Inhibition and Dissolution Crystals of Magnesium Ammonium Phosphate by *Acacia Radiana* (Bark) and (Sheet) in Vitro Study

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***Abstract*—**Struvites form or Ammonium Magnesium Phosphate Hexahydrate in humans as a result of urinary tract infection with ureolithic urea splitting micro organisms. In the present study, the inhibitory effect of aqueous extract of bark and sheet *Acacia Radiana* was investigated against struvite crystals by using the classical model for artificial urine This technique can be utilized as a simplified screening model to study the growth and dissolution of urinary stones in vitro. Corresponding series of experiments at concentrations of 1, 5, 10, 15 and 20 ml in extracts planst was performed. Mixing two equal volumes 50 ml of solutions A and B (containing the volume of inhibitor) at pH 8 at 37 ° C, magnetically stirred at constant speed.

Our results also with the extracts plants we studied expression of this inhibition on the crystal sizes the effect of bark and sheet acacia radiana 59.5% , the effect on the aggregate reaches 73%. . The two bodies are able to significantly reduce phosphate crystallization in vitro, it can be seen clearly from optical and electronic microscope, the aqueous extracts of: Acasia Radiana produce a significant amount of growth inhibition of phosphate crystals . This in vitro study provides useful information for in vivo studies.

***Keywords*—**Inhibition struvite, urinary stone, artificial urine, extracts plants.

I. Introduction

MAGNESIUM ammonium phosphate hexahydrate (MgNH4PO4·6H2O) is among the most important phosphates involved in urinary stone disease [1-2] . These stones are developed in urinary tracts infected by urea-splitting bacteria. The hyperammonuria and alkalinization of urine by bacterial urease are considered to be etiologic factors in the formation of these stones [3]. An elevated urinary pH reduces the solubility of magnesium ammonium phosphate and favors precipitation of Struvite crystals. Higher intake of phosphate (from Proteins) and magnesium based food and lower intake of water gives rise to the PO4 3-and Mg2+ ions in the supersaturated urine, which leads to the conditions of formation of Struvite [4]. The pathophysiology of struvite urolithiasis is different from that of other forms of urolithiasis. In general, the factors that influence the formation of a calculus include urine pH, renal mineral excretion, the presence of promoters, the absence of inhibitors, and the presence of infection or inflammation. In struvite urolithiasis, the concentration of struvite crystals free to react with other solutes in a solution, called the struvite activity product(SAP) also influences calculus formation. When the SAP increases to the point at which the urine becomes supersaturated, the crystals aggregate, forming uroliths. With further increases in SAP, spontaneous crystallization can occur [5]. Appropriate antibiotic therapy is continued in cases of infection-induced struvite urolithiasis for 1 mo beyond the apparent dissolution of the stones on radiographs for several reasons. Although the surface of the stone and the urine may be sterile during dissolution, bacteria under the surface of the urolith may be protected from antimicrobial drugs, and may be released during stone dissolution [6-7]. Currently, there is no established treatment for prevention of urolithiasis. Therefore, there is need to establish a medical treatment for prevention of recurrent stone formation. Indigenous plants have been used as a potential source of medicine since ancient times. Although many plants have been evaluated for anti-urolithiatic effect, search for medical treatment for renal calculi is still going [8].we reported the characterization of the structure of struvite crystals and results on the effect of Acacia Radiana its cristalysation in artificial urine. In this study, we evaluate associated with Acacia to extend the use of this plant in the case of struvite crystallization properties To this end, we performed in vitro experiments struvite crystals mineralization artificial urine. To our knowledge, our study is a study adding to evaluate the activity of medicinal plant extracts in the context of the formation of urinary stones.

II. Material And Method

A. Synthetic urine

Artificial urine is the classical model for the study of phosphate 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 : 11.02 g/l $Na_2SO_4.10H_2O$, 1.46 g/l $MgSO_4.7H_2O$, 4.64 g/l NH_4Cl, 12.13 g/l KCl et 0.24 g/l Ca^{2+}

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B : 2.65 g/l $NaH_2PO_4.2H_2O$, 18.82 g/l $Na_2HPO_4.12H_2O$, 13.05 g/l NaCl, 1 g/l $_2Na_3C_6H_5O_7.2H_2O$ et 0.05 g/l $C_2O_4^{2-}$.

The solution in C_2O_4 is prepared from oxalic acid 0.05 g.

The precipitation of the solid phase of phosphates from artificial urine at different initial pH value (pH = 8) 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 . The pH of solution B was adjusted to required value by adding either HCl or NaOH as appropriate. Mixture agitation was maintained to prevent sedimentation. The crystal size development was monitored by polarized microscopy at different time intervals by proceeding as follows: Sample drops were examined every five minutes by polarising optical microscopy. Crystals were identified with x 40 magnifying lens. After crystallization time, the mixture was filtered, the recovered dried precipitates were analyzed by FTIR spectroscopy, electronic microscopy scanning analysis.

B. Preparation of medicinal plants

Acacia radiana was collected in spring near to Bechar (ahara of Algeria). The extract was prepared according to a similar procedure used often by patients. Fresh herb was dried at 45 °C overnight, boiled in distilled water. The powder was reconstituted to prepare a solution of 15g/l in distilled water.

C. Formula of inhibition

Percentage of inhibition of crystallization (I%) was calculated as previously described [9] and based on the formula, I% = [(TSI- TAI) / TSI] x 100, in which TSI and TAI represent numbers of struvite crystals in absence and presence of inhibitors (plant extracts), respectively. Nucleation, growth and aggregation of crystals were visually assessed under the microscope.

III. RESULTS

The results of the evolution of the size of struvite crystals, in the presence of different concentrations of the bark Acasia radiana, and the percent inhibition (% I) are presented in the following table:

TABLE 1
EVOLUTION OF THE SIZE OF STRUVITE CRYSTALS IN THE PRESENCE ACACIA RADIANA (BARK).

Times mn	Size of struvite crystals in µm and I% inhibition										
	SI	1ml	I%	5ml	I%	10ml	I%	15ml	I%	20ml	I%
5	30	16	46.7	16	46.7	28	6.7	28	6.7	16	46.7
10	38	24	37	20	46.7	28	26	28	26	16	57.9
15	45	24	46.7	20	55.5	28	37.8	20	55.5	12	73
20	45	28	37.8	24	46.7	32	28.9	28	37.8	12	73
30	45	28	37.8	24	46.7	40	11	32	28.9	12	73
40	45	24	46.9	24	46.7	28	37.8	40	11	12	73
50	45	32	28.9	24	46.7	40	55.5	44	2	16	64
60	45	32	28.9	24	46.7	40	11	32	28.9	16	64
120	45	36	20	24	46.7	28	37.8	32	28.9	24	46.7
180	45	36	20	28	37.8	40	11	32	28.9	32	28.9
240	45	36	20	28	37.8	40	11	24	46.7	32	28.9

I %: inhibition. SI : without inhibitor.

We note that in the crystallization occurs rapidly at all different volumes recorded although a gradual decrease in the size of the crystals according to the volumes of the plant and time.The effect of the bark of the Acasia radiana struvite crystals at pH = 8 is not important. After 4 hours the crystal size has reached a minimum value of 24µm for a volume of 15 ml, a decrease of 46.7% relative to its value without inhibitor.It is noted that the crystal size remains constant from 50 min to a volume of 1 ml and after 20 min the height is in a constant volume of 5 ml. As against the effect of the on Acasia radiana aggregate size (Table 2) elevate.

TABLE II
EVOLUTION OF THE SIZE OF STRUVITE AGREGAT IN THE PRESENCE ACACIA RADIANA (BARK)

Time nm	Size of struvite agregate in µm and I% inhibition										
	SI	1ml	I%	5ml	I%	10ml	I%	15ml	I%	20ml	I%
5	60	G	-	G	-	G	-	24	60	52	13
10	92	G	-	G	-	G	-	80	13	64	30
15	104	G	-	G	-	G	-	40	61.5	60	42
20	92	G	-	G	-	G	-	92	0	68	26
30	112	G	-	G	-	G	-	72	35.7	68	39
40	104	G	-	32	69	G	-	60	42	104	0
50	140	G	-	36	74.3	G	-	68	51	80	43.0
60	140	60	57	60	57	G	-	88	37	104	25.7
120	168	60	64.3	60	64	G	-	88	47	104	38
180	168	72	57	68	59.5	48	71	100	40	104	38
240	168	72	57	68	59.5	80	52	104	38	104	38

G : granulation

The effects of the plant Acassia radiana (sheet) on the size of the crystals, and aggregates and the percent inhibition is given in Tables 3 and 4.

TABLE III
EVOLUTION OF CRYSTAL SIZE IN THE PRESENCE OF STRUVITE ACASSIA RADIANA (SHEET)

Times mn	Size of struvite crystals in µm and I% inhibition										
	SI	1ml	I%	5ml	I%	10ml	I%	15ml	I%	20ml	I%
5	30	G	-	G	-	G	-	28	6.7	28	6.7
10	38	16	57.9	20	47.4	20	47.4	28	26	28	26
15	45	24	46.7	20	55.5	20	55.5	36	20	24	46.7
20	45	24	46.7	20	55.5	20	55.5	32	28.9	24	46.7
30	45	32	28.9	20	55.5	20	55.5	32	28.9	20	55.5
40	45	24	46.7	24	46.7	20	55.5	40	11	20	55.5
50	45	28	37.8	24	46.7	24	46.7	36	20	24	46.7
60	45	24	46.7	20	55.5	28	37.8	36	20	24	46.7
120	45	24	46.7	20	55.5	24	46.7	40	11	24	46.7
180	45	28	37.8	20	55.5	28	37.8	24	46.7	20	55.5
240	45	28	37.8	20	55.5	28	37.8	36	20	20	55.5

The study by optical microscopy showed that 20 ml the case of crystal, had partially struvite decreases. The presence of granules, we suggested that it was apatite.

TABLE IV
EVOLUTION OF THE SIZE OF THE AGGREGATES IN THE PRESENCE OF STRUVITE ACASSIA RADIANA (SHEET)

Time nm	Size of struvite agregate in µm and I% inhibition										
	SI	1ml	I%	5ml	I%	10ml	I%	15ml	I%	20ml	I%
5	60	G	-	G	-	G	-	24	60	52	13
10	92	G	-	G	-	32	65	56	39	40	56.5
15	104	16	84.6	20	80.8	36	65.4	76	26.9	36	65
20	92	36	60.9	36	60.9	40	56.4	76	17	44	52
30	112	32	71.4	44	60.7	40	64	80	25	44	52
40	104	44	57.7	44	57.7	60	42	80	23	44	60.7
50	140	40	71.4	40	71.4	68	51	76	45.7	44	57.7
60	140	44	68.6	56	60	64	54	96	31	40	68.6
120	168	48	71.4	52	69	68	59.5	84	50	48	71
180	168	56	66.7	44	73.8	64	61.9	64	61.9	48	71
240	168	60	64.3	52	69	68	59.5	108	35.7	64	61.9

Fig 1. After 5 min without inhibitor

Fig. 2.After 60 min w. inhibitor

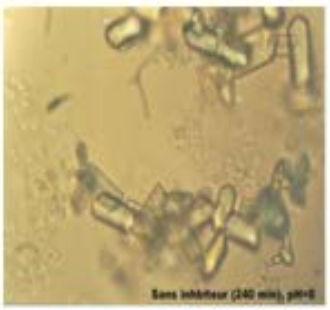


Fig. 3. After 240 min w. inhibitor

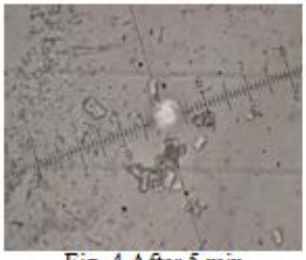


Fig. 4 After 5 min
1 ml of Acacia Radiana

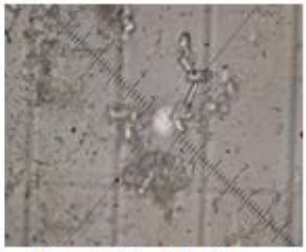


Fig. 5 After 60 min
10 ml of Acacia Radiana

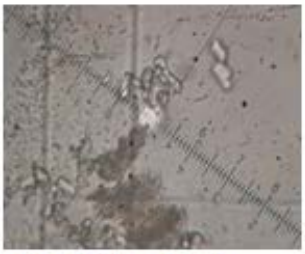
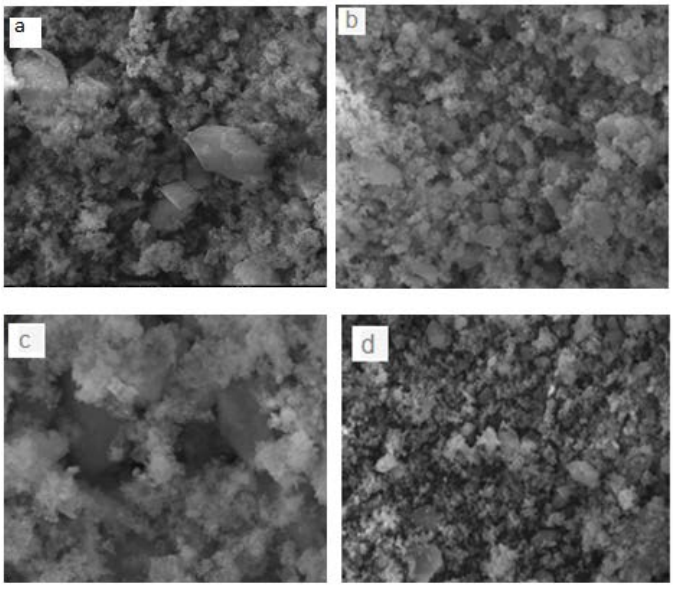


Fig. 6 After 240 min
15 ml of Acacia Radiana

It is observed that 1 ml can aggregates appeared from 1 to 2 in field, 15 ml against the number increased by 3 to 4 in field. The addition of 1 to 5 ml of inhibitor delay the onset of the aggregates at 10 minutes. The minimum size of the aggregates is 52 microns is 69%. of inhibition. The results of our series of experiments of crystal growth of struvite are represented in microscopic images. The images on the left and right, present in process struvite crystals grown from artificial urine in the time of the growth, in the absence and presence of the acacia radiana respectively.

Micrographs of this sample were performed under secondary electron mode high voltage 30KV. These images include those obtained at low magnification (Sample1_008 and 010) show aggregates of crystallized forms ranging in size ENTERED 2 and 10µm. These crystallites. Insofar as the X energy dispersive analysis is (EDX) is shown on these crystallites appear to those consisting mainly of calcium and phosphorus with a lower proportion of carbon and manganese.



The effect of extracts plant used in this work was seen in a remarkable manner. In assessing the inhibitory Acacia Radiana in electron micrographs of crystals Action we found. (Fig b) shows crystals of struvite have made a considerable difference in the nucleation stage. These crystals in nucleation rate and the characteristic morphology observed struvite comparing with the growth (Fig a) without inhibitor.To achieve equality of uncomplexed phosphate ions were matched (as in (Fig. d) and had a great concentrations in the absence and presence of hexagonal face at rest. Present, the total concentration of crystals rate should be,however, have wide and were significantly raised compared with platform (Figure c) in absence of inhibitor.

IV. Discussion

We explain these results due to the fact that the extract of Acacia Radiana (bark) has inhibitory activity on crystal sizes better than (sheet) : 73% and 55.5 % inhibition , respectively, the best results , unlike the effect on aggregate (sheet) has an inhibitory capacity 68% better than (bark), which reached 43 %. Several studies are performed using a microscope to confirm the results obtained by the turbidimetric model [10] . Then , we observe that the reduction of the number and size of crystals and aggregates are important for photographs 4, 5 and 6 comparing photographs. Experiment with the inhibitor 1 ml of extract Acacia Radiana (bark) has allowed us to reduce the size of the crystals is important that the inhibitor after 5 minutes crystallization stage of germination, which explains the importance inhibitory capacity. In photography in step 4 corresponding to the aggregation assays with the inhibitor 10 ml after 1 hour aggregates are reduced compared to the photo 2 without inhibitor , the effectiveness of Acacia Radiana inhibiteur (sheet) is more effective in picture 6 after 4 hours and 15 ml of concentration corresponds to the point of aggregation for assays without inhibitor in photo 3 , which explains a significant amount of inhibition on the aggregation of crystals. Many works have shown that extracts of medicinal plants are capable of producing significant products crystallization crystals [11] . Other researchers confirmed our results prevention and inhibition of phosphate calculi in human body. However, the occurrence of phosphate calculi in the body is a much more complex phenomenon that is occurring under dynamic conditions in which urine continuously flows. Nevertheless, this in vitro study provides basic information to identify the potent inhibitors. Both of these aqueous extracts contain many complex macro-bimolecular and the roles of these molecules are very important in the growth and inhibition study[12].

V. Conclusion

In conclusion, the different results obtained in our work (the percentage inhibiting, the microscopic photographs) show that the extract of Acacia Radiana bark and sheet effect is

important in the vitro study. photographs obtained by microscope, clearly show that the extracts clear at the stage of germination and aggregation. This study is useful to formulate the necessary dosages to prevent and cure urinary calculi. In this investigation, medicinal plant proved to be a good inhibitor.

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