

## Review Article

### Antiuro lithiatic activity of InjiRasayanam:

### A Siddha Formulation

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#### ABSTRACT

Aqueous extract of Inji Rasayanam, was carried out to investigate antiuro lithiatic activity by calcium oxalate crystallization was induced by the addition of 0.01M sodium oxalate solutions in synthetic urine and nucleation method.. The effect of Inji Rasayanam on calcium oxalate crystallization (200, 400, 600, 800 and 1000 µg/ml) was studied by time course measurement of turbidity in presence or absence of inhibitor (extract) at 620 nm for ten minutes by means of a spectrophotometer. Aqueous extract of Inji Rasayanam exhibited a concentration dependent inhibition of nucleation.

**Key-words:** InjiRasayanam, Antiuro lithiatic activity, Nucleation,

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## Introduction

Nephrolithiasis is common, affecting up to 10% of the population at some point during their lifetime<sup>1</sup>. Calcium-containing stones are the most commonly occurring to an extent of 75-90% followed by magnesium ammonium phosphate (Struvite) to an extent of 10-15%, uric acid 3-10% and cystine 0.5-1%<sup>2</sup>. Calcium oxalate stones are found in two different varieties, calcium oxalate monohydrate (COM) or Whewellite, and calcium oxalate dihydrate (COD) or Weddellite. COM, the thermodynamically most stable form, is observed more frequently in clinical stones than COD and it has a greater affinity for renal tubular cells, thus responsible for the formation of stones in the kidney<sup>4</sup>.

Inji Rasayanam, is an siddha formulation which contains ginger, ghee and sugar. Fry pieces of cleaned ginger in, ghee and powder. Slightly fry cumin and make a powder. Mix fine powders of these to powdered sugar. It is used traditionally in vomiting, biliousness, burning sensation, inflammations in chest, stomach and indigestion.

The present investigation on the antiurolithiatic activity by Inji Rasayanam by *in vitro* methods.

**Dose:** 5 g twice daily with honey

## Experimental Protocol

The effect of extract on CaOx crystallization was determined by the time course measurement of turbidity changes due to the crystallization in artificial urine on addition of 0.01M sodium oxalate solution. The Precipitation of calcium oxalate at 37°C and pH 6.8 has been studied by the measurement of turbidity at 620 nm using UV/Visible spectrophotometer.

### Preparation of artificial urine

The artificial urine (AU) was prepared according to the method Burns and Finlayson<sup>3</sup> (1980) and had the following composition: sodium chloride 105.5 mM, sodium phosphate 32.3 mM, sodium citrate 3.21 mM, magnesium sulfate 3.85 mM, sodium sulfate 16.95 mM, potassium chloride 63.7 mM, calcium chloride 4.5 mM, sodium oxalate 0.32 mM, ammonium hydroxide 17.9 mM, and ammonium chloride 0.0028mM. The AU was prepared fresh each time and pH adjusted to 6.0.

### Study without inhibitor

A volume of 1.0 ml of AU was transferred into the cell and 0.5 ml of distilled water added to it and blank reading was taken. The 0.5 ml of 0.01M sodium oxalate was added, to the previous volume, and the measurement is immediately started for a period of ten minutes.

### Study with inhibitor

The aqueous extract of Inji Rasayanam was dissolved in distilled water, filtered through membrane filter and the concentration of 200, 400, 600, 800 and 1000 µg/ml was obtained. A mixture of 1 ml of AU and 0.5 ml of plant extract solution is versed in the cell. A blank reading was taken and then 0.5 ml of 0.01M sodium oxalate solution was added and immediately the absorbance was measured for a period of ten minutes at 620nm<sup>5</sup>. The percentage of inhibition was calculated using the following formula:

$$\% \text{inhibition} = \{1 - [\text{Ab Test} / \text{Ab Control}]\} \times 100$$

Where; Ab Test: Absorbance in the presence of inhibitor (Extract), Ab Control: Absorbance of graph without inhibitor (Control).

### Nucleation Assay

The method used was similar to that described by Hennequinet al<sup>6</sup> with some minor modifications. Solutions of calcium chloride and sodium oxalate were prepared at the final concentration of 3 mM and 0.5 mM, respectively, in a buffer containing Tris 0.05 M and NaCl 0.15 M at pH 6.5. Both solutions were filtered through a 0.22 µm filter; 33 mL of calcium chloride solution was mixed with of the aqueous extract of Inji Rasayanam of different concentrations. Crystallization was started by adding 33 mL of sodium oxalate solution. The final solution was magnetically stirred at 800 rpm. The temperature was maintained at 37° C. The absorbance of the solution was monitored at 620 nm. The percentage inhibition produced by the herb extract was calculated as  $[1 - (T_{si}/T_{sc})] \times 100$ ,

Where Tsc was the turbidity slope of the control and Tsi the turbidity slope in the presence of the inhibitor.

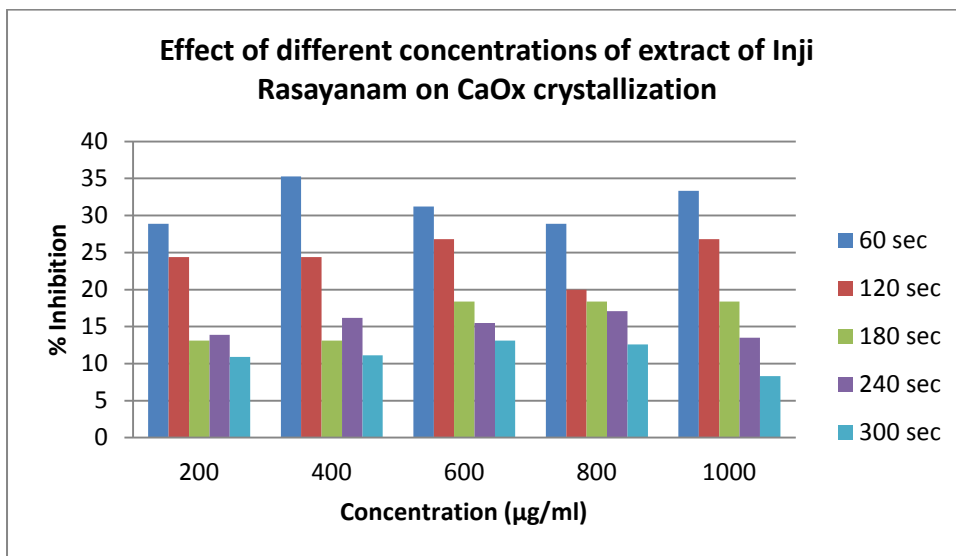


Figure 2: Effect of Inji Rasayanam on Nucleation of CaOx

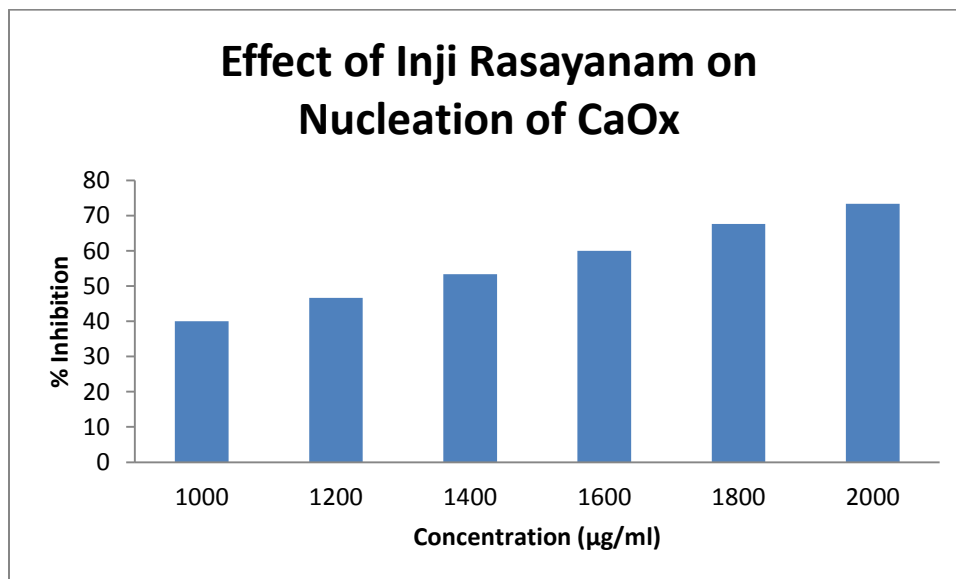


Figure 2: Effect of Inji Rasayanam on Nucleation of CaOx

## Results

### Effect of different concentrations of aqueous extract of Inji Rasayanam on CaOx crystallization

Figure 1 showed the graph of percentage inhibition of the crystallization of CaOx with different concentrations of aqueous extract of Inji Rasayanam. It inhibited the crystallization in a concentration dependent pattern. The percent inhibition was calculated using the above mentioned formula.

### Inhibition of Nucleation of CaOx Crystals by Inji Rasayanam

Figure-2 displays the effect of the different concentration of the aqueous extract of Inji Rasayanam on the nucleation of calcium oxalate crystals. The increase in the concentration of Inji Rasayanam extract, percentage inhibition was also increased. Maximum nucleation 73.33 observed at concentration of 2000 µg/mL.

## Discussion

In the present study, the anticalcifying properties of Inji Rasayanam were explored by *in vitro*. After nucleation, crystal growth is the next major step of stone formation. The driving force for crystallization is a reduction in the potential energy of the atoms or molecules when they form bonds to each other. The crystal growth process starts with the nucleation stage when several atoms or molecules in a supersaturated liquid start to form clusters<sup>7</sup>. Nucleation is the formation of a solid crystal phase in a solution. It is an essential step in renal stone formation<sup>8&9</sup>. The inhibitory potency of the Inji Rasayanam was tested on the nucleation and growth of the most commonly occurring kidney stones, calcium oxalate monohydrate. A concentration dependent inhibition was observed using Inji Rasayanam.

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