

# Antiuro lithiatic Activity of Gokhsuradi Churan, an Ayurvedic Formulation By *In Vitro* Method

Ashok Kumar Bagepalli Srinivasa<sup>1\*</sup>, Lakshman Kuruba<sup>2</sup>, Saleemulla Khan<sup>3</sup>, Gopi Setty Saran<sup>1</sup>

<sup>1</sup> Department of Pharmacognosy, Sri K.V.College of Pharmacy, Chickballapur, Karnataka, India.

<sup>2</sup> Department of Pharmacognosy, PES College of Pharmacy, Bangalore, Karnataka, India.

<sup>3</sup> Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, Karnataka, India.

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## ABSTRACT

**Purpose:** Gokhsuradi churna is an ayurvedic formulation, was investigate for antiuro lithiatic activity. **Methods:** Calcium oxalate crystallization was induced by the addition of 0.01M sodium oxalate solutions in synthetic urine and nucleation method. **Results:** The effect of Gokhsuradi Churna exhibited a concentration dependent inhibition of on calcium oxalate crystallization and nucleation. **Conclusion:** The present studies suggest that Gokhsuradi churna has a potential inhibition of calcium oxalate crystallization exhibited and nucleation. Gokhsuradi Churna showed potent antiuro lithiatic activity.

## Introduction

Gokhsuradi Churna contains Gokshura (*Tribulus terrestris* Linn), Kokilaksha (*Asteracantha longifolia* Nees), Satavari (*Asparagus racemosus* Willd), Kapikachu (*Mucuna pruriens* Baker), Naagabala (*Sida cordata*) and Atibala (*Abutilon indicum* Linn).

In Ayurvedic Gokhru is indicated for the treatment of urinary disorders, kidney diseases, diseases of the genito-urinary system and impotence. It is used for regulation of heart functions, reduction of inflammation, chronic cough and asthma also. The leaves of this medicinal plant of India are considered to possess stomachic properties, and a paste prepared from them is used in the treatment of bladder stones.<sup>1</sup> *Asteracantha longifolia* roots, leaves and seeds have been used in Indian systems of medicine as diuretics and also employed to cure jaundice, dropsy, rheumatism and diseases of the urinogenital tract. Root of *A. racemosus* has been referred as bitter-sweet, emollient, cooling, nervine tonic, constipating, galactagogue, aphrodisiac, diuretic, rejuvenating, carminative, stomachic, antiseptic<sup>2</sup> and as tonic. Beneficial effects of the root of *Asparagus racemosus* are suggested in nervous disorders, dyspepsia, diarrhoea, dysentery, tumors, inflammations, hyperdipsia, neuropathy, hepatopathy, cough, bronchitis, hyperacidity and certain infectious diseases.<sup>3</sup> *Sida cordata* traditionally used for arthritis, leucorrhoea, urinary infection, asthma, cough,

bronchitis, diarrhea, burning sensation, and general debility.<sup>4</sup> *Mucuna pruriens* has a long history of being used in the Indian Ayurvedic medicine. It has been used for dysentery, stone in the bladder, diarrhea, snakebite, sexual debility, cough, tuberculosis, impotence, rheumatic disorders, muscular pain, sterility, gout, menstrual disorders, diabetes, and cancer.<sup>5</sup>

*Abutilon indicum* Linn is used as a demulcent, aphrodisiac, laxative, diuretic, and sedative (leaves). The bark is astringent and diuretic; laxative, expectorant and demulcent (seeds); laxative and tonic, anti-inflammatory and anthelmintic (plant); analgesic (fixed oil); diuretic and for leprosy (roots). The leaves can also be used to treat ulcers, headaches, gonorrhoea and bladder infection.<sup>6</sup>

## Materials and Methods

### Preparation of Gokhsuradi churna

Gokhsuradi churna contains Gokshura, Kokilaksha, Satavari, Kapikachu, Naagabala and Atibala were made in to fine powder and passed through 100 No. sieve and mixed. Gokhsuradi churna was extracted with water for antiuro lithiatic study.

### Experimental Protocol

The effect of extract on CaOx crystallization was determined by the time course measurement of

\*Corresponding author: Ashok Kumar Bagepalli Srinivasa, Department of Pharmacognosy, Sri K.V.College of Pharmacy, Chickballapur, Karnataka- 562101, India. Tel: +919986946780, Email: ashok4vani@gmail.com

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 absorbance 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>7</sup> with slight modification and 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 0.0028 mM. 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 Gokshuradi churna 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>8</sup> The percentage of inhibition of calcium oxalate crystal formation was calculated using the following formula:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}} \times 100$$

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

### Nucleation Assay

The method used was similar to that described by Hennequin *et al.*,<sup>9</sup> with slight 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 Gokshuradi churna 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

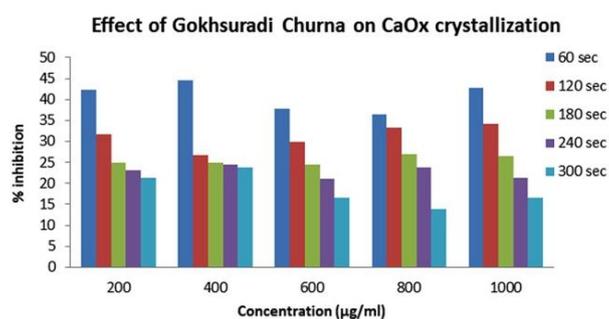
620 nm. The percentage inhibition produced by the herb extract was calculated as:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}} \times 100$$

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

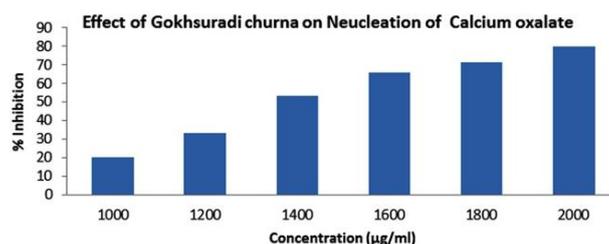
## Results and Discussion

Figure 1 showed the graph of percentage inhibition of the crystallization of calcium oxalate (CaOx) with different concentrations of aqueous extract of Gokshuradi churna. It inhibited the crystallization in a concentration dependent pattern. The percent inhibition was calculated using the above mentioned formula.



**Figure 1.** Effect of different concentrations of extract of Gokshuradi churna on CaOx crystallization.

Figure 2 displays the effect of the different concentration of the aqueous extract of Gokshuradi churna on the nucleation of calcium oxalate crystals. The increase in the concentration of Gokshuradi churna extract showed increase in the inhibition of nucleation. Maximum inhibition of nucleation 80 % observed at concentration of 2000 µg/ml.



**Figure 2.** Effect of aqueous extract of Gokshuradi churna on nucleation of calcium oxalate.

Nephrolithiasis is common, affecting up to 10% of the population at some point during their lifetime.<sup>10</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>11</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>12</sup>

In the present study, the anticalcifying properties of Gokshuradi churna 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>13</sup> Nucleation is the formation of a solid crystal phase in a solution. It is an essential step in renal stone formation.<sup>14,15</sup> The inhibitory potency of the Gokshuradi churna was tested on the nucleation and growth of the most commonly occurring kidney stones, calcium oxalate monohydrate. A concentration dependent inhibition was observed using Gokshuradi churna.

### Conclusion

The present study provides scientific proof for traditional claim of Gokshuradi churna as antiuro lithiatic. So further *in vivo* studies are required to support the ethnomedicinal claim.

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### Conflict of Interest

The authors report no conflicts of interest.

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