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Reactions of Calcium Phosphate and Calcium Oxalate with Kurthi (*Dolichos bifluorus*) Extract (Fresh and Hydrolysed)

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An attempt has been made to study the reactivity of Kurthi (*Dolichos bifluorus*) on urinary stones as the natural polyphosphates have never been harnessed for their value in the dissolution of urinary stones.

INTRODUCTION

Inhibitory activity results from the net effect of the action of most substances in artificial urine on the formation of calcium oxalate. Calcium and oxalate ions accelerate the reaction and increase the amount of material produced, whereas other ions contribute to the ionic strength and so increase the solubility of calcium oxalate. It slows down the reaction and decreases the amount of calcium oxalate produced. Pyrophosphate is adsorbed on to crystal faces and inhibits further deposition on them.

A few polyphosphates, mainly pyrophosphates, have been found to be potent inhibitors in the formation of calcium oxalate renal stone. Natural polyphosphates occur as phosphate side chains in phosphoproteins of a large number of natural products. A pulse (Kurthi) has been found to contain polyphosphate, connected probably to the protein moiety.

EXPERIMENTAL

(a) Dissolution of calcium phosphate and calcium oxalate in kurthi (*Dolichos bifluorus*) extract solution (fresh and hydrolysed)

Preparation of Kurthi (Dolichos bifluorus) extracts and its acid hydrolysation: 25 g of Kurthi (*Dhlichos bifluorus*) was suspended in 50 mL of water overnight. The amounts of water and Kurthi were so adjusted that the extract remained saturated. The extract was decanted and filtered off. The filtrate was treated with about 10 mL of 2 N HCl and refluxed on a water bath. Necessary amount of Kurthi (*Dolichos bifluorus*) extract was added to the hydrolysed extract and again refluxed to bring the pH back to approximately 6. Intermittent warming was done during the neutralisation with Kurthi (*Dolichos bifluorus*) extract.

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Dissolution of calcium phosphate and calcium oxalate: Solubilities of calcium phosphate and calcium oxalate in the natural product extracts were determined. 125 g of kurthi were suspended in 250 mL of water and processed for extracting juice in the manner mentioned above and the extract made up to 200 mL. To a 100 mL fraction, calcium salt (phosphate/oxalate) was added and stirred for 30 minutes and then filtered; the filtrate was then evaporated to dryness followed by decomposition at 150–200°C. The decomposed material was extracted with water and a few drops of hydrochloric acid, again filtered and made 100 mL. Calcium, in the filtrate, was estimated by using EDTA solution. A similar processing was done for another 100 mL of extract, without any calcium salt. The difference of calcium content was used for calculating the amount of dissolution of calcium salt in the extract.

Dissolution of calcium salt (oxalate/phosphate) in the hydrolysed Kurthi (*Dolichos bifluorus*) extract was determined by the same method.

(b) Inhibitory effect of kurthi on the mineralisation of calcium oxalate

The model system used to study the inhibitory effect of Kurthi (*Dolichos bifluorus*) extract (fresh and hydrolysed) on the mineralisation of calcium oxalate bears some resemblance to the one (model) used by Kabra[1]. The model consisted of two beakers of 100 mL capacity. In one of them 70 mL of 0.1 M solution of calcium chloride was taken, to it 20 mL Kurthi (*Dolichos bifluorus*) extract (fresh/hydrolysed) inhibitor was added. In the second beaker 70 mL of 0.1 M solution of sodium oxalate was placed and 20 mL of Kurthi extract (fresh/hydrolysed) was added to it. Two filter papers (Whatman 41) were folded in a square shape, so that both the wicks had equal surface area. Both of them were separately weighed and weights noted. These filter paper wicks were then suspended separately into the solution of the above two beakers. Suspension was done with the help of copper wires and glass rods. This set of two beakers was termed 'experimental set'. Next, a similar assembly of two beakers with suspended filter paper wicks (weighed and noted separately) were arranged at the side of the experimental set. In this case, however, the contents of the beakers were a little different from the one in the experimental set. One of the beakers of this set contained 90 mL of 0.1 M solution of $CaCl_2$ and the other beaker contained 90 mL of 0.1 M $Na_2C_2O_4$ solution. No inhibitor was added in this case. This set was termed 'blank set'.

Next, the two filter paper wicks suspended in the two beakers of 'experimental set' were interchanged at the end of every five minutes interval. This meant that the filter paper wick suspended in $CaCl_2$ solution was transferred to $Na_2C_2O_4$ solution in the second beaker and the wick suspended originally in $Na_2C_2O_4$ solution was transferred to the beaker containing $CaCl_2$ solution. This interchange of wicks, at the end of every five minutes, was continued for 4 h.

Similarly, the filter paper wicks suspended in the beakers of 'blank set' were also interchanged at the end of every 5 min for 4 h. Work was carried out at room temperature. At the end of experimentation, the filter paper wicks of 'experimental', as well as 'blank' set after careful washing were dried at 90°C in an air oven, cooled to room temperature and weighed separately. From the weights

of filter papers, the amount of crystalloid (CaC_2O_4) deposited on each paper was determined. Average deposition of oxalate in the experimental set was calculated. Similarly average deposition on the blank set papers was also calculated. Further, the difference between the average deposition of oxalate in experimental and blank set was calculated; This was termed 'net inhibition'. This net inhibition value was divided by the average amount deposited in the blank set and multiplied by 100 to get 'percentage inhibition' value. similar 'experimental' and 'blank' sets under all experimental conditions were run for different concentrations. Inhibitor concentration was, however, kept the same in all the runs.

(c) Estimation of phosphate as orthophosphate from kurthi (*Dolichos bifluorus*) extract

100 g of Kurthi (*Dolichos bifluorus*) was suspended in 300 mL of distlled water for 24 h and Kurthi (*Dolichos bifluorus*) extract was filtered out. Same Kurthi (*Dolichos bifluorus*) was suspended in another 300 mL of distilled water for 24 h and extract was filtered out. Both the extracts were acidified with concentrated nitric acid. Reddish brown precipitate was shown in solution. The solution was heated on water bath for complete precipitation and filtered out. Excess of nitric acid was added and heated on water bath for complete precipitation of reddish brown precipitate and filtered out. The solution was cooled and freshly prepared ammonium molybdate reagent was added in excess and stirred for some time. The solution was heated on water bath and kept for 6 h. A yellow precipitate was precipitated and filtered in weighed sintered glass crucible. Again, two or three times ammonium molybdate reagent was added in excess for complete precipitation. The yellow precipitate was filtered in the same crucible, dried at 100°C and weighed.

(a) Solubilities of calcium phosphate and calcium oxalate in various natural product extracts

TABLE-1

Natural product	Nature of phosphate	Solubility mg/100 mL of that extract	
		Cal. phosphate	Cal. oxalate
Kurthi (Fresh) (*Dolichos bifluorus*)	Pyrophosphate	15.50	12.00
Kurthi (Hydrolysed) (*Dolichos bifluorus*)	Pyrophosphate	21.07	14.47

(b) Kurthi (*Dolichos bifluorus*) extract on mineralisation of calcium oxalate

Contents of beakers: Beaker I 70 mL $CaCl_2$ + 20 mL inhibitor solution (Kurthi extract).

Experimental set: Beaker II 70 mL $Na_2C_2O_4$ + 20 mL inhibitor solution (Kurthi extract)

Blank set: Beaker I 90 mL $CaCl_2$ solution; Beaker II 90 mL $Na_2C_2O_4$ solution

TABLE-2
pH range 5–7

No. of observation	Inhibitor	Strength of salt forming solutions	Accretion of Cal. oxalate (mg)		Net inhibition	Percentage inhibition
			Blank set	Expt. set		
1.	Kurthi (*Dolichos bifluorus*) (Fresh)	0.10 M	572.0	449.0	123.0	21.5
2.	Kurthi (*Dolichos bifluorus*) (Fresh)	0.04 M	450.0	284.0	166.0	36.8
3.	Kurthi (*Dolichos bifluorus*) (Hydrolysed)	0.10 M	568.2	384.5	183.7	32.3
4.	Kurthi (*Dolichos bifluorus*) (Hydrolysed)	0.04 M	446.0	154.0	292.0	65.5

(c) Estimation of phosphate as orthophosphate from Kurthi (*Dolichos bifluorus*) extract

TABLE-3

Weight of Kurthi (*Dolichos bifluorus*)	Nature of phosphate	% weight of phosphate (g)
100 g	orthophosphate	4.340

RESULTS AND DISCUSSIONS

Our findings in the present work with Kurthi (*Dolichos bifluorus*) suggest that both calcium oxalate and calcium phosphate have appreciable solubilities in Kurthi (*Dolichos bifluorus*) extracts. The solubilities are more in acid hydrolysate of Kurthi (*Dolichos bifluorus*). This is perhaps due to increased polyphosphate content in acid hydrolysate of Kurthi (*Dolichos bifluorus*). The inhibitory action of Kurthi (*Dolichos bifluorus*) extracts in the mineralisation of calcium oxalate has also been found to be appreciable. Results show that in the inhibition experiment, the accretion of calcium oxalate in the experimental sets is appreciably less than that in the Kurthi (*Dolichos bifluorus*) corresponding blank sets. Presence of Kurthi (*Dolichos bifluorus*) extracts in the experimental sets is responsible for this inhibition of calcium oxalate accretion. The percentage inhibition increases as the dilution of the salt forming solutions increases. In the digestive system, where the dilution of salt forming solutions would be very high, the inhibitory effect of Kurthi (*Dolichos bifluorus*) can also be expected to be very high.

Our present findings with Kurthi (*Dolichos bifluorus*) suggest that it is a physiologically non-toxic natural product and a good solubiliser of calcium oxalate. It does so by virtue of its chain polyphosphate groups.

REFERENCE

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