ORIGINAL ARTICLE

EFFECT OF HERBAL EXTRACTS OF TETRACLINIS ARTICULATA AND CHAMAEROPS HUMILIS ON CALCIUM OXALATE CRYSTALS IN VITRO

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ABSTRACT

Background: A large number of people are suffering from problems due to urinary stones. Calcium oxalate monohydrate containing stones are the commonest ones. We studied the effect of herbal extracts of Tetraclinis articulata and Chamaerops humilis on these crystals in vitro.

Material and Methods: Calcium oxalate monohydrate crystals were grown by the classical model for the study of oxalate crystallisation. Crystallisation was studied and compared without and with inhibitor. Extracts of Tetraclinis articulata and Chamaerops humilis were studied as inhibitors.

Results: The crystallisation of calcium oxalate monohydrate occurred in the absence of inhibitors and were calculated at 5, 10, 15, 20, 25 and 30 minutes, by polarised light microscopy. The same procedure was followed for the study of calcium oxalate monohydrate crystallisation in the presence of Tetraclinis articulata and Chamaerops humilis extracts. A series of concentrations of 25, 50, 75, 100% of these extracts were studied. The follow-up of the crystal size by polarised light microscopy was carried out at 5, 10, 15, 20, 25 and 30 minutes and the percentage inhibition was calculated. With the addition of Tetraclinis articulata, the best inhibitory concentrations 87.94% and 84.12% were encountered at the concentration of 100% and 50% respectively. For Chamaerops humilis **t**he best inhibitory concentrations 94.86% and 93.07% were encountered at the concentration of 25% and 50% respectively after 30 minutes.

Conclusion: Extracts of Tetraclinis articulata and Chamaerops humilis inhibit the growth of calcium oxalate monohydrate crystals in vitro.

Key words: Oxalate, Herbal extract, Urinary calculi, Crystallization.

INTRODUCTION

A large number of people are suffering from problems due to urinary stones. There are many areas of high incidence of urinary calculi, which include British Isles, Scandinavian countries, Northern Australia, Central Europe, Northern India and Pakistan and Mediterranean countries.[1] It has also an economic impact on the society.[2] Calcium containing stones are the most common comprising about 75% of all urinary calculi, which may be in the form of pure calcium oxalate (50%) or calcium phosphate (5%) or a mixture of both (45%). Many factors affect the growth of urinary calculi. Different mineral metabolisms are important in the formation of urinary stones or calculi.[3] Hypercalciuria has received attention from researchers on urolithiasis. The pathophysiology of calcium oxalate stone formation has been discussed by Menon et al.[1] The urinary calculi are composed of mainly crystalline components. Multiple steps are involved in the formation of crystals, which are nucleation, growth and aggregation. The stone formation begins from the occurrence of nuclei and the formation of these nuclei is from supersaturated urine. Super-saturation also depends on urinary pH, ionic strength, solute concentration of certain glycoproteins, complex anions and the pathogenatic factors, which are quite complex and well explained by Menon et al.[1]

Many inhibitors of growth of calcium oxalate calculi are studied.[1,4] The slow and controlled diffusion to the growing crystals in the gel medium is very useful to study the growth and inhibition of calcium oxalate crystals in vitro. This also helps to develop different inhibitory conditions, which can be extended to urinary calculi, by adding different inhibiting solutions.

In our study the effect of aqueous extracts of common medicinal plants, Tetraclinis articulata and

Chamaerops humilis was studied on the growth and inhibition of calcium oxalate monohydrate (COM) crystals in vitro.

MATERIAL AND METHODS

Commercial dry herb from Tetraclinis articulata (*Cupressaceae*) and Chamaerops humilis (*Arecaceae*) were purchased from a specialised company, Oran Co, Algeria and identified by the plant taxonomy unit of Oran University. Infusions were prepared daily just before handling by suspending a weighed amount of dry plant material in boiling tap water. The suspension was left at room temperature for 15 minutes and then filtered. The infusion was used at room temperature.

We chose the classical model for the study of oxalate crystallisation because of its simplicity and satisfactory reproducibility. This model includes the study of crystallisation without inhibitor and with it, in order to assess the inhibiting capacity of any chemical species used.

Two solutions were prepared by dissolving chemicals of reagent-grade purity in deionised and redistilled water. Solution A was $Na_2C_2O_4$ (2 mmol/1) and Solution B $CaCl_2$ $2H_2O$ (10 mmol/1). Artificial urine was prepared by mixing and stirring equal volumes 50 ml of solutions A and B at 37°C in capped vessels.[5]

The crystal size development was monitored by polarised microscopy at different time intervals by proceeding as follows: Sample drops were examined every five minutes by polarising optical microscopy. Crystals were identified with x40 magnifying lens under microscope of the Zeiss type equipped with a camera WINDER M 476079.

RESULTS

Study of the oxalate crystallisation without inhibitors: Crystallisation of oxalate in the absence of inhibitors, led to the formation of COM crystals by polarised light microscopy. The process of calcium oxalate crystallisation in the control without the addition of inhibitors is shown in Table-1 and Fig-1.

Study of oxalate crystallisation in the presence of inhibitors: We followed the same experimental procedure for the study of crystallisation in the presence of inhibitors in order to assess the inhibiting potential of these substances and to understand the mechanism of action of these inhibitors on oxalate crystallisation.

We tested the effectiveness of extracts of medicinal plants Tetraclinis articulata and Chamaerops humilis. The same procedure as above was followed. However, we added the inhibitory amounts corresponding to the physiological concentrations to the synthetic urine prepared by mixing solutions A and B at 37°C.

The same parameters (size and number of crystals, time of crystallisation) were investigated. A series of experiments corresponding to the physiological concentrations of 25, 50, 75, 100% of Tetraclinis articulata and Chamaerops humilis were carried out in order to cover the physiological excretion range. The follow-up of the crystal size development by polarised light microscopy was carried out at 5, 10, 15, 20, 25 and 30 minutes.

Calculation of the Percentage of Inhibition (1%) was based on the formula:[6] $I\% = [(TSI - TAI) / TSI] \times 100$

TSI represents the number of COM crystals without inhibitors and TAI the number of COM crystals after addition of inhibitor.

Tetraclinis articulata: We followed the experimental procedure as mentioned above. We focussed on crystallisation because it may be the nucleating factor for many stones.[7] The inhibitory effect was not dose dependent; the best inhibitory concentrations (87.94% and 84.12 %) were encountered at the concentration of 100% and 50% respectively. The results of addition of 1 ml of Tetraclinis articulata to the mixture are given in Fig-2. The inhibitory effect involved the crystal growth phase. (Fig-3)

Chamaerops humilis: Results show that crystal morphology depends considerably on the concentration of medicinal plant. Size of the crystal

Table-1: Study of the calcium oxalate crystallisation without inhibitors.

Time (min)	5	10	15	20	25	30
Number of COM/mm^3	663	725	840	704	690	762
COM agregation /mm^3	101	104	115	114	87	76
Total	764	829	955	818	777	838

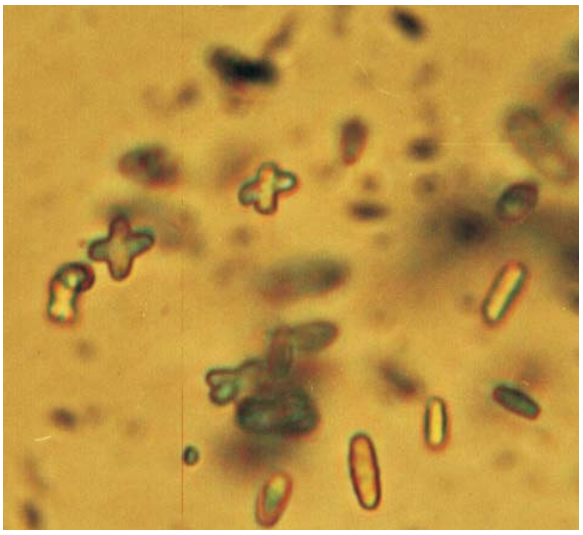

Fig. 1: Photograph of calcium oxalate monohydrate crystal formation without inhibitors

was found to decrease with increase in the concentration of medicinal plant and disintegration of the crystals was also observed. The addition of Chamaerops humilis worked on the aggregation phase of calcium oxalate crystallisation and to a

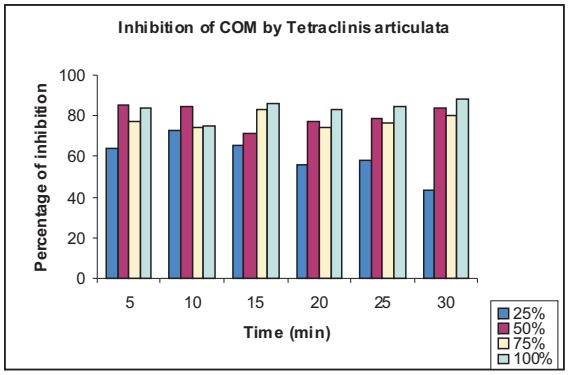

Fig. 2: Inhibition of COM crystal after addition of Tetraclinis articulata.

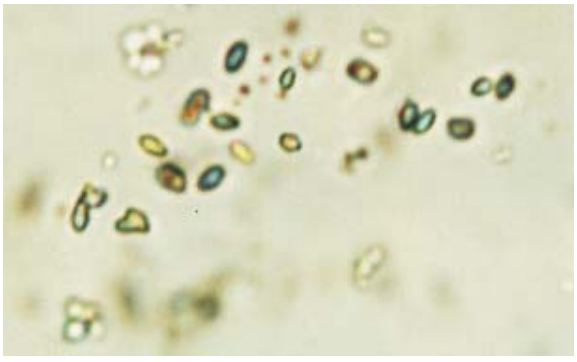

Fig. 3: Photograph of COM crystals after addition of Tetraclins articulata

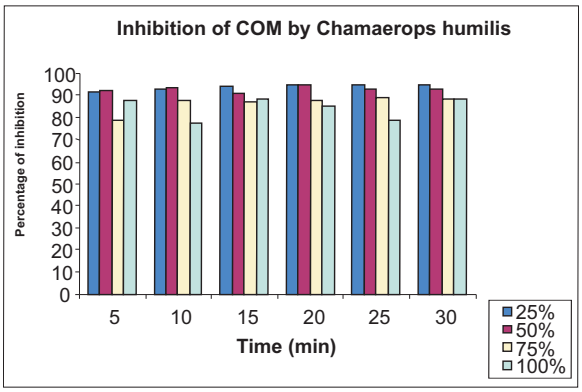

Fig. 4: Inhibition of COM crystals after addition of Chamaerops humilis

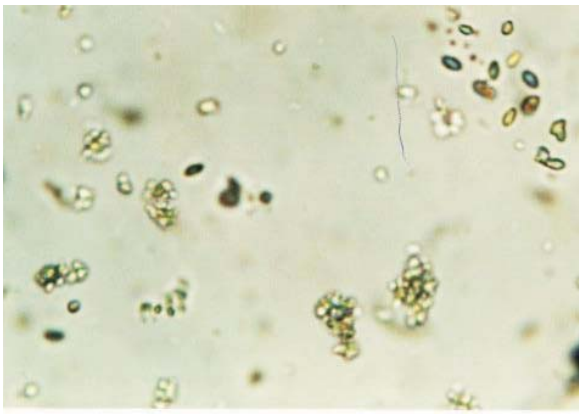

Fig. 5: Photograh of COM crystals after addition of Chamaerops humilis

lesser extent on crystal growth but did not affect the nucleation stage of calcium oxalate crystallisation. (Fig-5)

The best inhibitory concentrations (94.86% and 93.07%) were encountered at Chamaerops humilis concentration of 25% and 50% respectively after 30 minutes. The results of addition of 1 ml of Chamaerops to the mixture are given in Fig-4.

DISCUSSION

The present work was performed to study, in vitro, the inhibitory effects of certain plant extracts on calcium oxalate crystallisation. The tested substances included extracts of Tetraclinis articulata and Chamaerops humilis. Utilising polarised light photography, we were able to demonstrate the various phases of crystallisation, i.e. nucleation, growth and aggregation, which eventually lead to urolithiasis. Calcium stone formation involves different phases of increasing accumulation of Calcium oxalate; nucleation, crystal growth, crystal aggregation and crystal retention. On the other hand, in vitro studies showed that Tetraclinis articulata inhibited both the growth phases with a

maximal inhibitory effect of 87.94%. Growth is the formation of a solid crystal phase in a solution. It is an essential step in renal stone formation.[8,9]

Our study has proved the inhibitory effects of Chamaerops humilis mainly affecting the aggregation phase and to a lesser extent the crystal growth but has no effect on crystal nucleation with a maximal inhibitory effect of 94.86%. After nucleation, crystal growth is the next major step of stone formation. What causes crystals to "grow"? The driving force for crystallisation is a reduction in the potential energy of the atoms or molecules when they form bonds to each other. The crystal growth process starts with the nucleation stage. Several molecules in a supersaturated liquid start forming clusters; the bulk free energy of the cluster is less than that of the liquid.[10] Finally, the inhibitory effect of Tetraclinis articulata and Chamaerops humilis on the growth and aggregation processes in the case of COM appear to overlap during the initial lag-phase of precipitation. This later hypothesis was confirmed in recent work (Atmani et al.) showing that aqueous extract obtained from *Herniaria hirsuta* significantly inhibited COM crystal.[11] Such effect has been observed in vitro when crystallization was induced in the urine in presence of herb extract, and suggest that the plant may contain substances that inhibit COM crystallization.[12] Plants in Mediterranean region are widely used in Algeria to treat lithiasis patients and may have possible therapeutic potential as preventive agents hindering the formation crystals.[13] The absence of a realistic concept regarding the generation of urinary lihiasis in a large number of cases, up to a considerable degree, is proved by the fact that we cannot directly observe this process in vivo; all hypotheses are based on in vitro experiments.[14]

CONCLUSION

Extracts of Tetraclinis articulata and Chamaerops humilis inhibit the growth of calcium oxalate monohydrate crystals in vitro.

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