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In-vitro Inhibition of Mineralisation of Urinary Stone Forming by Some Dry-fruit Extracts

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Water extracts of some dry fruits, *viz.*, raisins, dried dates, currants or cashew have been studied as inhibitors in the mineralisation of urinary stone forming minerals, *viz.*, calcium phosphate, oxalate or carbonate. Inhibition efficiency has been studied in different experimental models. Utility of these dry fruits in urolithiasis inhibition has been discussed.

INTRODUCTION

Epidemiological studies[1–4] indicate that urolithiasis disease exists in 'endemic' proportions in some parts of our country. Urinary stones contain both crystalloid and colloid components. The crystalloid components are mainly calcium oxalate, calcium phosphate, calcium carbonate, magnesium ammonium phosphate, uric acid and cysteine.[5] Stone formation is apparantly related to the lever of urinary crystalloid and also to the level of inhibitors of calculogenesis in urine.[6–8] Quest for physiologically non-toxicant natural inhibitors of urolithiasis would be helpful in the prevention of this disease. As a part of our systematic study on inhibitors of urinary calculogenesis, we are presently reporting on the inhibition efficiency of some dry-fruit extracts on the mineralisation of calcium phosphate, oxalate and carbonate in different experimental models.

EXPERIMENTAL

Crystalloid forming solutions, *viz.*, solutions of calcium acetate, trisodium phosphate, disodium oxalate and sodium carbonate, were prepated in distilled water. Dry-fruit extracts were prepared by crushing and suspending 100 g of the dry fruit in 300 mL water for 1 h in case of raisins, currants and cashew. In case of dried dates 100 g fruit were cut into small pieces and suspended in 500 mL water for 2 h. The suspensions were filtered through ordinary filter paper and used as such. Four experimental models, namely, 'simultaneous flow static model' (S.S.M.), 'simultaneous flow dynamic model' (S.D.M.), 'Reservoir static model' (R.S.M.) and 'Reservoir dynamic model' (R.D.M.) were designed. In the S.S.M. model the two salt forming solutions, *e.g.* sodium phosphate and calcium acetate (for calcium phosphate) and the inhibitor (dry-fruit extract) were taken in three separate burettes (50 mL) and were allowed to fall simultaneously into a 250 mL beaker with a slow (dropwise) and equal speed. The whole operation took about 40 min. At the end the mixture was digested in a hot water bath for 10 min, cooled to room temperature and the precipitate was collected in a pre-weighed centrifuge tube by centrifuging small volumes at a time and rejecting the supernatant liquid. Next, the tube with the precipitate was dried in an air oven at 120°C, cooled to room temperature and weighed till constant weight. Weight of the precipitate was determined.

In the S.D. model, the process was same except that the reaction mixture in the beaker was continuously stirred in a magnetic stirrer during the flow of salt-forming solutions and the inhibitor. In the R.S. model, the whole amount of inhibitor solution (50 mL) was placed in the beaker in the beginning itself and the two salt-forming solutions were allowed to run into it dropwise through burettes. Thus, a reservoir of inhibitor was created into which the salt- forming solutions ran down. Rest of the operation was same as in other models.

In the R.D. model the process was same as R.S. model except that the reaction mixture was stirred continuously on a magnetic stirrer during experiment. Simultaneous blank experiments with water in place of inhibitor were also carried out for evaluating the inhibition efficiency of inhibitors compared to water. All experiments were conducted at room temperature (20–25°C).

RESULTS AND DISCUSSION

pH of all the final solutions after experimentation were found to be around 7. Percentage efficiency of inhibition of the inhibitor was calculated using the formula:

$$\text{Percentage inhibition} = \frac{\text{wt. of ppt. in blank set} - \text{wt. of ppt. in exptl. set}}{\text{wt. of ppt. in blank set}} \times 100$$

Inhibition efficiency of different dry-fruit extracts towards the precipitation of calcium phosphate, calcium oxalate and calcium carbonate are recorded in Tables 1-3, respectively.

TABLE-1
INHIBITION OF CALCIUM PHOSPHATE MINERALISATION BY DRY-FRUIT EXTRACTS

Salt-forming solution : —0.01 M $(CH_3COO)_2Ca$ and 0.01 M Na_3PO_4

Dry fruit	Inhibition efficiency (%)			
	S.S.M.	S.D.M.	R.S.M.	R.D.M.
Raisins (Kismis)	80	81	83	88
Currants (Munakka)	73	76	86	88
Cashew (Kaju)	78	82	87	87
Dried dates (Chhuhada)	50	50	54	55

Study of the Tables 1–3 suggests that the dry-fruit extracts. are moderate to good inhibitors of mineralisation of calcium phosphate, oxalate and carbonate minerals. Particularly in case of phosphate the inhibition is very good on an average. The dried date extract shows good inhibition only in case of carbonate but seems to be relatively poor inhibitor for phosphate and oxalate. On the other hand raisins extract shows poor inhibition for carbonate but good inhibition for phospate and oxalate. Currants show a uniformaly good inhibition for all the three minerals. Thus it is seen that the inhibitory power of dry fruits is a function of their components. The active inhibiting components might be effective calcium chelating agents present in the fruits. Currants contain citric, malic and tartaric acids which are well known calcium chelating agents. The sequestering of insoluble calcium salts might be involving either single or mixed ligands chelation[9].

TABLE-2
INHIBITION OF CALCIUM OXALATE MINERALISATION
BY DRY-FRUIT EXTRACTS

Salt forming solution –0.01 M $(CH_3COO)_2Ca$ & 0.01M $Na_2C_2O_4$

Dry fruit	Inhibition efficiency (%)			
	S.S.M.	S.D.M.	R.S.M.	R.D.M.
Raisins (Kismis)	60	60	74	77
Currants (Munakka)	78	81	84	88
Cashew (Kaju)	68	71	77	77
Dried Dates (Chuhada)	30	32	44	45

TABLE-3
INHIBITION OF CALCIUM CARBONATE MINERALISATION
BY DRY-FRUIT EXTRACTS

Salt-forming solution –0.01 M $(CH_3COO)_2Ca$ and 0.01 M Na_2PO_3

Dry fruit	Inhibition efficiency (%)			
	S.S.M.	S.D.M.	R.S.M.	R.D.M.
Raisins (Kismis)	50	50	53	55
Currants (Munakka)	84	86	95	95
Cashew (Kaju)	51	53	57	61
Dried dates (Chuhada)	70	73	79	81

A comparative study of different models indicates that the reservoir dynmaic model is the most effective one in the inhibition of mineralisation. This might be due to the mass effect. An *ab-initio* presence of large concentration of exract (in the reservoir) coupled with continuous stirring might be favouring complexation of Ca^{2+} ions and thus making them less available for precipitation as insoluble salts.

All the dry fruits, that we have presently studied are commonly edible ones. Their inhibition efficiency towards the mineralisation of calcium phosphate, oxalate and carbonate suggests that the increased intake of these dry fruits would be helpful in urinary stone prophylaxis.

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