International Journal of Medicinal Plants Research ISSN 2169-303X Vol. 8 (7), pp. 001-005, July, 2019.
Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Inhibition of calcium oxalate monohydrate crystal growth using Algerian medicinal plants

M. Beghalia[1,2*], S. Ghalem[1], H. Allali[1], A. Belouatek[2] and A. Marouf[3]

[1]Laboratory of Organic Chemistry, Natural Products and Analysis (COSNA), Department of Chemistry, Faculty of Sciences, University Aboubekr Belkaïd BP 119, 13000 Tlemcen, Algeria. [2]Department of Biology, Faculty of Sciences, University of Abdel Hamid Ibn Badis, 27000 Mostaganem, Algeria. [3]Department of Biology, Faculty of Sciences, University of Senia, 31000 Oran, Algeria.

Accepted 18 April, 2019

A large number of people in this world are suffering from urinary stone problem. Calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) containing stones (calculi) are commonly found. In the present study, COM crystals were grown by artificial urine is prepared by mixing and stirring two equal volumes of solutions A and B. A screening of plant extracts from wild species antilithic agents using a urolithic could lead to the urinary risk factors of urolithiasis. Ten extracts of different parts of plant species from West and South of Algeria, belonging to different botanical families, were harvested in 2006 and 2007. From each sample aqueous extract is obtained using decoction. Activity was tested in bioassays using different parts of the plants according to the traditional medicine way. Crystal of calcium oxalate growth inhibition was significantly induced when extracts were incorporated at 100%. The most active plants were: *Ammodaucus leucotrichus*, *Ajuga iva, Erica multiflora* and *Stipa tenacissima* . However, only with *A. leucotrichus* inhibition was 97.94% and 97.68% at (75%) and (100%) respectively. The extracts of *Globularia alypum*, *Atriplex halimus, Tetraclinis articulata, Chamaerops humilis* and *Erica arborea* give percentages of inhibition which varies with the concentration of plant and the time to crystallisation.

Key words: Plants extracts, inhibition, calcium oxalate, crystallization

INTRODUCTION

A large number of people in this world are suffering from problems due to urinary stones. There are many areas of high incidence of urinary calculi which include British Isles, Scandinavi an countries, northern Australia, central Europe, northern India and Pakistan and Mediterranean countries (Menon et al 1988).It has also an economic impact on the society (Clerk, 1995). 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%) and a mixture of both (45%). Calcium oxalate stones are found in two different varieties, calcium oxalate monohydrate (COM) or Whewellite, and calcium oxalate dihydrate (COD) or Weddellite (Menon et al 1988). Urolithiasis is a very painful disease that has afflicted a wide sector of human population since ancient times (Grases, 1998). Many inhibitors of growth of calcium oxalate calculi are studied (Ryall, 1997).Several authors have attempted to grow calcium oxalate crystals by gel growth technique (Deepa, 1993; Srinivasan and Natarajan 1996). In the present investigation, COM crystals were grown in the artificial urine technique. The effect of aqueous extracts of common medicinal plants, *Globularia alypum , Atriplex halimus, Tetraclinis articulata, Chamaerops humilis, Erica arborea, Ammodaucus leucotrichus, Ajuga iva, Erica multiflora and Stipa tenacissima* (in herb decoction form,) was studied on the growth and inhibition of COM crystals. In this work, we performed an in vitro crystallization study enabling the specification of kinetic and thermodynamic conditions of formation and growth of crystalline species. The slow and controlled diffusion of species to the growing crystals is very useful to study the growth and inhibition of Whewellite crystals *in vitro*. Different experimental procedures have been proposed using synthetic, diluted or natural supersaturated aqueous solutions of

*Corresponding author: E-mail:Beghalia_moh@yahoo.fr.

Table 1. Plant material harvested from the wild in West and south of Algeria,

Species (Family)	Part used
Ajuga iva (Lamiaceae)	aerial part
Ammodaucus leucotrichus (Apiaceae)	fruits
Atriplex halimus (Chenopodiaceae)	leaves
Chamaerops humilis (Arecaceae)	sheath
Erica arborea (Ericaceae)	leafed branch
Erica multiflora (Ericaceae)	leafed branch
Globularia alypum (Globulariaceae)	flowers - Roots
Stipa tenacissima (Poaceae)	leaves
Tetraclinis articulata (Cupressaceae)	leafed branch

urine (Jungers et al., 1989).Crystallization can be triggered by adding, to reaction medium calcium, oxalates or phosphates, or by crystalline germination of the species under investigation. Crystallization can also take place, by changing the pH of substances having pH - dependent solubility (Jungers et al., 1989, Daudon, 1997).Therefore, it is worthwhile to look for an alternative to these means by using medicinal plants (Gogte, 2000; Bisaz, 1984) In this regard, many plants have been used to treat kidney stones and showed to be effective among them medicinal plants. The plants studied are Mediterranean traditional medicinal plants widely used in Algeria to treat lithiasis. Our in vitro study showed that extract from plants are used in urine owing to its therapeutic potential as preventive agent hindering the formation calcium oxalate crystals.

MATERIALS AND METHODS

Plants and extracts

Ten extracts are prepared from species belonging to several families (Table 1). parts were harvested from natural resources in 2006 and 2007, mainly at flowering stage. Voucher specimens were deposited in the laboratory of Plant biochemistry, Oran University. Decoctions were prepared daily just before experimentation by suspending a weighed amount (10 g) of dry plant material in boiling tap water. The suspension was stored at room temperature for 15 min and then filtered through filter paper.

Synthetic urine

We chose the classical model for the study of oxalate crystallization because of its simplicity and satisfactory reproducibility. This model includes the study of crystallization without inhibitor and with it, in order to assess the inhibiting capacity of any chemical species used. Two solutions of following composition were mixed: A: $Na_2C_2O_4$ (2 m mol/1) and B : Ca Cl $_2$ $2H_2O$ (10 m mol/1) . The two solutions were prepared stake NaCl 9 g to obtain the ionic force like the Indoor environments. The formation and growth of the COM crystals of oxalate from artificial urine at different concentration was the object of our investigation. Artificial urine is prepared by mixing and stirring two equal volumes of 50 ml of solutions A and B at constant temperature (37°C) in capped vessels to give final artificial urine.. Mixture agitation was maintained to prevent sedimentation.

Simulation of the sedimentary crystal formation

The crystal size development was monitored in sample drops every five minutes by polarized microscope. Crystals were identified with x 40 magnifying lens. Catches of sight of the samples under microscope of the Zeiss type equipped with a camera WINDER M 476079. A series of experiments corresponding to the physiological concentrations of 25, 50, 75, 100% of plants extracts used in this work were carried out in order to cover the physiological excretion range. The follow-up of the crystal size development by polarized light microscopy was carried out at time intervals of 5, 10, 20, 25 30 min of formation of crystals. Calculation of the percentage of Inhibition (1 %) was based on the formula: (Hennquine, 1993).

I%=[(T I-TAI)/TSI].100

T I- represents the number of calcium oxalate monohydrate crystals without inhibitor.
TAI - represents the number of calcium oxalate monohydrate crystals after addition of inhibitor.

RESULTS

Study of the oxalate crystallization without inhibitors

Kidney oxalate stone is the result of supersaturation of urine with certain urinary salts such as calcium oxalate. Since crystallisable oxalate species are pH independent, The crystallization of oxalate in the absence of inhibitor, led to the formation of (C O M) calcium oxalate monohydrate monitored by polarized light microscopy ,the process of calcium oxalate crystallization in control without the addition of inhibitors is shown in (Table 2, Figures 1, 2 and 3).

In the crystal growth experiments sown, (nucleation, growth and aggregation), the rate of crystallization is usually controlled by the number of crystals of calcium oxalate as a function of time, Following the introduction of seed crystals. Entitled constant volume against time in the composition COM experiments determined that the rate of growth of crystals were made in the absence and presence of plants extract.

Study of oxalate crystallization in the presence of inhibitors

We followed the same experimental procedure for the

Table 2.Study of the calcium oxalate crystallization without inhibitors

Time (mn)	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

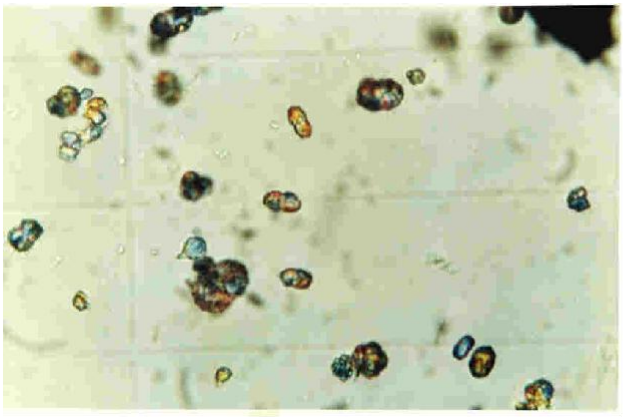

Figure 1. COM crystal nucleation *in vitro*

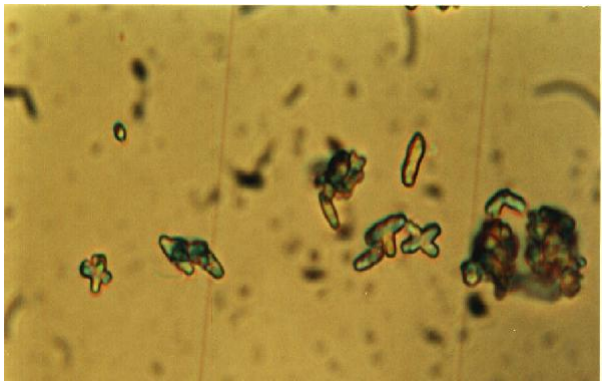

Figure 2. COM crystal growth *in vitro*

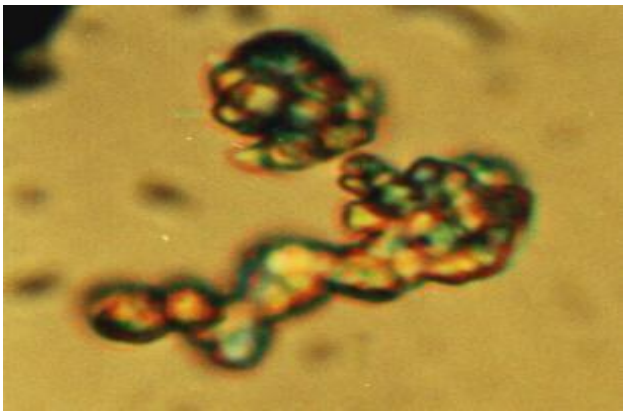

Figure 3. COM crystal aggregation *in vitro*

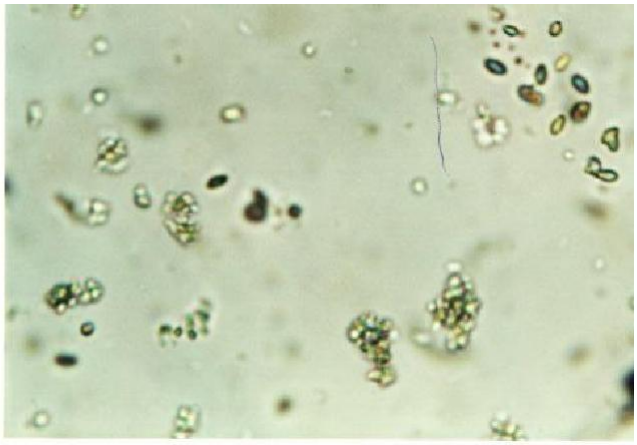

Figure 4. Extracts plants inhibited the nucleation phase of calcium oxalate crystallization.

study of crystallization in the presence of inhibitors. In order to assess the inhibiting potential of substances for oxalate crystallization and understand the mechanisms of action of these inhibitors on oxalate crystallization steps (nucleation, growth, aggregation), we tested the effectiveness of medicinal plants (Table 3).

In the presence of plants extracts, the length and the width of the crystals were reduced. The average length of the crystals grown in the presence of the inhibitors was less than that of the control sample. It was found that extracts plants used in this study inhibited potently the nucleation, growth and aggregation phases, the inhibitory effect on crystallization of calcium oxalate is given in Table 4 and Figure 4, 5 and 6.

After 30 min of reaction kinetics of the crystallization calcium oxalate some cases activity was found in all bioassays like for:

E. multiflora: 98.09% in concentrations 50 and 75% and values very important addition to 95.58% in concentrations 25 and 100%.

- Important values with the rest of the species are as follows:

Ammodaucus: in 97.85% inhibition at[100%] and 97.25% at [75%]; *Ajuga iva* 97.01% inhibition at [75%]; *Stipa tenassicina, Globularia* roots and flowers more than 96% inhibition. They can be classified according to the frequency increasing COM: *E. multiflora*> *E. arborea*> *Ammodaucus*> *A. iva*> *Globularia* (Roots) > *S. tenacis*

Table 3. Effect of extracts on deferent stages of crystallisation

	Nucleation	Growth	Aggregation
Globularia (Flowers)	+	+	-
Globularia (Roots)	-	+	-
Ammodaucus	+	+	+
Atriplex halimus	-	+	+
Ajuga iva	+	+	+
Tetraclinus articulata	-	+-	+
Chamaerops humilis	-	+	-
Stipa tenacissima	+	+-	-
Erica arborea	+	-	+
Erica multiflora	+	+	-

(+)Action on the stage (-)No action on the phase (+-) More or less action

Table 4. Results of inhibition after 30 min

	% Inhibition [25%]	% Inhibition [50%]	% Inhibition [75%]	% Inhibition [100%]
Globularia (Flowers)	75.53	90.45	89.73	96.09
Globularia (Roots)	88.78	95.58	96.77	95.22
Ammodaucus	94.98	96.87	97.25	97.85
Atriplex halimus	63.12	67.18	91.64	89.26
Ajuga iva	89.97	95.22	97.01	96.06
Tetraclinus articulata	42.84	84.12	80.42	87.94
Chamaerops humilis	94.86	93.07	88.54	88.66
Stipa tenacissima	96.18	84.12	95.58	95.58
Erica arborea	94.51	93.91	93.91	97.61
Erica multiflora	95.58	98.09	98.09	96.77

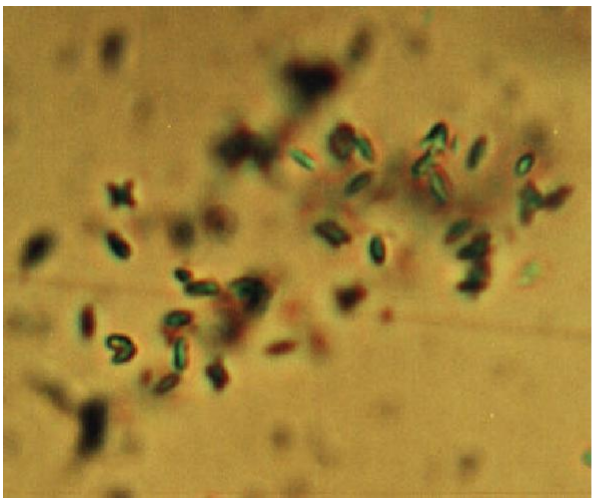

Figure 5. Extracts plants inhibited the growth phase of calcium oxalate crystallization

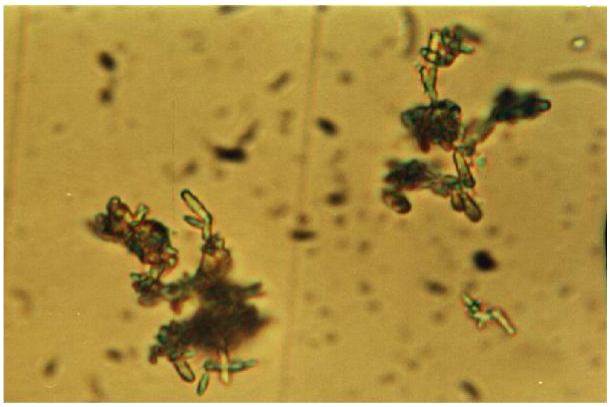

Figure 6. Extracts plants inhibited the aggregation phase of calcium oxalate crystallization

sim>Globularia (Flowers) > *C. humilis> A. halimus> T. articulata.*

DISCUSSION

The supersaturation of urine with CaOx, the most common component of kidney stones (Finlayson, 1974; Khan, 1992), is an important factor in crystallization, with later

factors being nucleation, growth and aggregation. Thus if supersaturation or later steps in crystallization can be prevented, then lithiasis should be avoided. Algeria, as in many less developed countries, phytotherapy is a common method of primary health care, because pharmaceutical products are expensive and the `folk' pharmacopoeia provides apparently effective remedies for many diseases. These results could be considered positives because the herb extract inhibits crystallization and prevents stones formation (Table 4). However, crystal-luria alone is not a risk factor for lithiasis because it is common in both healthy subjects and stone-formers (Robertson et al., 1976; Werness et al., 1981). The limit-ing factors in stone formation could be those processes that affect the size of the particles formed, because particles may become large enough to occlude the uri-nary tract, leading to stone formation. The extracts of medicinal plants induced more crystals in whole urine, thereby reduced supersaturation and the size of the particles (Table 3). This property of the extracts is therefore advantageous, preventing urinary stone formation by inducing the excretion of small particles from the kidney and reducing the chance of retention in the urinary tract. The herb extract may contain substances that inhibit the growth of COM crystals. This property of plants extract may be important in preventing kidney stone formation; crystals induced by urinary macro-molecules were less tightly bound to epithelial cell sur-faces than were COM particles (Wesson et al., 1998). The plants extracts may also contain substances that inhibit CaOx crystal aggregation (Figures 4, 5 and 6); the agglomeration of particles is a critical step in urinary stone formation, as larger crystals are less likely to pass spontaneously in urinary tract (Kok et al., 1990; Kok and Khan, 1994). If the extract keeps CaOx particles dispersed in solution they are more easily eliminated. Unfortunately, neither urinary chemistry nor crystalluria were assessed in that study. The mechanism by the pre-sent medicinals plants exerts there effects remains unknown and will be the objective of our next study.

REFERENCES

Bisaz S, Felix R, Nelman WF, Fleesch (1984). Quantitative determination of inhibitors of calcium phosphate precipitation in whole urine. Nephrologie, 5 :175-179.

Bruneton J (1987). El´ements de phytochimie et de pharmacognosie, Technique & Documentation Lavoisier, Paris, pp.136–162.

Daudon M ,Promat MF, Reveillaude ERG (1983). Etude de la cristallurie spontanée par spectroscopie infrarouge.Recherche de corrélation entre les cristaux, les calculs, les germes et le sexe des malades.Ann Biol LIN 41/199-207.

Daudon M. Plaidoyer pour une meilleure exploration des lithiases rénales. Leurobiologiste (1997), Tome XXXI, N°227; pp. 17-75

Finlayson B (1978). Symposium in renal lithiasis. Renal lithiasis in review. Urol Clin North Am 1974; 1: 181-212

Finlayson B (1978). Physicochemical aspects of urolithiasis. KidneyInt; 13: 344–60

Grases F, Costa-Bauza A, Garcia-Ferragut L (1998). Biopathological crystalization:a general view about the mechanisms of renal stone formation. Adv Colloid Interface Sci 74;169-94.

Jungers P, Daudon M, Le Duc A, La lithiase urinaire (1989). Médecine Sciences Falmmarion Paris, pp. 1-34.

JY Clerk, IM Thomson, SM Optenberg (1995). J. Urol. 154 2020–2040.

Khan SR, Byer KJ,Thamilselvan S, et al. (1999). Crystal-cell interaction and apoptosis in oxalate-associated injury of renal epithelial cells. J Am Soc Nephrol;10(Suppl 14):S457– 63.

Kok DJ, Khan SR (1994). Calcium oxalate nephrolithiasis, a free or ®xed particle disease. Kidney Int; 46: 847-854

M Deepa, K Rajendra Babu VK, Vaidyan J (1993) Mater. Sci. Lett. 132 617

MD Menon, BG Parulkar, GW Drach (1988). Campb ell's Urology,seventh ed.,W.B. Saunders Company, New York, medicinal plants,Bhartiya Vidya Bhavan's SPARC,Mumbai,

N Srinivasan, S Natarajan (1996). Indian J. Phys. 70A 563.

PC Hallson, SKS. Choong, GP Kasidas, CT Samuell (1997). Br. J. Urol. 80:533.

Qiu SR, Wierzbicki A, Orme CA, et al. 2004. Molecular modulation of calcium oxalate crystallization by osteopontin and citrate. Proc Natl Acad Sci USA. 101:1811–1815.

R.L. Ryall,World J. Urol. 15 (1997) pp. 155–164.

Robertson WG, Peacock M, Marshall RW, Marshall DH, Nordin BEC. (1976). Saturation inhibition index as a measure of the risk of calcium oxalate stone formation in the urinary tract. N Engl J Med; 294: 249-252

Saravanan N, Senthil D, Varalakshmi P (1995). Effect of L-cysteine on lipid peroxidation in experimental urolithiatic rats. Pharmacol Res; 32:165–9.

Scheid C, Koul H, Hill A, Luber-Narod J, Kennington L, Jonassen J, Menon M. (1996). Oxalate toxicity in LLC-PK1[r] cells: Role of free radicals. Kidney Int. 49:413–19.

VM Gogte (2000). Ayurvedic Pharmacology and Therapeutic uses of

Werness PG, Bergert JH, Smith LH. Crystalluria (1981). J Cryst Growth; 53: 166-181

Wesson JA, Worcester EM, Wiessner JH, Mandel NS, Kleinman JG. (1998) Control of calcium oxalate structure and cell adherence by urinary macromolecules. Kidney Int; 53: 952-957