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Evaluation of *In Vitro* Anti Urolithiatic Activity of *Clerodendrum inerme*

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ABSTRACT

The present study explores that evaluation of *in vitro* antiurolithiatic activity of *Clerodendrum inerme*. It was observed that the highest calcium oxalate crystals dissolution was observed in the aqueous extract of *C. inerme*. It was found that aqueous extract of *C. inerme* has more efficient to dissolve calcium oxalate. In this study Neeri was used as standard drug.

KEYWORDS: *Clerodendrum inerme*, Urolithiasis and Aqueous extract

INTRODUCTION

Urinary stones Nephrolithiasis or urolithiasis, commonly known as kidney or renal stone, is a highly prevalent clinical problem that affects about 20% of the human population. A majority of urinary stones are composed of phosphates, oxalates, cystine, and uric acid. Almost 80% of these calculi are composed of calcium oxalate (CaOx). Urolithiasis -or else called Urinary Calculosis- is the formation of stones (calculi) anywhere in the urinary tract (in the renal pyelocalyceal system, ureter, bladder, urethra). It is the third most common urinary tract disease in humans, following urinary tract infections and prostatic diseases. According to localization, there is renal lithiasis (Nephrolithiasis), ureter lithiasis (Stones in the tubules through which urine flows from kidneys to the bladder), bladder lithiasis and urethral lithiasis (Stones in the tubule ejecting urine from the bladder outwards) [1]. Calcium-containing stones may be in the form of pure calcium oxalate (50%) or calcium phosphate (5%) and a mixture of both (45%) followed by magnesium phosphate [2]. Medicinal plants are always remained important source of drugs. Some medicinal plants and proprietary composite herbal preparations are reported to be effective in the treatment as well as prevention of recurrence of renal calculi with minimal side effects [3]. Calcium oxalate is one of the main constituents of deposits in urinary tract. Crystallisation of calcium oxalate is of particular interest not only from the theoretical point of view but also because of its biological importance. The exact mechanism of the initiation of the calcium oxalate stone formation is not completely understood. Factors leading to the nucleation, crystal growth and aggregation of various hydrates of calcium oxalate depend not only on the excess of calcium and oxalate concentrations but also on the presence of various foreign substances. A number of studies have been carried out to determine the effect of various additives such as metallic ions and their complexes.[4]Present day medical management of urolithiasis mainly involves endoscopic removal of stones and techniques such as Extracorporeal Shock Wave Lithotripsy (ESWL) has revolutionized the treatment of urolithiasis but do not prevent the likelihood of new stone formation [5]. They cause side effects such as haemorrhage, hypertension, tubular necrosis, subsequent fibrosis of the kidney and also increase in stone recurrence. Moreover, they are very costly [6]. Data from *in vitro* and *in vivo* clinical trials revealed that phyto-therapeutic agents could be useful as an alternative therapy in the management of urolithiasis. Medicinal plants and their products are more useful because they promote the repair mechanism in natural way. Pharmacological and phytochemical prospecting of medicinal plants based on traditional knowledge can lead to the discovery of new drug and development of pharmacologically important products for human health care. Green medicines were safe and more dependable than the costly synthetic drugs, many of which have side effects [7]. Though technological advancements have made dramatic improvement in the removal of urinary stones still some of the drawbacks of these methods exists which includes their being too costly for a common man and recurrence of stone formation along with a number of r0other side effects [8]. Strategies of dietary supplementation for preventing calcium oxalate stones formation Intake of oxalate-rich foods should be limited. Some items are oxalate rich in food such as spinach, rhubarb, beets, nuts, chocolate, tea, wheat bran, and strawberries which have been shown influence for raising oxalate levels and significant increase in urinary oxalate excretion [9].

Urinary stones are classified according to their chemical composition.

- ***Calcium-based stones.*** This is the most common type of stone occurring more frequently in men aged 20-30 years old. Calcium binds with other substances, such as oxalates and phosphorus, and finally the stone is formed.
- ***Cystine Stones.*** They occur in patients suffering from Cystinuria, a hereditary disease afflicting both genders.
- ***Struvite stones.*** They are usually formed in women suffering from urinary tract infections. These stones may become very large in size.
- ***Uric acid stones.*** They occur more frequently in men than women. Patients undergoing chemotherapy are a high risk group for developing uric acid stones.
- More rare types of stones are formed in patients taking drugs, such as Acyclovir, Indinavir and Triamterene.

What are the risk factors of Urolithiasis?

Related with various risk factors those have to do either with each person's temperament *(Endogenous risk factors)* or with the environment *(Exogenous risk factors).* Usually there is a combination of both endogenous and exogenous factors leading to the formation of stones.

Endogenous risk factors include:

- **Age -** Stones are more common at the age group 20-50 years old.
- **Gender -** Urolithiasis occurs 3 times more frequently in men than in women.
- **Family history/ Heredity -** About 25% of patients with urolithiasis report that there is or was another member of their family with the same problem.

Exogenous risk factors include

- **Climate conditions:** People living in warm climates are more likely to develop stones.
- **Nutrition:** High consumption of salt, animal albumen and fat is strongly associated with stone formation. Also, as opposed to what is widely believed, high calcium consumption (Dairy products) also favours the development of stones.
- **Obesity:** High body weight predisposes stone formation.
- **Fluid intake:** Low fluid consumption -particularly if it is less than 1.5 lt/day- increases the risk for the development of stones. Even water hardness is a factor related to high stone rate.
- **Vocation (job):** People with a more mental and sedentary vocation have higher risk for developing stones compared to those having a manual or more physical vocation.
- **Pharmacotherapy:** Specific antihypertensive, antacid agents and therapies for immunosuppressed patients (e.g. HIV patients) predispose the formation of stones.

Stone removal depends on the size and location of stone in the urinary tract. When the stone is less than 1cm in size and treated pharmaceutically, in 70% of cases the stone will automatically subside on its own within a period of 4-6 weeks. In the rest of the cases, there will be need for invasive therapy aiming at the fragmentation and removal of the stone/s.

Invasive methods include:

- **Extracorporeal Shock Wave Lithotripsy (ESWL):** The stone is broken with the use of shock waves. It is usually well tolerated by the patient and has 90% success rate. However, there are still some stones which cannot break with ESWL.
- **Ureteroscopy and Extracorporeal Lithotripsy:** It is used for removing stones located in the ureter. The ureter scope is inserted through the urethra into the ureter until localizing the stone and removing its fragments. The most modern method is Laser Lithotripsy.
- **Percutaneous Nephrolithotripsy (PNL):** It is applied for removing large or/and dense kidney stones that do not break with extracorporeal lithotripsy. The nephroscope is inserted through the skin into the kidney, localizes the stone and breaks it up with the use of special ultrasonic or ballistic devices.
- **Open Surgery:** Today it is used only for very few selected cases.

MATERIALS AND METHODS

Plant Materials

The leaves of *Clerodendrum inerme* collected from Khagazmaddur (Village), Narsapur (Mondel), Medak (District) of Telangana in the month of March 2019. The plant was authenticated by D. Venkateshwara Rao, Deputy Director, Telangana. Forest Academy, Dullapally, Hyderabad, Rangareddy District. The leaves were washed with tap water and dried under shade.

Preparation of Plant Extract

The leaves of plant were dried under shade and crushed in pulveriser and powdered. This powdered plant material was extracted with water by maceration process for 48 hours. After completion of the 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 ($KMnO_4$), Sulphuric acid (H_2SO_4).

Investigation of *In Vitro* Antiurolithiatic 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 H_2SO_4. Both were allowed to react in sufficient quantity of distilled water in a beaker, the resulting precipitate was CaOx. 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 10 mg of calcium oxalate and 100 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 0.1M Tris buffer. First group served as blank containing only 1 mg of CaOx. The second group served as positive control containing 1 mg of CaOx and along with the 10 mg standard drugs, i.e. Neeri. The 3rd group along with 10 mg of calcium oxalate contain aqueous 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 sulphuric acid to each test tube and titrated with 0.9494 N $KMnO_4$ till a light pink colour end point obtained. The amount of remaining un-dissolved 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 (Figures 1-1c).

RESULTS AND DISCUSSION

In the present study, Titrymetry method was used to assess the antiurolithiatic activity of aqueous extract of *C. inerme*. The dissolution percentage, i.e. 98% of CaOx dissolution was observed in aqueous extract (Table 1). From this study, it was observed that aqueous extract of *C. inerme* leaves showed antiurolithiatic activity. This study has given primary evidence for *C. inerme* the plants which possess lithotriptic property. This *in vitro* study has given lead data and shown that aqueous extract of *C. inerme* is quite promising for further studies in this regard.

Table 1: Shows % dissolution of calcium oxalate (CaOx) by *Clerodendrum inerme* leaves extracts

	% of Dissolution of calcium oxalate	
S. No.	**Groups**	***Clerodendrum inerme***
1	Blank	0
2	Positive control	81
3	Aqueous extract	98

Figure 1: *In vitro* experimental model setup to evaluate antiurolithiatic activity

Figure 1(a): Decalcification of egg shell in 10% Acetic acid overnight

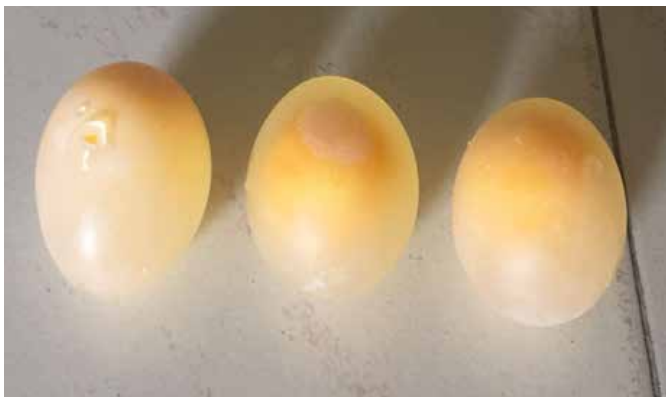

Figure 1(b): Decalcified Eggs

Figure 1(c): Egg membrane along with the contents suspended into the 0.1 M Tris buffer

CONCLUSION

In the present work, the dissolution of calcium oxalate crystals by aqueous extract of *C. inerme* was studied by using the standard drug, cystone. The work was performed by using *in vitro* antiurolithiatic

model for calculating percentage dissolution of kidney stone. This study has given primary evidence for *C. inerme* as the plant which possess antiurolithiatic property.

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