Short Communication

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INHIBITORY EFFECTS OF AQUEOUS EXTRACTS OF NATURAL PRODUCTS ON CRYSTALLIZATION OF URINARY LITHIASIS *IN VITRO*

Part I Carbonato Apatite

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Urinary obstruction and infection due to the formation of renal calculi of organic/inorganic nature is known since centuries [1]. In order to avoid the growth of these stones certain drugs were found to be effective [2-4]. Natural products and their aqueous extracts have also been quoted as potential inhibitors [5]. The present study comprises selection of some natural products the inhibitory effects of which have been checked on the growth of Carbonato apatite crystals.

Crystals of Carbonato apatite were grown by mixing calcium chloride dihydrate solution (1M) with a mixture of diammonium hydrogen phosphate (1M) and ammonium carbonate (1M) in ammonium chloride-ammonium hydroxide buffer solution (pH = 9.21). The technique for the growth of these crystals was similar to that described by Welshman and McGeown [6]. Each experiment was completed in seven days and solutions including buffer medium, aqueous extracts of natural products and wicks were replaced after every twenty four hours.

The aqueous extracts were obtained by refluxing a known weight of the natural product in 300 ml of distilled water for two hours and then filtering them through Whatman No. 40 filter paper after cooling to room temperature. Formaldehyde solution was added to these extracts to suppress the bacterial growth and were diluted to a fixed volume.

The deposits obtained on glass fibers, after seven days were dissolved in 0.25 N hydrochloric acid and the amounts of calcium were determined by the procedure of Williams and Moser [7]. The absorbancies were recorded at 506 nm using a spectronic 20 spectrophotometer.

Two experiments were run in parallel for each aqueous extract, one with the aqueous extract in the reaction beaker and the second with distilled water (pH = 6.9). The second experiment was run as control and the amount of carbonato appatite so deposited served as reference amount (100 %). The formation of carbonato apatite was confirmed by comparing its infrared spectrum with the one given in literature [8].

The growth retarding effects of certain compounds have been explained by factors like pH, formation of soluble chelates/ salts and adsorption of macromolecules like proteins and polysaccharides on the surface of calculus. The literature survey has indicated that aqueous extracts of the natural products used contain mostly the amino acids, proteins, carbohydrates, organic acids, vitamins, ionic species and mineral substances (Table 1). These compounds/ionic species can react with carbonato apatite forming either soluble salts like sodium carbonate, sodium phosphate etc. or soluble chelate complexes [9-11] with calcium ion.

The increase in solubility of carbonato apatite with the increase in pH has been observed during the present study as shown in Fig. 1. Amino acids should be very reactive at pH near neutral region (pH 6.5-7) because of their dipolar nature. They can react equally well with calcium and other ionic species of the apatite. The extent to which these amino acids could react in dissolving the apatite could be gained from the constituents of the aqueous extracts of these natural products (Table 1). Another way of inhibiting of crystal growth is achieved by adsorption of polysaccharides and proteins on the faces of the growing crystals.

The most efficient growth retarding effect was exhibited by Macrotyloma uniflorum, the aqueous extract of which contained amino acids. The relative solubility was found to be 64% more with respect to the control. The pH of this extract was almost neutral (6.8).

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Table 1. Solubility data of Carbonato apatite in the aqueous extracts of natural products

S. No	Natural product	Composition (aqueous phase)	Amount (gm/1)	pH	Increase in solubility %
1.	*Cucumis melo* (non-edible portion)	Calcium pectate, protein, carbohydrates, sodium, magnesium, ascorbic acid, methionine, phenylalanine.	10.0	6.0	37 ± 5
2.	*Eriobotyra japonica* (leaves)	Pectic acid, vitamin B_1	10.0	6.4	43 ± 7
3.	*Macrotyloma uniflorum* (seeds)	Arginine, phenylalanine.	10.0	6.8	64 ± 1
4.	*Pedalium murex* (fruit)	Hydroxybenzoic acid, glucose and alcohols, acids, esters (C_{28}-C_{32})	10.0	5.5	25 ± 0
5.	*Zea mays* (silk)	Aldose, ketose, glucose, organic acids, mineral substances and vitamin A & B.	5.0	5.4	14 ± 1

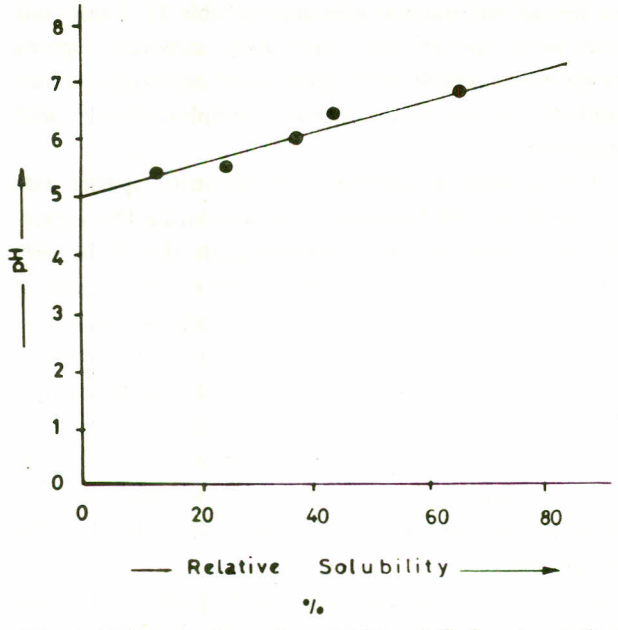

Fig. 1. Effect of pH on the solubility of Carbonato apatite.

The second most effective extract was that of *Eriobotyra japonica*, in which the adsorption of pectic acid on the crystal surfaces of the carbonato apatite and the complex formation capacity of vitamin B_1 might have reduced the quantity of carbonato apatite crystals. Sutor[12] has indicated this kind of reaction with vitamin B_6, resulting in the reduction of calcium oxalate crystals. Similar arguments could be advocated, though to a lesser significance, for growth retarding effects of *Cucumis melo*, *Pedalium murex* and *Zea mays* silk extracts.

Key words: Lithiasis urinary, Natural products, Carbonato apatite.

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