

## Human Stones: Dissolution of calcium phosphate and cholesterol by edible plant extracts and bile acids

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Tri-calcium phosphate (TCP) and cholesterol are major constituents of human stones. Dissolution of TCP by extracts of kulfa (*Portulaca oleracea*), pathari (*Trianthema monogyna*), tomato (*Lycopersicon esculantum*) and grape (*Vitis vinifera*) has been studied by monitoring Ca content in the filtrate by flame photometer. Grape extract is found to be most effective for dissolution of TCP amongst other extracts studied. All these extracts contain carbohydrate, which interacts with Ca to form a soluble complex. Dissolution of cholesterol has been studied in extracts of kulfa, pathari, tomato and grape and in alcoholic solutions of ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA). In dissolved portion, cholesterol was estimated by measuring optical density at  $\lambda_{\max} = 402$  nm using UV-visible spectrophotometer. Amongst all these extracts, tomato is found to be most effective for dissolution of cholesterol. UDCA is found to be more effective in dissolving cholesterol than CDCA.

**Keywords:** Biological apatite, Biomineralization, Cholesterol, Human stones, Tri-calcium phosphate

### Introduction

Calcium phosphate and calcium oxalate are most common crystalline constituents of human urinary stones. Importance of aggregation in calculus formation is that it constitutes the most effective mechanism to increase the size of particle composition and structure of urinary stone<sup>1</sup>. Using <sup>13</sup>C and <sup>13</sup>P MAS NMR spectroscopy, Bak *et al*<sup>2</sup> detected and quantified calcium oxalate, uric acid struvite and calcium phosphate that closely resemble brushite  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$  and calcium hydroxy apatite  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ . Chemical composition of 36 calculi was studied by X-ray diffraction analysis of their powder, out of which 19 stones had a homogeneous composition<sup>3</sup>. About 80% of gallstones are predominantly composed of cholesterol. Despite considerable progress in medical therapy, there is no satisfactory drug to treat kidney stones. Thus, most patients try to find an alternative therapy for many diseases including lithiasis<sup>4</sup>. Although dissolution and inhibition of calcium oxalate in presence of  $\alpha$ -keto glutaric acid<sup>5</sup>, amino acid<sup>6</sup>, polyhydroxy carboxylic acid<sup>7</sup> have been studied, dissolution of tri-calcium phosphate

(TCP) and cholesterol by edible plant and fruit extracts such as kulfa, pathari, tomato, grape and bile acids have not been reported so far.

Kulfa<sup>8</sup> (*Portulaca oleracea* L.) is a source of free oxalic acid, alkaloids, omega 3- fatty acids, flavonoids (cyanin), cumarines etc. Pathari<sup>8</sup> (*Trianthema monogyna* L.) is a naturally growing herb of northeastern UP. Tomato<sup>8</sup> (*Lycopersicon esculantum* L.) is rich in lycopene<sup>9</sup>. Grape<sup>8</sup> (*Vitis vinifera* L.) skin contains resveratrol<sup>10</sup> (50-100  $\mu\text{g/g}$ ). Chenodeoxycholic acid (CDCA) and Ursodeoxycholic acid (UDCA) are naturally occurring bile acids that may be given orally for gall stone dissolution and reduction in cholesterol saturation in the bile. CDCA is primary bile acid whereas UDCA is secondary bile acid<sup>11</sup>.

This study presents dissolution of TCP and cholesterol by edible plant extracts of pathari (*T. monogyna* L), kulfa (*P. oleracea* L), grape (*V. vinifera* L) and tomato (*L. esculantum* L). Dissolution kinetics of cholesterol in bile acids has also been studied.

### Materials and Methods

Cholesterol (Fluka), TCP (s.d.fine-chem Ltd), UDCA (Fluka), CDCA (Fluka), and leaves of *P. oleracea* and *T. monogyna* and fruits of *V. vinifera* and *L. esculantum* were used.

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### Preparation of Extracts and Dissolution of TCP and Cholesterol

Fresh and washed leaves of kulfa and pathari, and fruits of tomato and grape were crushed with distilled water (1:1 w/v) using mixer and grinder. Crushed material was squeezed, filtered by Whatmann filter paper and used as such.

Dissolution of TCP and cholesterol has been studied in different extracts by measuring [Ca] or [cholesterol] at different experimental conditions.

### Estimation of [Ca] by Flame Photometric Studies

Dissolution of TCP in aqueous extracts of leaves and fruits were studied by measuring calcium content in

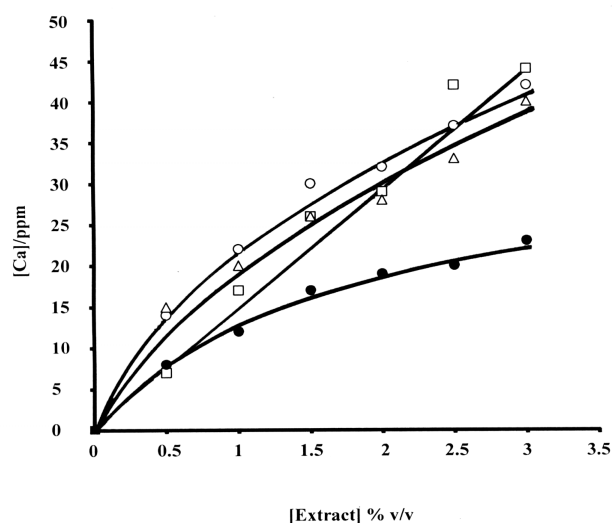


Fig. 1—Plot of calcium concentration vs volume of extracts for dissolution of tri-calcium phosphate in (O) kulfa (&#9633), (•) grape, ( $\Delta$ ) *Triantema monogyna* and ( $\bullet$ ) tomato extract; (10 mg of tri-calcium phosphate was mixed with 10 ml aqueous ct (10 mg of cholesterol was mixed with 10 ml aqueous solution of different extracts and kept for one week)

filtrate at different experimental conditions using flame photometer, attached with Ca filter (Toshniwal, India). TCP (10 mg) was mixed with distilled water (10 ml) containing additives in different volume in different test-tubes, wrapped with parafilm, stirred thoroughly and kept for one week. Ca content in each filtrate was determined. (Fig. 1, Table 1).

### Estimation of [Cholesterol] by UV-visible Spectrophotometer

Cholesterol concentration was estimated by measuring optical density at  $\lambda_{\max} = 402$  nm using UV-visible spectrophotometer. Optical density was measured at different concentrations of cholesterol and calibration curve was obtained (Fig. 2). Cholesterol concentration was estimated by measuring absorption and using the calibration curve. Dissolution kinetics of cholesterol was studied in extracts (Fig. 3) of kulfa, pathari, grape and tomato and bile acids (Fig. 4).

### Results and Discussion

Dissolution of TCP in extracts of edible leaves of kulfa and pathari, and fruits of grape and tomato have been studied by monitoring Ca content in the filtrate. [Ca] content in dissolved portion increased with increasing concentration of extracts, linearly in case of grape extract while in case of other extracts equation  $[Ca]^2 = mV + c$  is obeyed as evident by correlation coefficient values (Table 1); m and c are slope and intercept respectively and V is volume of extract (Fig. 1). For the dissolution of TCP, grape extract is found to be most effective amongst other extracts studied as grape > kulfa > pathari > tomato. All these extracts contain carbohydrate, which interacts with calcium ion to form a soluble complex<sup>12</sup> as

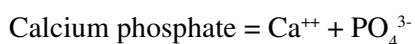


Table 1—Kinetic data for dissolution of tri-calcium phosphate in different extracts

Extract	Equation obeyed	Slope m	Intercept c	Correlation coefficient R
Kulfa	$[Ca]^2 = mV + c$	584.14	-56.64	0.995
Grape	$[Ca] = mV + c$	15.29	0.643	0.991
Pathari	$[Ca]^2 = mV + c$	493.72	-58.57	0.982
Tomato	$[Ca]^2 = mV + c$	176.86	-10.00	0.9924

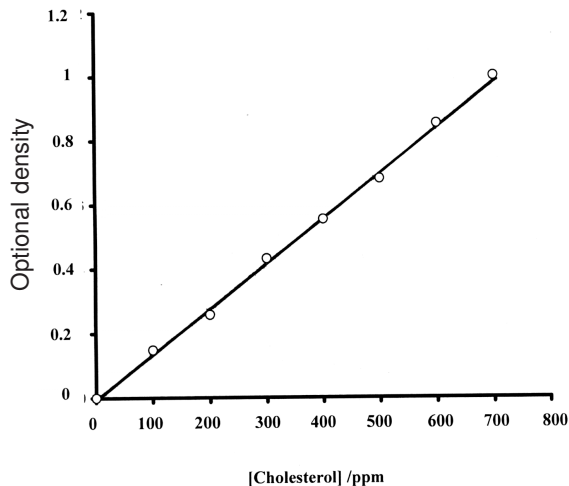


Fig. 2 — Calibration curve for determination of [Cholesterol]

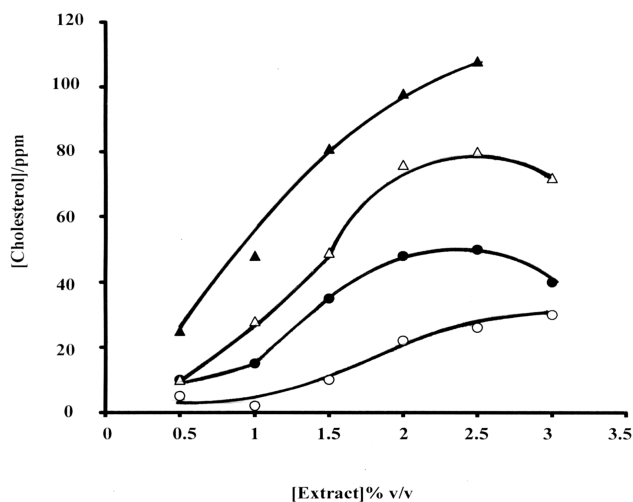
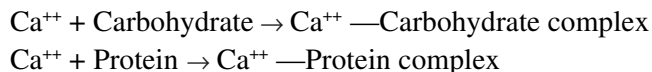


Fig. 3—Plots of [Cholesterol] vs volume of extracts for dissolution of cholesterol in (▲) tomato, (○) kulfa, (Δ) *Trianthema monogyna* and (●) grape extract (10 mg of cholesterol was mixed with 10 ml aqueous solution of different extracts and kept for one week)



Cholesterol insoluble in water is held in solution by detergent action of bile acids and phospholipids, with which it forms micelles. There was no induction period in case of tomato extract, however in other cases sigmoidal curves with induction period were observed. Induction period was found to increase as: Tomato < *Trianthema monogyna* < grape < kulfa. Among all these extracts, tomato is found to be most effective for the dissolution of cholesterol.

UDCA is found to be more effective in dissolving cholesterol than CDCA (Fig. 4). It increases linearly with

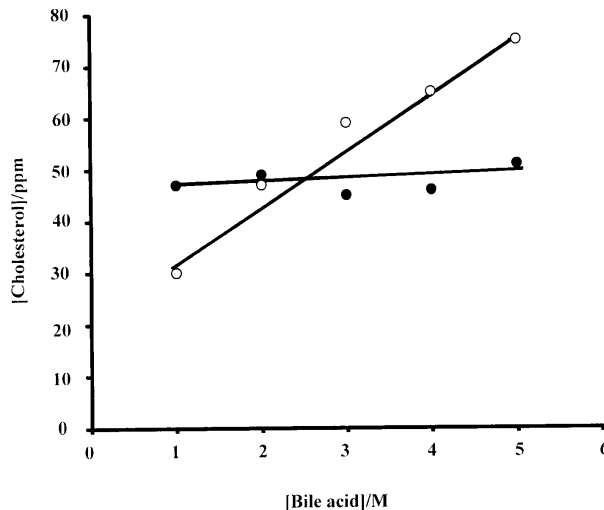


Fig. 4—Dissolution of cholesterol in different concentrations of (○) ursodeoxycholic acid and (●) chenodeoxycholic acid increase in bile acid concentration obeying the relation

$$[Cholesterol] = m [Bile acid] + c$$

where m and c are slope and intercept respectively.

### Conclusions

Kulfa, pathari, grape and tomato were found effective for dissolution of TCP. These biofunctional plant materials are enriched with bioactive molecules, which play major role in dissolution of TCP and cholesterol. Grape extract is found to be most effective in dissolution of TCP among all extracts. All extracts contain carbohydrate, which interact with  $Ca^{++}$  to form a soluble complex. Tomato extract was found to be most effective in dissolving cholesterol among all extracts. Dissolution of cholesterol increases with increasing concentration of bile acid.

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