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Experimental study of solubility of urinary stones in juice of *Chikku (Achras zapota)* fruit

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ABSTRACT

Urinary stone disease is global problem and is due to alteration in the normal crystallization environment of urine in the urinary tract systems. Major factors responsible for crystal formation are super-saturation of Ca^{2+} salts and level of crystallization inhibitors (Citrate & Phytate). Excessive citrate intake makes the urine more alkaline (pH between 6 and 7). This can be achieved by oral administration of alkalizer, citrus fruits and its juices. 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 Chikku (Achras zapota) fruit has been investigated and evaluated. The end result revealed that percentage solubility of powdered stones in Acid-hydrolyzed juice are 5.33%, 9.46% & 9.88% in 24 hrs, 48hrs and 72 hrs respectively which are not only significantly more than whole stone but also more than percentage solubility in natural fresh juice. Dissolution rate of powder form of stone are more in 1st and 2nd 24hr i.e. acid-hydrolyzation augment litholytic activity mainly in initial phase. This short term study would be helpful in designing of herbal formulation of acid-hydrolyzed fruit juice of Chikku for dissolving, at least partially 'the urinary stones'. However, additional studies are still being needed to evaluate the role of juice of Chikku (Achras zapota) fruit in long-term preventive and therapeutic management of Urolithiasis.

Key-word: Urolithiasis (Urinary stones), Solubility, Single stone (Bigger and Smaller), Powder stone, Chikku (Achras zapota), Natural-fresh juice, Acid-hydrolyzed juice.

INTRODUCTION

Kidney stones are typically over the age of 30 years. Sex ratio(male to female) is 4:1. Recent survey revealed that the increasing trend of urinary stones disease in pediatric age group also[1]. 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[2]. 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. Fewer occurrences of urinary calculi are found in southern India, which may be due to regular dietary intake of tamarind[3]. Most of kidney stones are Calcium salts, Uric acid, Cystine, and Struvite ($MgNH_4PO_4$) in the western hemisphere. About 75-85% of urinary stones are made of Calcium oxalate and Calcium phosphate but may be admixed in the same stone. Calcium phosphate in stone is usually in form of Hydroxyapatite [$Ca_5(PO_4)_3OH$] or, less commonly, Brushite($CaHPO_4.XH_2O$)[4]. 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. Factors that enhance the precipitation of calcium oxalate crystals in the urine are also responsible for the formation of renal stones. 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. 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 [7][8][9][10][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]. Insoluble ingredients of the kidney stones were solubilized with some extent with glycine, β-alanine and hippuric acid. Dissolution of stone ingredient in powdered form was much more than the whole stone[16]. Micronutrient metal ions increase or decrease of the inhibition efficiency of mineralization of urinary stone forming minerals. Micronutrient metal ions increase the inhibition up to some extent[17]. Acid-Hydrolyzation of Fruits Juice of Apple, Moushmi, Samras and Amla increases solubility of Urinary Stones more of powder form of stones[18] [19] [20].

In the present work we have estimated the solubility efficiency of both Natural-Fresh Juice and Acid-hydrolyzed Juice of Chikku (*Achras zapota*)fruit on Urinary Stones of different forms and sizes.

EXPERIMENTAL SECTION

Renal stones of patient Mrs Naheed Parween (HMRI reg. no-43091/2010) having operated on 14th Jan 2010 at Hai Medicare & Research Institute, Rajabazzar, Patna-18, was collected and washed properly with distilled water. Each stone was suspended separately in 20 ml of N/10NaCl solution for 24 hours then filtered and washed it with distilled water. Dried in air oven

at 80^{oC} for 2 hrs and cooled down. Two types of juice sample were 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 paper 41. Acid-hydrolyzed Juice was prepared from fruit juice by treatment of 100ml fresh juice with 20ml of 2N HCl and warmed on a water-bath, followed by neutralization with a dilute solution of 2N $NaHCO_3$ to a pH 7. The hydrolysate was filtered and made to 100 ml. Six different types of samples were prepared from fruit juice i.e. three from Natural fresh juice NFJ1, NFJ2 & NFJ3 and three from acid hydrolyzed juice AHJ1, AHJ2 & AHJ3 each contained 25 ml juice. Different sizes and weight of stones were suspended i.e. Bigger single stone, Smaller single stone and Powdered stone respectively for 24 hrs. Stones were again filtered, washed with distilled water, dried and weighed out. Filtrates were again suspended in 25 ml of each fruit juices for next 24 hrs. Stones were again filtered, washed with distilled water, dried and weighed out and further suspended in 25 ml of each fruit juices for next 24 hrs. Finally stones were filtered and washed with distilled water and then dried & 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 26.5^{oC} and Atm. pressure-673 mm of Hg. The principle of whole work is based on "existence of crystallization inhibitors present in fruit juice of Chikku(*Achras zapota*) which decreased the super-saturation of Ca^{2+} salts". Our main aim is to know the solubility efficiency of fruit juice (Natural fresh juice and Acid-Hydrolyzed juice) of Chikku (Achras zapota) fruit for different sizes and forms of urinary stones at different time interval.

Table -1 Observed data following experiment with Urinary stone in Natural fresh juice of Chikku (*Achras zapota*) Fruit

Sizes of stone	Sample No.	Wt. of whole stone (g) (a)	Wt. remained after N/10 NaCl treatment (g) (b)	Wt. remained after 24h treatment with juice (g) (c)	Solubility Difference (g) (b-c)	Wt. remained after 48h treatment with juice (g) (d)	Solubility difference (g) (b-d)	Wt. remained after 72h treatment with juice (g) (e)	Solubility Difference (g) (b-e)
BSS	NFJ1	0.2824	0.2710	0.2696	0.0014	0.2688	0.0022	0.2675	0.0035
SSS	NFJ2	0.2113	0.2008	0.2000	0.0008	0.1987	0.0021	0.1971	0.0037
PS	NFJ3	0.2446	0.2309	0.2288	0.0021	0.2251	0.0058	0.2248	0.0061

Table-2 Observed data following experiment with Urinary stone in Acid-hydrolyzed juice of Chikku (*Achras zapota*) Fruit

Sizes of stone	Sample No.	Wt. of whole stone (g) (a)	Wt. remained after N/10 NaCl treatment (g) (b)	Wt. remained after 24h treatment with juice (g) (c)	Solubility Difference (g) (b-c)	Wt. remained after 48h treatment with juice (g) (d)	Solubility difference (g) (b-d)	Wt. remained after 72h treatment with juice (g) (e)	Solubility Difference (g) (b-e)
BSS	AHJ1	0.2389	0.2272	0.2245	0.0027	0.2223	0.0049	0.2219	0.0053
SSS	AHJ2	0.1895	0.1701	0.1680	0.0021	0.1661	0.0040	0.1658	0.0043
PS	AHJ3	0.1623	0.1448	0.1368	0.0080	0.1311	0.0137	0.1305	0.0143

BSS=Bigger Sized Stone, SSS= Smaller Sized Stone & PS= Powder Stone

Weight reduction are observed following the suspension of different forms of urinary stone i.e. bigger single stone, smaller single stone & powdered stone in both natural fresh juice and Acid-Hydrolyzed juice at 24 hrs, 48 hrs and 72 hrs (Table-1 and Table-2).

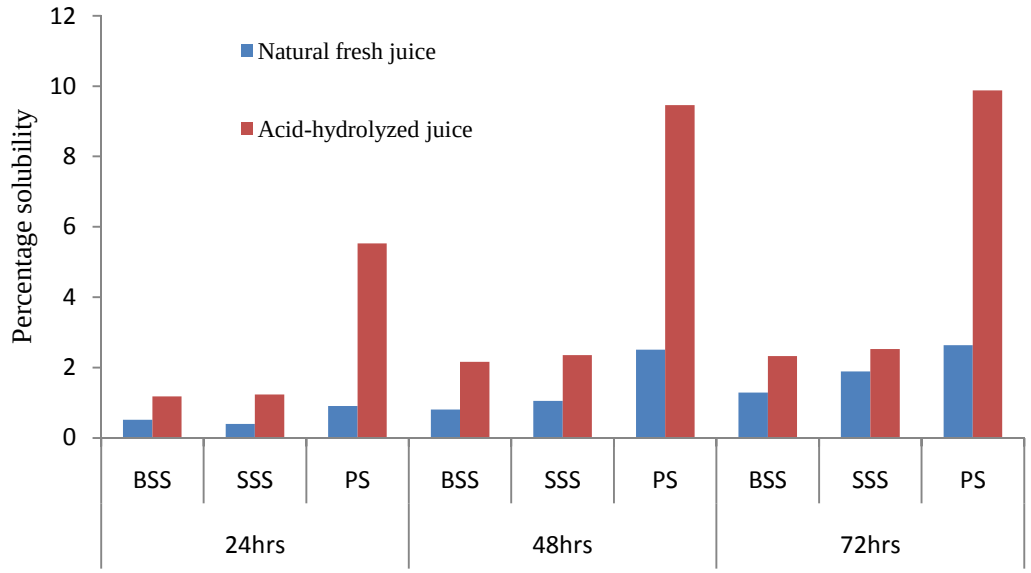
Table-3 Comparison of Percentage Solubility* of Urinary Stone per 25 ml of juice of Chikku (*Achras zapota*) fruit

Size of stones	In 24 hrs		In 48 hrs		In 72 hrs	
	Natural fresh juice	Acid-hydrolyzed juice	Natural fresh juice	Acid-hydrolyzed juice	Natural fresh juice	Acid-hydrolyzed juice
BSS	0.52	1.18	0.81	2.16	1.29	2.33
SSS	0.40	1.23	1.05	2.35	1.89	2.53
PS	0.91	5.53	2.51	9.46	2.64	9.88

$$*\text{Percentage solubility} = \frac{\text{Solubility difference}}{\text{Wt. of Stone before suspension}} X\ 100$$

Solubility difference (gram/25 ml of Fruit juice) of single urinary stone (Bigger and Smaller size) and powdered stone are 0.0014, 0.0008 & 0.0021 Vs 0.0022, 0.0021 & 0.0058 Vs 0.0035, 0.0037 & 0.0066 in Natural fresh fruit juice (Table-1) while

Fig-1. Comparative study of Percentage Solubility of Urinary stones in Natural fresh juices and Acid-hydrolyzed juices of Chikku (*Achras zapota*) fruit



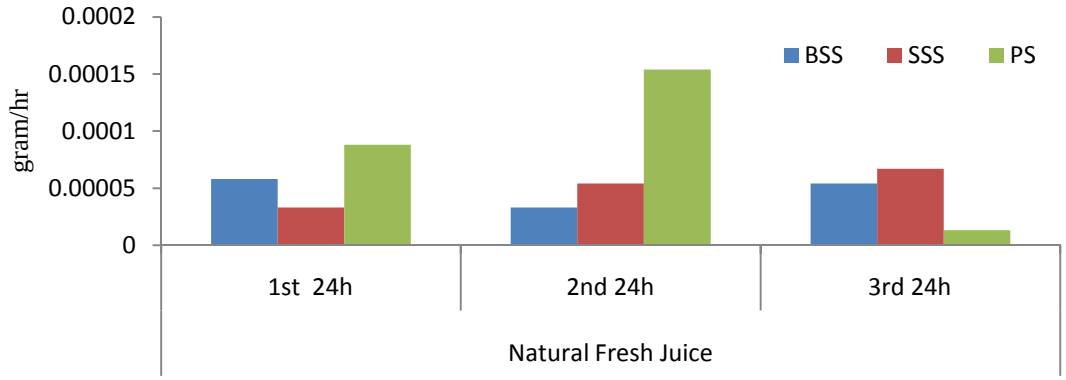
Solubility difference in Acid-hydrolyzed fruit juice are 0.0027, 0.0021 & 0.0080 Vs 0.0049, 0.0040 & 0.0137 Vs 0.0053, 0.0043 & 0.0143 in 24 hrs, in 48 hrs and in 72 hrs respectively (Table-2).

Percentage solubility of urinary stone of single stone (Bigger and Smaller size) and powdered stone are 0.52%, 0.40% & 0.91% Vs 0.81%, 1.05% & 2.51% Vs 1.29%, 1.89% & 2.64% in Natural fresh juice while 1.18%, 1.23% & 5.33% Vs 2.16%, 2.35% & 9.46% Vs 2.33%, 2.53%& 9.88% in Acid-hydrolyzed juice in 24 hrs, 48hrs and 72 hrs respectively (Table-3 and Fig.-1).

Table-4 Comparison of Solubility difference of Urinary Stone in juice of Chikku (*Achras zapota*) fruit

	Natural Fresh Juice			Acid-Hydrolyzed Juice		
	1st 24h	2nd 24h	3rd 24h	1st 24h	2nd 24h	3rd 24h
BSS	0.0014	0.0008	0.0013	0.0027	0.0022	0.0004
SSS	0.0008	0.0013	0.0016	0.0021	0.0019	0.0003
PS	0.0021	0.0037	0.0003	0.0080	0.0057	0.0006

Fig-2. Dissolution rate (gram/hr)of Urinary stones in Natural fresh juices of Chikku (*Achras zapota*) fruit



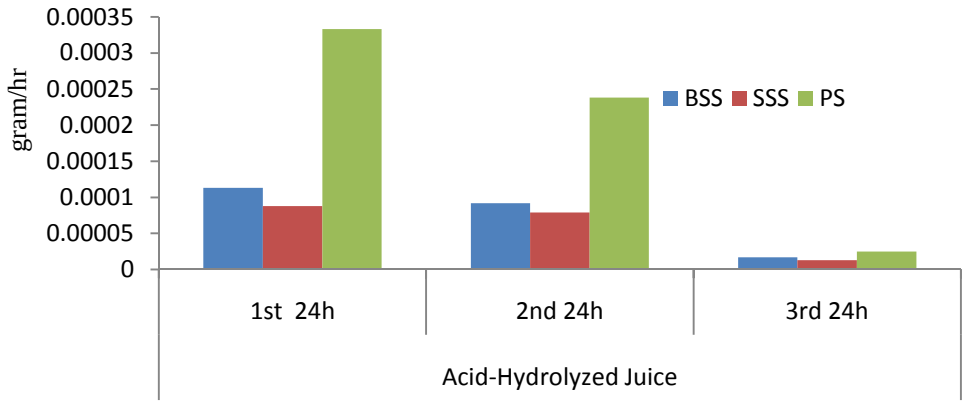
Solubility difference in different time frame i.e. in 1st 24hrs, 2nd 24 hrs and 3rd 24hrs of single urinary stone (Bigger and Smaller size) and powdered stone are 0.0014, 0.0008 & 0.0021 Vs 0.0008, 0.0013 & 0.0037 Vs 0.0013, 0.0016 & 0.0003 in Natural fresh fruit juice (Table-1) while 0.0027, 0.0021 & 0.0080 Vs 0.0022, 0.0019 & 0.0057 Vs 0.0004, 0.0003 & 0.0006 in Acid-hydrolyzed juice respectively (Table-4).

Table-5 Comparison of Dissolution Rate # (g/h) of Urinary Stone in juice of Chikku (Achras zapota) fruit

	Natural Fresh Juice			Acid-Hydrolyzed Juice		
	1st 24h	2nd 24h	3rd 24h	1st 24h	2nd 24h	3rd 24h
BSS	0.000058	0.000033	0.000054	0.000113	0.000092	0.000017
SSS	0.000033	0.000054	0.000067	0.000088	0.000079	0.000013
PS	0.000088	0.000154	0.000013	0.000333	0.000238	0.000025

$$^{\#}\textit{Dissolution Rate (gram/hr)} = \frac{Solubility\ difference\ (Gram)}{Time(hr)}$$

Fig-3. Dissolution rate(g/h) of Urinary stones in Acid-hydrolyzed juices of Chikku (*Achras zapota*) fruit



Dissolution rate (gram/hr) of urinary stone of single stone (Bigger and Smaller size) and powdered stone are 0.000058, 0.000033 & 0.000088 Vs 0.000033, 0.000054 & 0.000154 Vs 0.000054, 0.000067 & 0.000013 in Natural fresh juice while 0.000113, 0.000088 & 0.000333 Vs 0.000092, 0.000079 & 0.000238 Vs 0.000017, 0.000013 & 0.000025 in Acid-hydrolyzed juice in 1st 24hrs, in 2nd 24hrs and in 3rd 24 hrs respectively (Table-5,Fig.-2 and Fig.-3).

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 Chikku(Achras zapota) (Table-1 and Table-2). It has been observed that Percentage solubility of powdered form of urinary stone was more than single stone (smaller single stone > bigger single stone). It has been seen that solubility of stones was comparatively more in Acid-hydrolyzed juices than Natural-fresh juice in all time interval i.e. 24 hrs, 48 hrs and 72 hrs(Fig.-1) and it has been also noted that dissolution rate of powdered stones were faster in initial phase of reaction specially in 1st 24hrs and 2nd 24hrs of suspension(Fig.-2&3). Head to head comparative study clearly show that stone dissolving capacity of acid-hydrolyzed juice are more than non-hydrolyzed i.e. natural fresh juice of Chikku (*Achras zapota*).

CONCLUSION

Solubility of Powdered stone is much more than single piece of stone (smaller single stone > bigger single stone). Solubility efficiency of Acid-hydrolyzed juice is higher than Natural-Fresh (non-hydrolyzed) juice of Chikku fruit. Dissolution rate is faster in initial phase of reaction i.e.1st and 2nd 24hrs followed by it becomes slow. 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 of juice of Chikku (*Achras zapota*) fruit for dissolving, at least partially 'the urinary stones'.

REFERENCES

[1] Stamatelou, Kiriaki K.; Francis, Mildred E, et al., *Kidney International*, **2003**, 63, 1817-1823
[2] Ahamed J, Khalid N, and Jabeen E, *Pak. J. Sci. Ind. Res*., **1987**, 30, 205-07.
[3] Rao, T.V.K and Mytreye Dass, *Asian Journal of Chemistry*, **2000**, 12, 719.
[4] Vimal S. Joshi et.al, *Ind. J. Pure Applied Physics*, **2003**, 41, 183-192
[5] http://www.himalayahealthcare.com
[6] Mohamed Farook, N.A., Seyed Dameem and Sathya, *E-Journal of Chemistry*; **2004**, 1,137.
[7] Parmar; Malvinder S. *British Medical Journal* ,**2004,** 328 (7453): 1420-1424.
[8] John R; Asplin, et al, Nephrolithiasis, Harrison's Principle of Internal Medicine **15th** Edition, Chapter-279**.**
[9] Odvina; Clarita V. "Comparative Value of Orange Juice versus Lemonade in Reducing Stone-Forming Risk". Clinical Journal of the American Society of Nephrology, **2006**, 1 (6), 1269–74.
[10] Seltzer MA; Low RK et al, *J Urol*, **1996**, 1156, 907 –909.
[11] Curhan GC; Willett WC; Rimm EB, et al, *Am J Epidemiol*, **1996**, 143, 240 –247.
[12] Kessler T, et al, *Eur J Clin Nutr*, **2002**, 56,1020 –1023.
[13] Goldfarb DS, Asplin JR, *J Uro*; 2001 **166** :263 –267.
[14] Jenkins AD, Dousa TO, Smith LH, *Am J Physiol*, **1985**, 249 :F590 –595.
[15] Bala Shail., Reaction of naturally occurring substances with urinary stones, Ph.D-Thesis, **1985,** 101-116.
[16] Mahapatra B. et al, *Asian Journal of Chemistry*, 2008, 20(3),2457- 2458.

[17] Mahapatra B. et al; *Asian Journal of Chemistry*, **2008**, 20(3),2459-2460.
[18] Sinha MR, et al; *Asian Journal of Chemistry*, **2010**, 22(4),2783-2788.
[19] Sinha MR, et al; *Asian Journal of Chemistry*, **2010**, 22(6), 4840-4846.
[20] Sinha MR, et al: *Asian Journal of Chemistry*, **2011**,23(1), 293-296.