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Uricosuric effect of Roselle (*Hibiscus sabdariffa*) in normal and renal-stone former subjects

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ABSTRACT

Aim of the study: The Roselle (*Hibiscus sabdariffa*) was investigated for its uricosuric effect.
Materials and methods: A human model with nine subjects with no history of renal stones (non-renal stone, NS) and nine with a history of renal stones (RS) was used in this study. A cup of tea made from 1.5 g of dry Roselle calyces was provided to subjects twice daily (morning and evening) for 15 days. A clotted blood and two consecutive 24-h urine samples were collected from each subject three times: (1) at baseline (control); (2) on days 14 and 15 during the tea drinking period; and (3) 15 days after the tea drinking was stopped (washout). Serum and 24-h urinary samples were analyzed for uric acid and other chemical compositions related to urinary stone risk factors.
Results: All analyzed serum parameters were within normal ranges and similar; between the two groups of subjects and among the three periods. Vis-à-vis the urinary parameters, most of the baseline values for both groups were similar. After taking the tea, the trend was an increase in oxalate and citrate in both groups and uric acid excretion and clearance in the NS group. In the RS group, both uric acid excretion and clearance were significantly increased ($p < 0.01$). When the fractional excretion of uric acid (FEUa) was calculated, the values were clearly increased in both the NS and SF groups after the intake of tea and returned to baseline values in the washout period. These changes were more clearly observed when the data for each subject was presented individually.
Conclusions: Our data demonstrate a uricosuric effect of Roselle calyces. Since the various chemical constituents in Roselle calyces have been identified, the one(s) exerting this uricosuric effect need to be identified.

1. Introduction

Phytotherapy with medicinal plants is widely used worldwide as an alternative primary healthcare. Regarding the treatment of urinary stone disease, several medicinal plants are available (Nirdnoy and Muangman, 1991; Yasui et al., 1999; Selvam et al., 2001; Freitas et al., 2002; Premgamone et al., 2002; Atmani et al., 2004). Since the plants are claimed to be non-toxic, low-cost, available in rural areas and culturally acceptable, their effectiveness in the treatment of urinary stones has been widely studied. In Thailand, there are many herbal plants that act as antilithiasis agents, notably Roselle (*Hibiscus sabdariffa*), *Orthosiphon grandiflorus* and *Phyllanthus amarus* (Krissanapan, 1996; Muanwongyathi, 1996; Panutas, 2001).

Roselle (*Krachiap daeng* in Thai) belongs to the Malvaceae family and occurs widely throughout the tropics. Infusions of the leaf or calyx extracts are claimed to act as cholerectic, febrifugal and hypotensive agents as well as decreasing the viscosity of blood and stimulating intestinal peristalsis (Ali et al., 1991; El-Saadany et al., 1991; Chewonarin et al., 1999; Haji-Faraji and Haji-Tarkhani, 1999). Roselle extract is also reported to act as a diuretic and uricosuric substance in patients with urologic disorders and bladder stone disease (Farnworth and Bunyapraphatsara, 1992). Although some studies reported Roselle extract affects on urinary composition (Kirdpon et al., 1994) and acts as an anti-hypertensive (Herrera-Arellano et al., 2004), hypo-cholesterolemic and anti-oxidative substance (Hirunpanich et al., 2006), no scientific study has ever reported any antilithiasic and/or uricosuric effects, particularly in renal stone (RS) subjects. This study was therefore designed to study the effects of Roselle tea drinking on urinary excretions of uric acid and other compounds related to urinary stone risk factors in both normal and renal stone subjects.

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2. Materials and methods

2.1. Chemicals and reagents

The chemicals and reagents used in this study were commercially available and most were of an analytical grade.

2.2. Roselle tea preparation for drinking and analysis

A tea bag of dried Roselle calyx weighing 1.5 g is made commercially by the Lampang Medicinal Plant Conservation Assembly, Lampang province, Thailand. With regard to the herbal tea preparation for consumption, each cup of tea (~150 mL) was prepared (according to the directions) by steeping a bag of tea in a cup of hot water (98 °C) for ~10 min. Before drinking, the cup of tea (containing the bag) was stirred with a teaspoon for few seconds. The tea was provided to the subjects twice daily by trained village health volunteers at the local health center.

For content analysis, the tea was similarly prepared except that distilled water was used. The prepared tea was then filtered through filter paper (Whatman No. 4) and lyophilized. Prior to analysis, the powder was reconstituted to prepare a solution of 30 mg/mL in distilled water and assessed for calcium, sodium, potassium, magnesium, citrate, oxalate and glycolate using standard methods.

2.3. Participated subjects

This research protocol was reviewed by Khon Kaen University Ethics Committees and written informed consent was obtained from all subjects. We recruited two groups of healthy males, each nine, with no renal stones (non-renal stone, NS) and with a history of renal stones, between 36 and 65 years of age, residing in two rural communities of Khon Kaen province, Thailand.

Based on the urinary and blood examinations, both groups had normal renal and liver functions, and none of the participants had any diabetes mellitus or urinary tract infection.

After obtaining informed consent, all of the subjects were asked questions relating to urinary stone disease and then examined for stones by X-ray (plain KUB method). Fasting blood samples from the participating subjects were collected and analyzed for sugar and liver and renal functions. Spot urine samples were collected to investigate by culturing for any evidence of urinary tract infection.

2.4. Study protocol

This study was divided into three periods: (1) baseline (control), (2) during tea drinking, and (3) after the tea drinking was stopped (washout). Blood and urine samples were collected during each period. In the baseline period, after the collection of the first morning urine (without preservative), two consecutive 24-h urine samples (with thymol as a preservative) were collected from each subject. Clotted blood samples were collected on the last day of urine collection.

During the tea-drinking period, the subjects were assigned to take a cup of tea twice daily, morning and evening, for 15 consecutive days. The two 24-h urine and clotted blood samples were collected on days 14 and 15. During the tea washout period, the subjects were allowed to follow a normal lifestyle (without tea) for another 15 days during which blood and urine samples were again collected on days 14 and 15.

2.5. Analysis of blood and urine samples

Serum obtained from the clotted blood were analyzed for blood urea nitrogen (BUN), creatinine, uric acid, sodium, potassium, chloride and bicarbonate contents and assayed for aminotransferase (ALT) activity using automatic analyzer (Automatic Express Plus, E for L, USA).

After measuring volume and pH, the 24-h urine samples were centrifuged to remove cellular debris and analyzed for chemical composition. Creatinine was analyzed using the Jaffe reaction and urea by the direct condensation method (Smith, 1992). Calcium and magnesium were analyzed using atomic absorption spectrophotometry (AAS), phosphorus the phosphomolybdate reaction (Toffaletti and Jones, 1992) and uric acid the phosphotungstic acid method (Smith, 1992). Oxalate was determined using capillary electrophoresis (Holmes, 1995) and citrate by the citrate lyase method (Petrarulo et al., 1995).

2.6. Statistic analysis

All biochemical parameters were reported as the mean ± S.D. Differences between groups in the serum and urinary parameters were compared using the Student's *t*-test, whereas differences between serum and urine at baseline, during the tea drinking and washout periods were compared using the paired *t*-test on STATA version 8.0.

3. Results

3.1. Chemical composition of Roselle tea

The chemical composition of the extract of dried Roselle tea is presented in Table 1. The daily intake of each component varied from 1.2 mg/day for oxalate to 27.9 mg/day for potassium. These values were clearly lower than those of the daily urinary excretions of normal subjects (Toffaletti and Jones, 1992).

3.2. Serum analysis

The results of the serum analyses are presented in Table 2 and there were no significant differences in any of the serum parameters between the two groups of subjects or between the baseline and the tea drinking periods. Though the activity of ALT in the RS subjects tended to be higher, it was within normal range in both groups.

3.3. Urinary analysis

All the chemical parameters for the 24-h urine at baseline and the tea drinking periods for both groups are presented in Table 3. The baseline values of most parameters were similar between the two groups, except for calcium where the RS group tended to be higher. When the comparison between the two groups was analyzed, urinary volume and calcium tended to be higher in the RS group, both at baseline and during the tea drinking periods.

After the intake of tea, the trend was an increase in urinary parameters: (1) in both groups for oxalate and citrate; (2) in the

Table 1
Chemical components in Roselle tea intake per day compared to daily excretions in the urine of normal subject

Component	In Roselle tea intake (mg/day)	In normal urine (mg/day)
Calcium	27.0	<300
Magnesium	10.5	120–140
Potassium	27.9	1000–5000
Sodium	11.4	1000–5000
Uric acid	5.7	<700
Oxalate	1.2	<400
Citrate	3.3	<300
Glycolate	2.4	<105