J. Food and Dairy Sci., Mansoura Univ., Vol. 3 (5): 291 - 298, 2012

EFFECT OF GRAPEFRUIT JUICE ON CALCIUM OXALATE STONES FORMATION IN RATS KIDNEY

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ABSTRACT

To investigate the potential influence of grapefruit juice on urinary risk factors associated with the formation of calcium oxalate kidney stones as the product might effect the chemical composition of urine. Rats were rendered nephrolithic by providing drinking water containing 0.75% ethylene glycol (v/v) (EG) and 2% ammonium chloride (w/v) (AC) for 10 days. In addition to EG/AC treatment, three groups of rats were also gavage-administered solutions containing 100%, 75% and 50% grapefruit juice (v/v) (6 µl solution/g body weight). Positive control rats were treated with EG/AC but not grapefruit juice. Negative control rats were provided with normal drinking water and were administered normal water by gavage. Each group contained 6 rats. After 10 days serum samples were collected for analysis, the left kidney was removed and assessed for calcium levels using flame spectroscopy and the right kidney was sectioned for histopathological analysis using light microscopy. Analysis showed that the rats treated with EG/AC alone had higher amounts of calcium in the kidneys compared to negative control rats. This EG/AC-induced increase in kidney calcium levels was inhibited by the administration of grapefruit juice. Histology showed that rats treated with EG/AC alone had large deposits of calcium oxalate crystals in all parts of the kidney and that such deposits were not present in rats also treated with 100% or 75% grapefruit juice.

Keywords: Grapefruit juice, urinary risk, calcium oxalate, kidney stones.

INTRODUCTION

Most kidney stones are composed of calcium and oxalic acid, substances present in the urine that can crystallize inside the kidneys. Although these chemicals occur in everyone's urine, our natural biochemistry is usually able to prevent them from crystallizing. However, sometimes these protective methods fail and a stone develops. This article focuses mainly on these *calcium oxalate stones*. Less commonly, kidney stones may be made from calcium and phosphate, from another substance called struvite or, rarely, from uric acid or cystine (Curhan, *et al.,* 1998). A high fluid intake is the first general advice to patients in the prevention of stone recurrence, irrespective of stone composition. An increase of fluid intake is associated with a reduced risk for kidney stone formation, reported by several authors (Hesse *et al.,* 1993). Urine dilution causes a lowering of the concentration of constituent ions and thus a decrease of the super-saturation of the stone forming salts. Depending on the stone composition, several fluids have been found to be suitable, e.g. mineral water, orange juice, apple juice, fruit and herbal teas (Vahlensieck, 1986; Wabner and Pak, 1993). Alkalizing beverages such as orange juice are highly effective in the metaphylaxis of calcium oxalate (CaOx), uric acid and cystine lithasis.

Citrate, or citric acid, is an ordinary component of our diet, present in high amounts in citrus fruits. Citrate binds with calcium in the urine, thereby

reducing the amount of calcium available to form calcium oxalate stones. It also prevents tiny calcium oxalate crystals from growing and massing together into larger stones. Finally, it makes the urine less acidic, which inhibits the development of both calcium oxalate and uric acid stones.One form of citrate supplement, potassium citrate, was approved by the FDA in 1985 for the prevention of two kinds of kidney stones: calcium stones (including calcium oxalate stones) and uric acid stones (Trinchieri *et al.,* 2002). Curhan *et al.* (1998) investigated the influence of beverages on the risk of kidney stone formation in two prospective studies. Their investigations resulted in the postulation of grapefruit juice and apple juice being risk increasing beverages in respect to CaO_x formation. Their results were based on an evaluation of questionnaires without any measurement of urinary composition. These results, however, cannot be understood without any further information about the changes in urinary composition. Therefore, the results lead to the question: why does the ingestion of grapefruit juice causes an effect that is different form that of other juices with a high content of citric acid? There must be a constituent of grapefruit juice be responsible for this effect. To find an answer to this question some authors investigated the influence of grapefruit juice on urinary composition (Goldfarb and Asplin, 2000, 2001). The present study was undertaken to overcome these shortcomings by evaluating the influence of grapefruit juice on urinary composition and therefore on risk of crystallization.

MATERIALS AND METHODS

Materials:

Ten kg of grapefruit was obtained from local market at Kafr El-Sheikh city, Egypt. Then extraction juice from fruits and preserves without additions in the freeze until use.

Animal:

Thirty male rats weighing approximately 280 g were obtained from Faculty of Science, Tanta University then acclimated for 3 days in cages before experiments commenced. Experiments were conducted in accordance with internationally accepted standard guidelines for the use of animals. Rats had *ad libitium* access to standard chow and tap water and were kept under a controlled 12 h light/dark cycle at 22 $\pm$ 2^{o}C.

Methods:

Ethylene glycol-induced urolithiasis:

The thirty rats were divided into five groups comprising six animals per group. Each group underwent a different treatment protocol for 10 days. Group 1: negative control, *ad libitum* access to regular food and drinking water and administered 6 µl distilled water per 1 g of body weight by gavage (intra-gastric administration). Group 2, 3, 4 and 5: *ad libitum* access to regular food and *ad libitum* access to drinking water containing 0.75% (v/v) ethylene glycol (EC) and 2% (w/v) ammonium chloride (AC) in order to promote hypeoxaluria and CaO_x deposition in the kidneys(Khatib *et al*., 2010). Groups 2, 3 and 4 were also administered 6 µl grapefruit juice solution/g body weight

by gavage at the following concentration: Group 2, 100%(v/v) grapefruit juice; Group 3, 75% (v/v) and Group 4, 50%(v/v). Group 5 rats were administered 6 µ distilled water/g body weight by gavage (positive control). All rats were weighed daily.

Kidney and serum analysis:

After the 10 days experimental period, rats were anaesthetized and blood was collected from the retro orbital region, centrifuged at 10,0000 g for 10 min and the serum collected and analyzed for calcium, phosphorus, urea and creatinine using and automated system (Cobas Integra analyzer Model 400 plus) (Karadi *et al*., 2006). The rats were then sacrificed by cervical dislocation, the abdomen opened and both kidneys removed. The left kidney was dried with an oven at 100^{o}C for 24 h, after which the kidney was weighed and then minced in beaker containing 7 ml 0.5 N nitric acid. The mixture was then heated until the liquid became transparent. After calibration using a standard calcium solution, the calcium content of the mixture was determined using flame spectroscopy. The right kidney was fixed in bouine liquid (Cuzzolin *et al*., 1995; Nolte *et al*., 1995), Soaked in paraffin, cut at 3-4 µm intervals and the slices stained using hematoxylin and eosin (Cuzzolin *et al.,* 1995) tissue slices were photographed using optical microscopy under polarized light (Olympus Bx 41).

Statistical analysis:

Results are presented as mean $\pm$ standard (S.E.) A one-way ANOVA was used to determine the significance of differences among groups. Student's t-test was used to assess differences between means. Conventional Windows Software was used for statistical computations. AP value < 0.05 was considered to indicate a significant difference.

RESULTS AND DISCUSSION

Rats serum analysis:

Serum analysis showed that urea and creatinine levels were higher in groups 2, 3, 4 and 5 compared to group 1 (Tale 1). These data indicate marked renal damage in the EG/AC-treated rats. The data also showed that urea, creatinine, calcium and phosphorus levels were lower in rats treated with grapefruit juice (groups 2, 3 and 4) compared to rats treated with EG/AC alone (group 5, positive control). Grapefruit juices contain about 10 mg citric acid/g. The high content of citric acid causes an increased citric acid extraction and is responsible for the alkalizing effect. The alkalinzing effect and the increased citric acid excretion lead to a decreased relative super saturation (RS) CaO_x and may have mainly caused the decreasing of the BRI values. BRI is a newly established method for CaO_x crystallization risk determination from unprepared native urine samples (Laube *et al.,* 2002).

Table (1):Rats serum analysis of the treatments.

Treatment	Calcium (mmol/L)	Phosphorus (mmol/L)	Urea (mmol/L)	Creatinine (µmol/L)
Negative	2.4	2.3	3.0	45.0
Juice 100%	1.5 [a b]	1.0[ab]	5.0[ab]	46.0 [b]
Juice 75%	1.7 [a b]	1.2 [a b]	7.0 [a b]	51.0[b]
Juice 50%	1.9 [a b]	1.5[b]	8.0[ab]	60.0[ab]
Positive	4.6[a]	3.3[a]	18.0[a]	85.0[a]

Values represent mean ± SD for six rats in each group
a = Values are significantly different from the negative control group.
b = Values are significantly different form the positi*ve control group.*

Effect of treatments on body weight of rats:

EG/AC-treated rats (groups 2, 3, 4 and 5) weighted less than the negative control rats (group 1) at the completion of the experiment (Table 2). Weight loss increased with increasing of time experiment and low intake of grapefruit juice. This loss is probably due to defects in the kidney functions.

Table (2): Percentage of the loss in body weight in the various treatments during the ten days of experiment.

Treatment	Day									
	1	2	3	4	5	6	7	8	9	10
Negative	0	0.01	0.01	0.03	0.02	0.04	0.04	0.05	0.06	0.06
Juice 100%	0	-0.03	-0.05	-0.14	-0.16	-0.20	-0.23	-0.23	-0.25	-0.26
Juice 75%	0	-0.04	-0.06	-0.14	-0.17	-0.19	-0.24	-0.23	-0.27	-0.29
Juice 50%	0	-0.05	-0.06	-0.16	-0.16	-0.21	-0.23	-0.26	-0.29	-0.29
Positive	0	-0.07	-0.08	-0.17	-0.18	-0.20	-0.42	-0.29	-0.31	-0.34

Calcium levels in the kidneys of rats:

The left kidney was assessed for calcium levels. EG/AC treatment alone(group 5) resulted in increased kidney calcium levels compared to the negative control rats, while the administration of 100%, 75% and 50% grapefruit juice reduced this calcium accumulation to 34.5%, 31.0% and 28.9%, respectively (Table 3).

Table (3): Amount of calcium in left kidney (µg/g) of rats.

Treatment	Calcium
Negative	256
Juice 100%	247[b]
Juice 75%	260[b]
Juice 50%	268[b]
Positive	377[a]

Values represent mean ± SD for six rats in each group
a = Values are significantly different from the negative control group.
b = Values are significantly different form the positive control group.

Histological examination:

Examination of kidney paraffin sections showed that group 5 rats (EG/AC alone, positive control) had the greatest amount of CaO_x deposition.

The papillary tip (Fig. 1) in group 5 rats compared to the groups 2, 3 and 4 rats (EG/AC and grapefruit juice). Longitudinal sections showed the papillary tips were encrusted with CaO_x crystals (Fig. 6d and 6e). Analysis of portions of these crystalline deposits removed from the papillary tip showed they were composed of CaO_x monohydrate and CaO_x dehydrate. No papillary encrustations were seen in tissue from the negative control rats (group 1) (Fig. 6a) or rats treated with EG/AC and 100% grapefruit juice (group 2) (Fig. 6b). Major calcium deposits were observed on the surface of the papillary tips in 36% of the positive control rats (group 5) and 18% of the rate treated with EG/AC and 50% grapefruit juice (group 4) (Table 4). These morphological findings were consistent with the left kidney calcium level data. In contrast, no rats treated with grapefruit juice showed such papillary crystalline deposits. Rats treated with 100% or 75% grapefruit juice for les kidney calcification and lower renal tissue calcium levels than the positive control rats (group 5) (Table 4). No papillary encrustations were seen in 100%, 86% and 50% of rats treated with 100%, 75% and 50% grapefruit juice, respectively. These results clearly demonstrate the ability of the grapefruit juice to prevent the development of papillary calcifications on the kidney, consequently preventing the development of papillary calcium. Goldfarb and Coe (1999) postulated that grapefruit juice if it stimulates stone formation must have a significant effect on urinary composition different from that other citrus juice, or that the measurement of urinary composition in short-term studies fail to predict long-term outcomes accurately.

Table (4):Number and type of calcification observed.

Treatment	1	2	3	4
Negative	-	-	-	100
Juice 100%	-	-	-	100
Juice 75%	10	-	-	86
Juice 50%	18	-	12	50
Positive	36	34	24	19

1 =Percentage of rats with major calcifications on the papillary tip (> 90% of the papillary tip calcified).
2 = Percentage of rats with some area of the papillary tip calcified
3 = Percentage of rats with some calcified points on the papillary tip.
4 = Percentage of rats without calcifications on papillary tip.

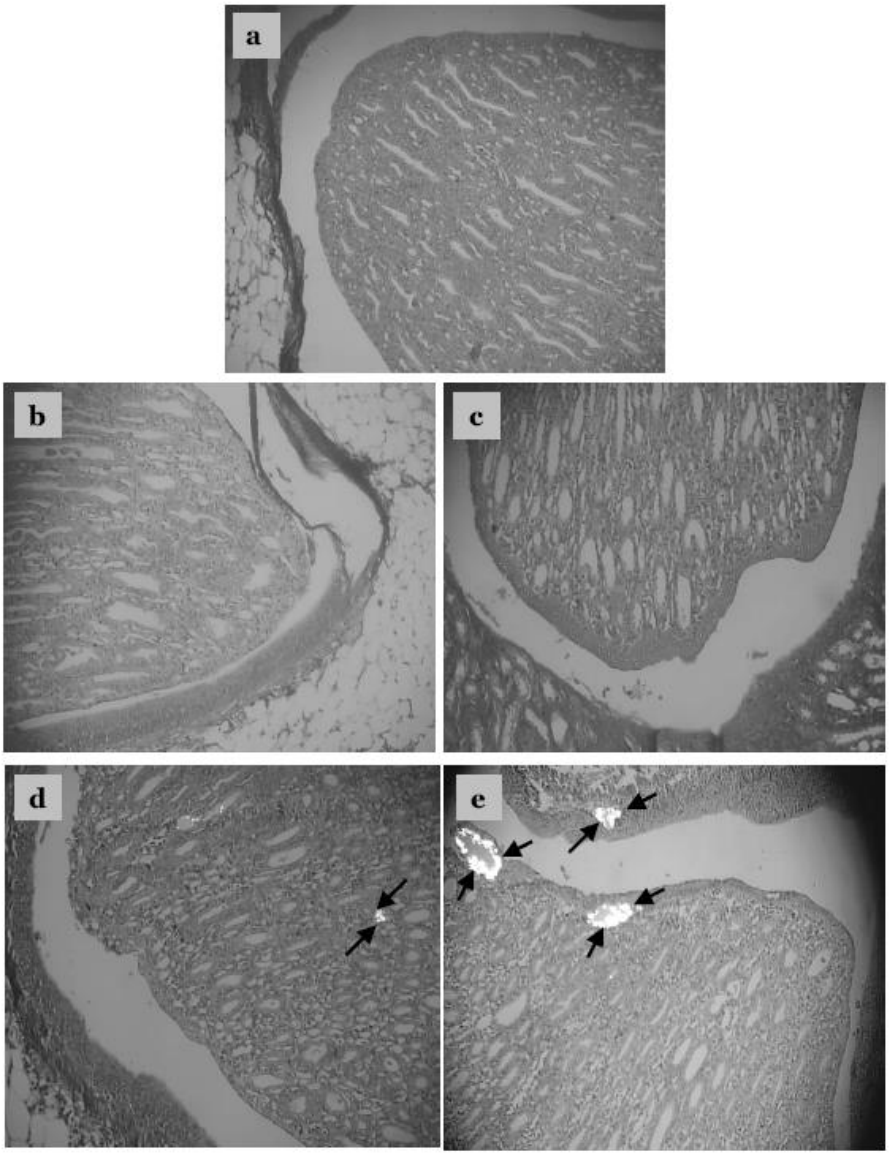

Figure 1: crystalline formations in the renal papilla tip.
a = tissue from negative control rats.
b = tissue from rats treated with EG/AC and 100% grapefruit juice.
c = tissue from rats treated with EG/AC and 75% grapefruit juice.
d = tissue from rats treated with EG/AC 50% grapefruit juice.
e = tissue from rats treated with EG/AC only (positive control).
Crystalline formations in the renal papilla are indicated by arrows.
Magnification x 100

Conclusion:

The present study found that the administration of grapefruit juice effectively prevented the development of urolithiasis in rats. These findings support the use of grapefruit juice as an alternative food to prevent urolithiasis. Further research is necessary to clarify the mechanisms underlying this preventative effect of grapefruit juice.

REFERENCES

Curhan, G.; Willett, W.; Speizer, P. and Stampfer, M., (1998). Beverage use and risk for kidney stones in women. Ann. Int. Med. 128, 534-540.

Cuzzolin, L.; Conforti, A.; Adami, A.; Lussignoli, S.; Menestrina, F.; Del Soldato, P. and Benoni, G. (1995). Anti-inflammatory potency and gastrointestinal toxicity of a new compound nitronaproxen. Phamacal Res., 31: 61-65.

Goldfarb, D. and Asplin, J. (2000). Effect of grapefruit on urinary lithogeniciity. In Urolithiasis, pp. 317-318 (Al Rodgers, BE Hibbert, B Hess, SR Khan and GM Preminger, editors). Cape Town: University of Cape town.

Goldfarb, D. and Asplin, J. (2001). Effect of grapefruit juice on urinary lithogenicity. J. Urol. 166, 263-267.

Goldforb, D. and Coe, F. (1999). Beverages, diet, and prevention of kidney stones. Am. J. Kidney Dis 33, 398-403.

Hesse, A.; Siener, R. and Jahnen, A. (1993). The influence of dietary factors on the risk of urinary stone formation. Scanning Microsc. 7, 1110-1128.

Karadi, R.; Gadge, N.; Alagawadi, K. and Savadi, R. (2006). Effect of *Moringa oleifera* Lam. root-wood on ethylene glycol induced urolithiasis in rats. J. Ethnaopharmaco., 105: 306-311.

Laube N.; Shneider, A. and Hesse, A. (2002). Comparison of laser-probe and photometer-based determinations of the urinary crystallization risk of calcium oxalate. Clin. Chem. Lab. Med. 40, 595-599.

Khatib N.; Dhaval G.; Hashilkar N. and Rajesh K. (2010). Antiurolithiatic potential of the fruit extracts of Carica papaya on ethylene glycol induced urolithiatic rats. J Pharm Res.; 3:2772-5.

Nolte, T.; Harleman, J. and John W. (1995). Histopathoalogy of chemically induced testicular atrophy in rats. Exp. Toxicol. Pathol. 47: 267-286.

Trinchieri, A.; Lizzano, R. and Bernardini, P. (2002). Effect of acute load of grapefruit juice on urinary excretion of citrate and urinary risk factors for renal stone formation. Dig. Liv. Dis. 34, pp. 160-161.

Vahlensieck, W. (1986). Review: The importance of diet in urinary stones. Urol. Res. 14, 283-288.

Wabner, C. and Pak, C. (1993). Effect of orange juice consumption on urinary stone risk factors. J. Urol. 149, 1405-1408.

تأثير تناول عصير الجريب فروت على تكوين حصوات أكسالات الكالسيوم فى كلى الفئران

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أجريت هذه الدراسة لمعرفة قوة تأثير تناول عصير الجريب فروت على عوامل الخطر فى البول والمسئولة عن تكوين حصوات أكسالات الكالسيوم بالكلى والناتجة من تأثير التركيب الكيمائى للبول. أجريت الدراسة على فئران مصابة عن طريق تناولها مياه شرب تحتوى على ٠,٧٥% اثيلين جليكول (وزن/وزن) (EG) بالاضافى الى ٢% كلوريد أمونيوم (وزن/حجم) (AC) لمدة عشرة أيام. ثلاث مجموعات من الفئران المعاملة بـ EG/AC أعطت ٦ ميكروليتر محلول يحتوى ١٠٠% ، ٧٥% ، ٥٠% (وزن/وزن) عصير جريب فروت عن طريق الفم لكل واحد جرام من وزن الجسم ، ومجموعة كنترول ايجابى معاملة بـ EG/AC بدون إعطائها محلول عصير الجريب فروت ، ومجموعة كنترول سالب غير معامة بـ EG/AC أعطت عن طريق الفم نفس الجرعة السابقة ٦ ميكروليتر/حجم من وزن الجسم ماء مقطر ، وكل مجموعة تحتوى على ستة فئران. بعد مرور عشرة ايام هى مدة التجربة جمع السيرم من كل مجموعة لتحليل الكالسيوم والفسفور واليوريا والكرياتينين ، وبعد ذبح كل مجموعات الفئران أخذت الكلية اليسرى لتقدير مستوى الكالسيوم بها ، والكلية اليمنى لعمل شرائح التحليل الهستولوجى باستعمال الميكروسكوب الضوئى. لوحظ من نتائج التحليل أن مجموعات الفئران المعاملة بـ EG/AC فقط احتوت على مستويات مرتفعة من الكالسيوم فى الكلى مقارنة بمجموعة الكنترول السالب. لوحظ ايضا أن المجموعات ا لمعالمة بـ EG/AC وعصير الجريب فروت قل مستوى الكالسيوم بكلياتها بزيادة تركيز العصير. كذلك التحليل الهستولوجى أوضح أن الفئران المعاملة بـ EG/AC فقط احتوت كلياتها على تجمعات كبيرة من بلورات أكسالات الكالسيوم فى كل أجزاء الكلية ، وعلى العكس من ذلك لم تظهر هذه التجمعات بالفئران المعالمة بتركيزات ١٠٠% أو ٧٥% من عصير الجريب فروت.

لذا نوصى باستخدام كوب او اثنين من عصير الجريب فروت خلال تناول وجبات اليوم وذلك لاعاقة تكون حصوات الكلى.

قام بتحكيم البحث

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