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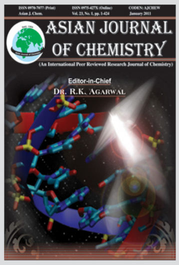

Influence of Acid-Hydrolyzation of Juice of Cape Gooseberry (*Physalis peruviana*) Fruit on Solubility of Urinary Stones

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Urinary stones are the consequence of an 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 and phytate). Increased citrate excretion decreases super saturation of calcium salt due to its capacity to make complex Ca^{2+} ions. Excessive citrate makes the urine more alkaline (pH between 6 and 7). This can be achieved by oral administration of alkalizer, citric 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 cape gooseberry (*Physalis peruviana*) fruit has been investigated and evaluated. The end result showed that solubility efficiency of natural fresh juice is higher than acid-hydrolyzed juice for urinary stones of different forms and sizes at all time intervals but more in beginning phase. Powder form of stone is more soluble than whole stone. Acid hydrolyzation augment litholysis activity only in initial phase, but later on, rather it increases the lithogenic effect. This short term study would be helpful in designing of natural (non-synthetic and non-hydrolyzed) herbal formulation of cape gooseberry (*Physalis peruviana*) fruit juice for dissolving, at least partially 'the urinary stones'. However, additional studies are still needed to evaluate the role of juice of cape gooseberry (*Physalis peruviana*) fruit in long-term preventive and therapeutic management of urolithiasis.

Key Words: Urolithiasis, Solubility, Single stone (bigger and smaller), Powder stone, Cape gooseberry (*Physalis peruviana*), Natural-fresh juice, Acid-hydrolyzed juice, Litholysis and Lithogenic.

INTRODUCTION

Urinary stone disease is a global problem. 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 ($CaHPO_4 \cdot XH_2O$)[1]. 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[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. 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[5-9]. 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[10,11]. 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[12]. 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[13]. 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[14]. 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[15]. Acid-hydrolyzation of fruits juice of apple, moushmi, samras and amla increases solubility of urinary stones more of powder form of stones[16-18].

In the present work we have estimated the solubility efficiency of both natural-fresh juice and acid-hydrolyzed juice of cape gooseberry (*Physalis peruviana*) fruit on urinary stones of different forms and sizes.

EXPERIMENTAL

Renal stones of patient Mrs. Muhari Devi (HMRI Reg. No. 42962/2010) having operated on 12th 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/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 paper No. 41. Acid-hydrolyzed juice was prepared from fruit juice by treatment of 100 mL fresh juice with 20 mL 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 hydrolyzate was filtered and made to 100 mL. Six different types of samples were prepared from fruit juice *i.e.*, three from fresh natural and three from acid hydrolyzed juice. Bigger single stone (NB), smaller single stone (NS) and powdered stone (NP) were suspended in each 25 mL of fresh natural juice while bigger single stone (HB), smaller single stone (HS) and powdered stone (HP) were suspended in each 25 mL of acid-hydrolyzed juice for 24 h. Stones were again filtered, washed with distilled water, dried and weighed out. Filtrates were again suspended in 25 mL of each fruit juices for further 24 h. 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 h. Finally stones were filtered and washed with distilled water and then dried and weighed out after due period. Whole procedure was done at room temperature (26 ºC) and atmosphere pressure (672.5 mm) of Hg in month of March.

RESULTS AND DISCUSSION

We have carried out whole procedure and reaction *in vitro* at room temperature (26 ºC) and atmosphere pressure (672.5 mm) of Hg. The principle of whole work is based on "level of crystallization inhibitors present in fruit juice of cape gooseberry which decreased the super-saturation of Ca^{2+} salts". The main aim of this study is to know the solubility efficiency of fruit juice (natural fresh juice and acid-hydrolyzed juice) of cape gooseberry (*Physalis peruviana*) fruit for different sizes and forms of urinary stones. 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 in 1st 24 h, 2nd 24 h and 3rd 24 h (Tables 1 and 2).

Solubility difference (g/25 mL of natural fresh juice) of single urinary stone (bigger and smaller size) and powdered stone are 0.0020, 0.0019 and 0.0054 in 1st 24 h, 0.0042, 0.0042 and 0.0105 in 2nd 24 h and 0.0048, 0.0047 and 0.0142 in 3rd 24 h, respectively (Table-1).

Solubility difference (g/25 mL of acid-hydrolyzed fruit juice) of single urinary stone (bigger and smaller size) and powdered stone are 0.0005, 0.0016 and 0.0049 in 1st 24 h, -0.0001, 0.0004 and 0.0006 in 2nd 24 h and -0.0005, -0.0008 and -0.0018 in 3rd 24 h, respectively (Table-2). Percentage solubility of single urinary stone (bigger and smaller size) and powdered stone in natural fresh juice are 0.82, 1.07 and 2.50 % in 1st 24 h, 1.73, 2.36, 4.87 % in 2nd 24 h and 1.97 %, 2.64 and 6.57 % in 3rd 24 h, respectively while in acid-hydrolyzed juice 0.13, 0.96 and 1.74 % in 1st 24 h, -0.02, 0.24 and 0.21 % in 2nd 24 h and -0.13, -0.36 and -0.64 % in 3rd 24 h, respectively (Table-3 and Figs. 1 and 2).

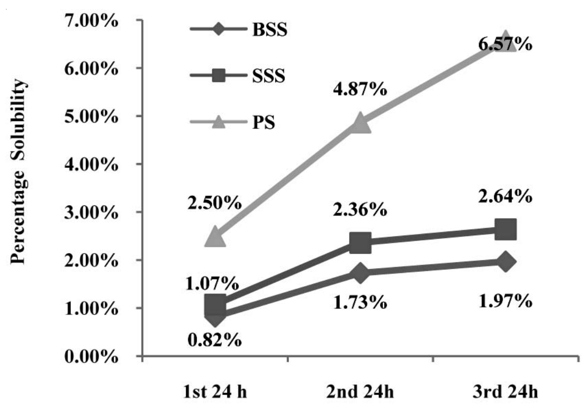

Fig. 1. Percentage solubility of urinary stone in natural fresh juice of *Physalis peruviana*

Change of weight (g) of single stone (bigger and smaller size) and powdered stone in natural fresh juice are 0.0020, 0.0019 and 0.0054 in 1st 24 h, 0.0022, 0.0023 and 0.0051 in 2nd 24 h and 0.0006, 0.0005 and 0.0037 in 3rd 24 h, respectively

TABLE-1
OBSERVED DATA FOLLOWING EXPERIMENT WITH URINARY STONE IN NATURAL FRESH JUICE OF CAPE GOOSEBERRY (*Physalis peruviana*) FRUIT

Sizes of stone	Sample No.	Weight of whole stone (g)	Weight remained after N/10 NaCl treatment (g)	Weight remained after 1st 24 h treatment with juice (g)	Solubility difference (g)	Weight remained after 2nd 24 h treatment with juice (g)	Solubility difference (g)	Weight Remained after 3rd 24 h treatment with juice (g)	Solubility difference (g)
		(a)	(b)	(c)	(b-c)	(d)	(b-d)	(e)	(b-e)
BSS	NS	0.2525	0.2433	0.2413	0.0020	0.2391	0.0042	0.2385	0.0048
SSS	NB	0.1866	0.1780	0.1761	0.0019	0.1738	0.0042	0.1733	0.0047
PS	NP	0.2293	0.2162	0.2108	0.0054	0.2057	0.0105	0.2020	0.0142

TABLE-2
OBSERVED DATA FOLLOWING EXPERIMENT WITH URINARY STONE IN ACID-HYDROLYZED JUICE OF CAPE GOOSEBERRY (*Physalis peruviana*) FRUIT

Sizes of stone	Sample No.	Weight of whole stone (g)	Weight remained after N/10 NaCl treatment (g)	Weight remained after 1st 24 h treatment with juice (g)	Solubility difference (g)	Weight remained after 2nd 24 h treatment with juice (g)	Solubility difference (g)	Weight remained after 3rd 24 h treatment with juice (g)	Solubility difference (g)
		(a)	(b)	(c)	(b-c)	(d)	(b-d)	(e)	(b-e)
BSS	HB	0.3871	0.3767	0.3762	0.0005	0.3768	-0.0001	0.3772	-0.0005
SSS	HS	0.1740	0.1664	0.1648	0.0016	0.1660	0.0004	0.1670	-0.0006
PS	HP	0.2933	0.2822	0.2773	0.0049	0.2816	0.0006	0.2840	-0.0018

TABLE-3
COMPARISON OF PERCENTAGE SOLUBILITY* OF URINARY STONE/25 mL OF JUICE OF CAPE GOOSEBERRY (*Physalis peruviana*) FRUIT

	In 1st 24 h		In 2nd24 h		In 3rd 24 h	
	Natural fresh juice	Acid-hydrolyzed juice	Natural fresh juice	Acid-hydrolyzed juice	Natural fresh juice	Acid-hydrolyzed juice
BSS	0.82	0.13	1.73	-0.02	1.97	-0.13
SSS	1.07	0.96	2.36	0.24	2.64	-0.36
PS	2.50	1.74	4.87	0.21	6.57	-0.64

*$\text{Percentage solubility} = \frac{\text{Solubility difference}}{\text{Weight of stone before suspension}} \times 100$; Bigger single stone = BSS, Smaller single stone = SSS, Powder stone = PS.

TABLE-4
CHANGE IN WEIGHT OF URINARY STONE AT DIFFERENT TIME INTERVAL IN NATURAL FRESH JUICE *Physalis peruviana*

Sample No.	Weight of stone before suspension (g)	Status in 1st 24 h Weight of stone (g)	Status in 2nd 24 h Weight of stone (g)	Status in 3rd 24 h Weight of stone (g)
BSS	0.2433	0.0020↓	0.0022↓	0.0006↓
SSS	0.1780	0.0019↓	0.0023↓	0.0005↓
PS	0.2162	0.0054↓	0.0051↓	0.0037↓

↓Decrease, ; ↑Increase

TABLE-5
CHANGE IN WEIGHT OF URINARY STONE AT DIFFERENT TIME INTERVAL IN ACID-HYDROLYZED JUICE *Physalis peruviana*

Sample No.	Weight of stone before suspension (g)	Status in 1st 24 h Weight of stone (g)	Status in 2nd 24 h Weight of stone (g)	Status in 3rd 24 h Weight of stone (g)
BSS	0.3767	0.0005↓	0.0006↑	0.0004↑
SSS	0.1664	0.0016↓	0.0012↑	0.0010↑
PS	0.2822	0.0049↓	0.0043↑	0.0024↑

↓Decrease, ; ↑Increase

while in acid-hydrolyzed juice 0.0005, 0.0016 and 0.0049 in 1st 24 h, 0.0006, 0.0012 and 0.0043 in 2nd 24 h and 0.0004, 0.0010 and 0.0024 in 3rd 24 h, respectively (Tables 4 and 5).

It was observed that there was definite reduction in weight of all form and size of urinary stones after treatment with natural fresh juice (non-hydrolyzed) in all time interval *i.e.* more with powder form than single stone (Table-4) on other hand, treatment with acid-hydrolyzed juice, weight reduction was observed only in 1st 24 h while increment in weight in later two interval *i.e.* 2nd and 3rd 24 h (Table-5). All phenomena were quantitatively more with powdered stone than single stone indicate the dissolution of some ingredient of the urinary stone in fresh natural juice of cape gooseberry (*Physalis peruviana*). It has been noticed that percentage solubility of powdered form of urinary stone in natural-fresh juice was higher than single stone in all time interval (Table-3 and Fig. 1) while in acid-hydrolyzed juice, initially it was increased followed by decreased in percentage solubility comparatively more with powdered stone than single (Table-3 and Fig. 2). It might be due to gradual decrement of inhibitory properties of fruit juice after acid hydrolyzation.

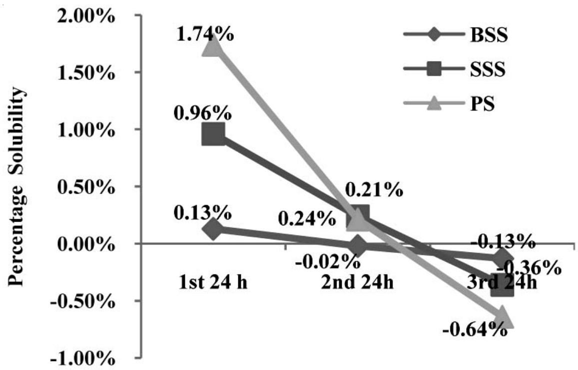

Fig. 2. Percentage solubility of urinary stone in acid-hydrolyzed juice of *Physalis peruviana*

Conclusion

Solubility efficiency of natural fresh juice is higher than acid-hydrolyzed juice for urinary stones of different forms and sizes. Powdered form of stones dissolve much higher than a large piece of urinary stone in natural fresh juice in all time intervals particularly in first 24 h followed by it become slow. Acid hydrolyzation augment litholysis activity only in initial phase *i.e.* in 1st 24 h, but later on, it increases lithogenic properties *i.e.* in 2nd and 3rd 24 h. Such studies would be helpful in designing of natural (non-synthetic and non-hydrolyzed) herbal formulation of cape gooseberry (*Physalis peruviana*) fruit juice for dissolving, at least partially 'the urinary stones'.

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