Evaluation of In Vitro Antiurolithiatic Activity of Acalfa indica

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INTRODUCTION

Urinary stone disease is a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 70-81% in males, and 47-60% in females. Occurrence of urolithiasis requires formation of nidus, its reaction and growth in the urinary tract which may cause obstruction of the ureter [1]. Lithiasis is the process of formation of stone and urolithiasis are the solid nonmetallic minerals in the urinary tract [2]. Stones result due to phase change whereby dissolved salts condense into solids because of super saturation [3]. The stone formation requires supersaturated urine which depends on urinary pH, ionic strength, solute concentration and complexations. Various substances in the body have an effect on one or more of the above processes, thereby influencing a person's ability to promote or prevent stone formation [4]. Dietary factors (Low intake of purine containing food), hereditary (cystinuria), diseased tissue (tuberculosis), less intake of vitamin A are some of the contributing factors for formation of kidney stones [5,6]. Recommended dietary life style which is vital for treatment of urolithiatic patients include increased water intake, limited tea, intake of less meat, limited dietary sodium and calcium, avoiding of certain antacids with calcium base. Various therapies include thiazide diuretics and alkali-citrate are used in attempt for treatment but scientific evidence is less influential [7]. Treatment procedures for renal stones such as surgical removal, percutaneous techniques and Extra Corporal Shock Wave Lithotripsy (ESWL) are prohibitively costly and with these procedures, recurrence is quite common. However, herbal remedies have been found to be effective in reducing the recurrence rate of renal stones [8].

Traditional plants are constantly being evaluated for possible antilithiac activity in a systemic manner. The present-day medical management of urolithiasis is either costly or not without side effects [9]. In recent times, focus on plant research has increased all over the world and large body evidence has collected to show immense of medicinal plants used in various traditional systems.

MATERIALS AND METHODS

Plant materials

The roots and stems of Acalfa indica were procured from the local areas of Narsapur, in the month of April. The plant was authenticated by M. Malla Reddy (M.Sc, M.Phil in Botany) retired lecturer in botany, Vikarabad, Telanagana. The roots and stems were washed with tap water and dried under shade.
Preparation of plant extracts
The roots and stems of plant were dried under shade and crushed in pulveriser and powdered. These powdered plant material was extracted with ethanol and water in a soxhlet apparatus for 72 h. After complete extraction, the extracts were cooled at room temperature and filtered and evaporated to dryness using rotary evaporator.

Chemicals used
Neeri, sodium oxalate, tris buffer, calcium chloride, Potassium Permanganate (KMnO₄), Sulphuric Acid (H₂SO₄).

In vitro antilithiatic activity test by titrimetry
The experimental kidney stones of Calcium Oxalate (CaOx) were prepared in the laboratory by taking equimolar solution of calcium chloride dehydrate in distilled water and sodium oxalate in 10 ml of 2N H₂SO₄. Both were allowed to react in sufficient quantity of distilled water in a beaker, the resulting precipitate was calcium oxalate. The precipitate was freed from traces of sulphuric acid by ammonia solution, washed with distilled water and dried at 60°C. The dissolution percentage of calcium oxalate was evaluated by taking exactly 1 mg of calcium oxalate and 10 mg of the extract, packed it together in semipermeable membrane of egg as shown in the model designed given below. This was allowed to suspend in a conical flask containing 100 ml of 0.1 M Tris buffer (Figure 1).

First group served as blank containing only 1 mg of calcium oxalate. The second group served as positive control containing 1 mg of calcium oxalate and along with the 10 mg standard drugs, i.e. Neeri. The 3rd, 4th groups along with 1 mg of calcium oxalate contain methanolic and Methanol, extracts. The conical flasks of all groups were kept in an incubator preheated to 37°C for 2 h. Remove the contents of semipermeable membranes from each group into separate test tubes, add 2 ml of 1N sulphuricacid to each test tube and titrated with 0.9494 N KMnO₄ till a light pink colour end point obtained. The amount of remaining undissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning to know the total quantity of dissolved calcium oxalate by various solvent extracts [10].

RESULTS AND DISCUSSION
This study evaluates the antiurolithiatic activity of different extracts of *A. indica*. The highest percentage, i.e.97.9%of calcium oxalate (CaOx) dissolution was observed in both ethanol and aqueous extracts and lowest percentage, i.e. 81% of calcium oxalate dissolution was observed in case of standard drug. From this study, it was observed that both ethanol and aqueous extracts of *A. indica* showed highest dissolution of calcium oxalate. This study has given primary evidence for *A. indica* the plant which possess lithotriptic property (Table 1). This *in vitro* study has given lead data and shown that both ethanol and aqueous extracts are quite promising for further studies in this regard.

CONCLUSION
In vitro antiurolithiatic activity of medicinal plant *A. indica* has been performed by using the *in vitro* models. The Ethanol and aqueous extracts of *A. indica* showed maximum activity when compared to the standard drug, Neeri. Thus, it showed that plant extracts have lithotropic activity.

Table 1: Shows % dissolution of calcium oxalate (CaOx) by *in vitro* antiurolithiatic activity of *Acalfa indica* ethanol and aqueous extracts

<table>
<thead>
<tr>
<th>S. No.</th>
<th>% of dissolution of calcium oxalate</th>
<th>Acalfa indica</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Blank</td>
<td>0</td>
</tr>
<tr>
<td>2.</td>
<td>Positive control</td>
<td>81</td>
</tr>
<tr>
<td>3.</td>
<td>Ethanol extract</td>
<td>97.9</td>
</tr>
<tr>
<td>4.</td>
<td>Aqueous extract</td>
<td>97.9</td>
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Figure 1: *In vitro* anti lithiatic activity test by titrimetry
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