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ORIGINAL PAPER

Effect of indigenous plant extracts on calcium oxalate crystallization having a role in urolithiasis

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Abstract Crystallization process has a major role in urolithiasis. In the present study, effect of two indigenous plants extracts namely *Boerhavia diffusa* and *Bryophyllum pinnatum* extract was determined on the crystallization of calcium oxalate crystals. Effect on the number, size and type of calcium oxalate crystals was observed. Results showed significant activity of both extracts against calcium oxalate crystallization at different concentrations ($P < 0.05$). Size of the crystals gradually reduced with the increasing concentration of both extracts. The number of calcium oxalate monohydrate crystals which are injurious to epithelial cells gradually reduced and at the highest concentration of extracts (100 mg/ml) completely disappeared ($P < 0.05$). These results confirm that *B. diffusa* and *B. pinnatum* extracts have antiurolithic activity and have the ability to reduce crystal size as well as to promote the formation of calcium oxalate dihydrate (COD) crystals rather than monohydrate (COM) crystals. Control of crystal size and formation of COD rather than COM crystals, in combination with the diuretic action of extracts is an important way to control urolithiasis.

Keywords Urolithiasis · Calcium oxalate crystallization · *B. diffusa* · *B. pinnatum* · Microscopy

Introduction

Urolithiasis is a common disorder and population studies conclude that 1 in every 1000 people pass a calcium oxalate calculus each year [1]. It is also characterized by a high rate of recurrence which is reported to range from 40% within 3 years of the first incidence of kidney stones to 74% within 10 years and 98% within 25 years [2]. Results show that a greater number of cases of patients suffering from urolithiasis occur in countries situated in the Afro-Asian tropical and subtropical regions [3].

Among the various types of stones, calcium oxalate is the most complex and difficult stone to treat. During the last decade, tremendous work has been done on calcium oxalate stones, in an attempt to disclose their etiology and to discover proper methods for its treatment.

Since urolithiasis is a multifactorial disease, its etiology is very complex and highly unpredictable. Various physicochemical factors contribute to the production of kidney stones. It is an accepted fact that kidney stone formation is a crystallization process which takes place in supersaturated urine [4]. Human urine is almost always supersaturated with a mixture of various ions and macromolecules, including calcium oxalate—the most common component of kidney stones. The process of crystallization involves crystal nucleation, growth and aggregation which ultimately results in the retention of these particles within urinary tract [5].

All the available surgical and medicament treatments for urolithiasis are highly associated with certain risk factors [6]. Stone recurrence is one of the side effects of these treatment modalities. Therefore, the present need is to find some better alternative to these conventional methods [7]. For the last few years, lot of emphasis has been given to explore some natural substances that could act as promising candidate for the prevention and treatment of urolithiasis [8]. Medicinal plants have always played a central role in the discovery of drugs, by providing a variety of active ingredients. This is the reason why significant amount of research

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work is taking place in the field of traditional phytotherapy all over the world [9].

Nowadays, herbal medicine has gained much popularity both in Europe and in the United States [10]. Use of Chinese herbal medicine has also been increased in China and Hong Kong [1]. Some herbal medicines such as Kampo medicine have been found to inhibit calcium oxalate crystallization in vitro [6]. Many plants like *Phyllanthus niruri, Aerva lanata*, *Crataeva nurvala* and *Herniaria hirsuta* have also shown effective antiurolithic activity [11–14]. The juices of lemon and orange are also widely used to inhibit crystallization [15].

Therefore, in light of all these facts, this study was carried out to determine the beneficial effects of indigenous plant extracts for the treatment of urolithiasis and possibly identifying the exact mechanism underlying the beneficial effects of these herbs [16].

Materials and methods

Plants of *Boerhavia diffusa* and *Bryophyllum pinnatum* were collected locally and dried under the shade. The dried plants were crushed and grinded to fine powder. This material was used to prepare ethanolic extracts. Different concentrations of each extract were prepared. Desired amounts of extract were weighed and dissolved in the required quantity of distilled water to get final concentrations of 1, 4, 8, 16, 32, 64, 80 and 100 mg/ml. Finally the solutions of different concentrations were filtered through 0.45 µm filter.

Fresh urine sample was collected from a healthy male individual of 30 years and checked by dipstick. Aliquots of 2 ml urine were taken in test tubes and pre-warmed at 37°C in a water bath. 50 µl of different concentrations of extracts were added in the respective tubes. In the control, extract was not added. 50 µl of 0.1 mol/l sodium oxalate solution was added to induce crystallization. The tubes were incubated in a shaking water bath at 37°C for 30 min [17].

After the completion of the crystallization assay, 10 µl from each tube was taken on to a slide and observed under the Nikon Eclipse light microscope and pictures were taken with Nikon digital sight camera. The size and types of calcium oxalate crystals, i.e.; calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD) were recorded.

The data was analyzed through Sigma Plot version 11 and expressed as a mean ± SEM. The differences between results were analyzed by using the Student's *t* test and a *P* value less than 0.05 was considered to be significant for all the analyzed data.

Results

In this study, it was found that the extracts of *B. diffusa* and *B. pinnatum* increased the crystallization process in a concentration dependent manner (Fig. 1, $R^2 = 0.970$; Fig. 2, $R^2 = 0.723$). The increasing concentration not only increased the number of crystals but interestingly the size of crystals as well got reduced (Figs. 3, 4, 5). Another important finding which is obtained from this study is that crystals of COD are formed rather than COM in the presence of both extracts (Figs. 7, 8).

The size of COM crystals was significantly reduced at the concentrations of 32, 80 and 100 mg/ml ($P < 0.05$) of *B. diffusa* extract (Fig. 4). COD crystals size was also significantly reduced at the concentrations of 16–100 mg/ml ($P < 0.05$) of *B. diffusa* extract (Fig. 3). *B. pinnatum* extract gradually reduced the size of COM crystals having significant effect at 64, 80 and 100 mg/ml ($P < 0.05$) (Fig. 5). Both the extracts have given surprising effects at their highest concentrations of 100 mg/ml that all the COM crystals were totally disappeared from the test system (Figs. 7, 8).

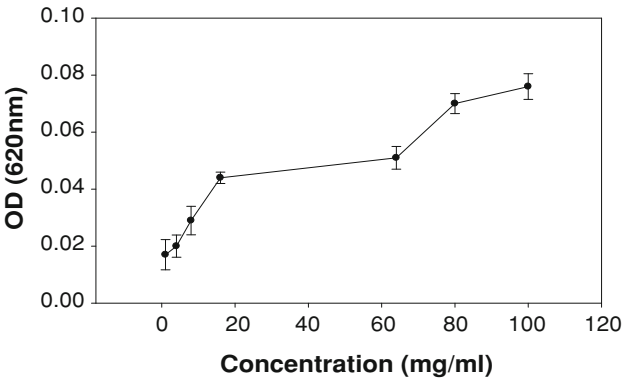

Fig. 1 Effect of increasing concentrations of *B. diffusa* extract on calcium oxalate crystallization measured by optical density (OD 620 nm). The *error bars* represent ±SEM

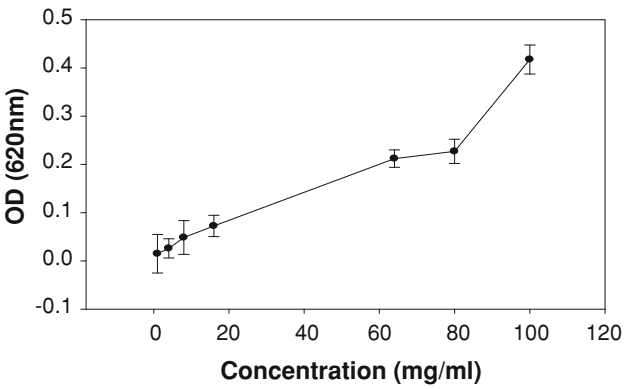

Fig. 2 Effect of increasing concentrations of *B. pinnatum* extract on calcium oxalate crystallization measured by optical density (OD 620 nm). The *error bars* represent ±SEM

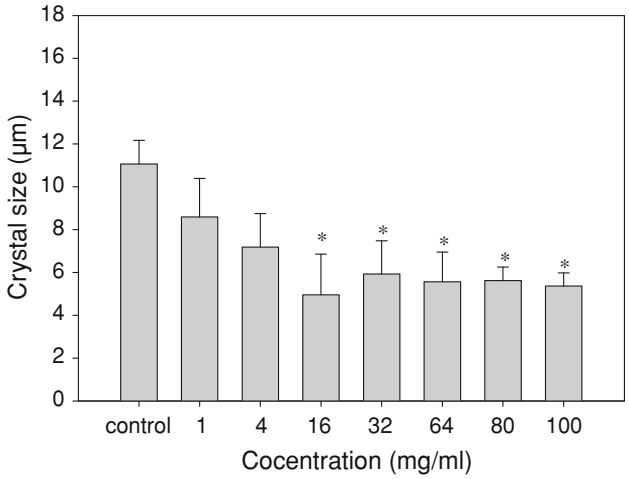

Fig. 3 The effect of increasing concentrations of *B. diffusa* extract on COD crystal size. *Error bars* represent ±SEM. (*$P < 0.05$)

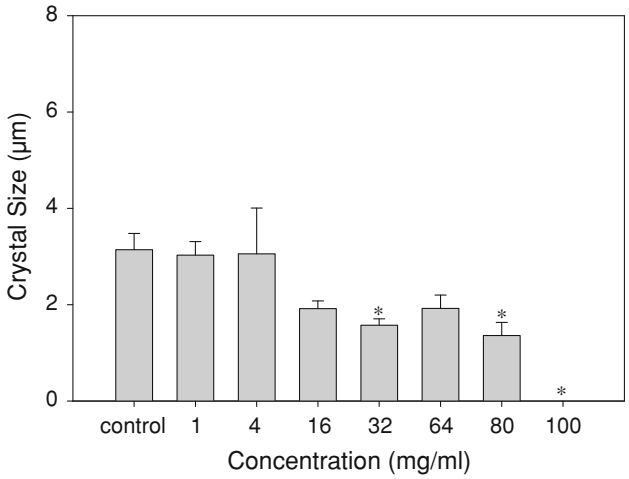

Fig. 4 The effect of increasing concentrations of *B. diffusa* on COM crystal size. *Error bars* represent ±SEM. (*$P < 0.05$)

Discussion

It is an accepted fact that kidney stone formation is the result of pathological crystallization events which takes place in supersaturated urine [17]. Therefore, for the prevention and treatment of urolithiasis, it is important to control the crystallization events [18]. Use of herbs can be a best source to overcome the pathological crystallization events as these have been widely used in folk medicine to treat kidney stones. Incorporation of the extracts of *B. diffusa* and *B. pinnatum* in the calcium oxalate crystallization assay has shown tremendous effect on the calcium oxalate crystal size and number.

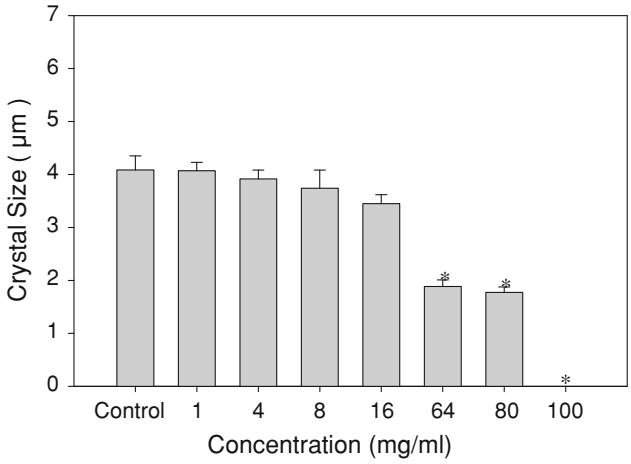

Fig. 5 The effect of increasing concentrations of *B. pinnatum* extract on COM crystal size. *Error bars* represent ±SEM. (*$P < 0.05$)

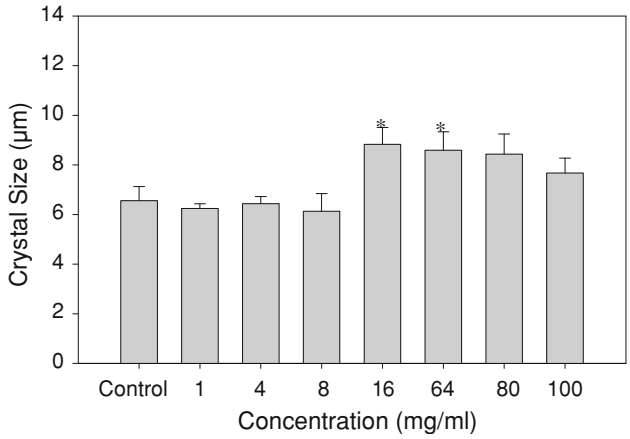

Fig. 6 The effect of increasing concentrations of *B. pinnatum* extract on COD crystal size. *Error bars* represent ±SEM. (*$P < 0.05$)

Boerhavia diffusa is a common indigenous herb which is widely used in this sub-continent for different ailment conditions. Its diuretic effects signify its importance as antiurolithic herb. In the calcium oxalate crystallization assay, the incorporation of its extract increased calcium oxalate crystallization but with reduce sized calcium oxalate monohydrate and dihydrate crystals. The small sized crystals can easily pass through the urinary tract as compared to the larger crystals because they have the least tendency of retention in the urinary tract. These findings are also in agreement with some previous findings by others [19]. Formation of COD crystals rather than COM in the presence of the extract is advantageous as COD crystals are considered as less urolithic than COD because these do not damage the epithelial lining of urinary tract [20].

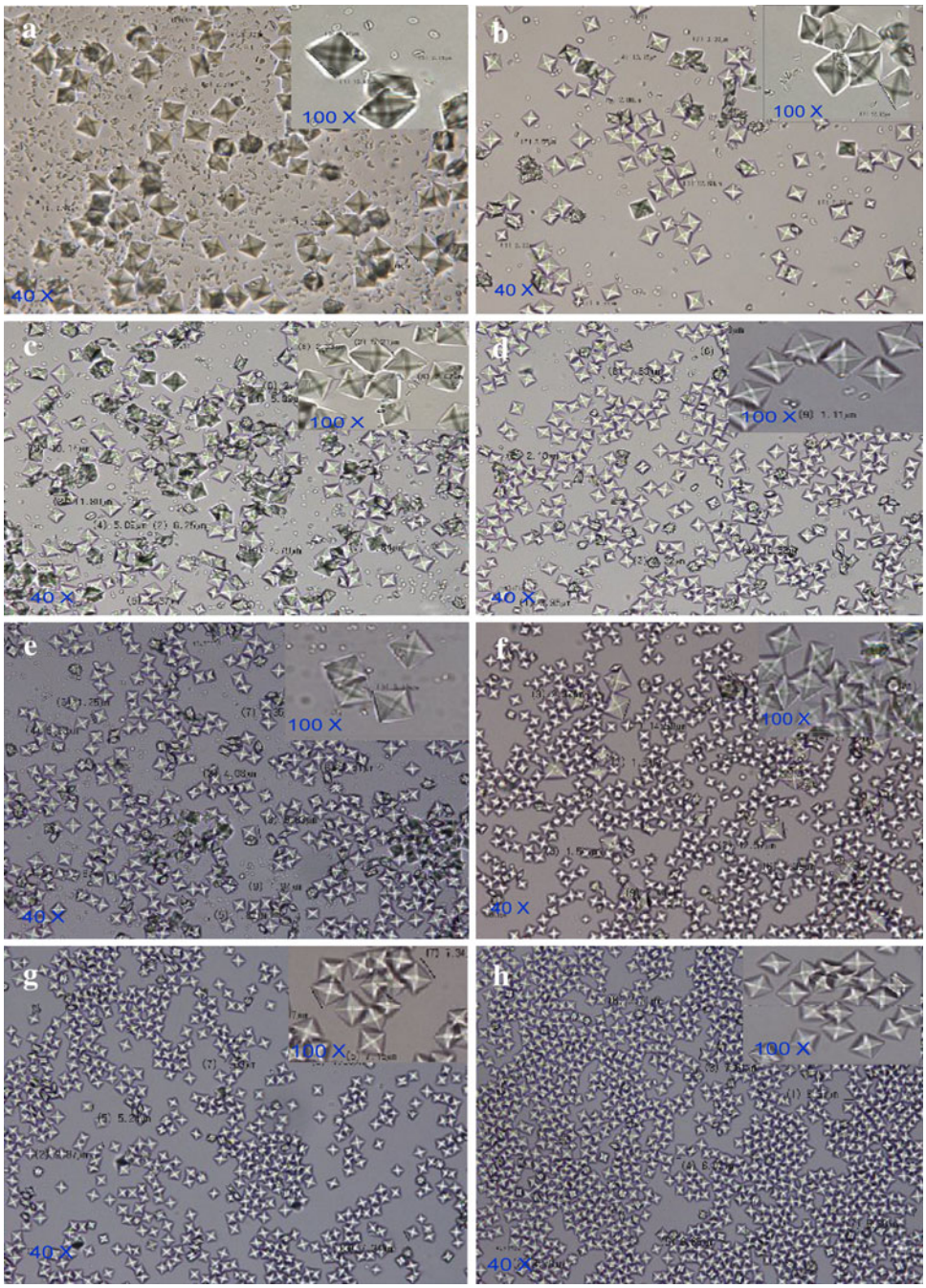

Fig. 7 Light microscopy of calcium oxalate crystals induced in in vitro, in the absence (**a**) and in the presence of different concentrations of *B. diffusa* extract, 1 mg/ml (**b**), 4 mg/ml (**c**), 16 mg/ml (**d**), 32 mg/ml (**e**), 64 mg/ml (**f**), 80 mg/ml (**g**), 100 mg/ml (**h**) at ×40 and ×100

Similarly, *B. pinnatum* which is selected in this study is commonly used by local people to expel kidney stones. Its common use assures its importance for the treatment of urolithiasis. *B. pinnatum* also showed significant activity against calcium oxalate crystallization. It increased calcium oxalate crystallization in a concentration dependant manner (Fig. 2, $R^2 = 0.723$). It also promoted the formation of COD crystals and gradually reduced the size of COM crystals and at its highest concentration totally inhibited the formation of COM crystals (Figs. 5, 6). The assertion of this research study rests on the belief that the natural compounds can be beneficial in treating renal stone formation by preventing super saturation of urine and the growth of crystals.

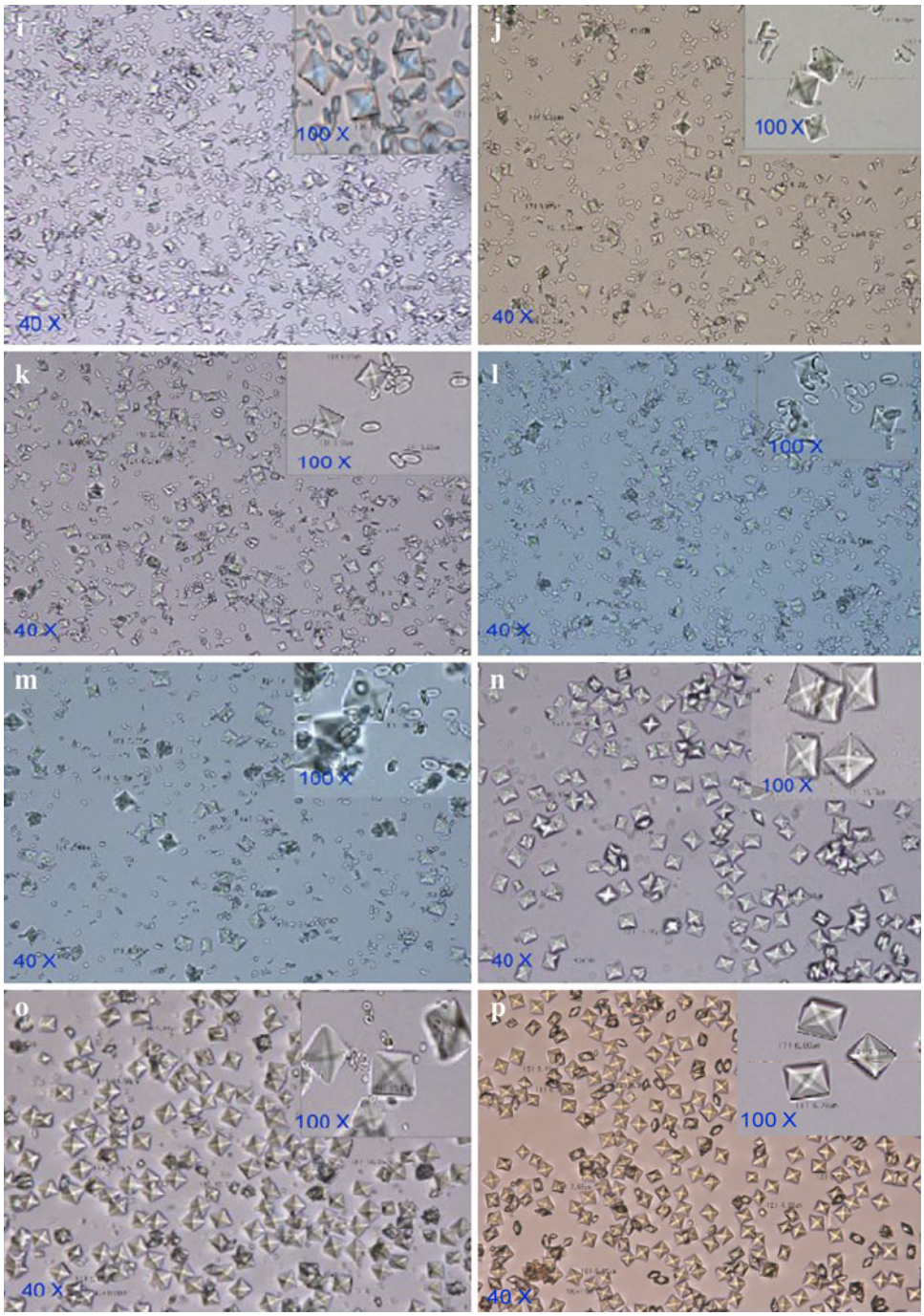

Fig. 8 Light microscopy of calcium oxalate crystals induced in in vitro, in the absence (**i**) and in the presence of different concentrations of *B. pinnatum* extract, 1 mg/ml (**j**), 4 mg/ml (**k**), 8 mg/ml (**l**), 16 mg/ml (**m**), 64 mg/ml (**n**), 80 mg/ml (**o**), 100 mg/ml (**p**) at ×40 and ×100

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