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Application of small caltrops (*Tribulus terrestris*) to inhibit calcium oxalate monohydrate crystallization

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ABSTRACT: Calcium oxalate (CaOx) can be crystallized in several forms and morphologies. Here we studied the crystallization of CaOx by the precipitation of calcium chloride and sodium oxalate in the absence and presence of small caltrops (*Tribulus terrestris*). The effects of small caltrops concentration and $[Ca^{2+}]/[C_2O_4^{2-}]$ on CaOx crystal forms and morphologies were investigated. The crystals were characterized by scanning electron microscopy, and the quantities of Ca^{2+} in aqueous solution were detected by atomic absorption spectrometry. The results show that small caltrops concentration and $[Ca^{2+}]/[C_2O_4^{2-}]$ affected the crystal morphologies that were mainly hexagonal, octahedral, and dendritic. Higher small caltrops concentration raised the formation of calcium oxalate dihydrate (COD) crystals in an octahedral shape. It was concluded that small caltrops acts as a good inhibitor for kidney stones since they induce COD crystals which are easily excreted in urine.

KEYWORDS: crystal morphology, herbal medicine, kidney stone, scanning electron microscopy

INTRODUCTION

Kidney stone diseases occur in 15–17% of the population in northeast Thailand and it is an important public health problem[1,2]. High concentrations of calcium carbonate in water and a low-nutrient diet are contributing factors to this disease[1]. Kidney stones are commonly composed of calcium oxalate monohydrate (COM), especially in the form of COM microcrystals[3–5]. COM is the most thermodynamically stable form of calcium oxalate (CaOx) at room temperature[4,6]. Ingesting foods such as spiny pigweed (*Amaranthus lividus*) and cocoa (*Theobroma cacao*) which contain high oxalic acid causes the formation of CaOx crystals in urine. These crystals are generally in the form of COM. Humans normally have biological control mechanisms to prevent COM crystallization in the urine by inducing inhibitors that decrease nucleation, growth, and aggregation of COM crystals[3,7]. In particular, inhibitors in urine will transform COM to calcium oxalate dihydrate (COD)[5,8].

In the past decade, interactions between stone crystals and organic matrices such as citrate, osteopontin, aspartate, glutamate, poly-(styrene-alt-maleic acid), and glycosaminoglycans have been investigated[3,4,6,9]. Only recently has the application of herbs been applied to the control of COM crystal development in vitro. Interaction mechanisms between organic molecular additives and inorganic crystals in vitro during the formation of stones remain poorly understood[6]. The applicability of herbs such as small caltrops (*Tribulus terrestris* L.) in inhibiting kidney stones remains unanswered. Small caltrops have been commonly used as a diuretic and against colicky pains, hypertension, and hypercholesterolemia in folk medicine in Iraq, Turkey[10], and Thailand[1,2,11,12]. Only in vivo studies with small caltrops have been made. For instance, they have an antiurolithic activity in experimentally induced urolithiasis in rats[12], reduce the amount of urinary oxalate in rats[13], and have the potential of propelling kidney stones in rats[10].

In the present study, we investigated the crystallization of CaOx crystals from aqueous solution in the absence and presence of small caltrops. The effects of small caltrops concentration and $[Ca^{2+}]/[C_2O_4^{2-}]$ on CaOx crystal formation and compositions were studied. In addition, we used atomic absorption spectrometry (AAS) for detecting the Ca^{2+} in the aqueous solution, and scanning electron microscopy (SEM) to describe the morphologies and surfaces of CaOx

crystals as well as their compositions with energy dispersive X-ray spectroscopy (EDS).

METHODS

$CaCl_2$ and $Na_2C_2O_4$ solutions

All preparations and experiments were conducted at room temperature (25 °C), and all chemicals were analytical grade. $CaCl_2 \cdot 2\,H_2O$ (ASP Ajax Finechem) and $Na_2C_2O_4$ (ASP Ajax Finechem) were from the same 1 M stock solutions in distilled (dI) water. CaOx crystals were produced by using the required concentrations of $CaCl_2$ (1 mM or 4 mM) and $Na_2C_2O_4$ (1 mM or 4 mM) diluted from the stock solutions with dI water. From the previous study[11], the appropriate value of $[Ca^{2+}]/[C_2O_4^{2-}]$ is 1 because surface characteristics cannot be classified when values of 2 or 3 are used.

Small caltrops-extracted solutions

Dried trunks of small caltrops were ground into a coarse powder that was extracted with ethanol. After 24 h equilibration, it was filtered and evaporated to form a small caltrops-extracted matrix. The matrix was diluted with dI water to 0.25, 5, 10, and 50 g/l, which were used as the crystal modifiers of the small caltrops-extracted solutions.

Crystallization of CaOx crystals

In a typical experiment, $CaCl_2$ (1 mM, 20 ml) was added to $Na_2C_2O_4$ (1 mM, 20 ml) in five well-beakers. Subsequently, the small caltrops were added to the solutions (0.25 to 50 g/l) and vigorously stirred for 1 min. Then the mixtures were covered with a glass plate for 24 h until the solutions crystallized. As a reference, the CaOx was prepared in the absence of small caltrops. The mixtures consisting of $CaCl_2$ (4 mM, 20 ml) and $Na_2C_2O_4$ (4 mM, 20 ml) were prepared in the same way.

Detection and characterization

Quantities of Ca^{2+} in solutions were determined after filtration of the mixtures by AAS (model AA-6501F, Shimadzu). Dried CaOx crystals were characterized for their morphologies by SEM (model 1450VP, LEO) with an accelerating voltage of 20 kV. Chemical compositions of crystals were also characterized by SEM with EDS as a detector.

RESULTS AND DISCUSSION

The effects of the varying amount of small caltrops on the morphology and the size of CaOx particles at 25 °C under the standard analysis conditions are shown in Fig. 1. Fig. 1a indicates that all CaOx particles were hexagonal and plate-like and hence were identified as COM[14,15]. The number of COM particles decreased but those of COD gradually increased with increasing small caltrops concentration. Most CaOx particles appeared as octahedral crystals of COD[8,14,15] and druses (Fig. 1e). This is because small caltrops contain mainly steroidal glycosides, steroidal saponins[16,17], linoleic acid, oleic acid, and stearic acid[18]. Hence, these functional groups of protein and acid could transform the structure of COM to COD. To summarize, the higher concentration of small caltrops inhibits the formation of COM and promotes the formation of COD.

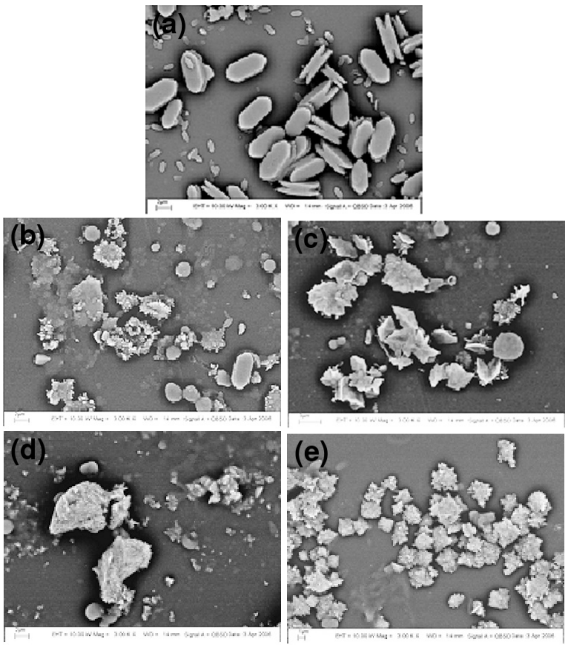

Fig. 1 SEM micrographs of CaOx particles. $[Ca^{2+}]$: 1 mM and $[C_2O_4^{2-}]$: 1 mM, [small caltrops]: (a) 0, (b) 2.5, (c) 5, (d) 10 and (e) 50 g/l. Scale bar: 2 μm.

After $CaCl_2$ reacted with $Na_2C_2O_4$ the crystallization of CaOx occurred so that the stable COM was the dominant phase. In the absence of small caltrops (Fig. 1a), Ca^{2+} concentration in the aqueous solution was very low. As small caltrops was added to the solutions, Ca^{2+} concentrations gradually decreased (Fig. 2). Due to the reaction between small caltrops and Ca^{2+} ions in the aqueous solutions, COD could be formed and its amount increased with the concentration of small caltrops.

Surface structures of COM and COD differ in their affinities for cell membranes[6]. COM has a higher affinity for renal tubule cells[19] and for cell membranes

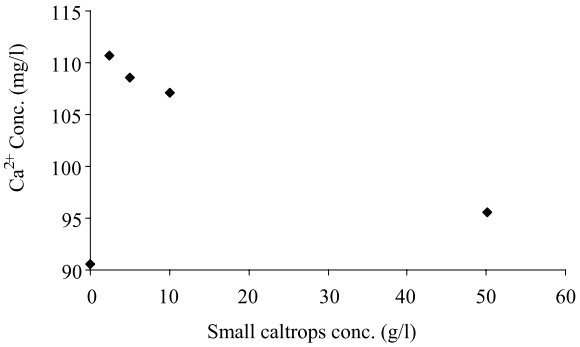

Fig. 2 Dependence of Ca^{2+} concentration on small caltrops concentration from the reaction between $CaCl_2$ and $Na_2C_2O_4$.

than COD[20]. Hence, a preferential adsorption to cell membranes of COM crystals induces kidney stones[6]. In contrast, COD prevents kidney stones because it is easily excreted in urine[19,21]. It is suggested that small caltrops act as a good inhibitor for kidney stones once they induce the formation of COD. This is consistent with the practice in Iraq, Turkey[10], and Thailand[1,2,11] of using small caltrops as a diuretic to excrete kidney stones. The presence of functional groups of protein and acid in small caltrops may inhibit the formation of COM by reacting with calcium instead of oxalate.

The compositions of crystals from $CaCl_2$ and $Na_2C_2O_4$ were characterized with EDS and found to consist of carbon (C), oxygen (O), calcium (Ca), and gold (Au). Au was found because the crystals had to be sealed with Au before detecting by SEM. The presence of C, O and Ca confirmed the SEM result.

Varying $[Ca^{2+}]$ and $[C_2O_4^{2-}]$ changes the CaOx crystals and the CaOx-modifier crystals reaction, leading to morphological variation of CaOx crystals[6]. At higher values of $[Ca^{2+}]$ and $[C_2O_4^{2-}]$, the COM particles were dendritic rather than hexagonal in shape (Fig. 3a) as was also shown in Refs. 5, 22. The amount of COD particles of octahedral shape gradually increased with small caltrops concentration. The compositions of crystals in the higher concentration case were also characterized by EDS. The chemical elements were found to be in the same ratio as for the lower concentration case.

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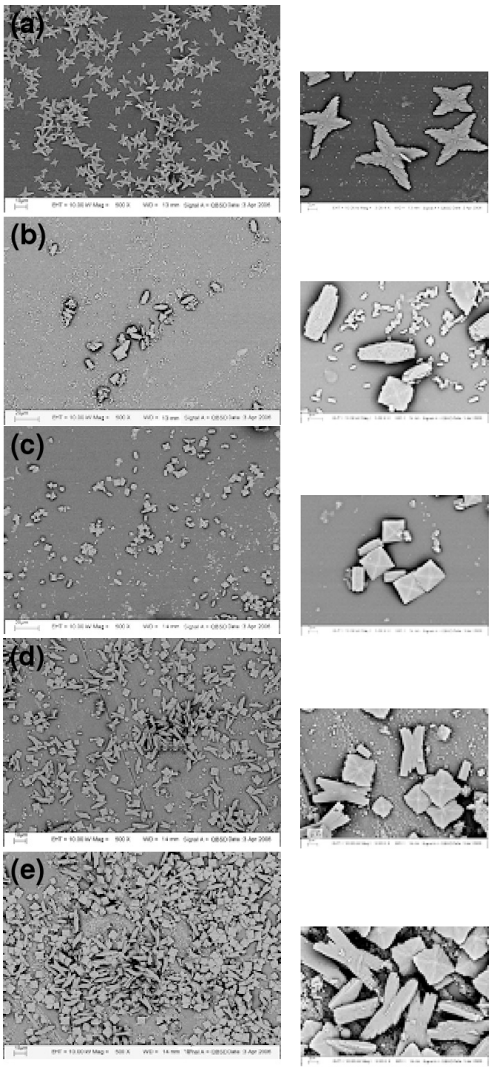

Fig. 3 SEM micrographs of CaOx particles with $[Ca^{2+}]$: 4 mM, $[C_2O_4^{2-}]$: 4 mM, [small caltrops]: (a) 0, (b) 2.5, (c) 5, (d) 10 and (e) 50 g/l. Magnified views on the right. Scale bars: 10 μm, 2 μm.

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