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Study of Solubility of Urinary Stone in both Natural-Fresh Juice and Acid-Hydrolyzed Juice of Samras (*Ribes grossubaria*) Fruit

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Urolithiasis is associated with alteration in micro-environment integrity of urine. High citrate content diet deliver high alkali load and hence reduce the risk of formation of urinary stone. The hardness of urinary stones varies from stone to stone, depending on size and their chemical compositions. The solubility of urinary stones of different forms (whole and powder) in natural-fresh juice and in acid-hydrolyzed juice of samras (*Ribes grossubaria*) fruit has been investigated. The solubility efficiency was studied. This study showed that the solubility of powdered form of urinary stone was much higher than the single whole stone and comparatively much higher in acid-hydrolyzed juice than non-hydrolyzed juice *i.e.*, natural-fresh juice of same fruits. This short term study suggests that acid-hydrolyzed formulation of fruits juice would be helpful in designing of herbal preparation for dissolving, at least partially 'the urinary stones'. However, additional studies are needed to evaluate the role of fruit's juice of samras (*Ribes grossubaria*) in long-term preventive and therapeutic management of urolithiasis.

Key Words: Urolithiasis (urinary stones), Solubility, Single stone (bigger and smaller), Powder stone, Samras (*Ribes grossubaria*).

INTRODUCTION

Large population suffer from problems due to urinary stones. Calcium salts, uric acid, cystine and struvite ($MgNH_4PO_4$) are the basic constituents of most kidney stones in the western hemisphere. Calcium oxalate and calcium phosphate stones make up 75-85 % of the total and may be admixed in the same stone. Calcium phosphate in stone is usually hydroxyapatite[$Ca_5(PO_4)_3OH$] or, less commonly, brushite($CaHPO_4{\cdot}XH_2O$)[1]. Kidney stones are typically over the age of 30 years. The ratio of incidence of urinary stone formation in male to female is 4:1. According to the recent survey it has been seen that the increasing trend of urinary stones in pediatric age group[2]. Areas of high incidence of urinary calculi include the Scandinavian countries, British Isles, Mediterranean countries, Northern Australia, Central Europe, Northern India, Pakistan and Saurashtra region, Gujarat has higher prevalence of urinary stones[3]. According to an estimate, every year 600,000 Americans suffer from urinary stones. In India, 12 % of the population is expected to have urinary stones, out of which 50 % may end up with loss of kidneys or renal damage. Also, nearly 15 % of the population of northern India suffers from kidney stones[4]. Fewer occurrences of urinary calculi are found in southern India, which may be due to regular dietary intake of tamarind[5,6]. Urinary stones contain both crystalloid and colloid components. The crystalloid components are mainly calcium oxalate, calcium phosphate, calcium carbonate, magnesium-ammonium phosphate, uric acid and cysteine. The most common type of kidney stone is composed of calcium oxalate crystals, which is found in about 80 % of cases and the factors that enhance the precipitation of crystals in the urine are also responsible for the formation of renal stones[7,8]. Earlier it was thought that consumption of too much calcium or calcium containing diets could promote the development of calcium oxalate kidney stones. However, the recent evidence suggests that the consumption of low-calcium diets is actually associated with a higher overall risk for the development of kidney stones[9,10]. This is perhaps related to the role of calcium in binding ingested oxalate in the gastrointestinal tract. As the amount of calcium intake decreases, the amount of oxalate available for absorption into the bloodstream increases; this oxalate is then excreted in greater amounts into the urine by the kidneys. In the urine, oxalate is a very strong promoter of calcium oxalate precipitation, about 15 times stronger than calcium[11]. Diet can help in the prevention of kidney stones and it is best to avoid oxalate-rich foods such as beets, beans, blueberries, celery, grapes, chocolate, strawberries, spinach, rhubarb, tea, nuts, bran, almonds and peanuts[12,13]. Citrate containing substances *i.e.*, potassium citrate as urine alkalizer, may also be used in kidney stone prevention. They are not only increases the urinary pH (makes it more alkaline), but also increases the urinary citrate level, which inhibits crystal growth and nucleation, though most of the stone inhibitory activity of citrate is due to lowering urine super-saturation *via* complexation of calcium[14]. Drinking plenty of citrus fruit juices especially orange, blackcurrant and cranberry, may reduce the risk of urinary stones formation this is because citric acid (citrate) protect against kidney stone formation. Fresh (non-hydrolyzed) and acid-hydrolyzed extract of Kurthi has a definite role on solubility of urinary stone[15].

In the present work, the solubility efficiency of both natural-fresh juice and acid-hydrolyzed juice of samras (*Ribes grossubaria*) fruit on urinary stones of different forms and sizes have been estimated.

EXPERIMENTAL

Renal stones of patient Kavitri Devi (HMRI reg. No-22271) having operated on 21st July 2006 at Hai Medicare and Research Institute, Rajabazzar, Patna-18, was collected and washed properly with distilled water. Each stone was suspended separately in 20 mL of N/10 NaCl solution for 24 h then filtered and washed it with distilled water. Dried in air oven at 80 ºC for 2 h and cooled down. Two types of juice sample was prepared *i.e.*, natural-fresh juice and acid-hydrolyzed juice. Natural-fresh juice was prepared by squeezing the fruit and then it filter with Whatman filter paper No. 41. Acid-hydrolyzed juice was prepared from fruit juice by treatment of 100 mL fresh juice with 20 mL of 2 N HCl and warmed on a water-bath, followed by neutralization with a dilute solution of 2 N $NaHCO_3$ to a pH 7. The hydrolyzate was filtered and made to 100 mL. Nine sample from three set of stone was made *i.e.*, bigger single stone of equal wt. (NF-1, NF-2, NF-3), smaller single stone of equal wt. (NF-4, NF-5, NF-6) and powder stone of equal wt (NF-7, NF-8, NF-9) and were suspended in 25 mL of natural fresh juice and kept for 24 h of samples (NF-1,NF-4 and NF-7), for 48 h of samples (NF-2, NF-5 and NF-8) and for 72 h of samples (NF-3, NF-6 and NF-9) then stones were filtered and washed with distilled water and then dried and weighed out after due period. Same way another nine samples of stone *i.e.*, bigger single stone of equal wt. (AH-1, AH-2, AH-3), smaller single stone of equal wt. (AH-4, AH-5, AH-6) and powder stone of equal wt. (AH-7, AH-8, AH-9) were suspended in 25 mL of acid-hydrolyzed juice and kept for 24 h of samples (AH-1, AH-4 and AH-7), for 48 h of samples (AH-2, AH-5 and AH-8) and for 72 h of samples (AH-3, AH-6 and AH-9) then stones were filtered and washed with distilled water and then dried and weighed out after due period whole procedure was done at room temperature 27 ºC and atm. pressure-673.5 mm of Hg in month of March.

RESULTS AND DISCUSSION

We have carried out whole procedure and reaction *in vitro* at room temperature 27 ºC and atm. pressure-673.5 mm of Hg. The principle of whole work is based on "the surface area of a substance increases; the rate of dissolving increases as well and therefore the substance is more soluble. For example, a powdered sample would dissolve much faster than a large piece of a sample". Our main aim is to know the solubility efficiency of urinary stones of different sizes and forms in fruit juice (natural fresh juice and acid-hydrolyzed juice) of samras (*Ribes grossubaria*) fruit containing inorganic and organic weak acids.

Weight reduction are observed following the suspension of different forms of urinary stone *i.e.*, bigger single stone, smaller single stone and powdered stone in both natural fresh juice and acid-hydrolyzed juice at 24, 48 h and 72 h (Tables 1 and 2).

Solubility difference (g/25 mL of fruit juice) of single urinary stone (bigger and smaller size) and powdered stone are 0.0008, 0.0010 and 0.0076 *versus* 0.0036, 0.0035 and 0.0085 *versus* 0.0046, 0.0043 and 0.0099 in natural fresh fruit juice in 24 h, in 48 h and in 72 h, respectively (Table-1).

Solubility difference (g/25 mL of fruit juice) of single urinary stone (bigger and smaller size) and powdered stone are 0.0021, 0.0031 and 0.0153 *versus* 0.0042, 0.0037 and 0.0168 *versus* 0.0057, 0.0041 and 0.0187 in acid-hydrolyzed fruit juice in 24 h, in 48 h and in 72 h, respectively (Table-2).

Percentage solubility of urinary stone of single stone (bigger and smaller size) and powdered stone are 0.49, 0.76 and 4.75 % *versus* 2.18, 2.67 and 5.31 % *versus* 2.79, 3.28 and 6.19 %

TABLE-1
OBSERVED DATA FOLLOWING EXPERIMENT WITH URINARY STONE IN NATURAL FRESH JUICE OF SAMRAS (*Ribes grossubaria*) FRUIT

Natural fresh juice	Sizes of stone	Sample No	Weight of whole stone (g)	Wt. remained after N/10 NaCl treatment (g)	Wt. remained after 24 h treatment with juice (g)	Solubility difference	Wt. remained after 48 h treatment with juice (g)	Solubility difference	Wt. remained after 72 h treatment with juice (g)	Solubility difference
			(a)	(b)	(c)	(b-c)	(d)	(b-d)	(e)	(b-e)
Samras (*Ribes grossubaria*)	Bigger single stone	NF-1	0.1728	0.1648	0.1640	0.0008	–	–	–	–
		NF-2	0.1728	0.1648	–	–	0.1612	0.0036	–	–
		NF-3	0.1728	0.1648	–	–	–	–	0.1602	0.0046
	Smaller single stone	NF-4	0.1411	0.1312	0.1302	0.0010	–	–	–	–
		NF-5	0.1411	0.1312	–	–	0.1277	0.0035	–	–
		NF-6	0.1411	0.1312	–	–	–	–	0.1269	0.0043
	Powder stone	NF-7	0.1746	0.1600	0.1524	0.0076	–	–	–	–
		NF-8	0.1746	0.1600	–	–	0.1515	0.0085	–	–
		NF-9	0.1746	0.1600	–	–	–	–	0.1501	0.0099

TABLE-2
OBSERVED DATA FOLLOWING EXPERIMENT WITH URINARY STONE IN ACID- HYDROLYZED JUICE OF SAMRAS (*Ribes grossubaria*) FRUIT

Acid-hydrolyzed juice	Sizes of stone	Sample No.	Weight of whole stone (g)	Weight remained after N/10 NaCl treatment (g)	Weight remained after 24 h treatment with juice (g)	Solubility difference	Weight remained after 48 h treatment with juice (g)	Solubility difference	Weight remained after 72 h treatment with juice (g)	Solubility difference
			(a)	(b)	(c)	(b-c)	(d)	(b-d)	(e)	(b-e)
Samras (*Ribes grossubaria*)	Bigger single stone	AH-1	0.2874	0.2782	0.2761	0.0021	–	–	–	–
		AH-2	0.2874	0.2782	–	–	0.2740	0.0042	–	–
		AH-3	0.2874	0.2782	–	–	–	–	0.2725	0.0057
	Smaller single stone	AH-4	0.2012	0.1947	0.1916	0.0031	–	–	–	–
		AH-5	0.2012	0.1947	–	–	0.1910	0.0037	–	–
		AH-6	0.2012	0.1947	–	–	–	–	0.1906	0.0041
	Powder stone	AH-7	0.1736	0.1641	0.1595	0.0153	–	–	–	–
		AH-8	0.1736	0.1641	–	–	0.1570	0.0168	–	–
		AH-9	0.1736	0.1641	–	–	–	–	0.1528	0.0187

in natural fresh juice while 0.75, 1.59 and 9.32 % *versus* 1.51, 1.90 and 10.24 % *versus* 2.05, 2.10 and 11.40 % in acid-hydrolyzed juice in 24, 48 and 72 h, respectively (Table-3 and Fig. 1).

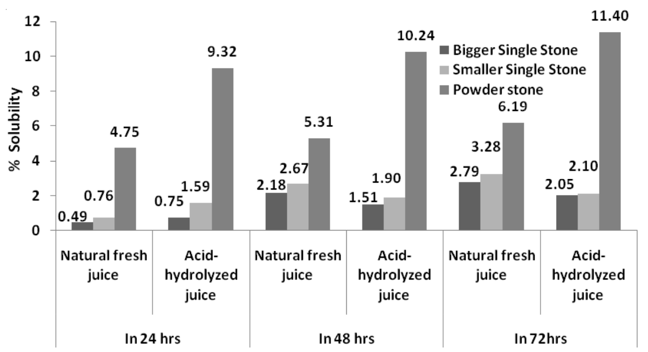

Fig. 1. Comparative study of percentage solubility of urinary stones in natural fresh juices and acid-hydrolyzed juices of samras (*Ribes grossubaria*)

Percentage solubility of urinary stone of single stone (bigger and smaller size) and powdered stone are 0.49, 0.76 and 4.75 % *versus* 2.18, 2.67 and 5.31 % *versus* 2.79, 3.28 and 6.19 % in natural fresh juice while 0.75, 1.59 and 9.32 % *versus* 1.51, 1.90 and 10.24 % *versus* 2.05, 2.10 and 11.40 % in acid-hydrolyzed juice in 24, 48 and 72 h, respectively (Table-3 and Fig. 1).

Solubility rate (g/h) of urinary stone of single stone (bigger and smaller size) and powdered stone are 0.000033, 0.000042 and 0.000326 *versus* 0.000117, 0.000104 and 0.000038 *versus* 0.000042, 0.000033 and 0.000058 in natural fresh juice while 0.000088, 0.000129 and 0.000638 *versus* 0.000088, 0.000025 and 0.000625 *versus* 0.000625, 0.000017 and 0.000079 in acid-hydrolyzed juice in 1st 24 h, in 2nd 24 h and in 3rd 24 h, respectively (Table-4).

It was observed that there was definite reduction in weight of urinary stones after treatment with both natural fresh juice (non-hydrolyzed) and with acid-hydrolyzed juices which indicate the dissolution of some ingredient of the urinary stone in fruit juice of samras (*Ribes grossubaria*) (Tables 1 and 2). It has been observed that percentage solubility of powdered form of urinary stone was higher than single stone (smaller single stone > bigger single stone) and comparatively more in acid-hydrolyzed juices than natural-fresh juice in all time interval *i.e.*, 24, 48 and 72 h (Table-3 and Fig. 1). It has also been noticed that solubility rate of powdered form of stone was comparatively faster in acid-hydrolyzed juice than in natural-fresh juice of samras (*Ribes grossubaria*) particularly in initial phase of treatment while whole stone has slower rate of solubility in early phase and faster in later phase (Figs. 2 and 3) of treatment. Head to head comparative study clearly show that stone dissolving capacity of acid-hydrolyzed juice are more than non-hydrolyzed *i.e.*, normal fruit juice.

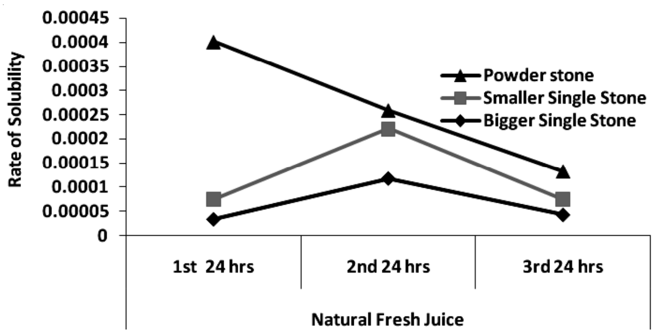

Fig. 2. Comparative study of rate of solubility in urinary stones in natural fresh juices of samras (*Ribes grossubaria*)

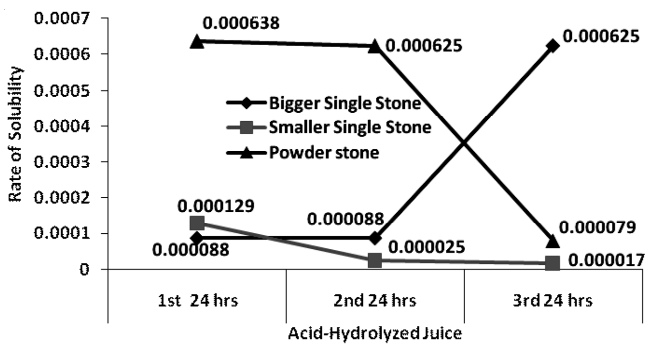

Fig. 3. Comparative study of rate of solubility of urinary stones in acid-hydrolyzed juice of samras (*Ribes arossubaria*)

TABLE-3
COMPARISON OF PERCENTAGE SOLUBILITY* OF URINARY STONE/25 mL OF JUICE OF SAMRAS (*Ribes grossubaria*) FRUIT

	In 24 h		In 48 h		In 72 h	
	Natural fresh juice	Acid-hydrolyzed juice	Natural fresh juice	Acid-hydrolyzed juice	Natural fresh juice	Acid- hydrolyzed juice
Bigger single stone	0.49	0.75	2.18	1.51	2.79	2.05
Smaller single stone	0.76	1.59	2.67	1.90	3.28	2.10
Powder stone	4.75	9.32	5.31	10.24	6.19	11.40

$$\text{*Percentage solubility} = \frac{\text{Solubility difference}}{\text{Weight of stone before supension}} \times 100 \cdot$$

TABLE-4
COMPARISON OF RATE OF SOLUBILITY# (g/h) OF URINARY STONE IN JUICE OF SAMRAS (*Ribes grossubaria*) FRUIT

	Natural fresh juice			Acid-hydrolyzed juice		
	1st 24 h	2nd 24 h	3rd 24 h	1st 24 h	2nd 24 h	3nd 24 h
Bigger single stone	0.000033	0.000117	0.000042	0.000088	0.000088	0.000625
Smaller single stone	0.000042	0.000104	0.000033	0.000129	0.000025	0.000017
Powder stone	0.000326	0.000038	0.000058	0.000638	0.000625	0.000079

$$\text{*Percentage solubility (g/h)} = \frac{\text{Solubility difference(g)}}{\text{Time (h)}} .$$

Conclusion

When the surface area of a substance increases the rate of dissolving increases as well and therefore the substance is more soluble. For example, a powdered sample would dissolve much faster than a large piece of a sample. Due to hard and stubborn nature of outer covering of urinary stone, it is too difficult to solubulize it easily. Solubility efficiency of acid-hydrolyzed juice is higher than natural-fresh (non-hydrolyzed) juice of samras (*Ribes grossubaria*) fruit for urinary stones of different forms and sizes. Powder form of urinary stone is much more soluble than whole stone (smaller single stone > bigger single stone). For powder form of stone, the solubilization is faster in first 24 h followed by it become slow while *vice-versa* with single stone. Acid hydrolyzation of fruit juice potentiates their dissolving properties against urinary calculi. Such studies would be helpful in designing of natural (non-synthetic) herbal formulation for dissolving, at least partially 'the urinary stones'.

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