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COMPERATIVE STUDY OF *RIVEA HYPOCRATERFORMIS*, *CYNODON DACTYLONE* AND *BALANITE AEGYPTICAE* USING ANTILITHIATIC ACTIVITY *IN VITRO*

Vina B. Patel *[1], Dipika G. Patel [1], Ashlesha G. Makwana [2], Jaymin.M.Patel[1], Maitreyee R. Brahmbhatt[1]

K. J. College of Pharmacy [1], Vadasma, Gujarat, India
K. B. Institute of Pharmaceutical Education and Research [2], Gandhinagar, Gujarat, India

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Correspondence to Author:

Vina B. Patel

K. J. College of Pharmacy, Vadasma, Gujarat, India

ABSTRACT

Ethanolic extracts of *Rivea hypocraterformis*, Methanolic extract of *Cynodon dactylone* and Water extract of *Balanite aegypticae* were tested for their *in vitro* antilithiatic /anticalcification activity by the homogenous precipitation method. The extracts were compared with an aqueous extract of cystone (a marketed preparation) for their activities. Extracts of Rivea hypocraterformis showed activity almost equivalent to cystone while Cynodon dactylone and Balanite aegypticae showed less activity.

INTRODUCTION: The problem of urinary stones is an ancient one, and still remains a common problem worldwide. The disease shows an almost world-wide distribution, with certain interesting patterns. Calcium oxalate or mixed calcium oxalate and calcium phosphate renal stones in adults are the predominant type in Europe, North America, Australia and South Africa.

Bladder stone disease in children, which is hyperendemic in the developing countries, is heterogeneous, urate being the main component in some parts of the Middle East, India and North Africa, while calcium oxalate stones predominate in some parts of South East Asia and India. *Rivea hypocraterformis*, *Cynodon dactylone*, *Balanite aegypticae* are the commonly used plants in folklore for their medicinal properties. The three plant drugs were evaluated alone. The stones normally consist of calcium oxalate and calcium phosphate and the activity of the extracts was evaluated by measuring the ability of the extracts to inhibit the formation of calcium and phosphate precipitates.

MATERIAL AND METHODS:

Plant materials: The leaf of *Rivea hypocraterformis* and the whole plants of *Cynodon dactylone* and fruits of *Balanite* aegypticae were collected from the local region of Gujarat, identified and authenticated by microscopically and physicochemical data. The dried plant materials were powdered, passed through a 60 mesh and stored in airtight containers prior to extraction.

Preparation of Plant extract: The leaf powder of *Rivea hypocraterformis*, the whole plants of *Cynodon dactylone*, and fruits of *Balanite* aegypticae were packed in a different soxhlet apparatus and extracted with Ethanol, Methanol and water respectively for 18 hrs. Appearance of colorless solvent in the siphon tube was taken as the termination of extraction. After each extraction, the solvents were recovered using distillation assembly, and the extracts were concentrated under reduced pressure. Then, the extracts were transferred into the different beaker and evaporated to a thick paste on the water bath, maintained at 50^{o}C for ethanol and methanol and 100^{o}C for water to get different extracts. The marc was finally air dried thoroughly to remove all traces of the solvents.

The aqueous extract of cystone was prepared by grinding a tablet to powder. This powder was mixed with 5mL water and kept for 2–3 h and then centrifuged at 1000 rpm. The clear supernatant was used for the study. The extracts of the plants were compared with the aqueous extract of cystone (a marketed herbal formulation for urolithiasis) for their antilithiatic activity [1].

Experimental set up: The experiment consisted of the following tubes for control and test, 25 mL each of 25 mM $CaCl_2.2H_2O$, 25 mM $Na_2HPO_4.2H_2O$ or 25mM $Na_2C_2O_4$. To the tubes of each set, the different extracts or an equal amount of vehicle was added. The tubes were incubated at 37°C for 4 h. The precipitates of calcium phosphate and calcium oxalate were generated as follows;

Calcium oxalate precipitate was generated by mixing 1 mL of solutions from the tubes having calcium chloride dihydrate and sodium oxalate solutions in TRIS buffer. Calcium phosphate precipitate was generated by mixing 1 mL of solution from the tubes having calcium chloride dihydrate and disodium hydrogen phosphate monohydrate in TRIS buffer. The buffer composition was: 0.1 M TRIS buffer; Solution A was 0.4 M TRIS [48.4 g of Tris (trihydroxymethyl) amino methane per 1000 Ml]; Solution B was 0.4 M hydrochloric acid. [33.6 mL of concentrated hydrochloric acid per 1000mL]; A working solution was made up of 25 mL solution A, 20.7 mL solution B madeup to 100 mL, the pH was 7.4 [2].

Calcium was estimated using titrimetry and phosphorus was estimated using colorimetric analysis. Comparisons were made between control with different extracts and cystone group. The amounts of precipitate of calcium and phosphate were determined in each of the sets by the methods of Clark and Collip [3, 4] respectively. The percent inhibition of the test was calculated in comparison with the control samples.

RESULT AND DISCUSSION: Our study clearly shows the utility of *Rivea hypocraterformis* in the treatment of renal and urinary calculi. It can be inferred that Rivea hypocraterformis is more active as a prophylactic than *Cynodon dactylone* and *Balanite aegypticae*. *Rivea hypocraterformis* showed good activity by inhibiting the formation of phosphate precipitate as well as calcium precipitate. Cystone which is a prescribed treatment for urinary and renal calculi, showed a good inhibitory effect on the formation of the precipitates of calcium and phosphate (Shown in **table 1**).

TABLE 1: PERCENT INHIBITION USING DIFFERENT EXTRACTS (QUANTITY: 2.5 ml OF 0.2 g/ml SOLUTION)

Extract	Amount of ppt. (µmol)		% inhibition	
	Phosphate	Calcium	Phosphate	Calcium
Control	7.45	7.2	------	------
Rivea hypocraterformis	2.068	4.96	72.24	31.11
Cynodon dactylone	2.68	5.37	64.02	25.41
Balanite aegypticae	3.51	4.34	52.88	39.72
Cystone	1.65	4.5	77.85	37.5

The exact mechanism of action of the plants is not known and the active components that may act through inhibition of calcium and phosphate accumulation. Compounds such as staurosporine and other substances that inhibit the increase of intracellular calcium either from extracellular or intracellular stores have been known to prevent lithiasis. It is interesting that L-type calcium channel blockers are ineffective in blocking lithiasis, contrary to the expectation that by blocking an increase in intracellular calcium they would be beneficial in the treatment of lithiasis [5].

CONCLUSION: *Rivea hypocraterformis* showed comparable activity to the marketed formulation in terms of inhibiting the formation of phosphate precipitate but showed a significantly better potential in preventing the formation of the calcium precipitate.

Our study definitely provides a basis for the continued use of Rivea hypocraterformis in the treatment of lithiasis.

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