In-vitro Inhibition of Mineralisation of Urinary Stone Forming Minerals by Fruit Juices

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Fruit juices, *viz.*, juice of lemon varieties, orange, sweet orange, grape, pineapple, mango and amla 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 fruits, in urolithiasis inhibition has been discussed.

INTRODUCTION

Urolithiasis disease exists in 'endemic' proportions in some parts of our country.[1] 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.[2] Stone formation is apparently related to level of urinary crystalloid and also to the level of inhibitors of calculogenesis in urine[3–5]. As a part of our systematic study on inhibitors of urinary calculogenesis we are presently reporting on the inhibition efficiency of some fruit juices on the mineralisation of calcium phosphate, oxalate and carbonate in different experimental models.

EXPERIMENTAL

Crystalloid forming solutions, *viz.*, solution of calcium acetate, trisodium phosphate, disodium oxalate and sodium carbonate were prepared in distilled water. Juices of the fruits were extracted with the help of an ordinary fruit juicer. In case of grapes the fruits were crushed. The thick juices, thus obtained, were passed through a mesh and then suction filtered through ordinary filter paper. All the juices were used undiluted, and were used out within the minimum possible time. In case of amla, the ripe fruits were crushed in distilled water to extract the juice. 60 g amla was used to make 50 mL of juice extract.

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 (fruit juice) were taken in three separate burettes (50 mL) and were allowed to fall simultaneously into a 250 mL beaker in 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 into 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 on 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 solution ran down. Rest of the operation was same as in other models.

In the R.D. model the process was same as in R.S. model except that the reaction mixture was stirred continuously on a magnetic stirrer during the 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 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 efficiencies of fruit juices 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 FRUIT JUICES

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

Inhibitor	Inhibition efficiency (%)			
	S.S.M.	S.D.M.	R.S.M.	R.D.M
Kagzi lemon	86	86	88	89
Jamiri lemon	92	92	94	96
Gagal lemon	93	94	95	96
Orange	85	87	88	88
Sweet orange	29	30	32	33
Grapes	87	87	89	89
Mango	28	28	30	32
Pineapple	81	82	83	83
Amla	95	97	100	100

TABLE-2
INHIBITION OF CALCIUM OXALATE MINERALISATION BY FRUIT JUICES

Salt forming solution 0.01 M $(CH_3COO)_2Ca$ and 0.01 M $Na_2C_2O_4$

Inhibitor	Inhibition efficiency (%)			
	S.S.M.	S.D.M.	R.S.M.	R.D.M
Kagzi lemon	50	51	53	53
Jamiri lemon	72	73	74	74
Gagal lemon	73	73	74	75
Orange	69	70	72	72
Sweet orange	22	22	24	25
Grapes	80	82	83	83
Mango	02	02	05	06
Pineapple	48	50	51	53
Amla	92	94	96	96

TABLE-3
INHIBITION OF CALCIUM CARBONATE MINERALISATION BY FRUIT JUICES

Salt forming solution 0.01 M $(CH_3COO)_2Ca$ and 0.01 M Na_2CO_3

Inhibitor	Inhibition efficiency (%)			
	S.S.M.	S.D.M.	R.S.M.	R.D.M
Kagzi lemon	25	26	28	30
Jamiri lemon	23	23	24	27
Gagal lemon	25	25	26	27
Orange	75	77	79	79
Sweet orange	25	27	28	28
Grapes	37	40	41	43
Mango	71	71	73	74
Pineapple	28	28	29	30
Amla	80	82	84	84

Study of the tables suggests that the fruit juices are moderate to good inhibitors of calcium phosphate and oxalate mineralisation. Sequestering of these insoluble calcium salts by the fruit juices might be due to effective single or mixed ligand chelation[6] by the hydroxy acids present in them (particularly the citrus fruits and grapes). The chelates so formed in solutions might be getting stabilised by effective hydrogen-bonding through the —OH groups of hydroxy acids. In case of amla fruit rich amount of ascorbic acid might be working as an inhibitor. Relatively poor inhibition in case of calcium carbonate precipitation by some fruits might be due to a higher pK value of carbonic acid leading to replacement

and precipitation of calcium salts of inhibitors rather than soluble mixed chelation. Calcium oxalate is a stubborn constituent of urinary calculi being highly insoluble. It has very poor solubility in the fruit juices as such; however, if the fruit juices are present in the milieu before the formation of calcium oxalate, they may prevent the precipitation of the latter by exerting the specificity of their inhibitor towards calcium ions.

A comparative study of different models indicates that the reservoir dynamic 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 juice (in the reservoir) coupled with continuous stirring might be favouring complexation of Ca^{2+} ions. In case of citrus fruits this might be shown as below:

$$Ca^{2+} + (Cit)^{3-} \rightleftharpoons Ca(Cit)^{-}$$

where Cit^{-} = Citrate

More the Cit^{3-} present more Ca^{2+} ions can be trapped as $Ca(Cit)^{-}$ and less Ca^{2+} ions will be free for precipitation as insoluble salts.

Most of the fruits that we have presently studied contain hydroxy polybasic acids, *viz.*, lemon varieties, orange and sweet orange contain citric acid, while grapes contain tartaric acid. Inhibitory power of these fruits towards precipitation of insoluble calcium salts might be vested in their hydroxy polybasic acids. Our present studies suggest that the increased intake of fruits, we have presently studied, would be helpful in urinary stone prophylaxis.

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