Effect of quince (*Cydonia oblonga* Miller) fruit extract against oxalocalcic crystallization

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Abstract. Kidney stone or Urolithiasis is a solid particle that forms in the urinary tract. In Morocco, as in many countries, most patients use medicinal plants as an alternative therapy for many diseases, including lithiasis. The fruit of *Cydonia oblonga* Miller. is traditionally used for the prevention and treatment of several diseases. The present study aims to explore the effects of aqueous extracts of pulp and peel quince on oxalocalcic crystallization. These results show that the aqueous extracts of *C. oblonga* M can inhibit calcium oxalate crystallization.

1 Introduction

Urinary calculi affect a large part of the population, it is evident that urine supersaturation is an important factor in the formation of these urinary concretions. Indeed, when the oversaturation is sufficient, it leads to the nucleation of crystals and their growth, then followed by aggregation, which lead to the formation of stones [1]. In general, kidney stones are composed of calcium salts, uric acid, cystine and struvite, depending on the main etiological entity. Each type of stone has its own group of causes, so the management of each entity is specific [2].

According to the literature, approximately 70% of human urinary stones contain calcium phosphate and calcium oxalate. Calcium oxalate monohydrate (COM) or Whewellite ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$), calcium oxalate dihydrate (COD) or Weddellite ($\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$), and calcium oxalate trihydrate (COT) or CaOxite ($\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$) are three forms of hydrated calcium oxalates, which can present in urinary stones [3].

Urine contains many molecules able to inhibit crystallization. Dietary factors appear to have an effect on the ability of urine to inhibit calcium oxalate crystallization[1]. The potential use of natural substances to inhibit crystallization is more widespread, we therefore tested the inhibitory effects of *Cydonia oblonga* Miller., the common Quince, belonging to the Rosaceae family, genus *Cydonia*[4].

In this paper, we report the effects of aqueous extracts of quince fruit on experimental calcium oxalate crystals.

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2 Material and Methods

2.1 Fruit collection and extract preparation

Healthy quince (*C. oblonga Miller*) fruit samples were collected in October 2019 from the region of Beni Mellal - khenifra, fruits were separated into peel, pulp and seeds, packed into plastic bags and frozen at -20 °C until used.

Aqueous extract of *C. oblonga Miller* was obtained by maceration of homogenized fruit material in water for three days in a refrigerated chamber at 4°C in the dark, then the mixture was centrifuged at 10000g for 15 min at room temperature, the supernatants were collected by filtration using Whatman Filter Paper (pore size 6 mm). Extracts were kept at 4°C in the dark, the extract's concentration was between 1 and 10 mg/ml.

2.2 Crystallization of CaOx

The inhibition of the crystallization was studied according to the method described by Beddada et al [5], In this respect, stock solutions of calcium chloride (CaCl_2) and ammonium oxalate $(\text{NH}_4)_2\text{C}_2\text{O}_4$ were prepared at a final concentration of 4 mmol/L, in a buffer containing sodium acetate (0.2 mol/L) and NaCl (0.15 mol/L) at pH 6. Before being used in crystallization experiments, both solutions were filtered through an 0.22 mm filter and warmed to 37 °C in order to approximate urinary physiological conditions.

2.3 Crystallization without inhibitor

CaCl_2 solution of 4 mM was transferred into in a beaker, to which we added an equal volume (10 ml) of equimolar solution of $(\text{NH}_4)_2\text{C}_2\text{O}_4$, to trigger the formation of the crystals. After incubating the mixture for 2 h under stirring in the water bath at 37 °C, the crystals were analysed morphologically under light microscope.

2.4 Crystallization with inhibitor

The inhibitor effect on crystallization was studied with several concentrations of the extract ranging from 1 to 10 mg/ml. Nevertheless, it should be mentioned that the inhibiting substance was added to the sodium acetate solution to adjust the pH value to 6. The experiments were carried out with 10 mL of CaCl_2 and 10 mL of the extract to which 10 mL of $(\text{NH}_4)_2\text{C}_2\text{O}_4$ was added, which promotes both the formation of the crystals.

2.5 Microscopic observation

The solutions were maintained at 37 °C in a water bath under constant stirring for 2 hours, then the number and the morphology of crystals in each concentration of extract was carried out using a polarized optical microscope. Each test was performed in triplicate.

2.6 Crystal characterization

The crystals were recovered through a 0.22 µm microfiltration membrane and dried under vacuum at 37 °C for 24 hours. The obtained solid samples were characterized by X-ray diffraction (XRD), also the chemical composition of the crystals was determined by Fourier transform infrared spectra (FTIR) between 4000 and 450 cm^{-1} with a resolution of 4 cm^{-1} .

3 Results

Fig. 1 Micrographs of calcium oxalate crystals in the presence of aqueous extract of *C. oblonga* Miller. pulp: (a)= 0 mg/ml, (b)= 1 mg/ml, (c)= 5 mg/ml, (d)= 8 mg/ml and (e)= 10 mg/ml (Gross×200)

3.1 Microscopic observation

The preventive effect of the aqueous extract of quince pulp and peel against the calcium oxalate's crystallization was investigated in vitro at a constant temperature of 37 °C using synthetic super saturated urine. The latter was prepared with calcium dichloride CaCl_2 and

ammonium oxalate $(\text{NH}_4)_2\text{C}_2\text{O}_4$. However, the development of calcium oxalate crystals was observed under a microscope after magnetic stirring of a synthetic urine according to the content of the aqueous extract of *C. oblonga* Miller pulp and peel in order to detect the minimum concentration of the aqueous extract which can inhibit the crystallization of calcium oxalate.

The light microscopic photographs taken of the control group reveals the presence of aggregates in addition to a predominance of the crystals as shown in figure 1(a). Furthermore, the incubation of the calcium oxalate crystals in the presence of the different concentrations fruits extracts caused significant decreases in the number and size of the latter compared to control group in a dose-dependent manner. Also, the analysis of the pictures taken for different samples in figure 1 and figure 2 shows that treatment with the *C. oblonga* Miller peel extract had a more notable effect than *C. oblonga* Miller pulp extract on CaOx crystals inhibition, significantly.

Fig. 2 Micrographs of calcium oxalate crystals in the presence of aqueous extract of *C. oblonga* Miller. peel: (a)= 1 mg/ml, (b)= 5 mg/ml, (c)= 8 mg/ml and (d)= 10 mg/ml (Gross×200)

Comparison of the images in both figure 1 and figure 2 shows that *C. oblonga* Miller peel and pulp extracts at a dose of 1 mg/mL decreases the number and size of crystals by reducing supersaturation. Increasing the extracts concentration shows a significant effect on crystal size and number. This result can be demonstrated by the inhibition of growth and crystal

aggregation by the polyphenols contained in the plant or by the dissociation of the aggregates by the bioactive molecules of the extracts.

3.2 XRD analysis of CaOx crystal's

The XRD pattern of the synthesized crystals was fitted by Rietveld refinement using Full prof software. Figure 3 shows the XRD experimental pattern presented by the black line, while the red line shows the calculated data and blue line provides the differences between both. It can be observed that the crystal phase composition of the solid shows diffraction peaks at 14.919° , 24.381° , 30.072° , 35.978° , 38.142° , 40.588° , and 43.478° which correspond to the (-101), (020), (-202), (112), (130), (202), and (321) [6], [7] (PDF #21-0838) respectively, revealing the presence of COM. In addition, we detected the peaks at 11.019° and 16.113° representing the index of reflective planes for (011) and (110) [8], suggesting the presence of DOC and TOC respectively. These data indicate clearly the monoclinic structure of the synthesized crystals.

Fig .3. XRD experimental pattern, calculated data and difference between both of synthesized calcium oxalate crystals.

3.3 Characterization by Fourier Transform Infrared Spectroscopy (FTIR).

The synthesized crystals were also examined by FTIR measurements in order to verify their chemical constitution. The obtained FIIR spectra of sample is illustrated in figure 4.

Fig. 4. FTIR spectra of the synthesized calcium oxalate crystals

According to Figure 4, multiple staircase bands at 3483 to 3057 cm^{-1} critical to a valence vibration of the OH stretching of water and corresponding to COM crystals. While the COD crystals show only one absorption peak at 3423 cm^{-1} [9], [10]. In addition, the absorption band due to the balancing vibration of the coordinated water plane appears at 780 cm^{-1} , also two bands around 949 cm^{-1} and 660 cm^{-1} appear, characterizing the formation of COD [7]. The absorption band at 885 cm^{-1} results from the C=C stretching mode and the band at 517 cm^{-1} is caused by the in-plane bending of O=C=O [10]. While absorption bands were observed at 1607 cm^{-1} and 1380/1315 cm^{-1} , the former are attributed to the antisymmetric carbonyl stretching band (vas (COO-)) and the latter are consistent with the symmetric metal carboxylate stretching band (vs (COO-)); these bands correspond to COT crystals [11]. XRD and FTIR measurements confirmed the presence of mixed crystals between COM + COD + COT.

4 Discussion

Numerous studies have shown that the species *Cydonia oblonga* Miller is an excellent natural source of phenolic acids and flavonoids. In this study, the in vitro inhibitory effect of aqueous extract of *C. oblonga* Miller peel and pulp on crystallization was evaluated at different concentrations of the extract. In order to valorise and verify the effectiveness of antilithiatic activity of *C. oblonga* Miller. The results of this investigation provide evidence that *C. oblonga* fruit can have a litholytic effect. In addition, our results are similar to those of Beddade *et al* [5], [12] have studied the effect of aqueous extract of *Z. Lotus* and *A. Unedo* on the crystallization of calcium oxalate in aqueous solution and in the Human in vitro, and in a recent study conducted by Kachkoul *et al* [13] two extracts of *Punica granatum* L. fruit

peel, it was found that they had an anticrystallization capacity. Another study suggested that the methanol soluble fraction isolated from *Hamulus lupulus L.* was the most effective on inhibition and dissolution of calcium oxalate crystals model [2]. The study carried out by Zaki *et al* [14] showed that the hydroalcoholic extract of *C. zeylanicum* has a significant inhibitory effect on the crystallization of CaOx, but not in a concentration-dependent manner.

5 Conclusion

In conclusion, we found that quince fruit extracts, particularly the pulp and peel, had significant effects. To our knowledge, this is the first report concerning the anticrystallization activity of this fruit. Nevertheless, such in vitro results need to be confirmed in vivo in order to develop a potent antilithic drug. In addition, the mechanism by which the drug exerts its effect in the animal model of lithiasis needs to be further studied.

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References

1. S. Kulaksizoğlu, M. Sofikerim, and C. Çevik **40** 589–594 (2008)
2. A. Frckowiak, T. Kozlecki, P. Skibinski, W. Gawe, E. Zaczynska, A. Czarny, K. Piekarska, R. Gancarz. *J. Cryst. Growth* **312**, 3525–3532 (2010)
3. V. Tszzorl and C. DOTVTBNECGBRRT. *Am. Mineral.* **65**, 327–334 (1980)
4. W. Zhou, A. Abdurahman, A. Umar, G. Iskander, E. Abdusalam, B. Berké, B. Bégaud, N. Moore. *J. Ethnopharmacol.* **154**, 163–169 (2014)
5. M. El Bir, M. Oubenali, M. Echajia, S. Rabi, and M. Berkani. **23**, 583–589 (2018)
6. Y. Zhang, Y. Tang, J. Xu, D. Zhang, G. Lu, and W. Jing. *J. Solid State Chem.*, 231, 7–12 (2015)
7. J. Gao, J. F. Xue, M. Xu, B. S. Gui, F. X. Wang, and J. M. Ouyang. *Int. J. Nanomedicine* **9**, 4399–4409 (2014)
8. Y. Shen, W. Yue, A. Xie, S. Li, and Z. Qian. *Colloids Surfaces B Biointerfaces.* **45**, 120–124 (2005)
9. R. Kachkoul, T. Sqalli Houssaini, Y. Miyah, M. Mohim, R. El Habbani, and A. Lahrichi. *Prog. en Urol.* 28, 156–165 (2018)
10. S. Bhattacharyya, A. K. Mandal, and S. K. Singh. *J. Postgrad. Med. Educ. Res.* **48**, 128–131 (2014)
11. R. Kachkoul, T. Squalli Housseini, M. Mohim, R. El Habbani, Y. Miyah, and A. Lahrichi. *J. Integr. Med.* **17**, 430–437 (2019)
12. L. Baddade, I. Elhadri, and M. Berkani. *Journal of Biomedical Research and Health Economics.* (2), 1–5 (2020)
13. R. Kachkoul, T. S. Houssaini, M. Mohim, R. El Habbani, and A. Lahrichi. *Evidence-based Complement. Altern. Med.*, **2020**, 1-14 (2020)
14. S. Zaki, N. Jahan, M. Kalim, and G. Islam. *J. Integr. Med.*, **17**, 273–281 (2019)