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Effect of Acid-Hydrolyzation of Fruits Juice of Apple (*Malus domestica*), Moushmi (*Citrus medica*) and Amla (*Emblica officinalis*) on Solubility of Urinary Stones

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The object of this study is to analyze the effect of acid-hydrolyzation of juices of common edible fruits *i.e.,* apple (*Malus domestica*), moushmi (*Citrus medica*) and amla (*Emblica officinalis*) on solubility of renal stones of different forms (whole and powdered). Attempts have been made to solubilize the whole stone (bigger- and smaller size) and powdered stone by treating them with acid-hydrolyzed juices of different fruits for 48 and 72 h. This study showed that the solubility of powdered form of urinary stone is much higher than the single whole stone and solubility of single smaller size stone is more than single bigger sized stone. The dissolving property of acid-hydrolyzed juice of amla and apple fruit was comparatively much higher than of moushmi fruit (in descending order). Comparative study showed that all forms and sizes of urinary stones have much higher solubility in acid-hydrolyzed fruits juice than in non-hydrolyzed juice *i.e.,* normal 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 such kinds of acid-hydrolyzed fruit's juice in long-term preventive and therapeutic management of nephrolithiasis.

Key Words: Urinary stones, Solubility, Dissolution, Apple (*Malus domestica*), Moushmi (*Citrus medica*), Amla (*Emblica officinalis*), Acid-hydrolyzed juice, Nephrolithiasis, Non-hydrolyzed juice.

INTRODUCTION

Urinary stones usually arise because of the breakdown of a delicate balance. The kidneys must conserve water, but they must excrete materials that have a low solubility. These two opposing requirements must be balanced during adaptation to diet, climate and activity. Urinary stones are firm and hard structure which can grow from crystals formed in the urinary systems; they cause pain in the back or the flanks which can radiate down into the groin area. Kidney stones are typically over the age of 30 years. About 10-15 % of adults in the US, are suffered with urinary tract stone[1]. The incidence rate is much more in those zones where chances of dehydration are high and climates are hot *i.e.,* 20-25 % in the Middle East. It has

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been found that recurrence rates are about 10 %/year, totaling of 50 % over a 5-10 year period and 75 % over 20 years[2]. Incidence of urinary stone formation is approximately 4 times more in men than that of women. According to the recent survey it has been seen that the increasing trend of urinary stones in pediatric age group. 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[3]. Many patho-physiological conditions promote the formation of 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[4-6]. 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 strong promoter of calcium oxalate precipitation, about 15 times stronger than calcium[7,8]. 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[9-11]. It is also best to avoid calcium supplements and foods which cause increased levels of urinary calcium such as animal protein from meat, dairy products, fish and poultry. It is for this reason that vegetarians suffer less from kidney stones. Potassium reduces urinary calcium excretion and by eating fruit and vegetables which are high in potassium the risk of suffering from kidney stones is reduced[7,12]. 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. Slowing of crystal growth increases the apparent upper limit of metastability because the critical growth of ion clusters into stable nuclei is hindered. As a consequence of the presence of these inhibitors, crystal growth in urine is slow compared with growth in simple salt solutions and the upper limit of metastability is higher[13]. 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[14.15]. Fresh (non-hydrolyzed) and acid-hydrolyzed extract of Kurthi has a definite role on solubility of urinary stone[16].

The present study is carried out to investigate the effect of acid-hydrolyzation of fruit's juice of three different kinds of common edible fruits (apple, moushmi and amla) on solubility of urinary stones (whole stone of different sizes and powdered stone).

EXPERIMENTAL

Renal stones of 2 patients (Rahiman and Manzoor having operated on 13th and 28th Sept., 2006, respectively at Hai Medicare and Research Institute, Rajabazzar, Patna, India) was collected and washed properly with distilled water. Each stone was suspended separately in 20 mL of 0.1 N NaCl solution for 24 h. Samples filtered and washed with distilled water. Dried in air oven at 80 ºC for 2 h and cooled down. Three different types of samples of acid-hydrolyzed juice were prepared from each fruit juice samples 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. First and second samples having the whole stone of different weight and third sample, powdered stone were suspended in each 25 mL of acid-hydrolyzed juice of different edible common fruits *i.e.,* apple (A1, A2 and A3), moushmi (B1, B2 and B3) and amla (C1, C2 and C3) and kept for 48 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. Whole procedure was done at room temperature (15 ºC) and pressure (738 mm of Hg) in month of December.

RESULTS AND DISCUSSION

The whole procedure and reaction have been carried out *in vitro* at room temperature (15 ºC). The main aim of present study is to break the hard crust of urinary stone by dissolving part of it and once a portion of crust is gone, the stone become susceptible to attack by inhibitors presents in acid-hydrolyzed juice of three different common edible fruits.

Keeping all these view, we have endeavored to solubulize the ingredients of urinary stone of different forms and weight with acid-hydrolyzed juice of natural sources 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 acid-hydrolyzed juice of three different common fruits at 48 and 72 h (Table-1).

Solubility difference (g/25 mL of acid-hydrolyzed fruit juice) of single urinary stone (bigger and smaller size) and powdered stone are 0.0063, 0.0066 and 0.0246 *versus* 0.0088, 0.0090 and 0.0251 in apple juice; 0.0101, 0.0101 and 0.0192 *versus* 0.0139, 0.0141 and 0.0199 in moushmi juice and 0.0039, 0.0039 and 0.0260 *versus* 0.0061, 0.0060 and 0.0266 in amla juice in 48 h and in 72 h, respectively (Table-2 and Fig. 1).

Percentage solubility of urinary stone of single stone (bigger and smaller size) and powdered stone are 4.50, 5.34 and 22.06 % *versus* 6.29, 7.28 and 22.51 % in apple juice; 6.39, 7.42 and 15.52 % *versus* 8.79, 10.35 and 16.09 % in moushmi juice and 3.01, 3.99 and 22.11 % *versus* 4.71, 6.15 and 22.62 % in amla juice in 48 and in 72 h, respectively (Table-3 and Fig. 2).

TABLE-1
SOLUBILITY OF URINARY WHOLE STONE IN ACID- HYDROLYZED JUICES OF DIFFERENT EDIBLE NATURAL SOURCES (INHIBITORS)

Acid- hydrolyzed juices	Sizes of stone	Sample No.	Initial wt. of stone (g)	Wt. remained after N/10 NaCl treatment (g)	Wt. remained after 48 h treatment with acid-hydrolyzed juice (g)	Wt. remained after 72 h treatment with acid- hydrolyzed juice (g)
			(a)	(b)	(c)	(d)
Apple	Single Bigger size	A1	0.1398	0.1363	0.1335	0.1310
	Single Smaller size	A2	0.1237	0.1201	0.1171	0.1147
	Powder stone	A3	0.1115	0.0926	0.0869	0.0864
Moushmi	Single Bigger size	B1	0.1581	0.1531	0.1480	0.1442
	Single Smaller size	B2	0.1362	0.1311	0.1261	0.1221
	Powder stone	B3	0.1237	0.1080	0.1045	0.1038
Amla	Single Bigger size	C1	0.1296	0.1271	0.1257	0.1235
	Single Smaller size	C2	0.0976	0.0950	0.0937	0.0916
	Powder stone	C3	0.1176	0.0941	0.0916	0.0910

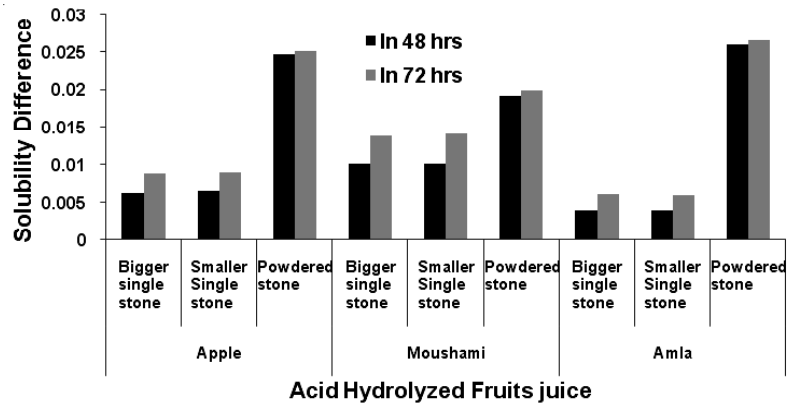


Fig. 1. Solubility difference of urinary stones in acid-hydrolyzed juice of different common edible frutis (per 25 mL of acid-hydrolyzed juice

TABLE-2
SOLUBILITY DIFFERENCE OF URINARY STONES IN ACID-HYDROLYZED JUICE OF DIFFERENT COMMON EDIBLE FRUITS (PER 25 mL OF ACID- HYDROLYZED JUICE)

Time period	Apple			Moushmi			Amla		
	Bigger single stone	Smaller Single Stone	Powdered stone	Bigger single stone	Smaller single stone	Powdered stone	Bigger single stone	Smaller single stone	powdered stone
In 48 h (a-c)	0.0063	0.0066	0.0246	0.0101	0.0101	0.0192	0.0039	0.0039	0.0260
In 72 h (a-d)	0.0088	0.0090	0.0251	0.0139	0.0141	0.0199	0.0061	0.0060	0.0266

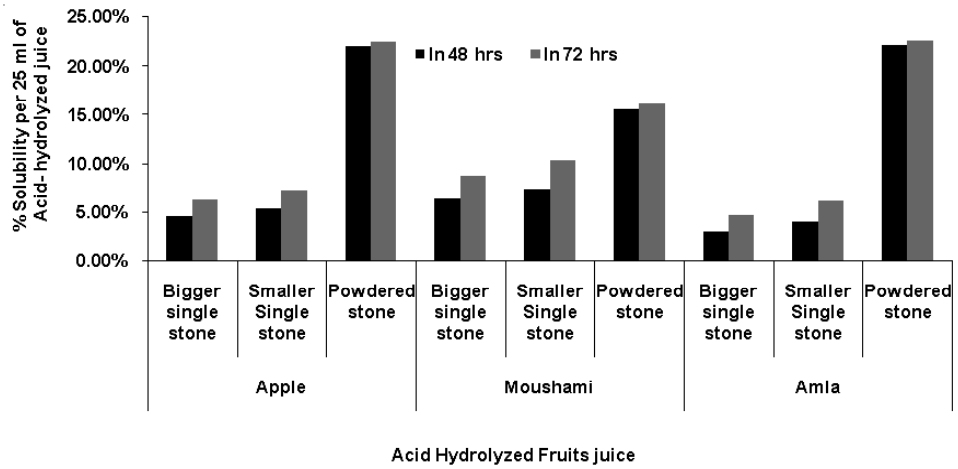


Fig. 2. Percentage solubility of urinary stones in acid-hydrolyzed juice of different common edible fruits (per 25 mL of acid-hydrolyzed juice)

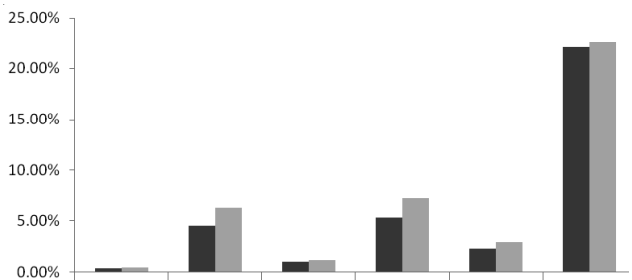
TABLE-3
PERCENTAGE SOLUBILITY* OF URINARY STONES IN ACID-HYDROLYZED JUICE OF DIFFERENT COMMON EDIBLE FRUITS (PER 25 mL OF ACID- HYDROLYZED JUICE)

Time period	Apple			Moushmi			Amla		
	Bigger single stone	Smaller Single Stone	Powdered stone	Bigger single stone	Smaller Single Stone	Powdered stone	Bigger single stone	Smaller Single Stone	Powdered stone
In 48 h	4.50	5.34	22.06	6.39	7.42	15.52	3.01	3.99	22.11
In 72 h	6.29	7.28	22.51	8.79	10.35	16.09	4.71	6.15	22.62

$$*: \text{Percentage solubility} = \frac{\text{Wt. remained after treatment with acid - hydrolyzed juice (g)}}{\text{Initial wt. of urinary stone (g)}} \times 100.$$

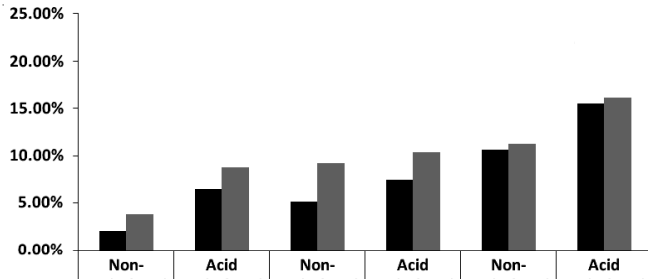
Solubility of stones is comparatively much higher in acid-hydrolyzed fruit juice than non-hydrolyzed juice *i.e.,* normal fruit's juice (Figs. 3-5).

It was observed that there was definite reduction in weight of urinary stones after treatment with acid-hydrolyzed juices which indicate the dissolution of some ingredient of the urinary stone in acid-hydrolyzed juices. It has also been observed that percentage solubility of powdered form of urinary stone was higher than smallsingle stone and much more higher than large single urinary stone in acid-hydrolyzed juices of amla-, apple- and moushmi fruit, that was, in descending order *i.e.,* powdered stone > smaller size single stone > bigger size single stone in both time interval *i.e.,* 48 and 72 h. It was noticed that solubility of single bigger size stone was comparatively higher in acid-hydrolyzed juice of moushmi fruit. 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.



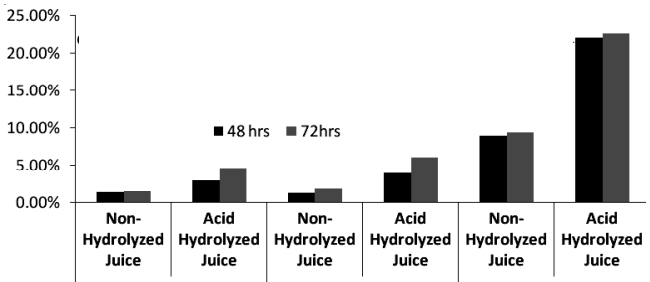
	Non-Hydrolyzed Juice	Acid Hydrolyzed Juice	Non-Hydrolyzed Juice	Acid Hydrolyzed Juice	Non-Hydrolyzed Juice	Acid Hydrolyzed Juice
	Bigger single stone		Smaller single stone		Powder stone	
48 hrs	0.40%	4.50%	1.01%	5.34%	2.27%	22.06%
72hrs	0.48%	6.29%	1.19%	7.28%	2.95%	22.51%

Fig. 3. Comparasion of percentage solubility of urinary stones in non-hydrolyzed juice (normal) and acid-hydrolyzed juice of apple fruit (per 25 mL of fruit juice)



	Non-Hydrolyzed Juice	Acid Hydrolyzed Juice	Non-Hydrolyzed Juice	Acid Hydrolyzed Juice	Non-Hydrolyzed Juice	Acid Hydrolyzed Juice
	Bigger single stone		Smaller single stone		Powder stone	
48 hrs	2.04%	6.39%	5.08%	7.42%	10.67%	15.52%
72hrs	3.77%	8.79%	9.27%	10.35%	11.30%	16.09%

Fig. 4. Comparasion of percentage solubility of urinary stones in non-hydrolyzed juice (normal) and acid-hydrolyzed juice of moushmi fruit (per 25 mL fruit juice)



	Non-Hydrolyzed Juice	Acid Hydrolyzed Juice	Non-Hydrolyzed Juice	Acid Hydrolyzed Juice	Non-Hydrolyzed Juice	Acid Hydrolyzed Juice
	Bigger single stone		Smaller single stone		Powder stone	
48 hrs	1.40%	3.01%	1.35%	3.99%	9.02%	22.11%
72hrs	1.57%	4.71%	1.91%	6.15%	9.47%	22.62%

Fig. 5. Comparasion of percentage solubility of urinary stones in non-hydrolyzed juice (normal) and acid-hydrolyzed juice of amla fruit (per 25 mL of fruit juice)

Conclusion

Due to hard and stubborn nature of outer covering of urinary stone, it is too difficult to solubulize it easily. Dissolution properties of fruit's juices for urinary stone are proportionate to the concentration of organic acids. Dissolving power of acid-hydrolyzed juice of amla and apple fruits against powder stone is much higher than moushmi fruit. Bigger sized stone comparatively more soluble in acid-hydrolyzed juice of moushmi fruit than acid-hydrolyzed juice of amla and apple fruits. Acid hydrolyzation of fruit juice potentiates their dissolving properties against urinary calculi. Such studies would be helpful in designing of herbal medicinal preparation for dissolving, at least partially 'the urinary stones'.

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