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Determination of components in traditional Chinese medicines associated with promoting or inhibiting urinary stone formation

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Abstract *Objective:* To measure oxalate, calcium, and sodium contents of traditional Chinese medicines (TCMs) that are commonly used to prevent and dissolve urinary stones to exclude the possibility that long-term use of such medicines promotes stone formation. The second objective was to measure citrate, potassium, and magnesium contents in the same medicines to provide possible clues about the mechanisms of prevention and dissolution of urinary stones by TCMs.
Methods: Ten of the most commonly-used TCMs for preventing and dissolving urinary stones were chosen and subjected to ion chromatography (IC) to measure water-soluble and total oxalate and citrate contents. Inductively coupled plasma atomic emission spectrometry (ICP-AES) was used to measure calcium, potassium, magnesium, and sodium contents in a water extract and in digestion liquid.
Results: Average contents of water-soluble oxalate, calcium, and sodium in the water extract were 41.92, 84.32, and 22.82 mg/100 g, respectively, far below the normal dietary intake of adults in China. The average contents of citrate and magnesium in water extracts were 268.99 and 66.65 mg/100 g, respectively, below the recommended intake for adults. These ion contents are therefore insufficient to inhibit the formation of urinary stones. The average content of potassium in the water extract was 867.71 mg/100 g, which was relatively abundant, so taking the prescription used in this experimental protocol can increase the body's potassium content to some extent.

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Conclusions: Long-term use of TCMs would not increase the risk of urinary stone formation. The potassium content in TCMs is high, which is one possible reason for the prevention of urinary stones by TCMs.

Introduction

Traditional Chinese medicines (TCMs) have been used for centuries in China to prevent and treat urinary stones (calculi). In Chinese medicine, prevention and treatment of urinary calculi follow three modes of action: expulsion, prevention, and dissolution. Mechanisms for preventing stones may involve crystallization inhibition and formation of complexes with stone-causing substances. Dissolution of stones may mainly involve altering urinary pH.[1]

In using TCMs to prevent and dissolve urinary stones, long-term administration is typically required. Despite centuries' worth of empirical application of these TCMs, there has been a lack of systematic research into their specific mechanisms behind prevention and dissolution of stones and identifying their effective monomer components. There is little evidence in the literature that can exclude the possibility that long-term use of TCMs can promote urinary stone formation. Therefore, it is both necessary and useful to measure components in these TCMs that might promote or inhibit stone formation. In this study, we measured the oxalate, citrate, calcium, potassium, magnesium, and sodium contents in TCMs that are commonly used to prevent or dissolve urinary stones, to identify factors that might promote or inhibit stone formation. The results may prove useful in clinical practice and provide a theoretical foundation for application of TCMs in preventing and treating urinary stones.

Materials and methods

Materials

Samples: plantago seed (*Plantago asiatica* L.), mallow fruit (*Malvaverticillata* L.), lygodium spore [*Lygodiumjaponicum* (Thunb.) Sw.], talcum, lysimachia plant (*Lysimachiachristinae* Hance), glechoma aerial part [*Glechoma longituba* (Nakai) Kuprian.], malva nut [Scaphium scaphigerum (Wall. ex G. Don) G. Planch], pyrrosia leaf [*Pyrrosia lingua* (Thunb.) Farw.], water plantain rhizome (*Alisma plantago-aquatica* L.), and gizzard lining (*Gallus gallusdomesticus* Brisson). All the samples were purchased from Affiliated Hospital of Nanjing University of Traditional Chinese Medicine. The samples were confirmed by Pro. Xunhong Liu, who works at Nanjing University of Traditional Chinese Medicine.

Instruments and reagents

The 883 Basic IC Plus ion chromatograph (Metrohm, Switzerland) was applied. OPTIMA5300DV inductively coupled plasma atomic emission spectrometer (PerkinElmer. Waltham, MA, USA). Sodium oxalate, sodium citrate, Na_2CO_3, NaOH, concentrated HNO_3, concentrated $HClO_4$, and concentrated HCl were of analytical grade. Ca, K, Mg, Na standard solutions (1000 mg/L) were produced by dissolving metal powder or metal oxide with a purity above 99.9% (mass fraction) in 1% HNO_3 solution (volume fraction), with dilution according to need. High purity, deionized water ($\geq$18.2 MΩ) was used in all experiments.

Experimental methods

Ion chromatography (IC) for measuring water-soluble oxalate and citrate content in TCMs

(1) Sample pretreatment: each sample was washed and dried [insolation and then oven-dried (60°C, 30 min)], then accurately weighed (5 g) and decocted (boiled) for 30 min in 500 mL of high purity water. The resultant decoction was strained, with both the filtrate and residue retained. The residue was decocted for 30 min in another 500 mL of water. This second decoction was strained, the filtrate retained, and the residue discarded. The two filtrate samples were combined by pouring into a volumetric flask making up to 1000 mL of liquid, which was reserved in a refrigerator at 4°C for experiments described below.
(2) 2 mL of the above extract was passed through a 0.25 μm microfiltration membrane, then water-soluble oxalate and citrate were measured by IC.
(3) Standard recovery tests: to examine the reliability of the method, standard recovery tests were conducted. Different amounts of oxalate and citrate standard solution were measured into a known amount of pyrrosia leaf extract, and standard recovery tests carried out.

IC to measure the total oxalate content in TCMs

To determine the total oxalate content, 10 mL of 0.5 mol/L HCl was added to 500 mL of pure water for sample pretreatment, then boiled for 30 min, together with the sample. Other steps were the same as above.

Inductively coupled plasma atomic emission spectrometry (ICP-AES) to measure calcium, potassium, magnesium, and sodium contents of the TCMs digestion liquid

(1) Sample pretreatment: the sample was washed and dried, then accurately weighed (5 g) into an