



EVALUATION OF IN VITRO ANTIUROLITHIATIC ACTIVITY OF TRIDAX PROCUMBENS

Pharma

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ABSTRACT

The present study was undertaken to evaluate the in vitro antiurolithiatic activity of the medicinal plant Tridax procumbens. Both Methanolic and Aqueous extracts showed their maximum efficiencies in the dissolution of calcium oxalate crystals. Aqueous extract was even more efficient than methanolic extract in dissolution of calcium oxalate crystals. Our results have clearly indicated that the aqueous and methanolic extracts of Tridax procumbens were quite promising for further studies in this regard. In this study Cystone was used as standard drug.

KEYWORDS

In vitro antiurolithiatic activity, aqueous extract, urolithiasis, Tridax procumbens.

1. INTRODUCTION:

Plants provide food, raw materials for medicine and various other requirements for the very existence of life from the origin of human beings¹. Even the current conventional medicine is using a lot of plant derived chemicals as therapeutic agents. The overuse of synthetic drugs results in higher incidence of adverse drug reactions has motivated humans to return to nature for safe remedies. Herbs and herbal drugs have created interest among the people by its clinically proven effects². Therefore, there is a compelling need for detailed scientific validation of all traditional medicinal plant drugs to establish their efficacy and safety in light of modern science¹.

Kidney stone disease is a multi-factorial disorder resulting from the combined influence of epidemiological, biochemical and genetic risk factors³. Urolithiasis is considered as the third most common affliction of the urinary tract. It refers to the solid non-metallic minerals in the urinary tract. It is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidney. The formation of kidney stones involves several phytochemical events beginning with crystal nucleation, aggregation and end with retention within the urinary tract. Among the several types of kidney stones, the most common are calcium oxalate stones representing upto 80% of the analyzed stones⁴. 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(15-20%), uric acid(10%) and cystine(1%)⁵.

It is estimated that at least 10% of the population in the industrialized part of the world is afflicted by urinary tract diseases and among these kidney stones are common with an annual incidence of 0.5-1.9%. About 12% of the population of India is expected to have urinary stones and out of that 50% of cases encounter loss of one or both kidneys with or without renal damage upto some extent².

Stone disease is 2-3 times more common in males, than in females. It has a recurrence rate of 70-81% in males and 47-60% in females⁵. In spite of substantial progress in pathophysiology and treatment of urolithiasis, there is no satisfactory drug being used in clinical therapy. Kidney dialysis, endoscopic stone removal and extra corporeal shock wave lithotripsy are prohibitively costly and recurrence is quite common with these procedures¹.

Data from in vitro and in vivo clinical trials revealed that phytotherapeutic agents could be useful as alternative therapy in the management of urolithiasis. Medicinal plants and their products are more useful, because they promote the repair mechanism in natural way¹. 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 care⁶. Green medicines were safe and more dependable than the costly synthetic drugs, many of which have side effects⁷.

The selected plant Tridax procumbens have occupied an important place in Indian culture and folk medicines. This plant have been extensively in ayurvedic system of medicine and is used throughout India. It is used in Ayurvedic medicine for liver disorders, hepatoprotection, gastritis and heatburn⁸. The plant shows various pharmacological activities like immunomodulatory, Anti-diabetic, Anti-hepatotoxic, Anti-oxidant, Anti-inflammatory, Analgesic etc⁹.

2. MATERIALS AND METHODS

Tridax procumbens was collected in the month of august 2017 from Jogipet vilage, Medak dist of Telangana, India. The whole plant was shade dried and powdered. The crude plant extract was prepared by Soxhlet extraction method. 50g of powdered plant material was extracted with 500ml of solvents methanol and water individually. The process of extraction was carried out up to 6 cycles, till the solvent in siphon tube of an extractor became colourless. The two extracts were filtered separately, the filtrates were placed in a beaker for evaporation. Further the dried extracts were maintained in a refrigerator at 4°C for further antiurolithiatic activity.

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 1mg of calcium oxalate and 10mg 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 1mg of calcium oxalate. The second group served as positive control containing 1mg of calcium oxalate, along with 10 mg standard drug, i.e. cystone. The 3rd and 4th groups along with 1mg of calcium oxalate contain 10mg of aqueous and methanolic extracts. The conical flasks of all groups were kept in an incubator preheated to 37°C for 2hrs. 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 KMnO₄ till a light pink colour end point is 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.

3. RESULTS AND DISCUSSION

Drug therapy has developed in response to population health care needs¹⁰. There are many crucial areas in medicine such as liver diseases, arthritis, old age related problems, certain viral infections and cancer where the conventional medicine is devoid of satisfactory treatment. These are among the promising areas of research and

development of medicines from the vast highly potential plant resources. Plants are also attractive sources for the development of novel and very effective and safe therapeutic agents against kidney procumbens¹¹. Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects¹¹. Unlike allopathic medicines which target is only one aspect of urolithiatic pathophysiology, most of plant based therapy have been shown to be effective at different stages of stone pathophysiology¹². About 80% of the world population rely on the use of traditional medicine which is predominantly based on plant material¹³. Plant based drug discovery programmes continue to provide an important source of new drug leads¹⁴.

Lithiasis (stone formation) is an important cause for acute and chronic renal failure, includes both nephrolithiasis (stone formation in kidney) and urolithiasis (stone formation in ureter or bladder or both). Among the various kinds of stones identified, calcium stones occur mainly in men, while phosphate stones formation is more in women¹⁵.

This study evaluates the antiurolithiatic activity of Methanolic and Aqueous extract of *Tridax procumbens*. The highest percentage i.e. 98% of calcium oxalate {CaOx} dissolution was observed in Aqueous extract followed by Methanolic extract which had a percentage dissolution of calcium oxalate was 95%. Both Methanolic and Aqueous extracts of *Tridax procumbens* were found to be more effective in dissolution of calcium oxalate.

From this study, it was observed that aqueous and methanolic extracts of *Tridax procumbens* showed their highest dissolution of calcium oxalate. Aqueous extract was found to be even more effective than methanolic extract in dissolution of calcium oxalate. This study has given primary evidence for *Tridax procumbens* as the plant which possess lithotriptic property. This in vitro study has given lead data and shown that aqueous and methanolic extracts are quite promising for further studies in this regard.

Table 1: Shows % dissolution of calcium oxalate (CaOx) by in vitro antiurolithiatic activity of *Acacia farnesiana* leaves extracts.

Sl.No.	% of dissolution of calcium oxalate	
	Groups	Tridax procumbens
1.	Blank	0
2.	Positive control	81
3.	Methanol extract	95
4.	Aqueous extract	98



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



Fig 1(b): Wasing of the left over CaOX in the egg membrane and Titration with 0.9494 N KMnO4.

4. CONCLUSION

In vitro urolithiasis has been performed on the selected plant *Tridax*

procumbens 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 *Tridax procumbens* as the plant which possess antiurolithiatic property.

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