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ORIGINAL PAPER

Prevention of renal crystal deposition by an extract of *Ammi visnaga* L. and its constituents khellin and visnagin in hyperoxaluric rats

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Abstract In Egypt, teas prepared from the fruits of *Ammi visnaga* L. (syn. "Khella") are traditionally used by patients with urolithiasis. The aim of this study was to evaluate whether oral administration of an aqueous extract prepared from the fruits of *A. visnaga* as well as two major constituents khellin and visnagin could prevent crystal deposition in stone-forming rats. Hyperoxaluria was induced in male Sprague-Dawley rats by giving 0.75% ethylene glycol and 1% ammonium chloride via the drinking water. The Khella extract (KE; 125, 250 or 500 mg/kg) was orally administered for 14 days. The histopathological examination of the kidneys revealed that KE significantly reduced the incidence of calcium oxalate (CaOx) crystal deposition. In addition, KE significantly increased urinary excretion of citrate along with a decrease of oxalate excretion. Comparable to the extract, khellin and visnagin significantly reduced the incidence of CaOx deposition in the kidneys. However, both compounds did not affect urinary citrate or oxalate excretion indicating a mechanism of action that differs from that of the extract. For KE, a reasonably good correlation was observed between the incidence of crystal deposition, the increase in citrate excretion and urine pH suggesting a mechanisms that may interfere with citrate reabsorption. In conclusion, our data suggest that KE and its compounds, khellin and visnagin, may be beneficial in the management of kidney stone disease caused by hyperoxaluria but that it is likely that different mechanism of action are involved in mediating these effects.

Keywords *Ammi visnaga* · Apiaceae · Nephrolithiasis · Hyperoxaluria · Calcium oxalate · Khellin · Visnagin

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Introduction

Hyperoxaluria is a common finding in patients with calcium oxalate (CaOx) kidney stones [10, 12]. Calcific stone formation is associated with various disorders, including renal tubular acidosis, hypercalciuria, hyperoxaluria, hypocitraturia, hypomagnesuria, and hyperusicosuria [20]. Hyperoxaluria is caused by either over production or intestinal over absorption of oxalate. Type 1 primary hyperoxaluria (PH1) and type 2 primary hyperoxaluria (PH2) are caused by rare autosomal recessive genetic disorders of oxalate synthesis. Idiopathic or mild hyperoxaluria is rather common among stone formers and may be caused by increased oxalate absorption fostered by a low-calcium diet. In these patients CaOx crystals form more readily with slight oxalate excess than with calcium excess [30].

Despite the major technical achievements for stone removal in the last three decades, the problem of recurrent stone formation remains. The recurrence rate of kidney stones is approximately 15% in the first year and as high as 50% within 5 years of the initial stone [27]. Effective kidney stone prevention is dependent on the stone type and the identification of risk factors for stone formation. When dietary modification is ineffective, pharmacological treatment should be initiated. However, none of the available treatment modalities are without any side effects. The most effective hypocalciuric agents are thiazide diuretics whose

hypocalciuric action enhances calcium reabsorption in the distal renal tubules [16]. However, long-term use in up to 50% of patients is limited because of side effects including fatigue, dizziness, impotence, musculoskeletal symptoms, or gastrointestinal complaints [21]. Potassium citrate (PCi) is effective in the treatment of patients who have calcium stones and normal urinary calcium. However, the main limitation for a more widespread use of alkali citrate preparations is the relatively low tolerability of available alkali citrate preparations. Adverse effects that reduce treatment compliance have been noted mainly in the gastrointestinal tract and include eructation, bloating, and diarrhea [17]. Data from in vitro, in vivo and clinical trials reveal (for review see [2]) that phytotherapeutic agents could be useful as either an alternative or an adjunctive therapy in the management of urolithiasis.

The fruits of *Ammi visnaga* L. (Apiaceae; "Khella") have traditionally been used in Egypt to relieve pain of kidney stone passage by drinking a tea prepared from the crushed or powdered fruits of Khella [8]. In a previous study, Khan et al. [14] investigated the effects of an *A. visnaga* extract in an animal model of nephrolithiasis and found that the plant extract significantly reduced the incidence of CaOx deposition in the rat kidneys. However, the extract in that study was not further phytochemically characterized leaving the question regarding possible active compounds open. We recently showed that an aqueous extract of *A. visnaga* L., as well as the main compounds khellin and visnagin could prevent cell damage caused by oxalate (Ox) or calcium oxalate monohydrate (COM) crystals in renal epithelial cells [29]. Our preliminary in vitro data are promising since they have shown for the first time a suppression of cell injury induced by oxalate exposure for KE and its major compounds khellin and visnagin. The extract as well as the single compounds could therefore play a potential role in the prevention of stone formation associated with hyperoxaluria. In the present study, the preventive effect of Khella extract and its two major components, khellin and visnagin, were investigated in stone forming rats.

Materials and methods

Chemicals

Khellin (97% purity), visnagin (97%) purity, ethylene glycol (EG), ammonium chloride (NH_4Cl), potassium citrate monohydrate (PCi) (96% purity), carboxymethylcellulose (CMC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Concentrations of calcium, citrate and oxalate were determined using commercial kits (Oxalate: Trinity Biotech, NJ, USA; citrate: R-Biopharm, MI, USA; calcium: Quantichrom, CA, USA) according to manufacturer's instructions. All other reagents were from Sigma (St. Louis, MO, USA) and were of analytical grade. Fruits of *A. visnaga* L. were obtained from a commercial supplier, Caesar and Lorentz, (Caelo) Hilden, Germany. A voucher specimen is deposited at the Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, USA (COP-21-2008). Since many extracts contain oxalate, citrate and calcium, the amount of these compounds were determined in order to exclude false positive effects. Oxalate ($\leq$0.01%), citrate ($\leq$0.01%) and calcium ($\leq$11.6%) concentrations in the extract were determined by the commercial kits mentioned above [29].

The Khella extract was prepared as described previously [29]. Briefly, an infusion of khella fruits was prepared using the following method: 20 g fruits were grinded (medium powder size) using a regular coffee grinder (Smart Grind CBG5, Black and Decker, Miramar, FL, USA). 200 mL of boiling water (the water was heated to 90–100°C) was poured over the grinded fruits and then steeped for 5 min at room temperature. The aqueous extract was filtered through No. 4 filter paper (Whatman International Ltd, Maidstone, England) and was freeze dried (FreeZone 6, Labconco, USA). It was kept in amber colored bottles under argon atmosphere and stored at −20°C until use. The marker compounds khellin and visnagin were quantitatively determined in an aqueous extract prepared using an HPLC method [29]. The quantitative amount of the marker compounds khellin and visnagin were 2.88 and 1.72 mg per 100 mg of the extract, respectively.

Animal model

Male Sprague-Dawley rats, weighing 150–200 g were purchased from Harlan (IN, USA). The animals were singly housed in plastic cages and were allowed to adapt to their environment for 1 week before being used for experiments. Animals were maintained on a 12 h/12 h light/dark cycle. They received a standard laboratory diet (8604 Teklad Rodent diet, Harlan^{TM}) and water ad libitum during the entire experiment. All animal experiments were performed according to the policies and guidelines of the Institutional Animal Care and Use Committee (IACUC) of the University of Florida, Gainesville, USA (NIH publication #85-23). The animals were divided into the following experimental groups ($N = 8$ per group): (1) control (vehicle group, healthy animals); (2) ethylenglycol (EG, 0.75%) + ammonium chloride (NH_4Cl, 1%) (nephrolithiasic group); (3) EG 0.75% + NH_4Cl 1% + PCi (2.5 g/kg; positive control); (4–6) EG 0.75% + NH_4Cl 1% + Khella extract in 125, 250 and 500 mg/kg, respectively. The extract was suspended in 0.8% CMC and given once daily orally using a feeding needle. Animals in the

control group as well as the nephrolithiasic group received 0.8% CMC as vehicle. All compounds were administered for a period of 14 days.

EG 0.75% and NH_4Cl 1% were added to the drinking water to induce chronic hyperoxaluria and to generate CaOx deposition in the kidneys [10]. In addition, the animals were placed in metabolic cages for 24 h urine collection at day 7 and 14. Urine volume as well as pH was measured and after that, the collected urine was centrifuged at 2,800 rpm for 10 min at room temperature and the supernatant was used for determining concentrations of oxalate and citrate as mentioned above. After 14 days, the animals were sacrificed by decapitation and the kidneys were harvested for histopathological investigation of CaOx deposition. The kidneys of animals that died before the end of the experiment were harvested and inspected for CaOx depositions as well.

Following the same experimental modalities as mentioned above a second animal experiment with the single compounds khellin and visnagin was performed. For this experiment, the animals were divided into the following experimental groups ($N = 8$ per group): (1) control; (2) EG 0.75% + NH_4Cl 1%; (3) EG 0.75% + NH_4Cl 1% + PCi (2.5 g/kg; positive control); (4–5) EG 0.75% + NH_4Cl 1% + khellin (5 and 10 mg/kg, respectively); (6–7) EG 0.75% + NH_4Cl 1% + visnagin (5 and 10 mg/kg, respectively).

Calcium oxalate crystal deposition in kidney

After harvesting, the kidney was kept in formalin, sliced vertically and embedded in paraffin and sent to histology service (Gainesville, FL, USA) for hematoxylin–eosin (H and E) staining. Paraffin-embedded sections were examined by light microscope after H and E staining with and without the use of polarizing optics. Crystal distribution within the kidneys was determined by counting crystal deposits in stained sections with a semiquantitative scoring system in which all crystal deposits visible at ×20 magnification were counted. On the basis of that examination, a severity grade was assigned: $<1 = 0$, $<10 = 1$, $<30 = 2$, $<50 = 3$, $<75 = 4$ and $75 = 5$ [13]. Even though a few crystals were seen in lumens of proximal tubules or papillary collecting ducts, most crystals were located in lumens of the collecting ducts of the outer medulla.

Statistics

Data were analyzed by one-way ANOVA and the Newman–Keuls test for multiple comparisons using GraphPad Prism (version 5.0; San Diego, USA). They are expressed as mean ± SEM. Results were considered significant if the probability of error was <0.05.

Results

Administration of EG and NH_4Cl resulted in the deposition of a large number of CaOx crystals in the rat kidneys. 6/8 rats in group B, the hyperoxaluria control, died in the first experiment. A combination of factors including significantly high urinary excretion of oxalate, significantly low urinary pH and lower urinary citrate levels may have led to copious CaOx crystal deposition and death of the animals in the group receiving 0.75% EG + 1% NH_4Cl. Ammonium chloride leads to severe metabolic acidosis which may also have played a part in death of the experimental animals. All animals in the hyperoxaluria control group B of the second experiment survived. Rats in all the other groups given citrate, extract of *A. visnaga*, khellin or visnagin in addition to the lithogenic diet had fewer crystal deposits in their kidneys and also survived the 2-week experiment. All urinary chemistry data are therefore shown after 7 days of treatment since not enough data points were available for the kidney stone group which would allow a statistical comparison of urinary parameters with other treatment groups after 14 days (Fig. 1).

In the present study, administration of 0.75% EG + 1% NH_4Cl in the drinking water to male Sprague-Dawley rats resulted in hyperoxaluria. As shown in Table 1, addition of 0.75% EG + 1% NH_4Cl significantly increased the urinary excretion of oxalate compared to the healthy control animals. Interestingly, administration of PCi also increased urinary excretion of oxalate, whereas treatment with KE dose dependently decreased oxalate excretion (Table 1). The increase in oxalate excretion for the hyperoxaluric group was accompanied by a significant decrease of urinary calcium and citrate (Table 1). In addition, the urinary pH was decreased in animals receiving 0.75% EG + 1% NH_4Cl. PCi significantly increased urinary pH; similar effects although to a lesser extent were observed for animals treated with KE which increased urinary pH in a dose-dependent manner (Table 1). If compared to the hyperoxaluric group the KE dose dependently increased calcium and citrate excretion but decreased oxalate excretion. KE also dose dependently increased the urine volume suggesting possible diuretic effects which was similar in the PCi group (Table 1).

The examination of paraffin kidney sections revealed that there was no incident of CaOx crystal deposition in the healthy control group whereas a high score of CaOx crystal deposition was counted in the hyperoxaluric group (Fig. 2). Animals receiving the positive control PCi or KE in doses of 125, 250 and 500 mg/kg, respectively, had a significantly lower CaOx crystal deposition score compared to the hyperoxaluric group.

A second set of experiments with the single compounds khellin and visnagin was performed. Table 2 presents the

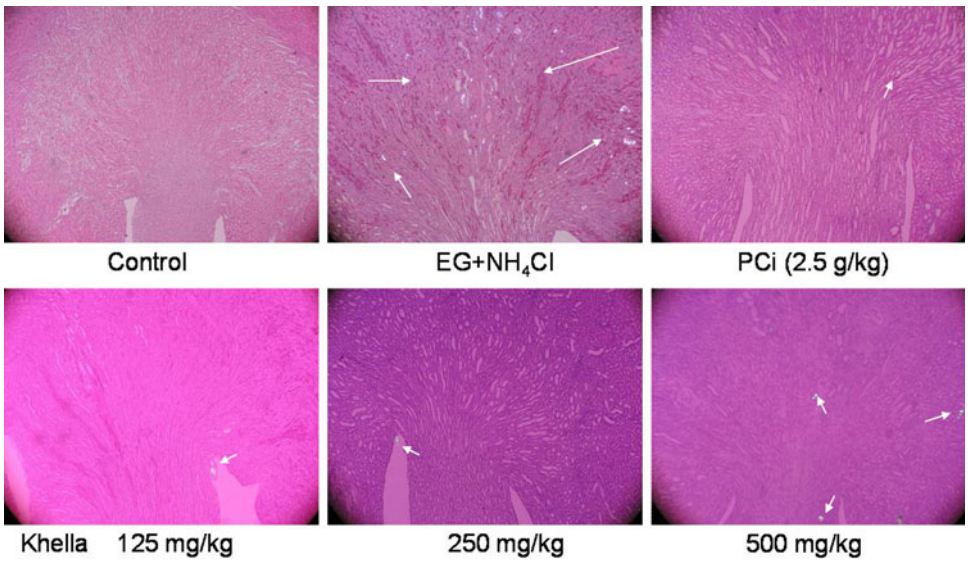

Fig. 1 Paraffin sections viewed under polarized light of a control rat kidney, a kidney from a rat that received EG 0.75% + NH_4Cl 1%, a kidney from a rat that received EG 0.75% + NH_4Cl 1%, and potassium citrate (PCi) 2.5 g/kg as positive control as well as EG 0.75% + NH_4Cl 1% and three different doses of Khella extract (125, 250 and 500 mg/kg, respectively). Note reduced crystal deposition compared to EG 0.75% + NH_4Cl 1% group

Table 1 Effect of *Ammi visnaga* extract (KE) on urinary chemistry in control and experimental animals (evaluated on day 7 of the experiment)

Group	Treatment	Calcium (mg/24 h)	Oxalate (mg/24 h)	Citrate (mg/24 h)	Volume (ml)	pH
A	Control	1.36 ± 0.12	0.44 ± 0.01	1.38 ± 0.09	9.31 ± 0.46	6.03 ± 0.04
B	0.75% EG + 1% NH_4Cl	0.52 ± 0.08$^{\#}$	1.01 ± 0.11$^{\#\#}$	1.06 ± 0.06	8.31 ± 0.42	5.63 ± 0.03$^{\#\#\#}$
C	B + PCi 2.5 g/kg	0.79 ± 0.07	1.50 ± 0.13**	3.16 ± 0.42**	11.75 ± 0.86*	6.23 ± 0.11***
D	B + KE 125 mg/kg	0.85 ± 0.13	0.79 ± 0.11	4.00 ± 0.23***	8.62 ± 0.47	5.84 ± 0.06*
E	B + KE 250 mg/kg	0.94 ± 0.05	0.58 ± 0.07**	4.02 ± 0.12***	10.44 ± 0.61	5.91 ± 0.06*
F	B + KE 500 mg/kg	1.12 ± 0.04*	0.57 ± 0.05*	5.85 ± 0.86***	12.38 ± 1.50*	6.05 ± 0.05*

Data are expressed as mean ± SEM of 8 animals per group

$^{\#}$ $p < 0.01$ versus A; $^{\#\#}p < 0.01$ versus A; $^{\#\#\#}p < 0.01$ versus A; *$p < 0.05$ versus B; **$p < 0.01$ versus B; ***$p < 0.001$ versus B

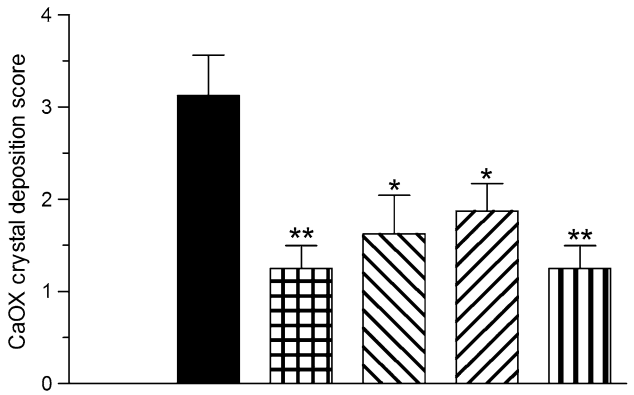

Fig. 2 Calcium oxalate crystal deposition score after treatment with ☐ control; ■ 0.75% EG + 1% NH_4Cl (hyperoxaluric group); ⊞ PCi 2.5 g/kg; ▨ 125 mg/kg KE; ▨ 250 mg/kg KE; ▥ 500 mg/kg KE; <1 = 0, <10 = 1, <30 = 2, <50 = 3, <75 = 4 and 75< = 5 [13]; *$p < 0.05$, **$p < 0.01$, compared to hyperoxaluric group; data are expressed as mean ± SEM of $N = 8$ animals per group

data of khellin and visnagin on urinary oxalate, calcium, citrate, pH and urine volume after 7 days of hyperoxaluria. Induction of hyperoxaluria with 0.75% EG + 1% NH_4Cl significantly increased oxalate excretion accompanied by a concomitant decrease of calcium and citrate excretion. There was no significant difference in urinary oxalate or calcium in groups receiving khellin or visnagin compared to the 0.75% EG + 1% NH_4Cl group. Urinary citrate levels significantly increased in animals which were treated with the positive control PCi but remained unchanged in groups receiving khellin or visnagin (Table 2). Similar effects were observed for urinary pH. Whereas PCi treatment increased the urinary volume, khellin and visnagin did not influence this parameter. Comparable results for khellin and visnagin were seen after 14 days of hyperoxaluria (Table 3). However, CaOx crystal deposition scores of animals that received PCi or khellin and visnagin (5 and 10 mg/kg, respectively) were significantly lower than in animals of the nephrolithiasic group (Fig. 3).

Discussion and conclusion

Kidney stone formation is a complex process and the result of a cascade of events, including crystal nucleation, growth, aggregation, and crystal retention within the renal

Table 2 Effect of khellin and visnagin on urinary chemistry in control and experimental animals (evaluated on day 7 of the experiment)

Group	Treatment	Calcium (mg/24 h)	Oxalate (mg/24 h)	Citrate (mg/24 h)	Volume (ml)	pH
A	Control	1.22 ± 0.10	0.42 ± 0.03	2.14 ± 0.08	12.25 ± 0.14	6.46 ± 0.05
B	0.75% EG + 1% NH_4Cl	0.46 ± 0.07$^{\#\#}$	0.93 ± 0.09$^{\#}$	1.39 ± 0.17	7.80 ± 0.20$^{\#\#}$	5.84 ± 0.03$^{\#\#}$
C	B + PCi 2.5 g/kg	0.71 ± 0.16	1.22 ± 0.08	2.55 ± 0.08**	13.33 ± 0.52***	6.27 ± 0.01**
D	B + khellin 5 mg/kg	0.71 ± 0.89	0.77 ± 0.06	1.57 ± 0.05	9.75 ± 0.13	5.27 ± 0.03
E	B + khellin 10 mg/kg	0.76 ± 0.20	0.77 ± 0.03	1.50 ± 0.16	10.19 ± 1.01	5.91 ± 0.08
F	B + visnagin 5 mg/kg	0.73 ± 0.13	1.03 ± 0.14	2.02 ± 0.12	9.12 ± 0.40	5.92 ± 0.11
G	B + visnagin 10 mg/kg	0.57 ± 0.09	1.18 ± 0.13	1.97 ± 0.30	9.12 ± 0.72	5.86 ± 0.04

Data are expressed as mean ± SEM of 8 animals per group

$^{\#}$ $p < 0.01$ versus A; $^{\#\#}p < 0.01$ versus A; $^{**}p < 0.01$ versus B; $^{***}p < 0.001$ versus B

Table 3 Effect of khellin and visnagin on urinary chemistry in control and experimental animals (evaluated on day 14 of the experiment)

Group	Treatment	Calcium (mg/24 h)	Oxalate (mg/24 h)	Citrate (mg/24 h)	Volume (ml)	pH
A	Control	2.02 ± 0.60	0.44 ± 0.05	2.19 ± 0.24	12.50 ± 0.18	6.39 ± 0.08
B	0.75% EG + 1% NH_4Cl	0.77 ± 0.15$^{\#\#}$	0.77 ± 0.19	2.14 ± 0.11	8.20 ± 0.20$^{\#\#}$	5.90 ± 0.04$^{\#}$
C	B + PCi 2.5 g/kg	0.81 ± 0.11	0.84 ± 0.14	2.49 ± 0.29	12.70 ± 0.11***	6.40 ± 0.14**
D	B + khellin 5 mg/kg	0.96 ± 0.08	0.61 ± 0.10	1.75 ± 0.15	9.70 ± 0.40	5.97 ± 0.05
E	B + khellin 10 mg/kg	1.17 ± 0.19	0.75 ± 0.10	2.30 ± 0.18	10.0 ± 0.91	5.76 ± 0.03
F	B + visnagin 5 mg/kg	0 .96 ± 0.24	0.49 ± 0.03	1.86 ± 0.21	9.21 ± 0.40	5.81 ± 0.08
G	B + visnagin 10 mg/kg	1.01 ± 0.25	0.74 ± 0.07	2.18 ± 0.23	9.30 ± 0.62	5.87 ± 0.04

Data are expressed as mean ± SEM of 8 animals per group

$^{\#}$ $p < 0.01$ versus A; $^{\#\#}p < 0.01$ versus A; $^{**}p < 0.01$ versus B; $^{***}p < 0.001$ versus B

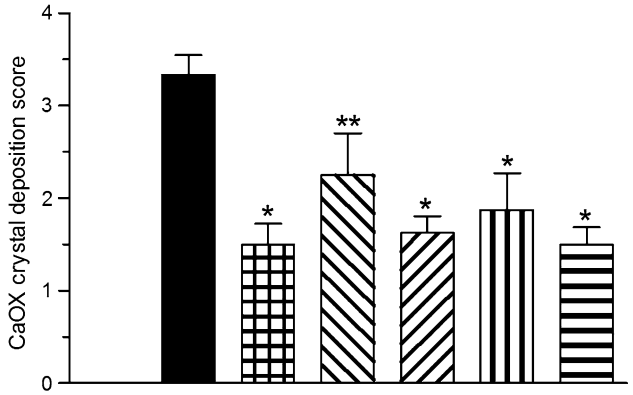

Fig. 3 Calcium oxalate crystal deposition score after treatment with ☐control; ■ 0.75% EG + 1% NH_4Cl (hyperoxaluric group); ⊞ PCi 2.5 g/kg; ▨ 5 mg/kg khellin; ▨ 10 mg/kg khellin; ▥ 5 mg/kg visnagin; ▤ 10 mg/kg visnagin; <1 = 0, <10 = 1, <30 = 2, <50 = 3, <75 = 4 and 75> = 5 [13]; $^{*}p < 0.05$, $^{**}p < 0.01$ compared to hyperoxaluric group; data are expressed as mean ± SEM of $N = 8$ animals per group

tubules [15, 20]. In the present study, hyperoxaluria was induced by administration of 0.75% EG + 1% NH_4Cl as aqueous solution in the drinking water to male Sprague-Dawley rats [10]. The biochemical mechanisms for this process are related to an increase in the urinary concentration of oxalate [1]. In the present study, hyperoxaluria was induced successfully since urinary excretion of oxalate increased rapidly and significantly, and citrate excretion decreased concomitantly. Interestingly, the increase in urinary oxalate excretion in the present experiments was further increased by treatment with the positive control PCi (2.5 g/kg). However, PCi also increased urinary citrate excretion along with an increase of urinary pH and urine volume. It has been shown that PCi reduces hypercalciuria by raising the soluble fraction of urinary calcium by chelation, thus preventing CaOx formation and therefore resulting in excretion of excessive oxalate in the urine [4, 6].

Our results further demonstrate that KE dose dependently prevented hyperoxaluria in rats by lowering in the urinary excretion of oxalate along with an increase of urinary excretion of citrate and, to some extent, the excretion of calcium. In addition, urine pH was increased in a dose-dependent manner which correlates with an increase of urinary citrate. Our data therefore indicate that KE seems to interfere with the citrate metabolism; however, the detailed mechanism of action has not been elucidated yet. It has been shown in literature that urinary citrate plays an important role in reducing recurrences of CaOx stones [19]. Its inhibitory effect on CaOx crystallization is generally recognized, the mechanism of action being a reduction of CaOx supersaturation by formation of

complexes with calcium and direct inhibition of crystal growth with aggregation and increasing urinary pH [3, 4, 24]. There are several factors that increase urinary citrate including a decrease of dietary protein intake, administration of alkali such as PCi, and an increase of hormone modulation turnover such as estrogen leading to an increase of citrate excretion [7, 25]. In addition, a systemic acid–base status is a dominant key to citrate excretion. It has long been known that acidosis decreases citrate excretion, whereas alkalosis increases it [25]. The proximal tubule reabsorbs approximately 75% of filtered citrate and thus is the primary determinant of final urinary citrate excretion [7]. A change in systemic pH would alter intracellular pH, resulting in changes in intracellular citrate metabolism, leading to alterations in citrate reabsorption and hence urinary citrate excretion [28].

Our data also demonstrated that KE increased the pH dose dependently along with an increase of urine volume. It can be speculated that KE changes the luminal pH, thus inhibiting the reabsorption of citrate which as a result could lead to the prevention of CaOx crystal formation in the kidney. The observed increase in urine volume also suggests a diuretic activity of KE. Khan et al. [14] showed in previous experiments that the prophylactic effects of *A. visnaga* on stone formation in rats are due to its diuretic effects. Diuretic activity reduces urinary saturation of stone-forming calcium salts and therefore dilutes promoters of CaOx crystallization. Recently, the authors Yuliana et al. [31] demonstrated that methoxyflavonoids from *Orthosiphon grandiflorus* act as antagonists at adenosine A_1 receptors. Some studies revealed that adenosine A_1 receptor antagonists can induce diuresis and sodium excretion [18]. Since adenosine A_1 receptors are expressed in the afferent arterioles, glomerulus, proximal tubules and collecting ducts adenosine antagonists could directly inhibit sodium reabsorption in the proximal tubules or indirectly by promoting afferent arteriole dilatation [22]. So far, there is no information available if *A. visnaga* or its compounds act on adenosine A_1 receptors but based on our present data it is also likely that KE interferes with citrate reabsorption. As pointed out previously, citrate is a known inhibitor of calcium based stones. Its presence in urine decreases the saturation of CaOx and calcium phosphate by forming soluble complexes with calcium. By its conversion through bicarbonate, citrate increases urinary pH which induces an additional citraturic response by slowing renal citrate metabolism and impairing citrate reabsorption [17]. However, pharmacological PCi supplementation requires a rigorous schedule of numerous tablets or liquid supplements taken routinely three to four times a day. Patient compliance significantly decreases when medications are administered more than once daily [5]. Patients therefore could benefit from intake of dietary supplements such as *A. visnaga* extract.

Interestingly, the experiments with the single compounds demonstrated that urinary calcium, citrate and pH were not affected after khellin and visnagin treatment whereas the CaOx crystal deposition scores were significantly decreased. The results indicate that the inhibition of CaOx stone formation of khellin and visnagin seem not to interfere with citrate reabsorption but could be explained by their calcium blocking activity [23]. This is of interest, as addition of α-antagonists or calcium channel blockers to standard therapy increases spontaneous expulsion of kidney stones. For example, verapamil administration was found to be effective to limit the re-growth of residual fragments and also to facilitate residual fragment clearance after shock wave lithotripsy. Patients receiving this medication seemed to pass the retained fragments easily in a shorter time than the others [26].

We recently showed that KE as well as khellin and visnagin could prevent renal epithelial cell damage caused by oxalate and COM [29]. Our in vitro data suggest that KE as well as khellin and visnagin suppress the production of radical oxygen species, which might be followed by suppression of stone formation due to the prevention of renal tubular epithelial cell injury. A positive correlation between urinary oxalate levels and renal tubular epithelial cell injury as well as between oxalate levels and lipid peroxides has been discovered in experimental urolithiasis rats [11] and patients with kidney stones [9].

In conclusion, based on our present results it seems likely that the *A. visnaga* extract contains several active compounds which in a multifunctional/synergistic approach operate on different points of action in the kidney. Our present in vivo experiment indicates that KE and its compounds, khellin and visnagin, are beneficial in the management of CaOx stone disease but since only a very limited number of studies have been performed with this particular plant, the overall benefits are not very clear yet and further studies are necessary to further investigate the reported effects. Studies in this direction are presently under way.

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