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Reactions of Urinary Stones with Hydroxy Acids

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Abstract : Stone diseases (gallstones and kidney stones) are extremely painful and often cause death. The aim of biomedical research in this area has been determination of factors resulting in stone formation inside the gallbladder and urinary tract. Many theories have been put forward to explain the mechanism of stone formation and their growth. However, their complete cycle of pathogenesis is still under debate. Several factors are responsible for stone formation; however, much emphasis is placed on the determination of elemental and molecular composition of the stones. In the present review article, we describe different kinds of spectroscopic techniques such as Fourier transform infrared spectroscopy (FTIR), X-ray fluorescence (XRF) spectroscopy, time-of-flight secondary ion mass spectrometry (TOF-SIMS), and laser-induced breakdown spectroscopy (LIBS) and highlight their use in the analysis of stone diseases. We have summarized work done on gallstones and kidney stones using these advanced techniques particularly over the last 10 years. We have also briefly elaborated the basics of stone formations inside the human body and their complications for a better understanding of the subject. An attempt has been made to solubilise the insoluble ingredients of the kidney stones (powdered stones and then whole stones) with plant acids (citric and malonic acids) and then by some fruit juices, e.g., tomato juice (which con ns these plant acids) and also with extracts of some natural products, e.g., Kurthi (*Dolichos bijliwrus*) containing inorganic and organic polyphosphates.

Keywords: Gallstones, Kidney stones, FTIR, WDXRF, EDXRF, TOF-SIMS, and LIBS.

I. INTRODUCTION

The ultimate shape of a renal calculus results from several forces working together. The basic crystalline characteristics of the stone areinfluenced by local factors: mobility or fixation, wall contact with resulting pressure and mucous coating, urine flow and stasis. Recognition of the active factors operating to form each stone separately, may help in prognosis, and provide a logical surgical attack and open avenues for preventing stone growth.

We do have some understanding of the factors which encourage stone formation:

1. Increase concentration in the urine of the stone forming crysta1Ioids
2. Abnormally low or high urine pH
3. Deficiency of sequestering substances, and
4. Urinary tract obstruction with stasis of urine.

The ‘relative probability’ of forming stones may be explained on the basis of a number of risk factors, viz., calcium oxalate, pH. Acid mucopolysaccarides (AMPS) and uric acid. Two main chemical factors seem to determine the risk of forming calcium containing stones:

1. The degree of saturation of urine with calcium oxalate and calcium phosphate

2. The level of protective inhibitory activity against crystallization of calcium salt of these factors calcium oxalate and pH together control the stimulation of urine with calcium salts; while AMPS and the uric acid largely determine the inhibitory activity of urine.

II. EXPERIMENTAL

Preparation of tomato extract and its acid hydrolysate

100g of fresh tomato was juiced on an ordinary juicer; the juice was treated with 20 ml of 2N HCl and warmed on a water bath. Then it was neutralized with a dilute solution of sodium bicarbonate to a pH<7. The hydrolysate was filtered and made 100 ml.

Preparation of Kurthi (Dolichos bifluorus) extract and its acid hydrolysate

25 g of Kurthi (Dolichos bifluorus) was suspended in 50 ml of water overnight. The amounts of water and Kurthi (Dolichos bifluorus) were so adjusted that the extract remained saturated. The extract was decanted and filtered off. The filtrate was treated with about 10 ml of 2N HCl and refluxed. Necessary amount of Kurthi (Dolichos bifluorous) extract was added to the hydrolysed extract and again refluxed to bring the pH back to approximate 6. Intermittent warming was done during the neutralization with Kurthi (Dolichos bifluorus) extract.

Experiment: Stone: were brought from P.G.I., Chandigarh.

(a) Powder Stone:

(i) 100 mg of powdered urinary stone (from P.G.I., Chandigarh, sample No. 458) was suspended in 20 ml of 0.1 N sodium chloride solution for 24 hrs. The sample was filtered, dried at 80°C and then cooled and weighed.

Weight amount of this powdered stone was suspended in 25 ml. of citric acid solution for 24 hrs. It was filtered out, washed with distilled water, dried at 80°C for 1 hr, cooled and weighed.

(ii) 30 mg of powdered urinary stone (from P.G.I., Chadigarh sample No. 458) was suspended in 20 ml. of 0.1 N NaCl solution for 24 hrs. The sample was filtered, washed with distilled water, dried at 80°C then cooled and weighed.

In this case, solubilites could not be determined because some precipitation took place from Kurthi (Dolichos bifluorus) extract, increasing thus the weight of the stone powder. Under similar conditions, further experiments were run for different stone powders, viz., Sample No. 434 from P.G.I., Chandigarh. Inhibitor concentration was kept the same in all the run.

(b) Whole stone: 100 mg of whole stone (from P.G.I., Chandigarh sample No. 458) was suspended in 20 ml. of 0.1 N NaCl solution for 48 hrs. the sample was filtered, washed with distilled water, dried at 80°C, cooled and weighed.

Weighed amount of this whole stone was suspended again in 25 ml. of Kurthi (Dolichos bifluorus) extract for another 48 hrs. the undissolved stone was taken out, washed with distilled water, dried at 80°C for 1 hr. cooled and weighed.

Similar experiments were carried on with a different whole urinary stone (from P.G.I., Chandigarh, sample No. 1279). In every experiment, different concentration of tomato fresh, tomato hydrolystate and Kurthi (Dolichos bifluorus) hydrolysate were used.

It was found that the dissolution of stone ingredient with Kurthi (Dolichos bifluorus) extract in powdered stone was much more than the whole stone. This led to infer that the outer surface of the stone is much stubborn and the extracts are not able to react so easily, to make soluble the ingredients of the stone.

Nevertheless, the dissolution of a part of the ingredient of the whole stone definitely loosens the hardness of the stone. The stone then became very much susceptible to attack; and the extracts then further dissolved the stone and the stone crumbled.

Table-1

Solubility of urinary powder stone in different inhibitor concentration

Inhibitor	Strength	Sample No.	Weight of powder stone (mg)	Weight remained after N/10 NaCl	Weight remained after inhibitor	Difference (solubility in mg/25ml.)	% Solubility in mg/25 ml. inhibitor
Citric acid	M/2.5	458	100	98.8	88.5	11.5	11.5
Tomato	Fresh	458	30	29.4	16.2	13.8	46.6
Kurthi (Dolchos bifluorus)	Hydro.	458	30	Solubility could not be detected because precipitation took place from Kurthi (Dolchos bifluorus) extract.			
Citric acid	M/2.5	431	100	99.2	81.0	19.0	19.0
Tomato	Fresh	431	30	29.5	22.5	7.5	25.0
Kurthi (Dolchos bifluorus)	Hydro	431	30	Solubility could not be detected because precipitation took place from Kurthi (Dolchos bifluorus) extract.			

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Table-2

Solubility of urinary whole stone in different natural product extracts

Natural product	Fresh/Hydrolysed	Sample No.	Weight of whole stone (mg)	Weight remained after N/10 NaCl treatment (mg)	Weight remained after inhibitor treatment in (i) first 48 h (ii) second 48 h (mg)	Difference (solubility in mg/25ml.)	% Solubility in mg/25 ml. inhibitor
Kurthi (Dolchos bifluorus)	Hydro.	458	100	100	(i) 85 (ii) 55	15 45	15.0 48.0
Kurthi (Dolchos bifluorus)	Hydro	431	115	112	(i) 72 (ii) 45	40 67	34.7 58.2

Similar experiments with tomato juice showed that the dissolution of powered ingredient was higher compared to whole stone. It has also been observed that for a particular stone, the Kurthi (Dolichos bifluorus) extracts (fresh and hydrolysed) dissolve more ingredients than the tomato extracts (fresh and hydrolysed). This is due to the fact that Kurthi (Dolichos bifluorus) extracts (fresh and hydrolysed) dissolve both the calcium oxalate and calcium phosphate part of the stone, whereas the tomato extracts are found to be only reactive towards the calcium-phosphate part o the stones.

Table-3

Solubility of urinary powder stone in different natural product extracts

Natural product	Fresh/Hydrolysed	Sample No.	Weight of powder stone (mg)	Weight remained after N/10 NaCl treatment (mg)	Weight remained after inhibitor treatment (mg)	Difference (solubility in mg/20ml.)	% Solubility in mg/20ml. of extract
Tomato	Fresh	1245	50.0	48.5	35.5	14.5	29.0
Tomato	Acid hydrolysed	1245	50.0	49.5	30.8	19.2	38.4
Tomato	Acid hydrolysed	1279	50.0	48.7	26.3	23.7	47.4

Table-4

Solubility of urinary whole stone in different natural product extracts

Natural product	Fresh/Hydrolysed	Sample No.	Weight of whole stone (mg)	Weight remained after N/10 NaCl	Weight remained after extract treatment (mg)	Difference (solubility in mg/20ml.)	% Solubility in mg/20ml. of extract
Tomato	Fresh	1245	71.0	70.4	67.30	3.70	5.1
Tomato	Acid hydrolysed	1245	87.0	86.5	80.20	6.80	7.8
Tomato	Acid hydrolysed	1279	35.0	35.0	30.00	5.00	14.3
Kurthi (Dolchos bifluorus)	Fresh	1245	70.0	69.3	65.80	4.20	6.0
Kurthi (Dolchos bifluorus)	Acid hydrolysed	1245	55.0	54.5	46.87	8.13	14.8

Next we carried out similar experiments to solubilise whole and powdered stone with citric acid and observed that in the case of whole stone, citric acid (M/2.5) did not dissolve significantly, compared to the other natural product extracts.

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