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EVALUATION OF ANTI–UROLITHIATIC ACTIVITY OF *COSTUS IGNEUS AND DOLICHOS BIFLORUS* LINN (HORSE GRAM) SEEDS.

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ABSTRACT The present study was undertaken to evaluate the *in vitro* antiurolithiatic activity of the *Costus igneus and Dolichos biflorus* Linn (Horse gram) seeds. Both alcoholic and Aqueous extracts of *Dolichos biflorus* showed their maximum efficiencies in the dissolution of calcium oxalate crystals than *Costus igneus* extracts. Alcoholic extracts of both the plants were even more efficient than aqueous extracts in dissolution of calcium oxalate crystals. Our results have clearly indicated that the aqueous and alcoholic extracts of *Dolichos biflorus* were quite promising for further studies in this regard. In this study Cystone was used as standard drug.

KEYWORDS : *In vitro* antiurolithiatic activity, aqueous extracts, urolithiasis, *Costus igneus Dolichos biflorus.*

INTRODUCTION

Urolithiasis is characterized by the formation of stone in the kidneys or urinary tract. Nowadays stone formation is the oldest and serious painful urologic disease with significant prevalence in the population due to change in lifestyle and dietary factors. Kidney Stone is a painful disease. It is usually found in individuals aged 30 to 60 years and four times more in men as compared to women. A large number of people, nearly 4-15% of the human populations suffer from urinary stone problem all over the globe (Khare et al., 2014). In India, 12% of the population is expected to have urinary stones, out of which 50% may end up with loss of kidneys or renal damage. In India the "stones belt" occupies parts of Maharashtra, Gujrat, Punjab, Haryana, Delhi and Rajasthan. The incidence of urolithiasis is very common in Northern India compared to southern state (Tiwari et al., 2012). Kidney stones are hard, solid particles that form in the urinary tract. The occurrence of moderately large urinary stones may cause severe pain, urinary tract obstruction and infection and can lead to complete renal damage. The formation of stones in the kidneys or urinary tract is not an uncommon disorder. The stones are formed from the chemicals usually found in the urine such as uric acid, phosphorus, calcium, and oxalic acid. Most kidney stones are composed either of calcium oxalate or phosphate, the latter being most common in the presence of infection. About ninety per cent of all stones contain calcium as the chief constituent.

The formation of stones in the kidneys is the result of defects in the general metabolism. They usually occur when the urine becomes highly concentrated due to heavy perspiration or insufficient intake of fluids. They are aggravated by a sedentary life-style. The other causes are a wrong diet, excess Intake of acid-forming foods, white flour and sugar products, meat, tea, coffee, condiments and spices, rich foods, and overeating. Lack of vitamin A and an excessive intake of vitamin D may also lead to formation of stones. Urinary calculi may cause obstruction, hydronephrosis, infection and hemorrhage in the urinary tract system. Surgical operation, lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. However; these treatments are relatively costly, painful and require expert hands with availability of appropriate equipments.

Management of stone disease depends on the size and location of the stones. Stones larger than 5mm or stones failed to pass through should be treated by some interventional procedures such as Extracorporial Shock Wave Lithotripsy (ESWL), Ureteroscopy (URS) or percutaneous nephrolithotomy (PNL). All these treatment options are, for stones found anywhere in the urinary tract but unfortunately there is no treatment option for stones found in gall bladder, the only option being removal of gall bladder. Unfortunately, the propensity for stone recurrence is not altered by removal of stones with ESWL and stone recurrence is still 50%. In addition, ESWL might show some significant side effects such as renal damage. However, these treatment options are costly and recurrence is quite common (Velpandian et al., 2012, Anupama and Handa, 1990). During the past decades, the use of herbal medicines continues to expand rapidly across the world. Many people now take herbal medicines or herbal products for their healthcare.

Now-a-days, however herbal medicines has gained much popularity because, herbal medicines are more effective, have less side effects and reduce recurrence rate of stone formation, hence search for antilithiatic drug from natural sources has assumed greater importance and is promising. In Ayurveda, many plants having the property of disintegrating and dissolving the stone are referred to as "Pashanbheda" (Agarwal and Varma, 2014). Herbal medicines have many phytoconstituents which may exert their beneficial effect in kidney stone treatment. Plant extracts contain phytochemicals that inhibit stone formation by inhibiting synthesis and agglomeration of crystals (Bhattacharjee et al., 2012).

Costus igneus commonly known as Spiral flag, is a member of Costaceae and a newly introduced plant in India from South and Central America. It is a perennial, upright, spreading plant reaching about two feet tall, with spirally arranged leaves and attractive flowers. In southern India, it usually grows as an ornamental plant and its leaves are used as a dietary supplement in the treatment of diabetes mellitus. Recently, a number of researches have been carried out to evaluate the anti-diabetic potential of this plant. Besides, it has been proven to possess various pharmacological activities like hypolipidemic, diuretic, antioxidant, anti-microbial, anti-cancerous.

Macrotyloma uniflorum Lam (Dolichos biflorus Linn) (family leguminoseae) commonly known as "horse gram" is grown mostly under dry land agriculture. Dolichos biflorus Linn is a type of lentil used in Indian and Asian cuisine. In Ayurveda medicine, it is known as "Kulthi". It occurs all over India up to an altitude of 5000 feet. It is used in various applications to treat digestive disorders, control blood sugar level and even to treat obesity and diabetes. It is a good herbal remedy for kidney stones. It is similar to urad. Its color is red. It can be given to the patient by making pulse. It is known as killer of stone. It is very useful for kidney stones and gall bladder stones. According to Ayurveda it has vitamin A. It fulfills the requirement of vitamin A in the body and prevents kidney stones. It is available in any grocery store. Therefore in the present study, an effort has been made to evaluate the Costus igneus and Dolichos biflorus. Linn (Horse gram) seeds extract for their possible potential to dissolve experimental kidney stone using a modified in vitro model.

MATERIALS AND METHOD

Fresh leaves of Costus igneus plant were collected from the garden and Dolichos biflorus linn. (Horse gram) seeds were bought from the local market. Shade dried leaves and Horse gram seeds were pulverized and about 150 gms of powder was extracted with water as well as 95% ethanol in a soxhlet.

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 H_2SO_4 were allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium oxalate. Both precipitates freed from traces of sulphuric acid by Ammonia solution. Washed with distilled water and dried at 60 0C for 4 hours.

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 for 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. All the content were squeezed out completely and placed in ammonia solution a while and then rinsed it with distilled water and stored in a refrigerator at a pH of 7.4

Estimation of Calcium oxalate by titrimetry.

10mg of calcium oxalate and 10mg of the extract/compound/standard and packed it together in semi permeable membrane by suturing. This was allowed to suspend in a conical flask containing 100ml 0.1 M TRIS buffer. One group served as negative control Blank (contained only 10mg of calcium oxalate). 500mg tablet of Cystone was placed in absolute ethanol for removing colour coating and 400mg was obtained. Cystone tablet was crushed into powder form and dispersed into 100ml of distilled water and filtered. Filtrate of Cystone was used as positive control (Standard) for in vitro anti-urolithiatic activity.

All the conical flasks of all groups were placed in an incubator, preheated to 37 0C for 2 hours, for about 7-8 hours.

Removed the contents of semi-permeable membrane from each group in a test tube, 2 ml 1N sulphuric acid added and titrated with 0.9404 N $KMnO_4$ till a light pink colour end point.

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

$$\text{Calcium content} = \frac{A \times N \times 2.004}{W}$$

Where A= Vol. in ml of std. $KMnO_4$ solution required
N = Normality of $KMnO_4$
W= weight of Ca in the sample

Weight of Ca reduced = (Wt. of Ca++ estimated)control – (Wt. of Ca++ estimated)test

$$\% \text{ dissolution} = \frac{\text{Wt. of Ca++ reduced}}{\text{Wt. of Ca++estimated control}} \times 100$$

RESULTS:

The nature and yield of successive solvent extracts were recorded and are given in Table 1. The preliminary screening for antiurolithiatic activity by using SPM of eggs was carried out on all the extracts accordingly. The percentage dissolution of Ca-Ox by the successive solvent extracts were found to be 60 %, 30%, 30%, 50%, 40%, for standard Cystone, alcoholic and aqueous extracts of Costus igneous and Dolichos biflorus respectively. The highest % dissolution of Ca i.e. 60% was observed with standard drug Cystone. Among all other extracts, the highest percentage of Ca dissolution i.e. 50% was observed in alcoholic extract of Dolichos biflorus followed by aqueous extract of it. Both the aqueous as well as alcoholic extracts of Costus igneous had shown 30% of Ca dissolution.

Table 1 : Calcium oxalate dissolution by Cystone, alcoholic and aqueous extracts

Group	Vol. of standard $KMnO_4$	Wt. of calcium estimated	Wt. of calcium reduced	% Dissolution
Control	1ml	0.190mg	---	---
Standard (Cystone)	0.4ml	0.076mg	0.114mg	60%
Alcoholic extract of Costus igneus	0.7 ml	0.133mg	0.057mg	30%
Aqueous extract of Costus igneus	0.7 ml	0.133mg	0.057mg	30%
Alcoholic extract of Dolichos biflorus	0.5ml	0.095mg	0.095mg	50%
Aqueous extract of Dolichos biflorus	0.6ml	0.114mg	0.076mg	40%

DISCUSSION

In India, phytotherapy is a primary health care, because from ancient time itself we are using plants as a medicine, it provides apparently effective remedies for many diseases and also pharmaceutical products are expensive compared to folk medicines (Atmani and Khan, 2000). Large numbers of plant products already exist in market as a prophylactic or curative agent. Development of standard drugs has some drawback because kidney stone disease is multifactorial in nature and it has different chemical composition (Butterweck and Khan 2009).

If the stone forming components exceeds their limit it leads to the formation of stone. Urolithiasis is the third most common disorder of the urinary tract, the others being frequently occurring urinary tract infections and benign prostatic hyperplasia/ prostate diseases. Since the main intention of treatment is enhancing excretion of stone forming constituent elements and altering the physico-chemical environment which influence formation, many drugs are lined up in the market. With current therapy the risks of reoccurrence are too high. Thus there is retrieval of interest towards safer plant drugs.

The present study on the in-vitro antiurolithiatic activity of Costus igneous and Dolichos biflorus (Horse gram seeds) shows that there is greater dissolution of Ca-Ox by the alcholic extract of Horse gram seeds than the other extracts. This study evaluates the antiurolithiatic activity of Ethanolic and Aqueous extracts of Costus igneous and Dolichos biflorus. Both Ethanolic and Aqueous extracts of Dolichos biflorus were found to be more effective in dissolution of calcium oxalate. Ethanolic extract was found to be even more effective than Aqueous extract in dissolution of calcium oxalate. This study has given the evidence for Dolichos biflorus as the plant which possess lithotriptic property. This in-vitro study has given lead data and shown that aqueous and ethanolic extracts are quite promising for further studies in this regard.

CONCLUSION:

In-vitro urolithiasis has been performed on the selected plant Costus igneous and Dolichos biflorus by using the standard drug, Cystone. The work was performed by using in-vitro antiurolithiatic model for calculating percentage dissolution of kidney stone. Ethanolic extract of Dolichos biflorus shows highest dissolution than the extract of leaves of Costus igneous. This study has given primary evidence for Dolichos biflorus as the plant which possess antiurolithiatic property.

REFERENCES:

1. Agarwal K and Varma R. (2014). Ocimum gratissimum L.: A Medicinal Plant with Promising Antiurolithiatic Activity. Int. J. Pharmaceut. Sci. Drug Res 6(1): 78-81.
2. Anupama, S. and Handa, S.S. (1990). Hepatoprotective activityof andrographolide from Andrographis paniculata against CCl4. Indian J. Med. Res.92,4, 276 - 283.
3. Atmani F, Khan S. R. (2000). Effects of an extract from Herniaria hirsuta on calcium

oxalate crystallization in vitro, Bri. J. Uro. Int.; 85: 621-625.
4. Bhattacharjee A., Shashidhara S.C. and Aswathanarayana (2012). Phytochemical and ethno-pharmacological profile of Cratraeva nurvala Buch-Hum (Varuna): A review Asian Pac. J. Trop. Biomed. 45: 1162
5. Butterweck, V. and Khan, S. (2009). Herbal medicine in the management of urolithiasis: alternative or complementary, Planta. Med.; 75: 1095-1103.
6. Khare, P., Mishra, V.K., Kakkar, A., Bais, N., Singh, R. (2014). Study on in-vitro anti-lithiatic activity of Phyllanthus niruri Linn. Leaves by Homogenous precipitation and turbiditory method. International Journal of Pharmacy and Pharmaceutical Sciences, 6(4): 124-127.
7. Tiwari A., Soni V. and Londhe V. (2012). An overview on potent indigenous herbs for urinary tract infirmity: urolithiasis. Asian Journal of Pharmaceutical and Clinical Research; 5: Suppl. 1.
8. Velpandian, S. S., Rajaprehidha, V., Bhaumathi, J., Anbu andAswini, A. (2012) Effect of Vediyuppu Cheyaneer inethylene glycol induced hyperoxaluria model in rats. Int. J. Life Sci. Pharma. Res.2, 63 - 67