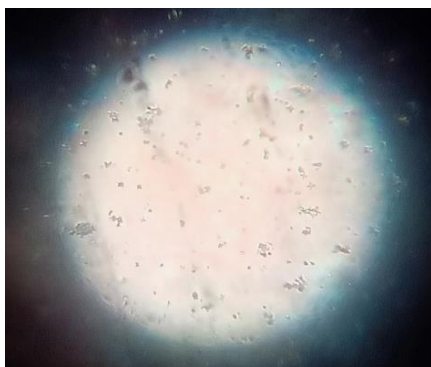


Fig 5: Formulation 600µg/ml – Decreased number of CaOx crystals

Discussion

For the management of renal calculi in siddha system of medicine the whole plant of *Aerva lanata* (Fig.1) and *Tribulus terrestris* (Fig.2) decoction is administered in empty stomach early morning. The decoction prepared is scientifically unexplored and in this research work, it is planned to formulate the traditional dosage form into modern dosage as simple syrup, a poly herbal syrup. The simple poly herbal syrup is developed and it is characterized by physical parameters (Table. 1 & 2). The values obtained from physical parameters gives a standard protocol for standardization of the polyherbal syrup.

In vitro urolithiatic activity is carried out by Nucleation assay. Calcium chloride added with sodium oxalate solution result in the nucleation of calcium oxalate crystals which is noted by turbidity. The formulation and the standard drug are incubated along with the reaction mixture solution and the percentage Inhibition of formation of calcium oxalate crystals is noted by decrease in turbidity estimated by UV spectrophotometry at 620nm.

In control mixture the percentage inhibition is null and the qualitative microscopical examination showed presence of a greater number of calcium oxalate crystals (Fig.3). Presence of Standard drug Cystone from 100 to 600µg/ml showed gradual increase in percentage inhibition of nucleation of calcium oxalate crystals in the reaction mixture, from 36.84 to 40.12% (Table.3, Graph.1). In the presence of formulation in the reaction mixture, of the same concentration the result showed the similar activity when compared to the standard drug Cystone from 34.22 to 41.6% (Table.3, Graph.1).

The qualitative microscopical examination under 10x showed a greater number of calcium oxalate crystals in the control group (Fig.3) and in the reaction mixture incubated with standard drug cystone and Formulation showed the decreased number of calcium oxalate nucleation when compared to control (Fig.4 & 5).

Conclusion

The study is aimed to design, develop and to formulate the polyherbal syrup using the decoction of *Aerva lanata* and *Tribulus terrestris* whole plants. The Formulation is made into simple syrup USP and it is characterized by physical parameters. The formulated polyherbal syrup is evaluated for urolithiatic activity by nucleation assay model followed by qualitative microscopic examination. From the nucleation assay the formulation showed the dose dependent activity similar to the standard drug cystone. Further *In vivo* studies

with molecular mechanism and clinical screening findings might give a potent drug formulation with antilithic potential for the management of patients with renal calculi.

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