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Formulation and *in-vitro* preliminary screening of polyphyto antilithiatic combination

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ABSTRACT

Objective: To study people suffered from urinary stone problem by calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) containing stones. **Method:** In the present study, CO crystals were grown by mixing and stirring two equal volumes of solutions A and B. Two solutions of following composition were mixed: A: $Na_2C_2O_4$ (2 mmol) and B: $CaCl_2 \cdot 2H_2O$ (10 mmol). The crystals after incubation in tris buffer were then subjected to the aqueous extract of the polyherbal formulation and cystone was taken as standard. **Result:** After the treatment with the extract the crystals were microscopically examined and elemental analysis for calcium was carried out. The formulation proved out to be very effective against the CO crystals. **Conclusions:** The results have come out to be very motivating and further pharmacological study could be carried out on the samples to reveal an effective drug for the urolithiasis. Data reveals that phytotherapeutic agents could be useful as either an alternative or a complementary therapy in the management of urolithiasis.

1. Introduction

Urolithiasis (nephrolithiasis) or kidney stone is formation of urinary calculi at any level of urinary tract. It is estimated that 12% of world population experiences renal stone disease with a recurrence rate of 70%-80% in male and 47%-60% in female[1,2]. Kidney stone formation or urolithiasis is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidneys.

The recurrence of urolithiasis represents a serious problem as patients who have formed one stone are more likely to form another[3]. Several reports have been published since Randall's first description of papillary calcifications and their possible active role in the genesis of COM papillary calculi[4].

Herbal drug therapy is the most trusted system of medicine in countries like India, where people strongly believe in Ayurveda as herbs are the part of rural Indian lifestyle. Most of the diseases which have no medicine in allopathic system can be cured successfully using traditional medicines[5].

The problem of urinary stones or calculi is an ancient problem and many remedies have been employed during the ages. These stones are found in all parts of the urinary tract, the kidney, the ureters and the urinary bladder and may vary considerably in size. Diet containing low amounts of inferior quality proteins and high intake of animal proteins might augment the risk of stone formation.

The incidence of urolithiasis is very common in Northern India compared to southern states. It is speculated that higher incidence may be due to wheat diets. People living in rocky areas, where the climate is hot and dry, seem to be more exposed to urinary calculi

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disease[6].

2. Material and methods

2.1. Polyphyto formulations

The plants were reviewed for their antilithiatic activity and the selected plants were then collected for the study. The plants were then authenticated from Botanical Survey of India, Dehradun.

2.2. Preparation of extract

Polyphyto combinations were subjected to maceration procedures for the preparation of the extracts. Similarly the extract of the marketed formulation, cystone was prepared, which is taken as standard in the present study.

2.3. Phytochemical screening of the formulation

Phytochemical analysis was carried out to determine the active ingredients of the extracts, were adopted for the detection of the presence of alkaloids, carbohydrates, flavonoids, glycosides, resins, saponins, sterols and tannins[7].

2.4. Standardization of Polyphyto antilithiatic formulation:

2.4.1. Pharmacognostic studies

2.4.1.1. Determination of extractive values

Extractive values of the plant leaves were determined by using various solvents like petroleum ether, chloroform, methanol and water by maceration. The extract was concentrated to dryness using Rotary evaporator and yields of each extracts were calculated individually.

2.4.1.2. Determination of ash values

A weighed quantity of leaf powder (3 g) was taken in a china dish and heated until it is free from carbon and then cooled to room temperature. The ash in the dish was weighed. The percentage of total ash was calculated with reference to air dried sample.

2.4.1.3. Determination of loss on drying (LOD)

The powdered leaf (2 g) was taken into a tared silica crucible and it was put into hot air oven at 105 ℃ for drying. It was then cooled and weighed. The powder will be heated till the constant weight obtained. The loss of weight in mg per gm was calculated with reference to the quantity of dried powder taken.

2.4.2. In-vitro antilithiatic activity

2.4.2.1. Preparation of CO crystals

We selected the classical model for the study of oxalate crystallization because of its simplicity and satisfactory reproducibility. This model includes the study of crystallization without inhibitor and with it, in order to assess the inhibiting capacity of any chemical species used.

Table 1
Information of formulation.

S.No	FLA-1	FLA-2	FLA-3
1	Tribulus terresteris (Fruit-10)	Tribulus terresteris (Fruit-10)	Tribulus terresteris(Fruit-5)
2	Berginea ciliate (Bark-10)	Kegelia africana (Fruit-10)	Berginea ciliate (Bark-5)
3	Kegelia africana (Fruit-20)	Withania somniferae (Leaf-5)	Kegelia africana (Fruit-10)
4	Withania somniferae (Stem-10)	Borrhevia diffusa (5)	Withania somniferae(Stem-10)
5	Santalum album (Stem-10)	Foenum gracum (Seed-5)	Valerian (10)
6	Valerian (Rhizome-10)	Curcuma longa (5)	Asparagus rosemosus(5)
7	Asparagus rosemosus (Stem-10)	Valeriana walichi (Rhizome-10)	Borrhevia diffusa (Bark-5)
8	Carica papaya (Root-5)	Gymnemus sylvester (Bark-5)	Curcuma longa (Root-10)
9	Cynodon dactylon(Leaf-15)	Carica papaya (Root-5)	Valeriana walichi (Rhizome-5)
10		Plumbago zeylanica (Fruit-5)	Carica papaya (Root-5)
11		Glycyrrhiza glabra (Fruit-5)	Plumbago zeylanica (Fruit-10)
12		Piper longum (Fruit-5)	Glycyrrhiza glabra (Fruit-5)
13		Achyrathes aspera (5)	Piper longum (Fruit-5)
14		Piper nigrum (Fruit-5)	Achyrathes aspera (5)
15		Sopindus mokurossi(5)	Cynodon dactylon(Leaf-5)
16		Butea monosperma (Flower-10)	

Table 2
Phytochemical screening of poly herbal formulation.

S. No.	Test	Aqueous	Methanolic	Ethanolic	Pet. Ether
FLA-1	Tannins	+ + ve	+ + ve	+ + ve	+ + ve
FLA-1	Glycosides	+ + ve	+ + ve	+ + ve	- - ve
FLA-1	Saponin	+ + ve	- - ve	- - ve	- - ve
FLA-1	Flavonoids	+ + ve	+ + ve	+ + ve	- - ve
FLA-2	Tannins	+ + ve	+ + ve	+ + ve	+ + ve
FLA-2	Glycosides	+ + ve	+ + ve	+ + ve	- - ve
FLA-2	Saponin	+ + ve	- - ve	- - ve	- - ve
FLA-2	Flavonoids	+ + ve	+ + ve	+ + ve	- - ve
FLA-3	Tannins	+ + ve	+ + ve	+ + ve	+ + ve
FLA-3	Glycosides	+ + ve	+ + ve	+ + ve	- - ve
FLA-3	Saponin	+ + ve	- - ve	- - ve	- - ve
FLA-3	Flavonoids	+ + ve	+ + ve	+ + ve	- - ve

Table 3
Standardisation of polyphyto antilithiatic formulation.

Formulation	Colour	Solubility	Bulk density (g/mL)	True density (g/mL)	Loss on drying (%w/w)	Angle of repose	Melting point	Water soluble Extractives value (%w/w)	Ethanol soluble extractive value (%w/w)	Pet. Ether soluble extractive value (%w/w)
Formulation-FLA-1	Light Brown	Soluble in water and insoluble in organic solvent	0.123	0.023	0.13	88°	90°	40	16	33
Formulation FLA-2	Light Brown	Soluble in water and insoluble in organic solvent	0.025	0.003	0.11	88°	92°	30	11	10
Formulation FLA-3	Light Brown	Soluble in water and insoluble in organic solvent	0.140	0.005	0.12	83°	89°	40	11	20

Two solutions of following composition were mixed: A: $Na_2C_2O_4$ (2 mmol/L) and B: $CaCl_2 \cdot 2H_2O$ (10 mmol/L). The formation and growth of the CO crystals was observed.

Artificial urine was prepared by mixing and stirring two equal volumes of 50 mL of solutions A and B at constant temperature (37 ℃) in capped vessels to give final artificial urine. Mixture agitation was maintained to prevent sedimentation.

2.4.2.2. Estimation of the antilithiatic activity

The crystal size development was monitored in the sample with the help of a microscope and quantitative elemental analysis for calcium was conducted by titrimetric method[8].

3. Results

3.1. Antilithiatic activity

Kidney oxalate stone is the result of supersaturation of urine with certain urinary salts such as calcium oxalate. The treatment of the crystals with the extracts of the polyphyto formulations showed a dramatic reduction in the crystal size and calcium level of the solution was increased proving the degeneration of the calcium-oxalate crystals.

The elemental calcium level in the sample number 27 and 29 has shown to be better in comparison with the sample containing cystone as the standard drug. The present results support sample number 27 and 29 to have Antilithic potential and can be used against urolithiasis.

Table 4
Calcium concentration of all samples.

S .N	Sample	Calcium (mg/dL)
1	Control	4.12±0.02
2	Std (cystone)	4.81±0.03***
3	Formulation FLA-1	4.76±0.04***
4	Formulation FLA-2	4.52±0.06***
5	Formulation FLA-3	4.44±0.07***

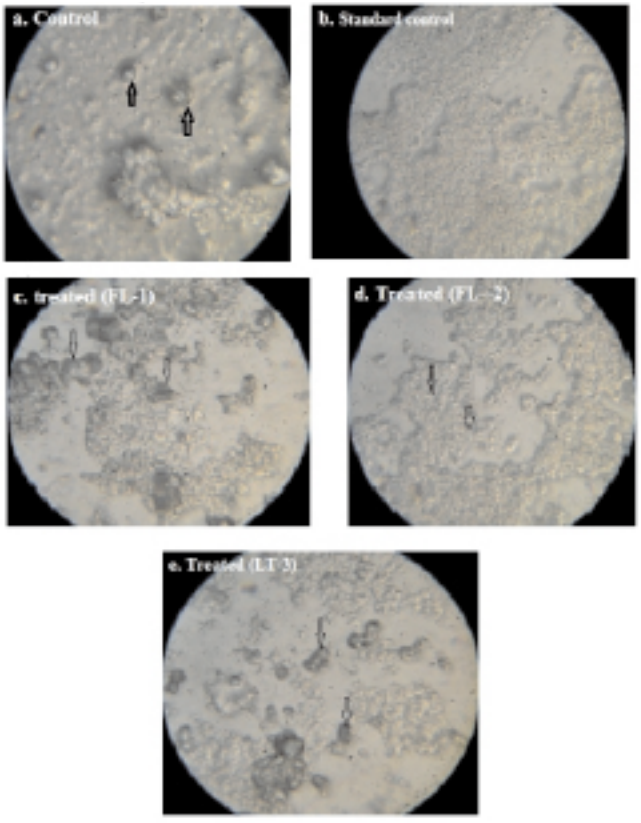

Figure 1. Microscopic study on formulation.

4. Conclusion

The use of the herbals as medicine is an age old tradition which will serve as a source of alternative medicine in future also and help to overcome the toxic effects of the synthetic drugs.

The results have come out to be very motivating and further pharmacological study could be carried out on the samples to reveal an effective drug for the urolithiasis.

Data reveals that phytotherapeutic agents could be useful as either an alternative or a complementary therapy in the management of urolithiasis.

Conflict of interest statement

We declare that we have no conflict of interest.

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