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ORIGINAL PAPER

Chinese herbal medicines and their efficacy in treating renal stones

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Abstract In herbal treatment of kidney stones, anti-lithics are used to ''dissolve'' the stones or aid their passing to guard against further retention. Diuretic action is also needed to increase the amount of fluid going through the kidneys and flush out the deposits. Previous clinical studies have shown that herbal medicines and their concoctions could be used to inhibit calcium oxalate crystallization. However, the pharmacodynamics and in-vitro effects of such medicines have not been established. Five Chinese herbal medicines were selected based on their usefulness in treating stone disease. A 96-well plate oxalate-induced turbidity in artificial urine was used to evaluate the efficacies of the different herbal medicines on calcium oxalate crystallization. The metastable limit was determined and the nucleation rate was derived from 12-min time-course measurement of turbidity at 405 nm. Phase-contrast microscopy was used to visualize the crystals. The results showed that with increasing concentrations of herbal extracts, smaller calcium oxalate crystal sizes were observed. Overall, the five herbal medicinal extracts tested were able to promote nucleation of calcium oxalate crystals while at the same time decreasing the size. This in-vitro crystallization confirms that prophylaxis of renal stones could be achieved by reducing overall supersaturation through promotion of small crystal nucleates and concomitant pharmacological diuretic action of herbal medicines. Clinical studies will provide more definitive conclusions.

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Introduction

The use of anti-lithic herbs and/or concoctions for treating renal stones has been practiced long before the use of western medicine. For instance, in China and Hong Kong the use of Chinese herbal medicine has been widely used and promoted. Nowadays, traditional Chinese medicine (TCM) have also become more popular in western countries such as in the UK where there are more than 600 TCM clinics [1]. Food and herbs are considered to play a role in maintaining people's health and homeostasis. While some foods are straightforward ''cold'' in nature (*yin*) for treatment of heat-syndrome such as fever and thirst, or ''warm'' in nature (*yang*) to treat cold-syndrome by activating blood circulation to warm the interior and expel cold [2, 3]. Some Chinese herbal medicines have been proven with actual pharmacological actions in humans, animals or in-vitro and shown efficacy in treatment or prevention of different diseases such as heart disease [4], arthritis [5] and hepatitis [6].

In-vitro studies of calcium oxalate crystallization using herbal medicines such as Kampo medicine containing Takusya (*Alismatis Rizoma*), Chorei (*Polyporous*), Bukuryo (*Hoelen*), Kasseki (*Talcum Crystallium*), Kinsensou (*Desmodii Herba*) and Kagosou (*Purunellae Spica*) demonstrated their abilities to inhibit calcium oxalate crystallization [7–11]. It was also shown that with the use of Takusya in rats, expression of osteopontin, a stone matrix protein was

lower than that in the stone-formers [10] implying the prevention of calculi formation. Likewise, extract of *Herniaria hirsute* promoted nucleation of calcium oxalate crystals and inhibition of crystal growth, and formation of calcium oxalate dihydrate crystals was promoted rather than the monohydrate forms [11, 12].

According to TCM criteria, renal stone diseases belong to the ''sha-lin'' (strangury from urolithiasis), ''shi-lin'' (strangury caused by urinary calculus) and ''xue-lin'' (strangury complicated by hematuria) [13]. While western medicine attributes kidney stone to the anatomical disposition and removal, TCM treatment encompasses the urinary system, balance of mineral electrolytes as well as the entire endocrine system including prostaglandins and the various neuro-transmitters [14, 15]. The decoction herbal medicine recipes are specific and promote the circulation of ''qi'' (vital energy—*yang*), inducing diuresis strangury—effective in removing stone of <1 cm (kidney and ureter) and <2 cm (bladder). Majority of decoction preparations for removal of urinary tract stones aim to achieve this in treatment and prevention [1, 13–15].

The implementation of the Chinese medicine ordinance in 1999 in Hong Kong provided for the regulation of the practice of Chinese medicine practitioners. The use, manufacture and trading of Chinese medicines has seen an increase in the establishment of TCM clinics in hospitals that are primarily treating through western medicine [16]. This has led to increase in the patients using either or both modes of treatments. This study purports to elucidate and evaluate the effectiveness of five commonly prescribed Chinese herbal medicines on calcium oxalate crystallization using an in-vitro oxalate-induced turbidity method [17].

Materials and methods

Chinese herbal medicines

Five Chinese herbal medicines were bought from a registered Chinese herbal shop under the supervision of practitioner (Cheung On Medicine Co. Ltd, Hong Kong). They are identified as Jin Qian Cao, Hai Jin Sha, Zhi Wei, Dong Kui Zi and Siaoniaotong. Table 1 shows the five medicines and their purported in-vivo effects.

Extraction of Chinese herbal medicines

Ten grams of the herbal medicine extract was boiled with 500 ml milli-Q water for 1.5 h to a final volume of 250 ml. The boiled extract was then filtered (Whatman No. 2). Mainly fibrous and coarse brown powdery material remained on the paper. The filtrate was clear with slight coloration (yellow-brown). For the Siaoniaotong pill, the sugar coating was removed by knife and 1 g of the powder was dissolved in 40 ml of milli-Q water and filtered.

Preparation of artificial urine

The artificial urine (AU) was prepared according to the method of Kavanagh et al. [18]. The AU was prepared fresh each day and pH adjusted to 6.0. Concentrations of the major ions were, calcium (6 mM), magnesium (3.3 mM), sodium (160 mM), potassium (82 mM) and citrate (2.2 mM).

Calcium, magnesium, oxalate and citrate determination

Calcium concentration in the herbal medicines was determined using a flame photometer (Corning 410) with calcium standards ranging from 0 to 12.5 mM. Magnesium level was determined by Beckman Synchron CX7 (courtesy of Kwong Wah Hospital, Hong Kong).

Oxalate was determined using a commercial oxalate kit (Sigma Diagnostics, USA) which relies on the enzymatic conversion (oxalate oxidase) of oxalate to carbon dioxide and hydrogen peroxide and the latter reacts with 3-methyl-2-benzothiazolinine acid in the presence of peroxidase to yield an andmine dye which has an absorbance at 590 nm. Citrate was determined by the Roche citric acid (UV-method) kit. All tests were measured by the Spectronic 20 Genesys spectrophotometer (Spectronic Instruments, Milton-Roy, USA).

Determination of the metastable limit

Nucleation is determined by the induction of crystallization at a minimum oxalate concentration (oxalate tolerance) which is termed the metastable limit (ML). Using a 96-well plate, a format of 24 oxalate concentration each in quadruplicate was tested [17]. In each well, 200 µl of AU and calcium (6 mM) were added and subsequently, differing concentrations of sodium oxalate (20 µl) was added with the concentrations of 0 (control), 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 20, 24, 28, 32, 36, 40 mM. (True level of calcium reaches 5.4 mM and oxalate 0.1–3.6 mM.) The plate was then placed in an ELISA plate reader (Spectrafluor Plus, Tecan, Austria) and turbidity (405 nm) read

Table 1 Pictures and description (or actions) of Chinese herbal medicines with their Chinese names

Chinese medicine	Root name (in Chinese)	Action
	*Jin Qian Cao* (金錢草) (*Desmodium styracifolium Merr.*)	Diuretic; acidification of urine; reduce ''heat'' and swelling
	*Hai Jin Sha* (海金沙) (*Pyrrosia sheareri*)	Remove heat and swelling; treatment of calculi; dissolution?
	*Zhi Wei* (石葦) (Abutilon seeds)	Diuretic; urinary tract infections; prevent urolithiasis
	*Dong Kui Zi* (冬葵子) (Lygodium spores)	Remove heat; detoxicant and lactogenic for urinary tract infections; prevent oliguria and calculi of urinary tract
	*Siaoniaotong* pills (消尿通)	Antipyretic; analgesic; elimination of calculi

at 30 s intervals for 25 cycles. The oxalate tolerance was the minimum oxalate concentration that brings about a rate significantly greater than the control. For oxalate concentrations above the ML, it meant that there was initiation of growth rate [17].

Determination of metastable limit with Chinese herbal medicines

The ML after adding the Chinese herbal medicines was determined in quadruplicates. In each well, 180 µl of AU plus calcium (6 mM) were added, and 20 µl of different Chinese herbal medicine extract of a range of concentrations (20, 40, 60, 80%, v/v), starting with the stock (100%, v/v) as a concentrate. As before, differing concentrations of sodium oxalate from 0 to 40 mM was added taking into account any endogenous oxalate (and calcium concentrations). The plate was placed in the ELISA plate reader and turbidity (405 nm) read at 30 s for 25 cycles.

Image analysis

The images of the crystals were captured by a phase-contrast microscope (Leica Model DM-IRB) connected to a computer and the magnification used was at 200× . Images were captured from five different fields and the most representative one is shown here. The purpose of imaging is two-fold: (1) to observe the sizes of the crystals and (2) observe any aggregates of crystals during the initial nucleation event. The pictures were captured immediately (<2-min) after the ELISA plate reader.

Statistical analysis

Comparisons between different herbal medicines was done by one-way ANOVA. All statistical comparisons, linear regressions, data analysis and graphs were done on two software, INSTAT and PRISM (Graphpad Software Inc., USA). Differences at a level $P \leq 0.05$ was considered significant.

Results

Calcium and oxalate content of the Chinese herbal medicines

Table 2 shows the basal levels of the calcium, magnesium and oxalate concentration in the stock solution in each of the herbal medicines. Citrate level was not detected (repeated thrice). Compared to the added calcium (6 mM) and magnesium (3.3 mM), it can be seen that the calcium and magnesium levels are small and insignificant to interfere with the results. It was found in a trial that the ML for the control (AU) and test [containing AU plus added distilled water with calcium (0.8 mM) and magnesium (1.5 mM)] was not statistically different. Hence, the effects of endogenous calcium and magnesium content of the herbal medicines was either nil or minimal. On the other hand, slight increases in oxalate may be important when compared to rises in calcium on calcium oxalate crystallization [19]. The ML was determined to be 14 mM and in comparison to the oxalate concentration found in the medicines, the latter was found to be lower. A separate investigation was carried out taking into account the oxalate concentration (Zhi Wei 0.8 mM) and there was no change in the ML.

Table 2 The basal level of calcium, oxalate, magnesium and citrate concentration as determined in the stock solution of each of the Chinese herbal medicines

Herbal medicine	Calcium (mM)	Oxalate (mM)	Magnesium (mM)	Citrate (mM)
Jin Qian Cao	0.77	0.21	1.41	Nil
Hai Jin Sha	0.54	0.02	0.64	Nil
Zhi Wei	0.77	0.80	0.28	Nil
Dong Kui Zi	0.61	0.30	0.54	Nil
Siaoniaotong	0.71	0.14	n/a	Nil

n/a not available, *Nil* not detected

Metastable limit

For oxalate concentrations above the ML, crystal growth can occur [20]. Experiments done in duplicates on separate occasion determined that the ML to be at 14 mM oxalate. The ML was tested again in the presence of the five herbal medicines at different concentrations. Whilst endogenous oxalate would contribute to the added oxalate, we found that in all the preparations, the ML to be between 14 and 15 mM oxalate concentration. For all subsequent experiments, 14 mM oxalate was used as the minimum oxalate concentration before initial nucleation occurs.

Effect of Chinese herbal medicines on calcium oxalate crystallization

When 12-min time-course experiments were conducted for each of the five herbal medicines with different solution (%) strength, it was found that the steepest slope was in the first 3 min which represents the initial nucleation events. Figure 1 shows one of the typical

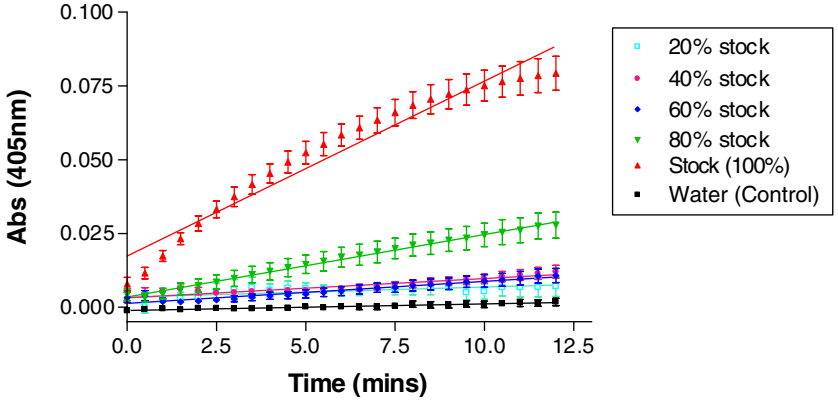

Fig. 1 Effects of different concentration of *Jin Qian Cao* on calcium oxalate nucleation rate for 12-min interval (means ± SEM of four wells for each point)

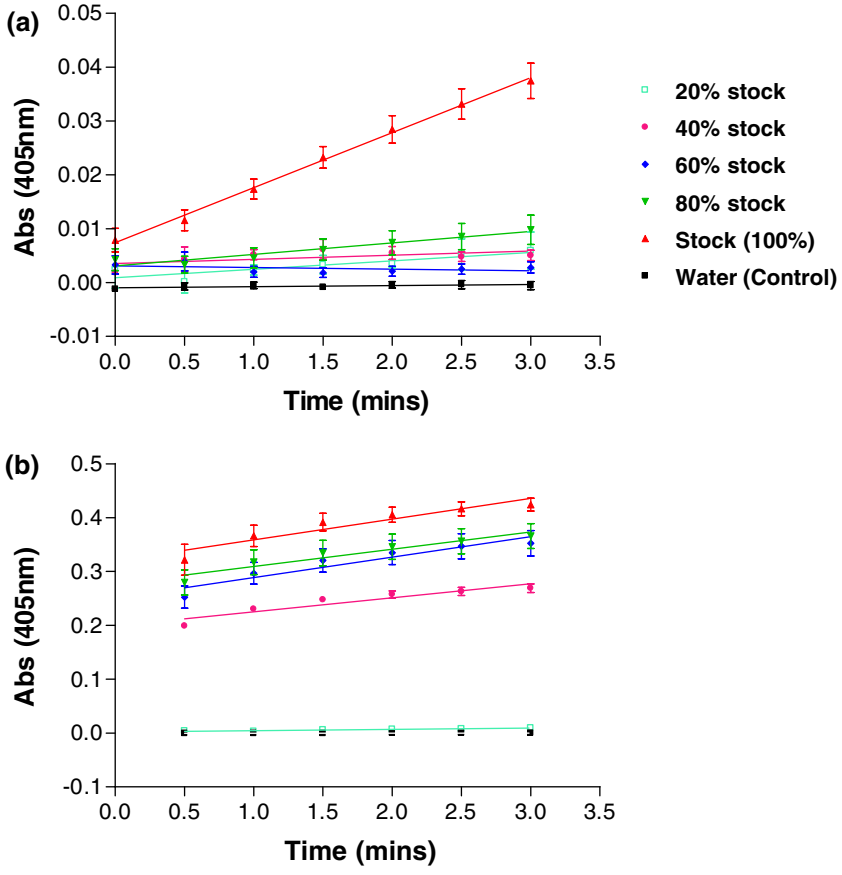

Fig. 2 Nucleation plots in the first 3-min for herbal medicines. **a** *Jin Qian Cao* as an example and **b** *Siaoniaotong* pills (means ± SEM of four wells each)

plots of the effect of herbal medicines on crystallization for a 12-min time course.

Since the initial events of nucleation occurs in the first few minutes, the graphs were re-plotted within the first 3-min for each of the herbal medicines (Fig. 2). As shown the stock solution shows the most appreciable rise and slope in nucleation for both the herbal medicines ($r^2 = 0.85$; Fig. 2a) and pills ($r^2 = 0.74$; Fig. 2b). Whilst the steepest slope is found in the stock solution in two of the four herbal medicines, in two other herbal medicines, namely *Hai Jin Sha* and *Zhi Wei*, it was found that even at 40 and 60% of stock solution maximum initial nucleation (slope) ($r^2 = 0.56$ and 0.69, respectively) was observed (*data not shown*). This suggests that the components (chemicals) or nucleating agents within the herbal medicines are different within this in-vitro tests.

Figure 3 shows the phase-contrast micrographs of the calcium oxalate crystal morphology, number and size. In all the tested herbal medicines and pills, calcium oxalate dihydrate form was observed. Concomitantly with increased nucleation of crystals, decrease

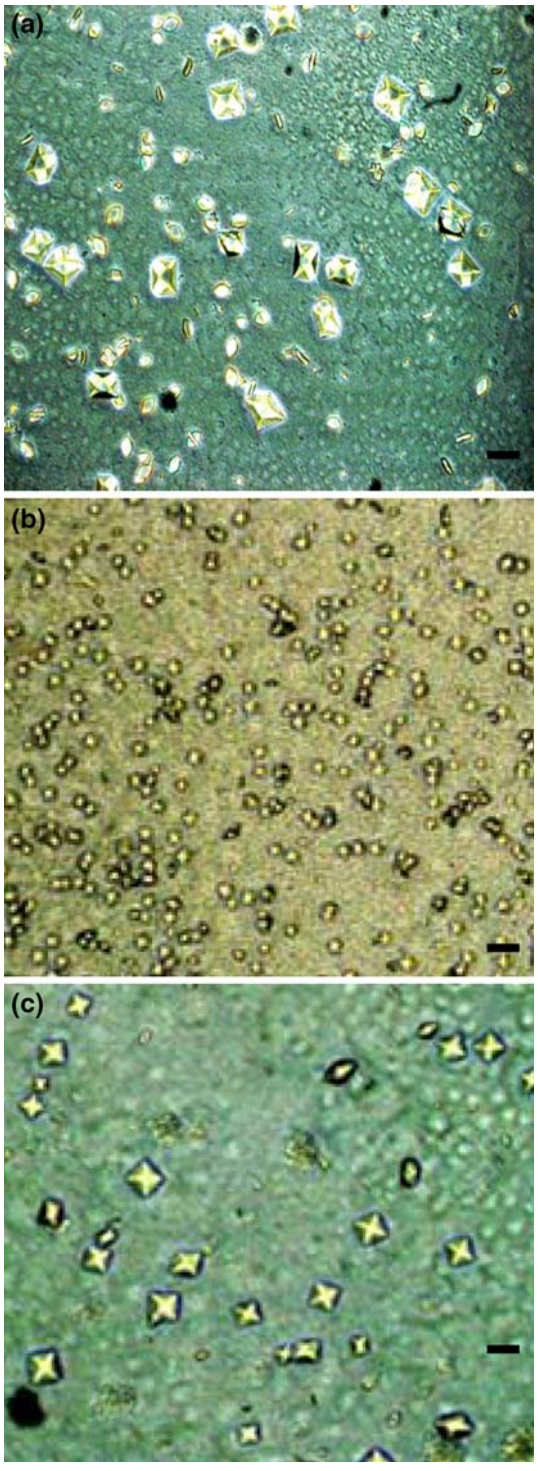

Fig. 3 Phase-contrast microscopy of **a** control—without herbal medicines (size of crystals = 25–50 μm), **b** stock solution of herbal medicines (2–10 μm) and **c** 20% solution of herbal extract (10–30 μm). Magnification, x200 (*black bar* = 25 μm)

in size was also observed. The Chinese herbal medicines had an effect of promoting nucleation of crystals and inhibition of growth. No aggregation (by microscopy) was observed during the 12-min time-course experiments.

Discussion

The use of alternative or Chinese herbal medicines have increased in the past few years and more so where both western and Chinese medical clinics are becoming more accessible to patients. Whilst the use of such alternative therapies are more in terminally ill patients, its use in recovering patients as well as for prevention cannot be ruled out [21–23]. The promotion of the use of Chinese medicine in Hong Kong is further encouraged through the Government policy in 1997 and the establishment of the Chinese Medicine Ordinance in 1999. The legislation regulated the Chinese medicine practitioners and the safe dispensing of quality herbal medicines [24]. The introduction of Chinese medicine into the public healthcare system has included providing out-patient Chinese medicine services in the public sector and selected public hospitals. This investigation stems from the increased usage of Chinese herbal medicines used by patients with kidney stones.

The five Chinese herbal medicines were selected after consultation with the practitioner of the specific herbal medicines dispensed for the treatment of renal stones. The *Siaoniaotong* pills are available over-the-counter without prescription. It should be realized that herbal medicines taken for a problem or imbalance are also relevant to aid the body's cleansing mechanism as a whole. All four of the prescribed herbal medicines have a claim to be anti-lithic and diuretic in action for renal stone patients [7]. To achieve efficacy, such that the active components of the decocted herbs reaches the intended site, kidney, ureter and bladder, the registered herbalists will prepare 5–20 g of dried herb for decoction. For severe cases, the dosage can be increased up to 60–80 g of dried weight. Although these dosages are empirically weighed and proportioned through a long history of traditional experience, they are now optimized for modern prescription to involve no serious side-effects [15, 25]. This is based on component analyses to ensure no toxicity exists. The overall aim in Chinese medicine is to restore the ''system'' or balance before the stone occurred rather specifically target the stone for treatment as in western medicine. Hence, although component analyses may reveal pharmacological active agent (for example, diuresis, analgesia), it may also have other agents to restore blood pressure, mineral balance and restore kidney function.

Among the several approaches for studying calcium oxalate crystallization, many different methods have been used. Some give a general indication of the crystallization potential [26–28] while others are more specifically aimed at measuring growth, nucleation and aggregation [17, 29]. For the current study, turbidimetry method of inducing crystallization was selected as a fast and reproducible method to measure nucleation, growth and aggregation of calcium oxalate crystals in a single experiment. The method of Kavanagh et al. [17] using the 96-well plate format allowed us to examine many different oxalate concentrations with sufficient replicates with speed and convenience. This method also gave an objective condition for oxalate tolerance, rather than relying on ''dramatic'' changes in absorbance.

The ML was achievable between 14 and 15 mM, the minimum oxalate concentration required to initiate nucleation of calcium oxalate crystals. The endogenous calcium and oxalate concentrations of the herbal medicines are considered to be negligible for this determination and did not affect the ML when added to the AU. For oxalate concentrations above 15 mM, it was found that nucleation was rapid with the graph leveling off or dipping lightly during a 12-min course experiment suggesting aggregation occurring which was confirmed under the phase-contrast microscopy. In this study, kinetic growth curves above 15 mM of oxalate were not done at this stage as the ML was fixed at 14 mM to derive nucleation data through turbidity rate and direct observation by phase-contrast microscopy for size and aggregation.

The [Ca] and [Ox] used here are derived from several studies [17, 20, 30]. Micropuncture data in animals and extrapolated to human [30] indicate that the [Ca] (range 0.21–7.5 mM) and [Ox] (range 0.01–3 mM) vary from the initial ultrafiltrate in the Bowman's capsule to the collecting duct. The [Ca] and [Ox] used here are representative of those found in the collecting duct [20, 30, 31]. The level of calcium is kept at 6 mM, at the higher end, since going any lower would require unphysiologically high [Ox] for nucleation [17].

In this study, five Chinese herbal medicines were used to evaluate their effectiveness or claims of stone prevention by using a 96-well plate oxalate-induced turbidity method together with phase-contrast micrographs. The concentration of the herbal medicine used in the in-vitro crystallization test used the full-strength decocted herbal extract (same as that would be ingested by an adult) and subsequent dilutions of the original decocted herbal extract. The rationale of this is that it would be unlikely the active component/s would reach the kidney in full concentrated state. There is no literature documenting renal clearance of Chinese herbal medicines and more clinical studies toward this are needed. Theoretically, an effective herbal medicine in treating stone disease is one with promotion of nucleation of crystals with inhibition of growth and aggregation to help in expelling of small crystals together with the aid of the diuretic action. Non stone-formers also form small crystals which are passed out harmlessly. Although some of the herbal medicines claim to ''dissolve'' stones, we did not observe any dissolution or reduction in mass (turbidity and hemocytometer counting of five fields for crystal number and size) of pure monohydrate calcium oxalate crystals when incubated with such medicines for over 5-days.

The nucleation rate was highest for the stock solution (100%) for each of the herbal medicine except for two, namely, *Hai Jin Sha* and *Zhi Wei* whereby the highest nucleation rate was achieved at lower concentrations. While we do not address the exact mechanism by which the Chinese herbal medicine affected crystallization process (in the absence of the actual chemical constituents), we suggest that mechanisms in these two herbal medicines are different. At issue (without any clinical evidence) is the bioavailability of the pharmacological active agents reaching the kidneys given that such preparations are taken once daily for a continuous period for 7–14 days. Most Chinese herbal preparations induce diuretic actions to facilitate the passage of the drugs to the site of action and consistently for a period of time [2, 3, 7]. A separate study is being undertaken by us to study the effects of such herbal medicines with volunteers of both stone-formers and non stone-formers.

From the results of this in-vitro crystallization study of the Chinese herbal medicine tested, it confirms that prophylaxis of renal stones could be achieved through promotion of nucleation and inhibition of growth and aggregation of calcium oxalate crystals concomitant with the pharmacological diuretic action of the herbal medicines. The resultant effect reduces the overall supersaturation and helps the patients to expel or discharge small crystals rapidly to prevent any retention or growth of existing stones. However, the effect of the herbal medicines after ingestion, absorption and metabolism remains to be investigated which is our current pursuit.

In conclusion, we demonstrate that of the Chinese herbal medicine dispensed by the practitioners for treating renal stones, their mode of action, as shown by the 96-well plate oxalate-induced turbidity method, is by promoting nucleation and inhibition of growth and aggregation of crystals. As a result of this study we are preparing clinical trials on treated (by western method) renal stone patients.

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