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Anti-Urolithiatic Activity of *Dolichos Biflorus* Seeds

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A kidney stone, also known as a renal calculus is a solid concretion or crystal aggregation formed in the kidneys from dietary minerals in the urine. Urolithiasis is a complex process that occurs from series of several physicochemical event including super-saturation, nucleation, growth, aggregation and retention within the kidneys. Data from in-vitro, in- vivo and clinical trials reveal that phytotherapeutic agents could be useful as either alternative or an adjunct therapy in the management of Urolithiasis. Medicinal plants / natural products are more useful for body because they promote the repair mechanism in natural way. Various plant species of *Dolichos biflorus*, have been reported to posses antiurolithiatic property. In this study aqueous, chloroform, benzene extracts of *Dolichos biflorus*.Linn and standard for dissolving kidney stones- calcium oxalate by an in-vitro model. To check their potential to dissolve experimentally prepared kidney stones- calcium oxalate by an in-vitro model for *Dolichos biflorus* seeds and cystone as a standard compound collected from market . Phenolic compound isolated from the benzene and aqueous, flavanoids and steroids from aqueous fraction of the seed. Aqueous fractions showed highest dissolution of stones as compare to others. Aqueous fraction was more effective in dissolving calcium oxalate (48.5±0.022%). Reference standard-formulation Cystone was found to be more effective (53.5±0.02 %) when compared to phenolic and flavanoids fraction.

Keyword: *Dolichos biflorus*, Polyherbal Formulation, Kidney Stones, Urolithiatic, Calcium Oxalate.

1. Introduction

Kidney stones are hard, solid particles that form in the urinary tract. In many cases, the stones are very small and can pass out of the body without any problems. However, if a stone (even a small one) blocks the flow of urine, excruciating pain may result, and prompt medical treatment may be needed. Recurrent stone formation is a common part of the medical care of patients with stone disease. Calcium- containing stones, especially calcium oxalate monohydrate, calcium oxalate dihydrate and basic calcium phosphate are the most commonly occurring ones 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% ^[1].

In most of the cases the commonly occurring stones are calcium oxalate or magnesium ammonium phosphate type. Helps in spontaneous passage of calculi by increasing urine volume, pH and anti-calcifying activity. Balance the Inhibitor and promoter of the crystallization in urine and affects the crystal nucleation, aggregation and growth (Crystallization inhibition activity). Relieves the binding mucin of calculi (lithotriptic activity) Improved renal function ^[2]. Herbs and herbal drugs have efficient pharmacological action and potent effects on body. Also, the overuse of synthetic drugs, which results in higher incidence of adverse drug reactions, has motivated humans to return to nature for safe remedies. The concept of 'Traditional' medicines for the developing countries. The problem of

urinary stones or calculi is a very ancient one. The incidence of urolithiasis is very common in Northern India compared to southern state [3].

Dolichos biflorus.Linn or *Vigna unguiculata* [L.] *Papilionaceae* (Fabaceae) is a branched, sub-erect and downing herb which belongs from fabaceae family. And it is native to most parts of India and is found up to altitudes of 1000 m. It is also known as kulthi beans or horsegram seeds. In Ayurveda, the seed is used in the treatment of piles, pain, constipation, wounds, urinary calculi, cough, edema, asthma etc [4]. It is also beneficial in enlarged liver and spleen. The seeds of herb have been reported to possess antilithiatic, antihepatotoxic and hypolipidemic activity and involved in lowering the level of blood sugar and total cholesterol [5, 6, 7, 8, 9, 10]. Traditional uses of *Dolichos biflorus*.L is also given into Ayurvedic books. The study have been undertaken to evaluate *Dolichos biflorus*.Linn different seeds extracts and cystone as a standard for their possible potential to dissolve experimental kidney stone using a modified in vitro model to isolate the chemical constituent responsible for the activity.

2. Materials and Methods

2.1 Plant Material

Dolichos biflorus.Linn seeds were collected from G.Y Hakim shop, Vadodara. It grows into an herbal garden in our college, Sigma Institute of Pharmacy and takes for my proposed work. The plant was identified and authenticated by Dr. Padamnabhi Nagar. Taxonomist, M.S. University, Herbarium number SIP/MPH-PCG/2013/01 was deposited in Sigma Institute of pharmacy, Vadodara. Voucher specimen was dried in shade and stored in air tight container at 25°C for further study.

2.2 Extraction and Isolation

The seeds are pulverised and about 60 gms of powder was extracted with chloroform and aqueous in soxhlet, also extracted successively with acetone, benzene, petroleum ether, Alcohol [11]. All extracts were concentrated on a water bath and residue was dried in a desiccator.

All the prepared extracts were subjected to qualitative chemical tests to detect the presence of different classes of phytoconstituents. TLC studies were done for identifying the presence of constituents which are detected in chemical tests and to know how many extracts are present in each extracts [12,13]. This separated parameter is subjected for physical, chemical and spectral study (UV). And positive results for two fractions were taken for pharmacological evaluation.

2.3 Evaluation for Anti-urolithiatic Activity [14]

Step-1: Preparation of experimental kidney stones (Calcium oxalate stones) by homogenous precipitation: Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Sodium oxalate (AR) in 10ml of 2N H₂SO₄ were allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium oxalate. Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Disodium hydrogen phosphate (AR) in 10ml of (2N H₂SO₄), was allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium phosphate. Both precipitates freed from traces of sulphuric acid by Ammonia solution. Washed with distilled water and dried at 60 °C for 4 hours.

Step -2: Preparation of semi-permeable membrane from farm eggs: The semi - permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin & yolk. Shell was removed chemically by placing the eggs in 2M HCl for an overnight, which caused complete decalcification. Further, washed with distilled water, and carefully with a sharp pointer a hole is made on the top and the contents squeezed out completely from the decalcified egg. Then egg membrane washed thoroughly with distilled water, and placed it in ammonia solution, in the moistened condition for a while & rinsed it with distilled water. Stored in refrigerator at a pH of 7- 7.4.

Step-3: Estimation of Calcium oxalate by Titrimetry: Weighed exactly 1mg of the calcium

oxalate and 10mg of the extract/compound/standard and packed it together in semi evaluation. Permeable membrane by suturing as shown in Model design Figure. This was allowed to suspend in a conical flask containing 100ml 0.1 M TRIS buffer. One group served as negative control (contained only 1mg of calcium oxalate). Placed the conical flask of all groups in an incubator, preheated to 37 °C for 2 hours, for about 7-8 hours. Removed the contents of semi-permeable membrane from each group

into a test tube. Added 2 ml of 1 N sulphuric acid and titrated with 0.9494 N KMnO_4 till a light pink colour end point obtained. 1ml of 0.9494 N KMnO_4 equivalent to 0.1898mg of 4 Calcium.

The amount of undissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate actually test substance(s) could dissolve.



Fig 1: *In vitro* Experimental model set-up to evaluate anti-urolithiatic activity

3. Results

Qualitative chemical tests indicated the presence of phenolic compounds, flavnoids, steroids and Saponin in different extracts of *Dolichos biflorus*.Linn. On basis of this fraction we

performed *in vitro* Anti-Urolithiatic Activity by comparing different extracts of *Dolichos biflorus* with standard. % Dissolution of Calcium oxalate table is given below:

Table : 1.1 % Dissolution of Calcium oxalate

SR. NO.	Group	% Dissolution Calcium Oxalate
1	Blank	0%
2	Chloroform extract	42± 0.024776781
3	Aqueous extract	48.5±0.022173558
4	Benzene	44±0.032015621
5	Standard (Cystone)	53.5±0.038297084

These graphical represent shows % Dissolution of calcium oxalate by *in vitro* Anti-Urolithiatic Activity of extracted fraction of *Dolichos biflorus*.L drug. An aqueous extract at 10mg concentration produced higher dissolution of calcium oxalate as compare to other fraction. Standard shows higher dissolution as compare to others.

4. Discussion

This study evaluate that antiurolithiatic activity of different extracts of *Dolichos biflorus*.L seeds and isolated phenolic compound, steroidal compound . The study of the urinary chemistry with respect to the stone-forming minerals will provide a good indication of the risk of stone formation. From the study results it is observed that aqueous fraction show highest dissolution of

calcium oxalate in comparison to other fractions. This study has given primary evidence for *Dolichos biflorus*.L. as plant which possess lithotriptic property. This in vitro study has given

lead data, and shown that phenolics and steroids form aqueous fraction is quite promising for further work in this regard.

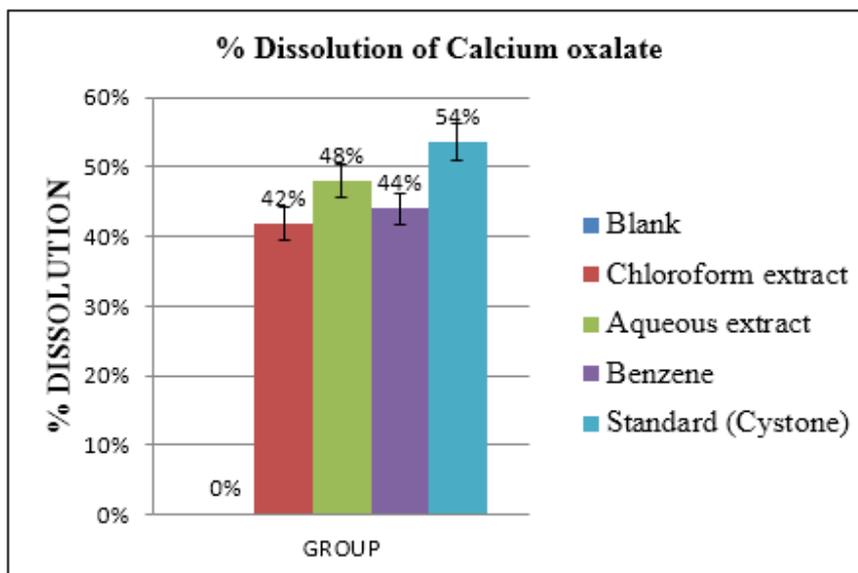


Fig 2: 1.2 % Graphical representation of dissolution of Calcium oxalate

5. Conclusion

Urolithiasis has been performed for *Dolichos biflorus*. Linn and two marketed polyherbal formulation Ural Tablet and Yastone Tablet sample purchased from ayurvedic industries Vasu Health Care, Vadodara and Yash Remedies, Ahmedabad. And perform work by using In Vitro Antilithiatic Model for calculating % dissolution of kidney stone. And aqueous extract is giving higher % dissolution of calcium oxalate crystal.

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