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IN-VITRO ANTI-UROLITHIATIC ACTIVITY OF *COMMELINA BENGHALENSIS* LINN

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ABSTRACT

Urolithiasis is one of the common condition, that the calculi are formed or located in the urinary system. It is a process of formation of the stone in kidney, bladder, ureter and also in the gall bladder. Since it is a multidactorial disease, its etiology is very complex and highly unpredictable. The urinary stone is an aggregation of solute materials from urine such as calcium oxalate, calcium phosphate, and uric acid. A wide range of population depends on traditional system of medicine. Number of plants is used in traditional system of medicine to treat many diseases. The remedy from herbal source that offer better protection and decreased relapse, because they promote the repair mechanism in natural way. Therefore investigation of a newherbal remedy for urolithiasis from mother natureis the goal of this study. After survey, *Commelina benghalensis* plant was selected for the present study. Aqueous extract of leaf of the plant showed good anti-urolithiatic activity when compare to the standard cystone.

KEYWORDS

Commelina benghalensis plant, Anti-urolithiatic activity and Standard cystone.

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INTRODUCTON

Obstruction to the urine out flow in the urinary tract increases the susceptibility to urinary stones and urinary tract infection. If the obstruction is complete and bilateral, acute renal failure may result. If the obstruction is relieved quickly, renal function returns to normal within days. On the other hand, if the obstruction is partial and prolonged, chronic renal failure may occur, which is irreversible and ends in uremia.

In childhood, urinary tract obstruction is usually caused by a congenital anatomical defect in the urinary tract. In adults, acquired intrinsic causes

include benign prostatic hyperplasia, cancer prostate, renal stone and stricture urethra[1]. Urolithiasis is a calculus formation at any level in the urinary collecting system, but most often the calculi arise in the kidney. They occur frequently, as evidenced by the finding of stones in about 1% of all autopsies. Symptomatic urolithiasis is more common in males. A familial tendency toward stone formation has long been recognized.

About 75% renal stones are composed of either calcium oxalate or calcium oxalate mixed with calcium phosphate. Another 15% are composed of magnesium ammonium phosphate, and 10% are either uric acid or cystine stones. In all cases there is an organic matrix of mucoprotein that makes up about 2.5% of the stone by weight. The cause of stone formation is often obscure, particularly in the case of calcium containing stones. Probably involved is a confluence of pre disposing conditions. The most important cause is increased urine concentration of the stones constituents, so that it exceeds their solubility in urine (super saturation). 50% of the patients who develop calcium stones have hypercalciuria that is not associated with hypercalcemia. Most in this group absorb calcium from the gut in excessive amounts (absorptive hypercalciuria) and promptly excrete it in the urine, and some have a primary renal defect of calcium reabsorption (renal hypercalciuria). In 5% to 10% of patients there is hypercalcemia (due to hyperparathyroidism, vitamin D intoxication, or sarcoidosis) and consequent hypercalciuria. In 20% of this sub group there is excessive excretion of uric acid in the urine, which favours calcium stone formation; presumably the urates provide a nidus for calcium deposition. In 5% there is hyperoxaluria or hypercitraturia, and in the remainder there is no known metabolic abnormality[2].

Commelina benghalensis Linn.is a plant belonging to the family *Commelinaceae.* It is an erect, rarely climbing, very rarely shrub found growing as a weed in moist regions of tropical Asia, Africa, Celon, China and throughout India. The plant generally attains a height of about 60 - 90 cm, flowering and fruiting takes place during the winter season, the flowers are bluish violet in colour. The rhizomes are starchy and mucilaginous. It was reported to be effective for night blindness, cataract, conjunctivitis, eye sores, improvement of eye sight and also used as emollient, demulcent, diuretic, antileprotic, refrigerant and laxative. The plant contain n-octacosanol, stigmasterol and campestrol[3,4].

MATERIAL AND METHODS

The Plant Material

Commelina benghalensis Linn. Leaves were collected from the moist regions of Kanyakumari district, Tamil Nadu in the month of July. The botanical identity has been confirmed by Research Officer-Botany, Central Council for research in Ayurveda and Siddha, Tirunelveli, Tamil Nadu. The voucher specimen is preserved in our laboratory for future reference.

Extraction

The leaves were air dried, pulverised and extracted separately with ethanol and water. Both the extracts were concentrated in water bath and stored in desiccator for anti-urolithiatic experiment[5,6].

Anti-urolithiatic activity

Anti-urolithiatic activity of aqueous and alcoholic extracts of leaves of *Commelina benghalensis* Linn.was screened by titrimetric and spectroscopic in-vitro models.

Preparation of molybdate-sulphuric acid reagent

Molybdate-sulphuric acid reagent was prepared by 5% w/v of sodium molybdate solution, 13 ml of conc. H_2SO_4 in 80 ml of distilled water. Finally, volume was adjusted to 100 ml with distilled water.

Preparation of reducing solution

1gm of p-phenylene diamine was dissolved in 100 ml of 3% w/v of sodium meta-bisulphite solution.

Preparation of artificial kidney stones

Usually, the human kidney stone is an aggregation of solute materials from urine such as calcium oxalate, calcium phosphate and uric acid. The kidney stones required for the present study was prepared artificially by homogenous precipitation method.

Preparation of Calcium oxalate stones

Equimolar solution of calcium chloride dihydrate in distilled water and sodium oxalate in 10 ml of 2N

Sulphuric acid were allowed to react in sufficient quantity of distilled water in a beaker to produce precipitate of Calcium oxalate stones.

Preparation of Calcium phosphate stones

Equimolar solution of calcium chloride dihydrate in distilled water and Disodium hydrogen phosphate in 10 ml of 2N Sulphuric acid were allowed to react in sufficient quantity of distilled water in a beaker to result precipitate of Calcium phosphate stones. Both the precipitates (stones) were freed from traces of sulphuric acid by the treatment of ammonia solution, washed with distilled water and dried at a temperature not exceeding 60ºC for four hours[7,8].

Preparation of semi-permeable membrane

The semi-permeable membrane was prepared with the aid of farm eggs. The eggs were placed in 2 M hydrochloric acid for an overnight, during which period the outer shell of the eggs were removed chemically, which caused complete decalcification. Further, the membrane layered eggs were washed with distilled water. A hole was made on the top and the contents were squeezed out completely from the decalcified eggs carefully with a sharp pointer. Again wash thoroughly with distilled water and placed it in ammonia solution, in the moistened condition for a while and rinsed it with distilled water and stored in refrigerator at a Ph of 7-7.4[9].

Estimation of calcium oxalate (Titrimetric method)

Weighed accurately about one milligram of calcium oxalate and 10 mg of the extract/reference standard and packed it together in semi permeable membrane pouch by suture. This was allowed to suspend in a conical flask containing 0.1 M TRISS buffer. One group served as negative control, which contain only calcium oxalate. All the groups were kept in incubator (pre heated to 37ºC for 2 hours) for about eight hours.

The contents of semi permeable membrane pouches were transferred to test tubes. Each test tube was added with 2 ml of 1N sulphuric acid and titrated against 0.9494 N $KMNO_4$. Light pink colour was obtained as end point. Each ml of 0.9494 N $KMNO_4$ is equivalent to 0.1898 mg of Calcium.

Estimation of calcium Phosphate (Colorimetric Method)

Follow the procedure as above up to incubation. Transfer the contents of each group into the test tubes. Each test tube was added with 2 ml of 1 N Sulphuric acid, 2.5 ml of Molybdicsulphuric acid reagent, 1 ml of reducing solution and made up the volume to 10 ml using distilled water. Standard solutions of Calcium phosphate was prepared by adding 2.5 ml of Molybdicsulphuric acid, 1 ml of Reducing solution and made the volume to 10 ml with distilled water. Absorbance was measured for groups and standard dilutions (200, 400, 600, 800 and 1000µg/ml) using colorimeter at 750 nm. The un dissolved calcium phosphate was determined from the standard calibration curve by extrapolation method[10].

RESULTS AND DISCUSSION

Aqueous and alcoholic extracts of leaves of *Commelina benghalensis* was screened for its anti-urolithiatic activty against the reference standard cystone by *in-vitro* model. Aquous extract shows more percentage of dissolution than alcoholic extract but less than the reference standard cystone.

Anti-urolithiatic activity of leaf extracts of *Commelina benghalensis*

S.No	Group	% Dissolution of Calcium oxalate	% Dissolution of Calcium phosphate
1	Control	-------	-------
2	Standard (Cystone)	61.2	47.9
3	Aqueous extract of *C.benghalensis*	49.3	31.2
4	Alcoholic extract of *C.benghalensis*	36.1	27.4

CONCLUSION

This study authorizes that the plant *Commelina benghalensis* Linn. Posses anti-urolithiatic property. Further studies are needed to detect the compounds present in the plant responsible for its anti-urolithiatic activity.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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