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ORIGINAL PAPER

Orthosiphon grandiflorum has a protective effect in a calcium oxalate stone forming rat model

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Abstract This study amied to investigate the effects of *Orthosiphon grandiflorum* on the renal tubular cell injury induced by oxalate and the inhibitory effects of *O. grandiflorum* on urinary deposit formation in an animal model and compared the results with those from a potassium citrate treatment. Rats were divided into three groups: an untreated stone-forming group, an *O. grandiflorum*-treated stone-forming group and a potassium citrate-treated stone forming group. Ethylene glycol (0.5%) was administered to the rats during the last week, and vitamin D3 (0.5 μm) was force fed to induce hyperoxaluria and kidney calcium oxalate crystal deposition. Twenty-four hour urine samples were collected before and after inducing crystal deposits. Rats were killed and both kidneys were harvested after 3 weeks. Bisected kidneys were examined under a polarized light microscope to determine the number of crystals. The renal tissue superoxide dismutase and catalase levels were measured by Western blot. Oxidative stress was examined by 8-OHdG immunohistofluorescence. *O. grandiflorum* and potassium citrate have the ability to alkalinize urine. Among all groups, the number of crystal deposits and the level of 8-OHdG staining decreased significantly in the *O. grandiflorum*-treated stone forming group, as compared to the other groups. Superoxide dismutase and catalase levels also increased significantly in the *O. grandiflorum*-treated stone-forming group, as compared with the untreated stone-forming group. The results indicate that *O. grandiflorum* has a significant inhibitory effect on crystal deposition in the calcium oxalate-stone-forming rat model.

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Introduction

Urolithiasis is an important health problem all over the world. Its prevalence differs in various parts of the world; 1–5% in Asia, 5–9% in Europe, 13% in North America, and 20% in Saudi Arabia [1]. Another report indicated that the prevalence of urolithiasis varies from 0.38% [2] to 16.9% [3]. Calcium oxalate is the most common stone component with a frequency of approximately 80% [4]. The northeastern part of Thailand has the highest incidence of urolithiasis [5]. The composition of surgically removed urinary stones in northeast Thailand was reported in 1983; all stones were of a mixed type, and calcium oxalate was the main component [6]. In Japan the incidence of urolithiasis has steadily increased since the 1950s, which is a similar trend to other developing countries in Europe and the United States [7]. Idiopathic calcium urolithiasis in the upper tract has become the most common type of urinary calculi [8].

Over the past two decades, revolutionary advances in the minimally invasive and noninvasive management of stone disease have greatly facilitated the ease with which stones are removed. However, treatments such as percutaneous nephrolithotomy and extracorporeal shock wave lithotripsy do not necessarily prevent the recurrence of stones. Furthermore, these techniques result in side effects such as hemorrhage, hypertension, tubular necrosis, and subsequent kidney fibrosis [9]. Hence, the development of a medical prophylactic program to prevent stone recurrence is desirable.

The use of natural medicine is a persistent aspect of present-day health care. Although modern medicine may be

available throughout the world, many people have turned to alternative or complementary therapy, including medical herbs [10].

Java Tea (*Orthosiphon grandiflorum*) is a medicinal herb trusted for many centuries to treat kidney ailments and bladder stones [11]. *O. grandiflorum* has synonyms such as *O. aristatus, O. stamineus*, and *O. spicatus*. This herb is also known by its vernacular names such as Java tea (English), Thé de Java (France), kumis kucing (Indonesia), kumis ucing (Sudanese), remuk jung (Japanese), kumis kucing or misai kucing (Malaysia), balbas-pusa and kabling-gubat (Philippines), kapen prey (Cambodia), hnwàd méew (Laos), yaa nuat maeo (Thailand) and r[aa]u m[ef]o in Vietnam.

O. grandiflorum belongs to the family Lamiaceae. It is occasionally planted throughout Thailand for medicinal and ornamental purposes. Three main groups of *O. grandiflorum* chemical constituents are flavonoids, organic acids, and terpenoids. For example, the flavonoids are eupatorin, sinensetin, salvegenin, ladanein, vomifoliol, 5-hydroxyl-6,7,3′,4′-tetramethoxylflavone, 6-hydroxy-5,7,4′-trimethoxyflavone, 7,3′,4′-tri-O-methylluteolin, tetramethylscutellarein, and scutellarein tetramethylether [12–15]. Caffeic acid and its derivatives including rosmarinic acid [14, 15] and 2,3-decaffeoyltartaric acid [16] are organic acids in aqueous extracts.

Flavonoids from plants or herbs decreased calcium oxalate stone formation in a rat hyperoxaluria-induced stone model [17, 18], and rosmarinic acid inhibited several complement dependent inflammatory processes, acting through stimulation of a host antioxidant response [19]. A clinical study in Thailand was performed to compare the efficacy of *O. grandiflorum* and sodium potassium citrate in treatment of renal calculi by ultrasound images and evaluated with rates of stone size reduction per year (ROSRPY). This study indicated that treating renal calculi with *O. grandiflorum* tea is an alternative method for management. These two means of ROSRPY were not significantly different [20].

The present study was undertaken to determine the protective effect of *O. grandiflorum* on urinary excretion factors and to determine the antioxidative effects on calcium oxalate urolithiasis formation in a rat hyperoxaluria-induced stone model, compared with sodium potassium citrate [21], for the treatment of renal calculi.

Materials and methods

Preparation of *O. grandiflorum* for gastric feeding

Fresh leaves of OG were collected from Thailand. The samples of leaves were washed thoroughly, dried in an oven at 45°C for 48 h. The dried samples were ground to powder, then passed through a sieve with mesh no.180 and kept in well-closed containers. In order to prepare a solution for gastric gavages, 100 g of finely powdered OG and 500 ml of doubly distilled water were heated until OG powder turned into solution. Then, the solution was stored at room temperature. Finally the feeding solution contained 200 mg of OG per milliliter.

Stone forming rat model and experimental design

All animal experimental protocols were approved by the Animal Research Committee of the University of Osaka. Sixteen male Sprague–Dawley rats, 7-weeks-of-age, and weighing 180–200 g, were used.

The rats were acclimated to room temperature and fed a standard commercial rat chow during the study. Ethylene glycol (EG, 0.5%) was administered to the rats in their drinking water during the last week, and 0.5 μm of vitamin D3 dissolved in 1 ml of salad oil was force fed by gastric intubation to induce hyperoxaluria and kidney calcium oxalate (CaOx) crystal deposition.

The rats were divided into three groups. All groups rested for 1 week before the start of the experiment. Group 1, EG group ($n = 6$): the animals received normal drinking water for 2 weeks and crystal deposits were induced in the last week. Group 2, potassium citrate (KCit) group ($n = 6$): the animals were force fed KCit (100 mg/kg daily) by gastric intubation [20, 21] for 2 weeks before the induction of crystal deposits with 1 week of 0.5% EG. Group 3, *O. grandiflorum* (OG) group ($n = 6$): the animals were force fed OG (200 mg/kg daily) by gastric intubation [20] for 2 weeks before the induction of crystal deposits with 1 week of 0.5% EG.

The experimental period was 3 weeks. At the end of the experiment, all rats were euthanized with anesthesia, killed, and both kidneys were harvested. One kidney was fixed in formalin, embedded in paraffin, and strained with hematoxylin and eosin solution, while the other kidney was immediately stored at −80°C for further study.

Measurement of urinary variables

Twenty-four hour urine samples were collected on day 14 before starting the crystal deposit induction and before killing. Rats were kept individually in metabolic cages for 24 h to collect urine, which was either analyzed immediately or stored at −80°C until further analysis. Drinking volume, urine volume, and urine pH were measure manually. Urinary calcium, potassium, sodium, phosphate, creatinine, and magnesium levels were measured using a Model 705 automate analyzer (SRL, Tokyo, Japan). A portion of the urine sample was acidified with 6 mol/L HCl to maintain

urine pH at <3 for measuring urine oxalate levels by capillary electrophoresis (SRL, Tokyo, Japan). Urine citrate was measured with a Citric Acid Enzyme Bio-Analysis kit (Boehringer Mannheim, Mannheim, Germany). Relative calcium oxalate supersaturation indices [SS(CaOx) Rat] were calculated by [SS(CaOx) Rat] = −0.3562 + 34.634 [Ox] + 0.394 [Ca] − 0.483 [Mg] + 0.101 [Cit] (all mmol/l) [22].

Evaluation of the severity of renal crystal deposition

Paraffin-embedded sections (5 μm) were stained with hematoxylin and eosin. A polarized light microscope was used to highlight the CaOx birefringent crystals at a magnification of 100. The number of crystal deposits (percent in renal tubules) was determined by randomly choosing a digital image from one of eight regions per slide, and 5 slides per kidney, so 40 image files represented one kidney. These digital pictures were quantitatively analyzed by Image J version 1.42 [23].

Immunohistofluorescence analysis of an oxidative stress biomarker

Anti-8-HOdG mouse monoclonal antibody (Japan Institute for the Control of Aging) and fluorescent antibody (Alex 567; anti mouse IgG, Invitrogen, Carlsbad, CA, USA) were used to localize 8-OHdG in the paraffin-embedded kidney slides. The sections (5 μm) were dewaxed and rehydrated in a graded alcohol series and distilled water. After deparaffinization, the antigen was retrieved by autoclaving the sections at 121°C for 10 min in zinc sulfate solution. The sections were incubated with anti 8-OHdG mouse monoclonal antibody (5 μg/ml) overnight at 4°C and then washed in phosphate-buffered saline containing Tween (PBS-T). Sections were probed with fluorescent antibody for 5 min at 37°C and then fixed with 4′,6′-diamino-2-phenylindole. The slides were examined by fluorescence microscopy (Olympus Inc., Center Valley, PA, USA).

Evaluation of superoxide dismutase (SOD) and catalase levels

The kidney samples were thawed and homogenized in 1 ml of 25 mM Tris–HCl, pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 1% SDS and centrifuged at 10,000g for 10 min at 4°C. The supernatant was recovered and the protein concentration was measured using Bradford protein assay. An equal protein concentration was electrophoresed by SDS PAGE and transferred to a PVDF membrane. Blots were blocked with 3% BSA overnight at 4°C, probed with appropriate dilutions of SOD1 antibody (EP1727Y, rabbit monoclonal to SOD1, Abcam Inc., Cambridge, MA, USA) or catalase antibody (EPR1928Y, rabbit monoclonal to catalase, Abcam) and incubated with a 1:1000 dilution of anti-rabbit IgG conjugated second antibody (Cell Signaling Technology, Inc., Danvers, MA, USA) for visualization by enhanced chemiluminescence. Blots were quantitatively analyzed using Image J version 1.42 [23].

Statistical analysis

All data are presented as the mean ± standard error (SE). The statistical analysis was performed with the Mann–Whitney *U*-test, and a $p < 0.05$ was considered significant.

Results

General variables

Table 1 presents the general variables obtained before and after we introduced the CaOx crystals. All groups had a similar increase in body weight after the experiment. There were no differences in water intake or urine output among the groups. The urine pH increased significantly in the KCit group rats compared with the EG group rats. Urine pH values among the stone-forming rats increased significantly in the OG group rats.

Urinary creatinine, calcium, and phosphate excretion were similar before and after inducing crystal deposition. Urine magnesium excretion in OG + EG group was reduced more than EG and KCit + EG groups but was not statistic significantly. Urine calcium excretion increased slightly after crystal induction. Urine oxalate excretion increased significantly in untreated stone-forming rats compared with normal drinking water rats. Urine citrate excretion tended to decrease after inducing crystals in untreated and OG treated rats, but the difference was not significant, whereas urine citrate excretion increased significantly in stone-forming rats treated with KCit compared with untreated stone forming rats (Table 2).

Calcium oxalate relative supersaturation indices

SS(CaOx) was not significantly different before stone formtion and after in stone-forming rats, but after inducing crystal formation, all groups had significantly increased SS(CaOx) compared with the normal drinking water group (Fig. 1).

Evaluation of the severity of renal crystal deposition

Hematoxylin- and eosin- stained kidney sections revealed crystal deposits (Fig. 2). There were increased levels of crystal deposits in the kidneys from untreated (EG) and

Table 1 General variables data

	Body weight (g)	Urine pH	24 h Water intake (cc)	24 h Urine output(cc)
Before				
Normal drinking water	266 ± 2.41	7.5 ± 0.20	32.5 ± 4.33	16.75 ± 2.13
KCit	273.5 ± 3.30	8.5 ± 0.00×	31.25 ± 4.26	15.75 ± 2.17
OG	280 ± 4.56	8.38 ± 0.12	39.75 ± 2.86	20 ± 2.38
After				
EG	317 ± 2.34	6.38 ± 0.23	38.5 ± 10.53	30.25 ± 6.98
KCit + EG	352.4 ± 4.58	7.25 ± 0.32	31.25 ± 4.26	33.75 ± 4.93
OG + EG	365.5 ± 14.56	8.25 ± 0.14*	47.75 ± 10.08	35.25 ± 7.59

× $P < 0.05$ vs. Normal drinking water

* $P < 0.05$ vs. EG

Table 2 Urine parameters before and after inducing crystal deposits

	Creatinine (mg/kg/24 h)	Calcium (mg/24 h)	Phosphate (mg/24 h)	Magnesium (mg/24 h)	Oxalate (mg/24 h)	Citrate (mg/24 h)
Before						
Normal drinking water	2.96 ± 0.18	0.82 ± 0.25	12.70 ± 3.53	2.44 ± 1.22	0.69 ± 0.06	77.22 ± 22.54
KCit	2.96 ± 0.11	0.66 ± 0.12	7.77 ± 1.01	0.77 ± 0.03	0.96 ± 0.15	64.56 ± 20.29
OG	3.02 ± 0.24	0.87 ± 0.16	12.53 ± 2.18	1.24 ± 0.69	0.95 ± 0.13	72.37 ± 22.92
After						
EG	3.26 ± 0.44	0.88 ± 0.38	13.71 ± 4.26	3.82 ± 1.69	2.99 ± 0.60×	62.9 ± 20.49
KCit + EG	3.95 ± 0.15	2.23 ± 1.91	23.56 ± 2.83	2.53 ± 1.32	3.84 ± 0.86	91.33 ± 43.07*
OG + EG	3.91 ± 0.52	2.00 ± 1.80	11.86 ± 2.12	0.25 ± 0.13	4.48 ± 1.25	42.65 ± 23.24

× $P < 0.05$ vs. Normal drinking water

* $P < 0.05$ vs. EG

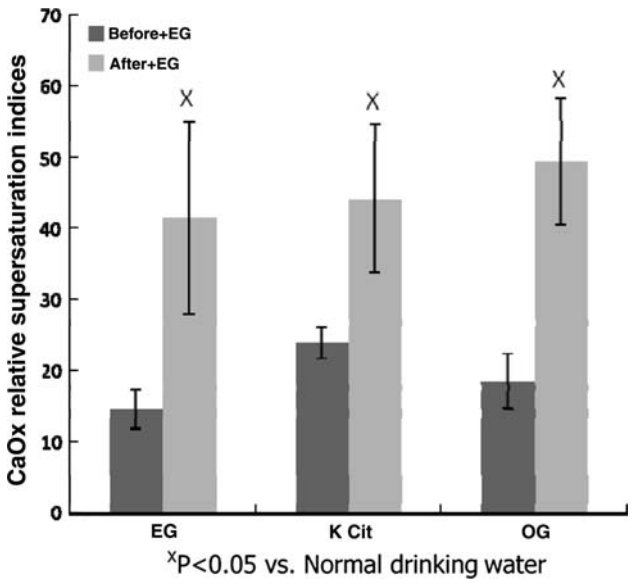

Fig. 1 SS (CaOx) increased significantly in the stone-forming rat group compared with the normal drinking water group before inducing crystal formation

KCit treated stone-forming rats, whereas there was decrease in crystal deposits in the kidneys from stone-forming rats treated with OG.

A quantitative analysis of the number of crystal deposits (Fig. 3) showed that the KCit-treated stone-forming rats tended to have fewer crystal deposits than untreated stone-forming rats, but it was not statistically significant, whereas in the OG-treated stone-forming rats there was significantly fewer crystal deposits than KCit-treated and untreated stone-forming rats.

Evaluation of oxidative stress biomarker

8-OHdG immunohistofluorescence is shown in Fig. 4. The greatest number of positive nuclear stains occurred in the untreated stone-forming rats. The KCit-treated stone-forming rats were moderately positive for nuclear stain, and the lowest positive nuclear stain occurred in the OG-treated stone-forming rats. KCit-treated and OG-treated stone-forming rats had a significantly lower percentage of positive

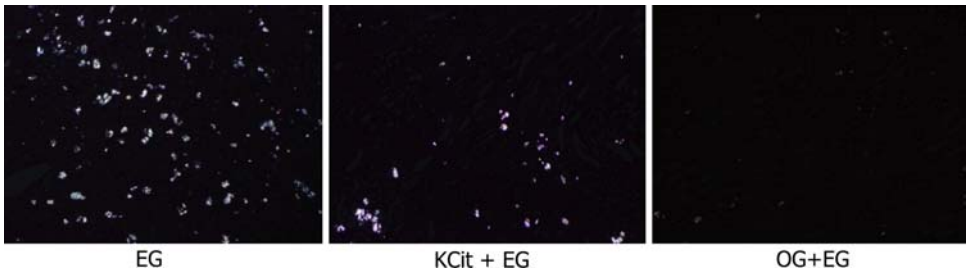

Fig. 2 Crystal deposits in the kidneys of the stone forming rat group. There was an increase in crystal deposits in EG-untreated stone forming rats and a slight increase in the KCit-treated stone forming rats, whereas there was a decrease in crystal deposits in OG-treated stone forming rats. H&E, under polarized light, reduced from ×100

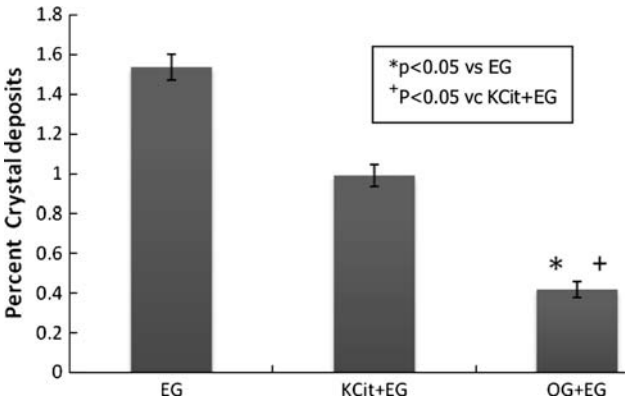

Fig. 3 The percentage of crystal deposits in the renal tubules were significantly lower in OG-treated stone forming rats than in KCit-treated and untreated stone forming rats

nuclei stained than untreated stone-forming rats. The OG-treated stone-forming rats tended to have fewer positive cells than the KCit-treated stone-forming rats, but the difference was not significant (Fig. 5).

Evaluation of SOD and catalase level

A SOD and catalase band were clearly detected on the Western blot. (Fig. 6a, c). A quantitative analysis of SOD and catalase revealed that the SOD level in OG-treated stone-forming rats increased significantly compared with untreated and KCit-treated stone-forming rats. The catalase level in OG-treated stone-forming rats was significantly higher than that in the untreated stone-forming rats but not significantly higher than that in KCit-treated stone-forming rats (Fig. 6b, d).

Discussion

To date, the development and increased use of minimally invasive and non-invasive stone managements such as extracorporeal shock wave lithotripsy, percutaneous nephrolithotomy, and endoscopy have greatly facilitated the removal of kidney stones; however, high recurrence rates have been reported: 10 to 23% per year [24] and 50% at 5 years [25].Thus, stone recurrence is a critical problem in stone formers and requires an effective prophylactic treatment. Potassium citrate therapy has become one of cornerstones of medical stone management [21] and has been prescribed as a prophylactic remedy aimed to lengthen the stone-free status [26]; therefore, the present study choose to compare results obtained usig this medicine. The physical process of stone formation is a complex mechanism. State of saturation, crystal growth, aggregation, retention, and inhibitors and promoters of crystal formation are still under discussion. We found that urine pH was increased by KCit under normal and stone-inducing conditions. The primary mechanism for KCit is to inhibit calcium oxalate and calcium phosphate stone formation [27], but OG had the ability to increase urine pH in a stone inducing condition. Urine citrate is an inhibitor of crystal formation, causing an increase in urine citrate, resulting in a decrease in stone formation [28], which corresponded with our result.

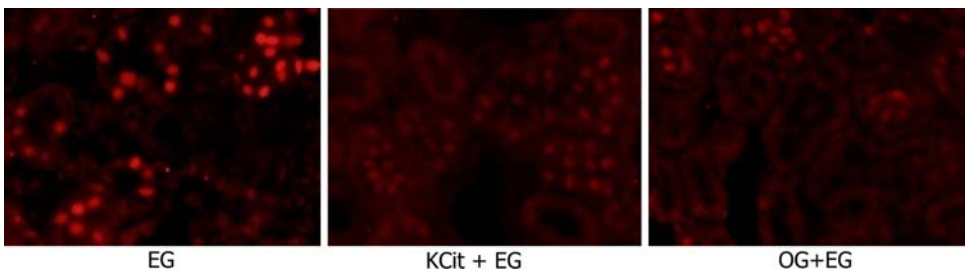

Fig. 4 8OHdG immunohistofluorescence examined using fluorescence microscopy. The greatest number of positive nuclear stained cells occurred in the untreated stone forming rat (EG).The least number occurred in the OG-treated stone forming rat

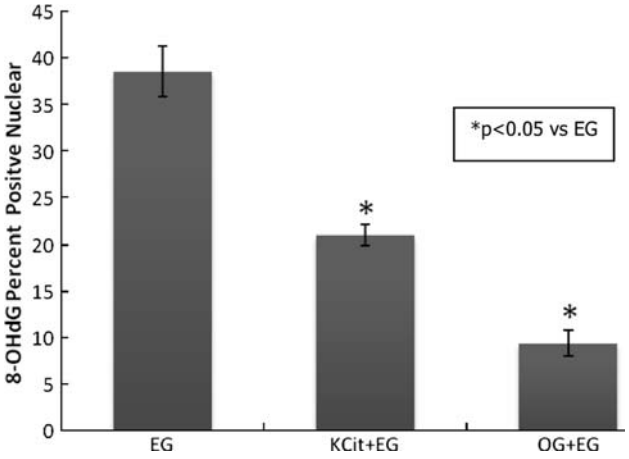

Fig. 5 8-OHdG in kidneys decreased significantly in KCit-treated and OG-treated stone forming rats

The risk of stone formation is determined by the level of urine supersaturation with CaOx and a balance between stimulatory and inhibitory factors. Generally, the urine of human CaOx stone formers is more supersaturated with CaOx than that of normal subjects, and there is a strong correlation between supersaturation level and disease severity [29]. Thus, our rat stone-forming model was effective for increasing the risk of stone formation by raising the relative CaOx supersaturation indices to the supersaturation zone [30]. However, among the stone-induced rat groups, there were approximate relative CaOx supersaturation indices. In contrast, the number of crystal deposits was significantly different and decreased in the OG-treated rats.

Renal calculi can be broadly classified in two large groups: tissues attached and unattached. Attached calculi are mainly integrated by calcium oxalate monohydrate (COM) renal calculi. Unattached calculi are developed in renal cavities of low or reduced urodynamic efficacy. At present, its seems clear that renal epithelial cell injuries play a decisive role in such a type of renal calculi development, and in fact the lithogenic effect caused by ethylene glycol must be mainly attributed to the oxidative damage caused by the high level of oxalate generated by ethylene glycol. Thus, epithelial cell injuries caused crystal aggregation and retention that contributed to the severity of crystal deposits in the present study [10].

One popular theory proposes that oxalate-induced injury to renal tubular epithelial cells is caused by the production of reactive oxygen species (ROS). Oxidative stress influences cell injury and inflammatory processes that promote aggregation and retention of CaOx crystals [31]. Another interesting study reported that renal tubular cell injury is mediated by free radical formation [32]. Not only are high concentrations of oxalate toxic to renal tubular cells, but CaOx crystals themselves also promote damage to cells [33]. In a previous animal experiment in which the administration of high oxalate loads lead to CaOx crystal formation, elevated urinary levels of cell injury enzyme markers, including *N*-acetyl-*β*-glucosidase and 8-OhdG, indicated renal tubular epithelial cell damage [34]. 8-OHdG is a

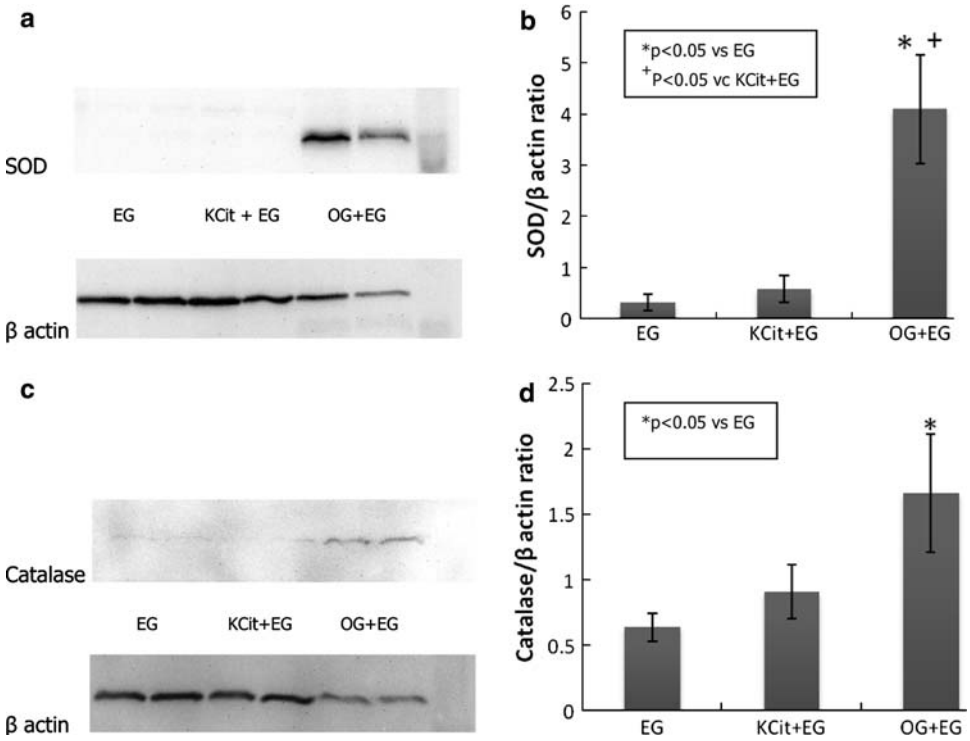

Fig. 6 Western blot perfomed to detect SOD and catalase levels. **a** SOD band in rat kidney tissues. **b** A quantitative analysis of the SOD level revealed a significantly higher level in OG-treated stone forming rats. **c** Catalase band in rat kidney tissues. **d** A quantitative analysis of catalase revealed a significantly higher level in in OG-treated stone forming rats than in untreated stone forming rats

protein that functions in damaged DNA, results in oxidative stress, and can be detected in the nucleus of affected cells. Thus, many pathological studies have used 8-OHdG as a biomarker for oxidative stress [35, 36]. In this study, the percentage of 8-OHdG positive nuclei in kidneys increased in the untreated stone-forming rats but decreased significantly in the KCit-treated and OG-treated stone forming rats, suggesting that KCit and OG have the ability to decrease renal tubular cell injury from oxidative stress and that OG is the most effective.

Flavonoids are anti-oxidants that are currently of interest and are contained in OG. Flavonoids are semiessential food components that are ubiquitously present in nature. Natural products with a source of flavonoids are fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine. The mechanism and sequence of events by which free radicals interfere with cellular functions are not fully understood, but one of the most important events seems to be lipid peroxidation, which results in cellular membrane damage. Free radicals attract various inflammatory mediators, contributing to a general inflammatory response and tissue damage. Flavonoids interfere with various free radical-producing systems including direct radical scavenging, reduction in ischemia–reperfusion injury by interfering with inducible nitric-oxide synthase activity, leukocyte immobilization during ischemia, inflammation conditions, and the xanthine oxidase pathway, which is an important oxidative injury route that is inhibited by flavonoids [37].

In a previous study, we noted that the anti-oxidative effects of flavonoids (catechin) in green tea decrease oxidative injury in renal tubular cells and CaOx deposition in the rat kidney [18]. Similar results were obtained in the catechin-treated oxalate-induced stone formation group compared with an oxalate-induced stone formation group [38]. In vitro and in vivo studies have concluded that the bioflavonoid quercetin has potential antioxidant effects to decrease the lipid peroxidation production induced by oxalate in MDCK cells [17]. The reduction or elimination of renal tubular cell damage that initiates the process of crystal deposition is associated with restoring anti-oxidation kidney defenses and increasing the activities of SOD, catalase, glutathione peroxidase (GPx) and/or free radial scavengers [39]. In this study, we demonstrated significantly increased SOD levels in OG-treated stone-forming rats compared with KCit-treated and untreated stone-forming rats. The catalase level increased significantly in OG-treated compared with untreated stone-forming rats but was not significantly higher than KCit treated stone-forming rats. These results and the result of positive 8-OHdG nuclear staining show that OG has an anti-oxidative effect of decreasing the number of crystal deposits in stone-forming rats, which corresponds with the result of percent crystal deposits that were significantly lower in the OG-treated stone-forming rats. Flavonoids are probably the main chemical component that promote this function, but further investigation is necessary.

Our study reported that OG treatment has good efficacy in reducing calcium oxalate crystal deposits. However, KCit has more urine inhibitory factors. Thus, in areas that have OG it can be used as a therapeutic tool in a combination therapy to prevent and treat calcium oxalate urolithiasis, or OG could be used alone in low socioeconomic cases for longer-term treatment.

References

1. Ramello A, Vitale C, Marangella M (2000) Epidemiology of nephrolithiasis. J Nephrol 3:45–50
2. Sriboonlue P, Prasongwattana V, Chatta K, Tungsanga K (1992) Prevalence of upper urinary tract stone disease in a rural community of northeast Thailand. Br J Urol 69:240–244
3. Yanakawa M, Kawamura J, Onishi T et al (1997) Incidence of urolithiasis in northeast Thailand. Int J Urol 4:537–540
4. Coe FL, Evan A, Worcester E (2005) Kidney stone disease. J Clin Invest 115:2598–2608
5. Unakul S (1961) Urinary stones in Thailand: a statistical survey. Siriraj Hospital Gaz 13:199–214
6. Prasongwattana V, Sriboonlue P, Suntarapa S (1983) Urinary stone composition in northeast Thailand. Br J Urol 55:353–355
7. Osamu Y, Akito T, Tadashi O, Yusaku O (1999) Nation trend of the incidence of urolithiasis in Japan from 1965 to 1995. Kidney Int 56:1899–1904
8. Pearle MS, Calhoun EA, Curhan GC et al (2005) Urologic diseases in America project: urolithiasis. J Urol 173:848–857
9. Aeckart KSJ, Schroder FH (1989) Effect of extra corporeal shock wave lithotripsy (ESWL) on renal tissue. Urol Res 17:3–7
10. Touhami M, Laroubi A, Elhabazi K et al. (2007) Lemon juice has protective activity in a rat urolithiasis model. BMC Urol 5:7–18
11. Chin JH, Hussin AH, Ismail S (2009) Effect of *Orthosiphon Stamineus* leaf extracts on hepatic cytochrome P450, UGT and GST activity in STZ-induced diabetic rats. J Adv Sci Arts 1(1)
12. Lyckender IM, Malterud KE (1996) Lipophillic flavonoids from *Orthosiphon Stamineus* prevent oxidative inactivation of 15-lipoxygenase. Prostaglandins Leukot Essent Fatty Acids 54(4):239–246
13. Malterud KE, Rydland KM (2000) Inhibition of 15-lipoxygenase from orange peel. J Agri Food Chem 48(11):5576–5580
14. Takeda Y, Matsumoto T, Terao H et al (1993) Orthosiphol D and E minor diterpenes from *Orthosiphon Stamineus*. Phytochemistry 33(2):411–415
15. Tezuka Y, Stampoulis P, Banskota AH et al (2000) Constituents of the Vietnamese medical plant *Orthosiphon Stamineus*. Chem Pharm Bull 48(11):1711–1719
16. Sumaryono W, Proksch P, Wray V et al (1991) Qualitative and quantitative analysis of the phenolic constituents from *Orthosiphon Stamineus*. Plant Med 57:176–180
17. RPark HK, Jeong BC, Sung MK, Park MY et al (2008) Reduction of oxidative stress in cultured renal tubular cells and preventive effects on renal stone formation by the bioflavonoid quercetin. J Urol 179(4):1620–1626
18. Jeong BC, Kim BS, Kim JI, Kim HH (2006) Effects of green tea on urinary stone formation: an in vivo and in vitro study. J Endourol 20(5):356–361

19. Kalidas S, Gopinadhan P, Anthony P, Robert EL (2006) 2.11 Rosmarinic acid biosynthesis and mechanism of action. In: Food biotechnology 2nd edn. Mortimer, London
20. Premgamone A, Sriboonlue P, Disatapornjaroen W et al (2001) A long-term study on the efficacy of a herbal plant, *Orthosiphon grandiflorus*, and sodium potassium citrate in renal calculi treatment. Southeast Asian J Trop Med Public Health 32(3):654–660
21. Robinson MR, Leitao VA, Haleblian GE, Scales CD Jr, Chandrashekar A, Pierre SA, Preminger GM (2009) Impact of long-term potassium citrate therapy on urinary profiles and recurrence stone formation. J Urol 181(3):1145–1150
22. Ogawa Y, Machida N, Ogawa T et al (2006) Calcium oxalate supersaturation in dialysis patients with and without primary hyperoxaluria. Urol Res 34(1):12–16
23. Luke MN (2007) Quantifying western blots without expensive commercial quantification software. In: Miscellaneous topics all vaguely related to science. http://www.lukemiller.org/journal/2007/08/quantifying-western-blots-without.html. Accessed 01 August 2007
24. Churchill DN (1987) Medical treatment to prevent recurrent calcium urolithiasis: a guide to critical appraisal. Miner Electrolyte Metab 13:294–304
25. Preminger GM (1992) Renal-calculi: pathogenesis, diagnosis, and medical therapy. Semin Nephrol 12:200–216
26. Mattle D, Hess B (2005) Preventive treatment of neprolithiasis with alkali citrate: a critical review. Urol Res 33(2):73–79
27. Preminger GM, Sakhaee K, Pak CY (1988) Alkali action on the urinary crystallization of calcium salts: contrasting responses to sodium citrate and potassium citrate. J Urol 139(2):240–242
28. Barcelo P, Wuhl O, Servitge E, Rousaud A, Park CY (1993) Randomized double-blind study of potassium citrate in idiopathic hypocitrauric calcium nephrolithiasis. J Urol 150(6):1761–1764
29. Stevenson AE, Robertson WG, Markwell P (2003) Risk factor analysis and relative supersaturation as tools for identifying calcium oxalate stone-forming dogs. J Small Anim Pract 44(11):491–496
30. Carvalho M, Vieira MA (2004) Changes in calcium oxalate crystal morphology as a function of supersaturation. Int Braz J Urol 30(3):205–209
31. Miller C, Kennington L, Cooney R et al (2000) Oxalate toxicity in renal epithelial cells: characteristics of apoptosis and necrosis. Toxicol Appl Pharmacol 162:132–141
32. Thamilselvan S, Khan SR (1998) Oxalate and calcium oxalate crystals are injurious to renal epithelial cells: results of in vivo and in vitro studies. J Nephrol 11(1):66–69
33. Khan SR, Byer KJ, Thamilselvan S et al (1999) Crystal-cell interaction and apoptosis in oxalate-associated injury of renal epithelial cells. J Am Soc Nephrol 10(14):457–463
34. Tsujihata M, Momohara C, Yoshioka I, Tsujimura A, Nonomura N, Okuyama A (2008) Atorvastatin inhibits renal crystal retention in a rat stone forming model. J Urol 180(5):2212–2217
35. Schriner SE, Ogburn CE, Smith AC et al (2000) Level of DNA damage are unaltered in mice overexpressing human catalase in nuclei. Free Radic Biol Med 29(7):664–673
36. Calişkan-Can E, Firat H, Ardiç S, Simşek B, Torun M, Yardim-Akaydin S (2008) Increased levels of 8-hydroxydeoxyguanosine and its relationship with lipid peroxidation and antioxidant vitamins in lung cancer. Clin Chem Lab Med 46(1):107–112
37. Nijveldt RJ, van Nood E, van Hoorn DE et al (2001) Flavonoids: a review of probable mechanism of action and potential applications. Am J Clin Nutr 74(4):418–425
38. Itoh Y, Yasui T, Okada A et al (2005) Preventive effects of green tea on renal stone formation and the role of oxidative stress in nephrolithiasis. J Urol 173(1):271–275
39. Khan SR (2005) Hyperoxaluria-induced oxidative stress and antioxidants for renal protection. Urol Res 33(5):349–357