Asian Journal of Chemistry Vol. 12, No. 3 (2000), 719–722

In-vitro Inhibition of Mineralisation of Urinary Stone Forming Minerals by Some Medicinal Plant Extracts

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Extracts of some medicinal plants and shrubs, *viz.*, horse gram (*Dolichose biflorus*), bark of sal tree (*Shorea robusta*), leaves of Pathhar chur (*Bryophyllum spp.*), bark of Ashoka tree (*Saraca indica*), Brahmi buti (*Centella asiatica*), leaves of Tulsi (*Ocimum sanctum*), leaves of Khatmithi (*Oxalis* spp.) and sticks of Chiraita (*Swertia chirata*) have been studied as inhibitors in the mineralization of urinary stone forming minerals, *viz.*, calcium phosphate, oxalate or carbonate. Inhibition efficiency has been studied in different experimental models. Utility of the results in urolithiasis inhibition has been discussed.

INTRODUCTION

Urinary calculogenesis is a result of mineralisation of insoluble calcium and magnesium salts or uric acid or cysteine over a suitable nidus in the urinary tract. Urinary stone forming disease, called urolithiasis, exists in 'endemic' proportion in some parts of our country[1–4]. Stone formation is apparently related to the level of urinary crystalloids and also to the level of inhibitors of calculogenesis in urine[5–7]. Plants and shrubs of medicinal value, used as 'folk-medicines', might contain inhibitors of mineralisation of insoluble stone forming compounds. As a part of our systematic study on the inhibitors of urinary calculogenesis we are presently reporting on the inhibition efficiency of some medicinal plants and shrub extracts towards the mineralisation of calcium phosphate, oxalate and carbonate in different experimental models.

EXPERIMENTAL

Crystalloid forming solutions, *viz.*, solutions of calcium choride, trisodium phosphate, disodium oxalate and sodium carbonate, were prepared in distilled water. Inhibitor extracts, *viz.*, extract of horse gram, bark of Sal tree, bark of Ashoka tree, Pathharchur leaves, Brahmi buti, leaves of Tulsi, leaves of Khatmithi and sticks of Chiraita, were prepared by suspending and crushing (in case of leaves and buti) 20 g of material in 200 mL of water for 2 h. The extracts were filtered through ordinary filter paper and used out immediately. In case of horse gram (kulthi) whole pulse was used for preparing the extract. Four experimental models namely 'simultaneous flow static model' (SSM), 'simultaneous flow

dynamic medel' (SDM), 'Reservoir static model' (RSM) and 'Reservoir dynamic model' (RDM) were designed. In the SSM model the two salt-forming solutions, *e.g.*, sodium phosphate and calcium chloride (for calcium phosphate) and the inhibitor (plant extract) were taken in three separate burettes (50 mL) and were allowed to fall simultaneously into a 250 mL beaker with a slow 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 preweighed 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 SD 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 RS model, the whole amount of inhibitor solutions (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 RD model the process was same as RS 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

pHs 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 plant extracts towards the mineralisation of calcium phosphate, calcium oxalate and calcium carbonate are recorded in Tables 1–3. A study of the Tables suggests that most of the plant extracts are moderate to good inhibitors of mineralisation of calcium phosphate, oxalate and carbonate. In case of carbonate all the extracts show high inhibition. Extracts of horse gram, Brahmi buti, bark of sal and khatmithi leaves have been found to be uniformly good inhibitors for all the three minerals. Even for calcium oxalate, which is a stubborn constitutent of urinary calculi, horse gram, brahmi buti and bark of sal show inhibition as high as 52–84%. The inhibition by the extracts might be due to active complexing agents of calcium present in the plants. Natural products containing well known chelating agents such as hydroxy acids have earlier been found[8] to be good inhibitors of insoluble calcium salt mineralisation.

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 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 salt.

TABLE-1
INHIBITION OF CALCIUM PHOSPHATE MINERALISATION BY MEDICINAL PLANT EXTRACTS

Salt forming solutions: 0.01 M $CaCl_2$ and 0.01 M Na_3PO_4
Inhibitor concentration: 10% w/v

Inhibitor	Inhibition efficieny (%)			
	SSM	SDM	RSM	RDM
Horse gram (*Dolichos biflorus*)	60	61	63	66
Bark of Sal tree (*Shorea robusta*)	91	91	92	100
Bark of Ashoka tree (*Saraca indica*)	41	48	50	50
Pathharchur leaves (*Bryophyllum* spp.)	60	62	65	66
Brahmi buti (*Centella asiatica*)	82	83	86	91
Khatmithi leaves (*Oxalis* spp.)	65	67	70	71
Tulsi leaves (*Ocimum sanctum*)	30	32	33	33
Chiraita sticks (*Swertia chirata*)	59	59	64	66

TABLE-2
INHIBITION OF CALCIUM OXALATE MINERALISATION BY MEDICINAL PLANT EXTRACTS

Salt forming solutions: 0.01 M $CaCl_2$ and 0.01 M $Na_2C_2O_4$
Inhibitor concentration: 10% w/v

Inhibitor	Inhibition efficieny (%)			
	SSM	SDM	RSM	RDM
Horse gram (*Dolichos biflorus*)	60	62	63	64
Bark of Sal tree (*Shorea robusta*)	50	51	52	52
Bark of Ashoka tree (*Saraca indica*)	41	43	46	48
Pathharchur leaves (*Bryophyllum* spp.)	42	42	43	44
Brahmi buti (*Centella asiatica*)	76	78	80	84
Khatmithi leaves (*Oxalis* spp.)	52	56	70	60
Tulsi leaves (*Ocimum sanctum*)	39	40	43	46
Chiraita sticks (*Swertia chirata*)	37	38	40	42

TABLE-3
INHIBITION OF CALCIUM CARBONATE MINERALISATION BY MEDICINAL PLANT EXTRACTS

Salt forming solutions: 0.01 M $CaCl_2$ and 0.01 M Na_2CO_3
Inhibitor concentration: 10% w/v

Inhibitor	Inhibition efficieny (%)			
	SSM	SDM	RSM	RDM
Horse gram (*Dolichos biflorus*)	80	82	85	88
Bark of Sal tree (*Shorea robusta*)	90	91	94	100
Bark of Ashoka tree (*Saraca indica*)	86	88	93	95
Pathharchur leaves (*Bryophyllum* spp.)	82	82	86	90
Brahmi buti (*Centella asiatica*)	82	84	90	92
Khatmithi leaves (*Oxalis* spp.)	89	91	95	95
Tulsi leaves (*Ocimum sanctum*)	84	86	91	91
Chiraita sticks (*Swertia chirata*)	81	82	90	92

All the plant extracts that we have presently studied have medicinal value. Horse gram, bark of Sal and Pathharchur leaves are used as folk-medicines in urinary stone disease. Bark of Ashoka and Chiraita sticks are used as blood purifier in folk-medicines. Brahmi buti finds use in stomach disorders and Tulsi leaf extract is useful in cough and colds. Inhibition of mineralisation of urinary stone forming minerals by the extracts of these medicinal plants and shrubs, as indicated by our present studies, suggests that they may find application in urinary stone prophylaxis.

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(*Received*: 1 January 2000; *Accepted*: 19 February 2000) AJC-1987