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To Study The Composition Of Human Stones In Gorakhpur Region, North-East Uttar Pradesh, India

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Abstract: The purpose of the present study was to investigate the composition of stones from the different parts of the body from the patients of age group of 16, 18, 44 &70 years. Samples were collected after surgery (buccal cavity, vesical calculi, gall bladder and urinary bladder calculi). The samples were analyzed by X-Ray diffraction method, FTIR Spectroscopy and Atomic Absorption Spectroscopy. Results show that these stone samples contain Ca^{++}, PO_4^{---}, Mg^{++} and $C_2O_4^{--}$ ions while urinary bladder vesical calculi samples contained uric acid. Samples were estimated by atomic absorption spectrometry shows calcium ion concentration in vesical calculi and urinary bladder in the range of 2.7% to 13.8 % while in gallbladder 4.2% calcium ion content. The study reveals that patients suffering from stone are mainly concerned with the deposition of calcium phosphate present in the local drinking water.
Key words: Composition Of Human Stones In Gorakhpur Region, North-East Uttar Pradesh, India.

Introduction

Urolithiasis is a medical term which denotes stone originating anywhere in the urinary tract including kidneys and bladder [1]. The stone formation in kidney is entirely different from the formation of stones in the bladder from pathological view point. Stones in kidney originate due to physic-chemical change leading to supersaturation of urine with stone forming salts. The fate of supersaturation is formation of phosphate containing stone which are commonly known as Brushite [2-5]. The urolithiasis related to urinary tract infection results in the formation of Struvite stone. These struvite stones are primarily composed of calcium salts [6]. It is interesting to note that most human do not form stones although the supersaturation of urine is a frequent process. This is probably due to presence of inhibitors in the normal urine which prevent the crystal growth. The present study involves FT-IR, X-ray diffraction and atomic absorption studies of stone samples of different age group patient, and also influence of certain inhibitors such as α-ketoglutaric acid, ascorbic acid and leucine on the precipitation and dissolution of calcium phosphate.

Materials And Methods

Collection and characterization of stone samples

Surgically removed stone sample from different part of the body dry on gauze and washed with deionizer distilled water. These samples were collected from BRD Medical College, Gorakhpur and characterized by different physic - chemical methods.

Qualitative Analysis

Qualitative analysis for Ca^{++}, Mg^{++}, PO_4^{---}, and $C_2O_4^{--}$ by usual methods and uric acid was analyzed by the following methods. To the substance added 2-3 drops of concentrate HNO3 in a small evaporating disc and evaporated to dryness by heating on a water bath. A reddish yellow residue was obtained which on cooling changed to purplish red in addition to dilute ammonium hydroxide. It is also known as Murexide test. The presence of cholesterol in the sample was tested by the following methods.

Powder X-ray diffraction studies

Powder X ray diffraction patterns of surgically removed stones from buccal cavity, vesical calculi, gall bladder and urinary bladder calculi were taken with a Phillips Analytical X ray diffractometer type 1710 equipped with PC -ADP diffraction software and using Cu K-α radiation in the 20 range 10-120. Powder X ray diffraction patterns of Calcium phosphate obtained by reacting $CaCl_2$ (0.01M) and ADP (0.01M) and Calcium phosphate were recorded.

Infra red spectroscopy

Infra spectra of different stone samples were taken with a I-R spectrophotometer spectrometer model FTIR spectrometer, perkin Elmer paragon 1000. Results were compared with those of calcium phosphate, calcium oxalate and uric acid.

Precipitation and characterization of Calcium phosphate

Efforts have been made carried out. Detailed investigation on calcium phosphate which is one of the major constituents of the calculi .Calcium phosphate was precipitated by mixing an equimolar mixture of aqueous Calcium chloride (0.01M) and ammonium di-hydrogen phosphate (0.01M) in gel media However for the characterization of product precipitation was made in the absence of gel media.

Qualitative and quantitative analysis

The reaction product which is obtained by the addition of 0.01 M $CaCl_2$ to 01 M ADP solutions was analyzed for Ca^{++} and PO_4^{---} ions. The calcium content was quantitatively estimated titrimatically as follows:

The $KMnO_4$ solution was standardized with standard ferrous ammonium sulphate. Took 0.5g of calcium phosphate in a few milliliter of concentrate HNO_3, boiled, evaporated it 2-3 times and dissolved in water. Ammonium oxalate solution and NH_3 solutions were added and filtered with Whatmann's filter paper. The filtrate was tested with oxalate ion by the addition of $CaCl_2$ solution, precipitate was poured along with the filter paper in a 400ml beaker. Added dil H_2HSO_4 and 30 ml distilled water. Warmed up to 400°C and finally titrated it with standardized $KMnO_4$ solution.

Results And Discussion

Variety of stone samples from different parts of body viz vesical calculi (patients from different aged group 16, 18, 44 &70 years), urinary bladder stone, buccal cavity and gall bladder stones were collected and placed on sterile gauze to dry in air and washed with distilled de – ionized water to remove the bile and debris and qualitatively analyzed for the presence of Ca^{++}, Mg^{++}, PO_4^{---}, uric acid etc. Results shows (Table 1) that these stone samples contain Ca^{++}, PO_4^{---}, Mg^{++} and $C_2O_4^{--}$ ions while urinary bladder vesical calculi samples also contained uric acid. A variety of physical techniques including X- ray diffraction, Infra-red spectroscopy, and Atomic absorption spectroscopy were employed. Powder X-ray diffraction patterns of stone samples and calcium phosphate (Fig 1-2) and (Table 2-5). d values and corresponding I/I0 value from ASTM data file and X- ray diffraction pattern indicate that buccal cavity stone contained the lines of $CaHPO_4.3H_2O$ and $CaC_2O_4.H_2O$. Vesical calculi samples obtained from 6 year old patients contained lines of ammonium phosphate. In Gall bladder stone however lines for the $CaC_2O_4.H_2O$ could not be detected. Generally, FT-IR spectra shows absorption bands (stretching or bending) at special wavelength representing particular functional group as a result of vibrational and rotational motion in the molecule. The absorption or intensity of a band is greatly influenced by the molecular composition and stretching of each constituent of a complex mixture. Therefore, in kidney stone each constituent could be differentiated by its absorption spectrum with specific stretching or bending bands. Commonly in FTIR the possible method for the analysis of composition of sample is the spectral matching technique through which an unknown sample spectrum is compared to a number of library spectra, installed within the FTIR and identified by the most similar spectrum. Similarities close to 100 means that the sample consists of the same constituent with the same ratio. Infrared spectra of different calculi samples shows that vesical calculi from (i) 6 year old patient contained peaks at 1000, 1125 for phosphate, 1310 for oxalate, 1375 for carbonate, 1600 -1700 cm-1for uric acid, (ii) 18 year old patient contained peaks at 1000

and 1120 for phosphate, 1310 for oxalate, 1625 for uric acid and 3050 cm-1for magnesium ammonium phosphate, (iii) 44 year old patients contained peaks at 1000, 1125 for phosphate, 1310 for oxalate, 1625 for uric acid and 3000-3600 cm-1 for magnesium ammonium phosphate. (iv) 70 year old patient contained peaks at 1025 and 1125 for phosphate, 2000 for uric acid and 2550 – 3250 cm-1 for magnesium ammonium phosphate which shows a broad band. Urinary bladder stone from 40 year old patient contained peaks at 1120, 1310, 1625, 2320, 3050 cm-1 for similar constituents as for other calculi maintained above. (vi) Gall bladder stone from patient contained peaks at 1000, 1080, 1325 1400, 1475, 1625, 2325, 2975, and 3450 cm^{-1} showing the presence of similar constituents as above (Table – 6, Fig 3). The interpretation of the most important peaks in the infrared spectra was carried out with the help of literature [7-10].

Total calcium concentration in different stone samples was estimated by atomic absorption spectrometry. Total calcium ion concentration in vesical calculi and urinary bladder sample were found in the range of 2.7 to 13.8 % while gallbladder stone contained 4.2% calcium ion content (Table 7-10 and Fig 4 -5). Calcium oxalate and calcium phosphate are the most Common crystalline constituents of human urine, Powder X-ray diffraction studies, Infrared and atomic absorption results suggest the presence of calcium phosphate in different stone samples. Calcium oxalate crystals are proposed to be induced by calcium phosphate. There is a considerable interest in the solubility of most wide spread calculi and it has been a subject of many investigations. Dissolution of vesical calculi samples in α-ketoglutaric acid, leucine and ascorbic acid have been studied (Table 11 and Fig 6). Results show that dissolution was quite effective in presence of leucine, ascorbic acid and α-ketoglutaric acid of equal concentration.

Table 1

Qualitative analysis of inorganic and organic constituents of different calculi samples obtained from different sources.

Sample	Qualitative analysis results
Vesical calculi of different age group patients	
6 years	Ca^{++} PO_4^{---} Mg^{++} $C_2O_4^{--}$ uric acid
18 years	Ca^{++} PO_4^{---} Mg^{++} $C_2O_4^{--}$
24 years	Ca^{++} PO_4^{---} Mg^{++} $C_2O_4^{--}$ uric acid
44 years	Ca^{++} PO_4^{---} Mg^{++} $C_2O_4^{--}$
70 years	Ca^{++} PO_4^{---} Mg^{++} $C_2O_4^{--}$ uric acid
Urinary bladder calculi	Ca^{++} PO_4^{---} Mg^{++} $C_2O_4^{--}$ uric acid
Buccal cavity stone	Ca^{++} PO_4^{---} Mg^{++} $C_2O_4^{--}$
Gall bladder stone	Ca^{++} PO_4^{---} Mg^{++} $C_2O_4^{--}$ Cholesterol

Table 2

X-ray diffraction data of buccal cavity stone.

Buccal cavity calculi		
Angle (2θ)	d-value (A^{o})	Rel.int (%)
10.125	8.7292	46.7
10. 245	8.6272	30.4
25.940	3.4320	57.6
28.310	3.1498	13.1
31.255	2.8594	57.6
32.275	2.7714	100.0
36.690	2.4474	15.7
39.720	2.2674	21.7
46.540	1.9498	17.1
49.590	1.8367	18.6
53.100	1.7233	25.0
87.135	1.1176	14.4
96.705	1.0308	8.6
117.953	0.8989	5.0

Table 3

X-ray diffraction data of vesical calculi obtained from 6 year old patient.

Vesical calculi		
Angle (2θ)	d-value (A^0)	Rel.int (%)
24.8	3.590	83.75
26.0	3.4269	75.0
28.6	3.1210	72.5
30.5	2.9308	100
35.2	2.5995	72.5
37.5	2.3982	62.5
42.5	2.1270	27.5
46.0	1.9646	27.5
48.6	1.8733	33.7
51.8	1.7648	23.75

Table 4

X-ray diffraction data of vesical calculi obtained from 70 year old Patient.

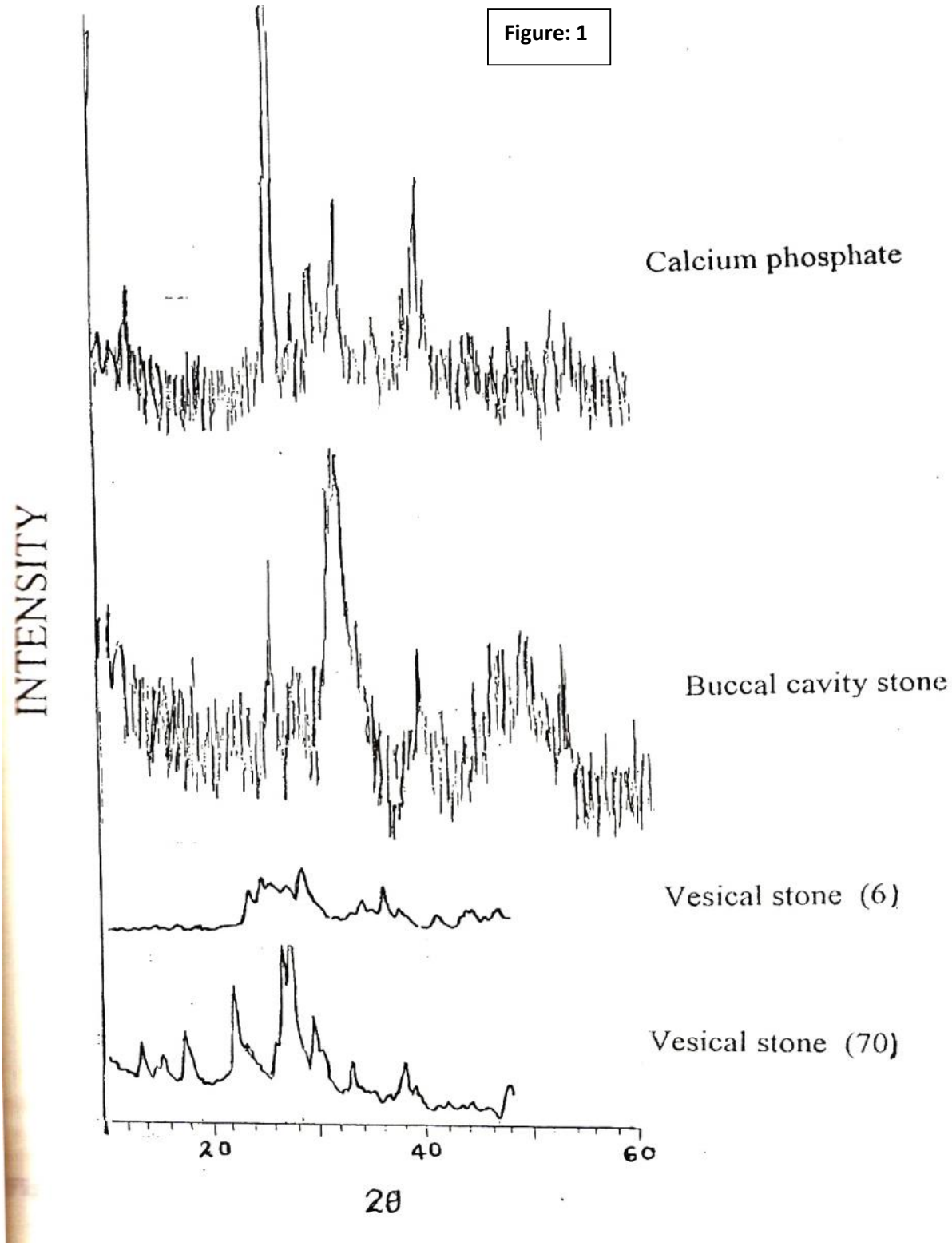
Vesical calculi		
Angle (2θ)	d-value (A^0)	Rel.int (%)
10.2	8.6720	10.8
13.6	6.610	8.7
15.0	5.906	32.6
15.8	5.6088	57.6
18.0	4.9279	26.9
23.0	3.867	47.1
24.5	3.6332	60.7
28.0	3.1865	22.3
29.0	3.0789	84.2
31.0	2.8847	100
32.0	2.7968	46.9
34.8	2.5779	13.4
40.2	2.2432	25.7
41.3	2.1859	26.1
44.5	2.0359	15.0
47.0	1.9333	7.6
50.9	1.7938	16.15

Table 5

X-ray diffraction data of urinary bladder calculi

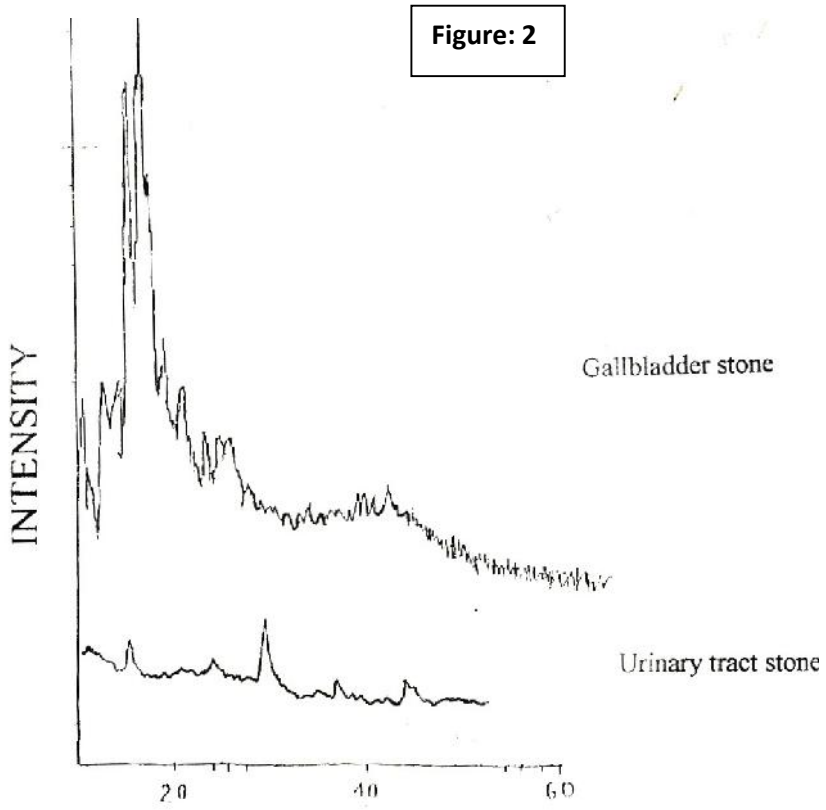
Urinary Bladder Calculi		
Angle (2θ)	d-value (A^0)	Rel.int (%)
15.2	5.8288	76.92
24.6	3.6187	57.1
30.5	2.9308	100
38.5	2.3382	31.8
40.2	2.2431	15.7
44.0	2.0579	13.7
46.2	1.9649	36.2

Figure: 1



X – Ray diffraction pattern of calcium phosphate, buccal cavity stone, vesical calculi from 6 year old patient, vesical stone from 70 years old patient.

Figure: 2



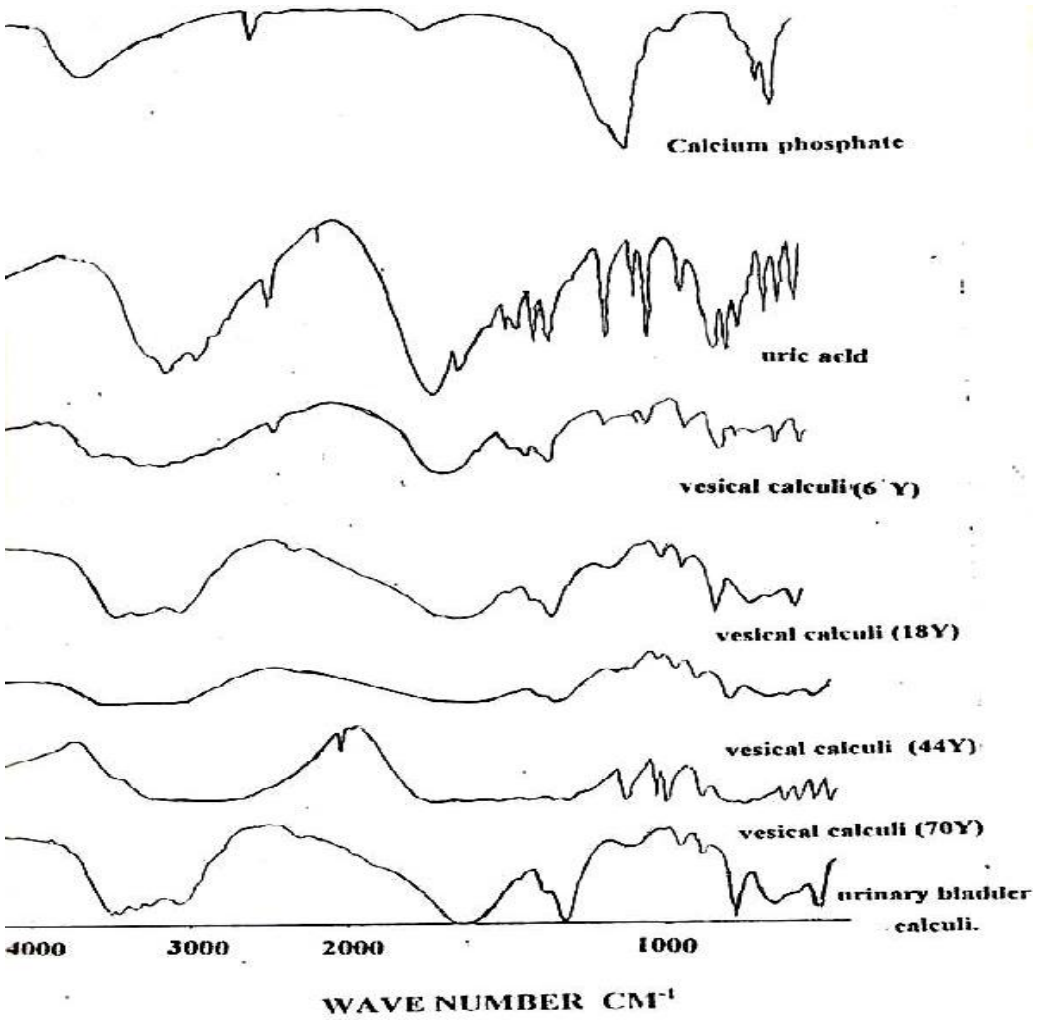
Powdered X – Ray diffraction pattern of gall bladder stone and urinary tract

Table 6

Infrared spectral results of surgically removed calculi obtained from different parts of the human body.

Vesical calculi obtained from different aged patients				Other stones and reference substances				
6 y	18 y	44 y	70 y	Urinary stone	gall bladder stone	calcium phosphate precipitate	calcium oxalate	uric acid
wave number cm^{-1}				wave number cm^{-1}				
1000	1000	1000	1025	1120	1000	1025	877	1000
1125	1120	1125	1125	1310	1080	1127	1000	1025
1310	1310	1310	2000	1500	1325	1305	1066	1100
1375	1625	1625-1700	2550-3250	1625	1400	1550	1132	1300
1600-1700	2320	2320	3000 -3600	2320	1475	1650	1350	1350
	3050	3000-3600		3050	1625	2340	1647	1575
					2325	2900	2366	1675
					2975	3450	2873	2000
					3430		3178	2300
							3301	
							3413	
							3483	
							3559	

Figure: 3



Infrared spectrum of Calcium Phosphate, uric acid, vesical calculi (6Y. 18Y, 44Y and 70 Y) and urinary bladder calculi

Table 7

Total calcium content in different stone samples.

Stone samples	Total calcium content (ppm)
Vesical calculi	
6 y	2761
44 y	1818
70 y	543
Gall bladder calculi	853
urinary bladder calculi	2701

Table 8

[α - ketoglutaric acid] / M	Ca content in the liquid phase (ppm)
0.0	0.0
2.0×10^{-4}	15.05
6.0×10^{-4}	23.8
1.0×10^{-3}	28.57
4.0×10^{-3}	40.12
8.0×10^{-3}	46.20

Urinary bladder calculi were obtained from a 70 year old patient. 50mg of the stone was mixed with 20ml of an additive and left for 24 hrs.

Table 9

[α - ketoglutaric acid] /M	Ca content in the liquid phase (ppm)
2.0×10^{-4}	46.02
6.0×10^{-4}	48.32
1.0×10^{-3}	57.92
2.0×10^{-4}	98.82
4.0×10^{-3}	115.6
8.0×10^{-3}	119.1

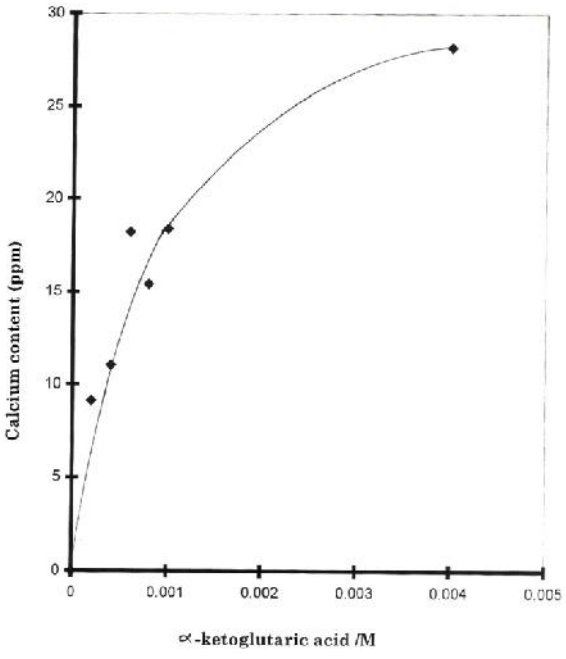
Gall bladder calculi were obtained from a 32 year old patient. 50mg of the stone was mixed with 20ml of an additive and left for 24 hrs.

Table 10

Samples obtained from different aged patients	calcium content in the liquid phase after 24 h (ppm)
6 years	21.84
18 years	22.12
24 years	15.75
44 years	22.75
70 years	16.87

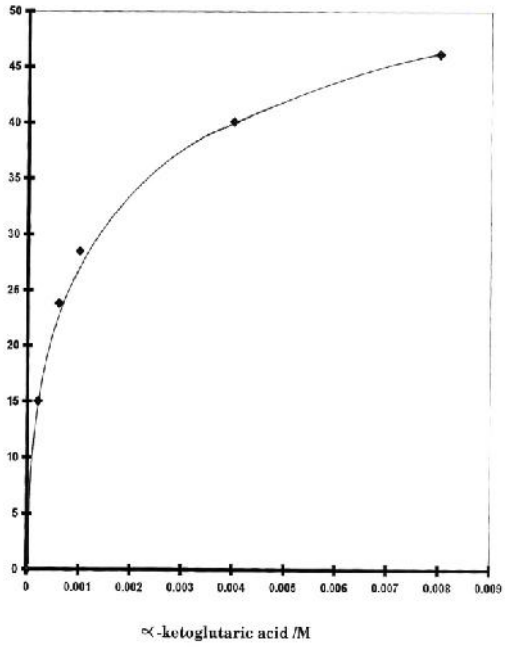
Vesical calculi were obtained from different age group patient. 50mg of the stone was mixed with 20ml of α-ketoglutaric acid (6mM).

Figure: 4



Dissolution of vesical calculi at different concentration of α-ketoglutaric acid

Figure: 5



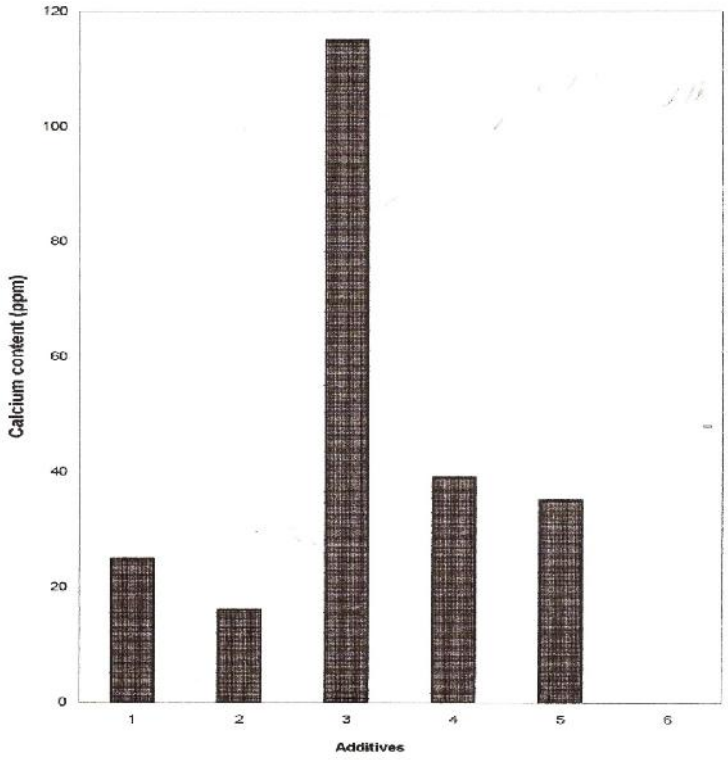
Dissolution of urinary bladder at different concentration of α-ketoglutaric acid

Table 11

Stone samples	Additives	Concentration	Ca content in the liquid phase (ppm)
Vesical calculi	Tulsi extract	1%	3.85
	Bel extract	1%	61.42
	α - ketoglutaric acid	2.0×10^{-4}	9.120
		4.0×10^{-4}	11.02
		6.0×10^{-4}	18.2
		8.0×10^{-4}	15.4
		1.0×10^{-3}	18.37
		4.0×10^{-3}	28.17
	Leucine	1.0×10^{-3}	35.17
	Ascorbic acid	1.0×10^{-3}	31.50
Urinarybladder calculi	α - ketoglutaric acid	0.0	0.0
		2.0×10^{-4}	15.05
		6.0×10^{-4}	23..80
		1.0×10^{-3}	28.57
		4.0×10^{-3}	40.12
		8.0×10^{-3}	46.20
Gall bladder calculi	α - ketoglutaric acid	0.0	0 .00
		2.0×10^{-4}	46.02
		6.0×10^{-4}	48.32
		1.0×10^{-3}	57.92
		2.0×10^{-3}	98.82
		4.0×10^{-3}	115.6
		8.0×10^{-3}	119.5
Vesical calculi	α - ketoglutaric acid	6.0×10^{-3}	
18Y			22.12
24Y			15.75
44Y			22.75
70Y			16.87
Calcium phosphate	0.0	0.0	0.0
	Neem extract	1%	25
	Bel extract	1%	16
	α - ketoglutaric acid	2.0×10^{-4}	54
		4.0×10^{-4}	66
		6.0×10^{-4}	93
		1.0×10^{-3}	115
	Leucine	1.0×10^{-3}	39
	Ascorbic acid	1.0×10^{-3}	35

Atomic Absorption spectroscopic results for the dissolution of vesical calculi in different media

Figure: 6



Dissolution of Calcium Phosphate in presence of different activities (1) Neem extract 1%, (2) Tulsi extract 1%, (3) Bel extract 1%, (4) α-ketoglutaric acid, (5) Leucine, (6) Ascorbic acid (0.001M)

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