Original Research Article

Inhibition of mineralization of urinary stone forming minerals by some natural acids and their derivatives

Nilam Kumari[1], Naresh Kumar[2*]

[1]M.SC Ph. D scholar, [2]Professor, Department of Chemistry, B. N. Mandal University, Madhepura, Bihar, INDIA.
Email: nilam.nkd@gmail.com

Abstract

Background: Most stones form due to a combination of genetics and environmental factors. Risk factors include high urine calcium levels; obesity; certain foods; some medications; calcium supplements; hyperparathyroidism; gout and not drinking enough fluids. Stones form in the kidney when minerals in urine are at high concentration. **Methods:** It was Observational Study. The participants in the study included 10 calculi patients with CaOx stones urinary stones. The Study Conducted in the surgical department of M.G.M. Medical College and L.S.K. Hospital, Period between January 2018-December 2019 and all participants provided informed consent. **Results:** the inhibitors are relatively less effective in the inhibition of calcium oxalate. However, malic and tartaric acids in higher concentration are effective in inhibiting even this stubborn crystalloid to good extant (80-88%). Once formed calcium oxalate has practically no solubility in hydroxyl acid solution but if they are present before the formation of calcium oxalate, they may prevent the precipitation by exerting specificity towards calcium ions. Most of the inhibitors have been found to effectively inhibit calcium carbonate precipitation. A higher pk value of carbonic acid compared to that of inhibitors might be a factor. **Conclusion:** All of our observations in the present study are in-vitro and from chemical point of view. In vivo studies in animal systems and also human trials can only prove the affectivity of these acids in inhibition and dissolution of urinary stones. Our present in-vitro studies, nevertheless, would definitely form foundation for designing drugs for chemodissolution of urinary calculi.
Key Word: urinary stone, calcium oxalate, calcium phosphate, mineralisation

[*]**Address for Correspondence:**
Dr Naresh Kumar, Professor, Department of Chemistry, B. N. Mandal University, Madhepura, Bihar, INDIA.
Email: nilam.nkd@gmail.com
Received Date: 06/11/2019 Revised Date: 12/12/2019 Accepted Date: 19/01/2020
DOI:https://doi.org/10.26611/10021636

Access this article online	
Quick Response Code:	Website: www.medpulse.in
	Accessed Date: 24 December 2020

INTRODUCTION

A solution is saturated when the activity product of solutes supports neither growth nor dissolution of crystals composed of those solutes. However, spontaneous crystallization often does not occur when the activity product is higher than this value. Such a solution is said to be supersaturated with respect to these moieties or metastable. Urine is virtually always metastable with respect to calcium oxalate in most individuals, whether they are stone formers or not, and it is metastable at least some of the time with respect to other stone constituents, such as uric acid, other urates, and calcium phosphate. Tubular fluid supersaturation estimates based on rat data have been made, which suggest that supersaturation for calcium phosphate regularly occurs in the loop of Henle.[1] If true for humans, it could provide the driving force for the development of interstitial calcium phosphate deposits known as Randall's plaques. The role of these deposits in stone disease is discussed later. Although the urine of calcium stone formers is often more supersaturated with respect to calcium oxalate and calcium phosphate than normal individuals, most of the latter are also supersaturated. However, nucleation of calcium oxalate or phosphates usually does not occur and, even if it does, the crystals produced do not grow or aggregate to a sufficient size to

be retained in the kidney on the basis of size alone.[2] Consequently, urine or, more properly, tubular fluid likely contains inhibitors of crystal formation, specifically, nucleation, growth, or aggregation. Inhibition of all of these aspects of crystallization has been observed in vitro with urine itself, with some low molecular weight components of urine, and with some macromolecules isolated from urine.[3-5] This inhibitory property has been reported to be defective in some stone formers. Supersaturation is expressed as the ratio of urinary calcium oxalate or calcium phosphate concentration to its solubility, which is the driving force in stone formation. Supersaturation is generally higher in patients with recurrent kidney stones than in those without, and the type of stone that is formed correlates with urinary supersaturation. At supersaturation levels above 1, crystals can nucleate and grow, promoting stone formation, while they dissolve at levels below 1. Calcium oxalate supersaturation is independent of urine pH; however calcium phosphate supersaturation increases rapidly as urine pH rises from 6 to 7. Since calcium oxalate stones may form over an initial calcium phosphate layer, treatment optimally should lower the supersaturation of both chemical species.[6]

METHODOLOGY

It was Observational Study. The participants in the study included 10 calculi patients with CaOx stones urinary stones. The Study Conducted in the surgical department of M.G.M. Medical College and L.S.K. Hospital, Period between January 2018- December 2019 and all participants provided informed consent. Crystalloid forming solutions, viz., solution of calcium acetate, trisodium phosphate, disodium oxalate and sodium carbonate were prepared in distilled water. Four experimental models namely simultaneous Flow Static Model (S.S.M), Simultaneous Flow Dynamic Model (S.D.M), Reservoir Static Model (R.S.M), and Reservoir Dynamic Model (R.D.M), were designed. In the S.S.M model the two salt forming solution, e.g. Sodium Phosphate and Calcium Acetate (for calcium phosphate) and the inhibitor (hydroxyl or dibasic acid) were taken in three separate burettes (50 ml) and were allowed to fall simultaneously into a 250 ml beaker in a slow (dropwise) and equal speed. The whole operation took about 40 minutes. At the end the mixture was digested in a hot water bath for 10 minutes cooled to room temperature and the precipitate was collected into a pre-weighed centrifuge tube by centrifuging small volumes at a time and rejecting the supernatant liquid. Next the tube with the precipitate was dried in an air oven at 120° C, cooled to room temperature and weighed till constant weight. Weight of the precipitate was determined. Surgically removed urinary stones were procured from local hospital/ nursing homes. Small piece of stones were cut off and subjected to qualitative analysis by adopting standard methods[7]. Ten such stone samples were selected which were mainly composed of calcium, magnesium, oxalate and phosphate. Some of them were mixed calculi. The stones were labelled separately as sample S-1 to S-10. Each stone sample was carefully cut into six pieces. The sis pieces from each sample were placed in 100ml of 0.001M aqueous solution of naturally occurring acids viz. , lactic, malic, tartaric, citric or succinic acids separately in 250ml conical flasks. One piece of each sample was placed in 100ml distilled water for blank purpose. Each of the stone piece was weighed out on a sensitive monopan balance before dropping into the solution. Each flask was labelled indicating the number of the sample, the initial weight of the stone and the nature of solution. A pinch of sodium chloride was added to each of the flask, a bit of camphor was also added to each of the flask as a preservative. After every 24 hours the stone pieces were carefully removed from the solution with the help of a stainless steel spatula and washed carefully with distilled water and dried in an air oven at 100° C for 0.5 hours. Next the pieces were colled to room temperature and weighed out carefully. The stone pieces were then dropped back into their respective earlier solutions. The process of weighing after every 24 hours was continued till 10 days or till crumbling into small pieces whichever was earlier. From the weights of stone dissolved every 24 hour, the percentage dissolution was calculated out using the formula:

$$\% \text{ dissolution} = \frac{\text{Initial wt. Of stone} - \text{wt. Of stone dissolved}}{\text{Initial wt. Of stone}} \text{X } 100$$

RESULTS

Table 1: Inhibition of CALCIUM PHOSPHATE Mineralisation by naturally occurring acids

Salt forming solutions : 0.01M $(CH_3COO)_2$ Ca and 0.01M Na_3 PO_4									
	Conc.(M)	Wt. Of ppt (mg)				Inhibition efficiency (%)			
		S.S.M	S.D.M	R.S.M	R.D.M	S.S.M	S.D.M	R.S.M	R.D.M
Water (Blank)	-	60.0	60.0	60.0	60.0	-	-	-	-
Lactic acid	0.01	0.0	0.0	0.0	0.0	100	100	100	100
Lactic acid	0.001	32.1	31.0	31.0	25.0	49	50	50	60
Malic acid	0.01	0.0	0.0	0.0	0.0	100	100	100	100
Malic acid	0.001	31.8	30.8	31.0	28	48	49	50	58

Tartaric acid	0.01	9.0	8.8	8.2	7.5	87	88	88	89
Tartaric acid	0.001	43.0	40.8	40.2	38.0	31	32	33	36
Citric acid	0.01	0.0	0.0	0.0	0.0	100	100	100	100
Citric acid	0.001	32.1	31.1	31.0	25.0	49	50	50	60
Succinic acid	0.01	3.3	3.3	0.0	0.0	95	95	100	100
Succinic acid	0.001	40.0	39.1	30.0	28.0	35	37	50	55

Table 2: Inhibition of CALCIUM OXALATE Mineralisation by naturally occurring acids

Salt forming solutions : 0.01M $(CH_3COO)_2$ Ca and 0.01M $Na_2C_2O_4$									
	Conc.(M)	Wt. Of ppt (mg)				Inhibition efficiency (%)			
		S.S.M	S.D.M	R.S.M	R.D.M	S.S.M	S.D.M	R.S.M	R.D.M
Water (Blank)	-	90.0	90.0	90.0	90.0	-	-	-	-
Lactic acid	0.01	61.2	60.8	52.2	51.0	34	35	44	46
Lactic acid	0.001	83.5	83.8	81.6	79.8	07	07	10	12
Malic acid	0.01	18.5	17.2	11.6	10.9	80	82	89	89
Malic acid	0.001	56.8	55.9	51.8	40.0	39	40	43	44
Tartaric acid	0.01	15.3	15.3	11.3	11.4	84	84	89	89
Tartaric acid	0.001	61.0	57.0	55.7	54.0	33	35	39	40
Citric acid	0.01	58.0	58.6	51.3	51.2	36	36	43	43
Citric acid	0.001	74.2	74.2	69.8	59.4	18	19	23	25
Succinic acid	0.01	46.0	45.2	39.7	38.2	50	51	57	58
Succinic acid	0.001	80.1	80.2	77.6	76.5	12	11	14	15

Table 3: Inhibition of CALCIUM CARBONATE Mineralisation by naturally occurring acids

Salt forming solutions : 0.01M $(CH_3COO)_2$ Ca and 0.01M Na_2CO_3									
	Conc.(M)	Wt. Of ppt (mg)				Inhibition efficiency (%)			
		S.S.M	S.D.M	R.S.M	R.D.M	S.S.M	S.D.M	R.S.M	R.D.M
Water (Blank)	-	45.0	45.0	45.0	45.0	-	-	-	-
Lactic acid	0.01	9.2	8.5	7.8	7.2	82	84	92	94
Lactic acid	0.001	17.0	16.8	4.9	4.5	62	64	91	91
Malic acid	0.01	1.6	1.6	0.0	0.0	97	97	100	100
Malic acid	0.001	9.9	9.5	4.5	3.8	79	81	90	92
Tartaric acid	0.01	15.8	15.6	4.5	4.5	66	66	90	90
Tartaric acid	0.001	23.2	21.5	9.0	8.2	50	53	80	82
Citric acid	0.01	0.0	0.0	0.0	0.0	100	100	100	100
Citric acid	0.001	18.0	17.5	3.5	3.4	60	63	93	93
Succinic acid	0.01	0.0	0.0	0.0	0.0	100	100	100	100
Succinic acid	0.001	15.9	15.4	5.6	5.5	66	66	88	88

Table 4: Qualitative composition and anatomical location of selected stone sample

Sample	Sex	Age(Year)	Anatomical Location	Qualitative Composition
S-1	Female	35	Upper Calyceal system	Ca^{++}, Mg^{++}, PO_4^{--}
S-2	Male	42	Bladder	Ca++, PO_4^{---}
S-3	Male	33	Palvis	
S-4	Male	35	Bladder	Ca++, PO_4^{---}
S-5	Female	36	Palvis	Ca++, PO_4^{---}
S-6	Male	29	Bladder	Ca++, PO_4^{---}
S-7	Female	31	Palvis	Ca++, PO_4^{---}
S-8	Male	35	Bladder	Ca^{++}, Mg^{++}, PO_4^{--}
S-9	Male	27	Bladder	Ca++, PO_4^{---}
S-10	Male	21	Upper Urinary tract	Ca^{++}, Mg^{++}, PO_4

DISCUSSION

As mentioned earlier, formation of urinary stones is a function or several factors operating in the urinary tract, as well as the prevailing chemical milieu. Three most important factors are:

1. Level of calculogenetic crystalloids in the urine.
2. Level of inhibitors of calculogenesis.
3. Availability of a suitable nidus or matrix in the urinary tract.

A disbalanced state between the first two factors with crystalloid dominating, would indicate the state of formation of stone. Once a small stone has formed, it grows on and on by the deposition of salts drawn from the solution bulk. The small stone or initially, however, is very important in all the stages. The nature of chemical bonds formed between the inhibitor and crystalloid, the stability of the resultant compound as compared to the lattice energy of the crystal formation and various physical and chemical forces operating in the stone, would be the overall deciding factors in calculogenesis. In fact, it looksthat the formation of stone is a reult of complex chemical equilibria prevailing in the urinary tract. Any factor that would favour the growth of a crystal would induce the formation of stone. Crystal growth is a very complex process, since, both, the surface area and the supersaturation, varies continuously throughout the period of the growth. The nucleus for crystal growth is the "embryo" that consists of a small number of atoms and that its rate of formation varies with the super saturation according to mass laws. Again it has also been demonstrated that crystal growth may occur by aggregation of stable groups or sub-microns formed from a large number of ions and similar to the "micelle" of an insoluble metal salt.[8,6] The effects of protecting agents cannot be directly explained by application of mass action law. Two main processes determine the growth of nuclei and crystals from a solution, the transfer of material from the bulk of the solution to the region of the growing nucleus or crystal and the deposition of solute ions on to a growing phase from material in the close vicinity. Inhibitors of crystallisation would interfere in the above proceses by sequestering such ions which would from insoluble precipitates by combining with opposite ions and deposit on to the nidus or already growing crystal. The process of sequestration might involve either weak or strong chemical combinations ranging from simple hydrogen bonding interactions to stable complexation. Sequestration by complexation would be most effective in inhibiting the mineralisation of insoluble salt. In urolithiasis it is the calcium ions that form stubborn insoluble minerals (phosphates, oxalates) and an effective inhibitor has to be a good calcium complexing/ sequestering agent. Presently we have studied some naturally occurring acids as inhibitors for calcium phosphate, oxalate or carbonate mineralisation. These naturally occurring acids are also common plant acids and are capable or forming aqueous soluble complexes with calcium ions in different stoichiometries[8,7]. In complexes containing larger number of ligands the Ca^{++} concentration would be quite lower as compared to that in insoluble calcium phosphate, oxalate or carbonate. As such these acids are likely to be sequestrants and inhibit mineralisation of insoluble calcium salts. Our present results concerning the inhibition efficiency of different naturally occurring acid solutions towards the precipitation of calcium phosphate, calcium oxalate and calcium carbonate are recorded in table 1,2 and 3 respectively in 'Results' section. Study of the tables suggest that the hydroxy acids are moderate to good inhibitor of calcium phosphate and carbonate mineralisation. The inhibition efficiency varies in the range 30 – 100% forphosphate and 50 – 100% for carbonate. Sequestering of these insoluble calcium salts by the hydroxyl acids might be due to complexation coupled with effective hydrogen-bonding through the –OH groups. It is observed that the inhibitory capacity decreases with a decrease in the strength of inhibitor solution. Mass effect might be playing role here . As the concentration of inhibitor decreases the equilibrium might be favouring the precipitation of insoluble salts. As shown for citrate.

$$Ca^{++} + (Cit)^{---} \rightleftharpoons Ca\,(Cit)^{-}$$

Lesser the cit $^{---}$ present lesser Ca^{++} ions can be trapped as $Ca(cit)^{-}$ complex and more Ca^{++} ions will be free for precipitation as insoluble salt.

A comparative study of Table 1, 2 and 3 suggest that the inhibitors are relatively less effective in the inhibition of calcium oxalate. However, malic and tartaric acids in higher concentration are effective in inhibiting even this stubborn crystalloid to good extant (80-88%). Once formed calcium oxalate has practically no solubility in hydroxyl acid solution but if they are present before the formation of calcium oxalate, they may prevent the precipitation by exerting specificity towards calcium ions. Most of the inhibitors have been found to effectively inhibit calcium carbonate precipitation. A higher pk value of carbonic acid compared to that of inhibitors might be a factor. A comparative study of different models indicate that the reservoir dynamic model is the most effective one in the inhibition of mineralisation. This may again be due to the mass effect. An ab- initio presence of large concentration of inhibitor coupled with continuous stirring might be effectively chelating the calcium ion and screening from precipitating anions like phosphate, oxalate or carbonate. Dissolution of urinary stone by a chelating agent is a function of reaction of the later with the crystals

embedded in the former (Stone). More than one type of crystals are present in the mixed calculi. In fact, calcareous calculi mostly consists of calcium phosphate, calcium oxalate and sometimes magnesium ammonium phosphate also. Dissolution of an ionic crystal involves a series of steps: ions must be attached from the crystal lattice, solvated, than removed from the vicinity by diffusion into the solution phase. Where crystal lattice forces are powerful, so that a large activation energy is required for dissolution to proceed, the first step is likely to be the rate determining one but experimental conditions will usually determine whether the reaction is controlled by a surface reaction, a diffusion process or both. For example, depending on the stirring rate, dissolution of powered hydroxyl apatite and human dental enamel involved both diffusion and surface processes.[8] Gardener and Nancollan[9] found the dissolution of calcium oxalate monohydrate crystals in aqueous solution to have a rate limiting step involving diffusion of lattice ions away from the crystal surface. Presently our studies on the dissolution of urinary stones in different chelating agents suggest that the rate of dissolution seem to be a function of many factors, viz, the composition of the calculi, the surface area of the calculi, the cementation of crystals within the stones, the time factors as well as the composition of the particular layer of the stone(ultrastructure) to which the chelating agent is exposed t. However the net total dissolving power of a chelating agent seem to be a factor of specificity of the agent towards th Ca^{++} ions. In other words, the chelating ability of the agent with the Ca^{++} ions and its stabilisation in solution is the most important factor in the process of dissolution. The non-oxalate stones have been found to have a better solubility as compared to the oxalate ones. The average dissolution of non-oxalate calculi is highest (55.53%) with tartaric acid, closely followed by citric (49.07%) and malic (45.97%). Lactic and succinic acids show rather low dissolution. The higher average dissolution by tartaric acid can be explained on the basis of its high specificity[10] for Ca^{++}. Citric and malic too have good chlating ability for calcium ions. Banerjee *et al.*[11] have synthesised a number of aqueous soluble calcium complexes of these hydroxyl acids. The dissolution of calcium phosphate of the stone by the hydroxyl acids might also be due to mixed ligand calcium complexation by the phosphate and hydroxyl acid anions. In fact Rao[12] has evidenced such complexes in solution and also isolated them from aqueous solutions. The aqueous solubility (12.62%) of the stone might be due to slight decementation and crumbling as well as slight salt solubility. Amongst the non- oxalate stones, the ones containing magnesium also show a better dissolution percentage, which may be explained on the basis of higher solubility of Magnesium salts compared to those of calcium. The oxalate containing calculi, in general showed a low dissolution compared to the non-oxalate stones. This might be due to a slightly lower specificity of hydroxyl acid anions compared to the oxalate ions for Ca^{++}. It seems the extraction of Ca^{++} by the chelating agents from calcium oxalate lattice required a higher activation energy than from calcium phosphate lattice. The lattice energy values of calcium oxalate and phosphate seem to be important ruling factors in the chemodissolution of urinary stones. For oxalate containing stones, the trend of dissolution power of chelating agent has not been found to be significantly different. The overall range varies only slightly from 23 to 31%. Only citric acid has somewhat better ability (31.9%). The average aqueous dissolution of oxalate stones is a meagre 8.8%. The process of dissolution has been observed to be slow in the beginning. The rate picked up after 2 to 3 days, perhaps, after good initial surface activity. In non- oxalate as well as oxalate stones, there has been crumbling of the calculi in some cases, particularly in citric, malic or tartaric acids. It seems continuous removal of calcium ions from the surface layers by the chelating agents effects the cementation of the entire stone. This might be for exposition of new inner ca^{++} by the chelating anions. In fact a crumbling agent would also serve the purpose in urolithiasis, because the crumbled small pieces can easily be passed out through urine. A suitable crumbling agent would from an alternative to lithotripsy. Explanation of the mechanism of dissolution of urinary stone by a chelating agent is a tough nut to crack. A number of chemical and physical forces might be simultaneously operating. We suggest that the surface effects are likely to be of increasing importance. This is because the ligand ions can attack lattice calcium ions and whereas, at the same time, ensure negligible concentration of free calcium ions in the solution (Urine) phase. In the presence of chelating agent, dissolution begins with the loss of calcium ions lying in the surface of the crystals of stones. These are coordinatively less saturated than calcium ions inside the crystals and hence are prone to attack by the chelating agent. Removal of a calcium ion as its chelate would leave negatively charged oxalate/phosphate ions on the surface, bound only at one end to further calcium ions. The oxalate/ phosphate ions, in turn, would tend to diffuse exposing new surfaces.

CONCLUSION

As observed presently these hydroxyl polybasic acids have also good dissolving power for surgically removed urinary stones. Thus, if these acids could be cycled into the urinary tract, the onset of stone formation process might be inhibited, and also the already formed stone (if any) could be slowly dissolved in due course of time. Partial dissolution of at least the phosphate part of a mixed calculi by these acids would result in de-crustation/de-

cementation of stones and would cause the stone to crumble. The resulting small pieces can then be flushed out through urine. All of our observations in the present study are in-vitro and from chemical point of view. In vivo studies in animal systems and also human trials can only prove the affectivity of these acids in inhibition and dissolution of urinary stones. Our present in-vitro studies, nevertheless, would definitely form foundation for designing drugs for chemodissolution of urinary calculi.

REFERENCES

1. Vos T, Allen C, Arora M, Barber RM, Bhutta ZA, Brown A, *et al.*. (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators) (October 2016). "Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015". *Lancet*. 388 (10053): 1545–1602.
2. Vos T, Allen C, Arora M, Barber RM, Bhutta ZA, Brown A, *et al.*. (GBD 2015 Disease and Injury Incidence and Prevalence Collaborators) (October 2016). "Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the Global Burden of Disease Study 2015". *Lancet*. 388 (10053): 1459–1544.
3. Schulsinger DA (2014). *Kidney Stone Disease: Say NO to Stones!*. Springer. p. 27. Archived from the original on 8 September 2017.
4. Preminger: Stones in the Urinary Tract". In Cutler RE (ed.). *The Merck Manual of Medical Information Home Edition* (3rd ed.). Whitehouse Station, New Jersey: Merck Sharp and Dohme Corporation GM (2007). "Chapter 148.
5. *Nephrolithiasis~Overview* at eMedicine § Background.
6. Pearle MS, Calhoun EA, Curhan GC (2007). " Ch. 8: Urolithiasis" (PDF). In Litwin MS, Saigal CS (eds.). *Urologic Diseases in America (NIH Publication No. 07–5512)*. Bethesda, Maryland: National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, United States Public Health Service, United States Department of Health and Human Services. pp. 283–319.
7. Kalpana Devi V, Baskar R,Varalakshmi P. Biochemical effects in normal and stone forming rats treated with the ripe kernel juice of Plantain (Musa Paradisiaca). Ancient Science of Life, 3 and 4, 1993, 451 – 461.
8. Coward RJ, Peters CJ, Duffy PG, Corry D, Kellett MJ, Choong S, van't Hoff WG. Epidemiology of paediatric renal stone disease in the UK. Arch Dis Child. 2003;88:962–5.
9. Biociç M, Saraga M, Kuzmiç AC, Bahtijareviç Z, Budimir D, Todoriç J, Ujeviç RM. Pediatric urolithiasis in Croatia. Coll Antropol. 2003;27:745–52.
10. Edvardsson V, Elidottir H, Indridason OS, Palsson R. High incidence of kidney stones in Icelandic children. Pediatr Nephrol. 2005;20:940–4.
11. Baker K, Costabile RA. Demographics, stone characteristic, and treatment of urinary calculi at the 47th Combat Support Hospital during the first 6 months of Operation Iraqi Freedom. Mil Med. 2007;172:498–503.
12. Ali SH, Rifat UN. Etiological and clinical patterns of childhood urolithiasis in Iraq. Pediatr Nephrol. 2005;20:1453–7.

Source of Support: None Declared
Conflict of Interest: None Declared