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Antilithiatic effect of watermellon *(Citrullus lanatus)* juice on Calcium Oxalate kidney stones

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Abstract

Background: Nephourolithiasis is a term refers to kidney stones formation, due to aggregation of minerals leading to the development of crystalluria in kidneys or any part of the urinary tract system. Many herbs were used to fragment and crush renal calculi. Habitually, it is noted that people who suffer from kidney problems are advised to eat large quantities of watermellon fruit then, the patient feels healing, without knowing the scientific reason for this improvement.

Objectives The current study was designed to investigate the antilithiatic effects of Fresh Watermelon Juice (FWJ) on calcium oxalate kidney stones.

Methods Direct exposure technique between FWJ and enucleate stons was used. The pH, and electrical conductivity (EC) of the exposure solution in addition to the chemical and morphological properties of the renal calculi before and after exposure using X-ray diffraction method (XRD) and scanning electron microscope (SEM) were investigated.

Results: after 24 hours of submerging renal calculi with FWJ, the calculi appeared dissolved and fragmented with absence of solidity, rigidity and crystallization. The Ph Value and electrical conductivity (EC) of FWJ increased significantly after 24 hours of submerging the calculi increasing total soluble solids and ion concentrations in the solution. X-ray diffraction showed sharp peaks and intensity of of calcium oxalate calculi indicating a significant change in calculus compositionn, shape and crysallization within 48 hours of exposure FWJ.Scanning electron microscope images of treated and untreated renal calculi showed a significant difference in the shape of the crystals that have been transformed from their regular shape as bipyramids with tetragonal crystal point group symmetry to sculpted and un coordinated particles with the absence of a regular geometric shape of crystals with no clear crystallization patterns.

Conclusion: Fresh watermellone juice appears to have a strong effect on the shape and composition and integrity of renal calculi which may aid its expulsion from the body.

Keywords: *Renal calculi; Calcium oxalate; X-ray diffraction; Watermelon; Antilithiatic.*

1. Introduction

Renal calculi are common and a widespread health problem around the world. Between 4% to 15% of population are affected with renal calculi causing serious sickness and severe pain (Agarwal and Singh, 1991; Stamatelou etal, 2003; Nakata, 2003; Parks et al, 2003; Minevich 2001). Calculi may consist of oxalate, uric acid or phosphate crystals. The largest percentage of renal calculi are located in the kidney while the rest proportion is lodged in the urethoura or urinary bladder. The real cause of kidney stone is still unclear and not fully understood (Selvam, 2002; Huang, 2003; Wesson and Ward, 20097; Farmanesh etal, 2014). Many factors contribute to the formation of renal calculi, including food habits, life style, geographical and environmental factors, soil and water quality in addition to morph-anatomy of the urinary system. About 80% of renal stones are calcium stones, calcium phosphate (CaP) or calcium oxalate (CaOx) (Sheng etal, 2005; Borissova etal 2010; Gardner, 1975). Calcium oxalate (CaOx) has thouree types, calcium oxalate monohydrate COM (wheelie), calcium oxalate dihydrate COD (weddellite) and calcium oxalate trihydrate COT.

Traditionally, medicinal plants were used to treat many types of diseases and health disorders (Haleem etal,2013; Haleem, 2013). Many medicinal plants and their extracts were used to prevent renal stone synthesis or mitigate their effects extracts of *Herniaria hirsute* (Atmani etal, 2006) watery extracts of *Foeniculum vulgare* and *Cymbopogon proximus* (Ibrahim and Al-Khateeb, 2013) roots watery extract of *Alhagi maurorum* (Hassan and Al-Abasi, 2013), *Nigela sativa* (Mahdi etal, 2017), watery extracts of *zea mays* hair (Jassim, 2017), *Ammodaucus leucotrichus, Ajugaiva* (Beghalia, 2017) have been all used to alleviate the effects of renal calculi around the world. In this study, we used fresh juice of Watermelon *(Citrullus lantu*s) on calcium oxalate stones *In Vitro.* Watermelon belongs to *Cucurbitaceae* family cultivated in Africa and South East Asia. The fleshy part of watermelon is a good sources of vitamin C, precursor of vitamin A, amino acids, potassium, beta carotene, lycopene and citrulline. It is also low in fats, with no cholesterol, and high watery content which make it a favorite food for diets and hypertension patients because of its diuretic and antihypertensive nature (Alim etal, 2012; Okafor etal, 2015; Fuhourman etal 2000, Perkins etal, 2006). Watermelon has many therapeutic roles as diuretic agent (Somia etal 2014), and its seeds have been used to treated induced hyperglycaemia in Wistar Rats, besides antimicrobial activity of its seeds extracts (Omigie and Agoreyo, 2014; Godwin etal, 2015), as well as the ability of watermelon juice in reducing the toxicity of drugs (Olaniyan etal, 2016).

2. Materials and Methods

2.1. Preparation of watermelon juice

Watermelon fruits were collected from local markets of Baghdad city from October 2018 to February 2019. All fruits were washed by running tap water to remove suspended dust and cut by sharp knife to small pieces after removing of peels and seeds. Colored parts of the fruit were then mixed by an electrical blender for five minutes. The mixture was passed thorough gauze to get rid of large pieces, another filtration step was performed by Wattman filter paper, and the above procedure was repeated whenever needed.

2.2. Renal calculi samples

Twenty-five samples of kidney stones were obtained from surgical clinics, private and general hospitals from patients who underwent surgical removal off kidney stones. The contents of the stones were analyzed using a specialized laboratory kit (LTA kidney stone analyses kit, (Analitica, Italy). Calcium oxalate stones were recorded in 64% of the samples followed by uric acid stones in24% of the samples as shown in the Table (1).

Table (1): Percentages of kidney stones

Stone type	Number	Percent%
Calcium Oxalate Stones	16	64
Uric Acid Stones	6	24
Calcium Phosphate Stones	2	8
Cystine Stones	1	4

2.3. Effects of watermelon juice on renal calculi

Calcium oxalate stones (n=16) were in the study. The stone weights ranged between 0.17 to 0.32 gm with a dimension ranged between 3-9 mm Each stone was placed in (100) mL polyethylene container and submerged with (25) mL of the watermelon juice. The morphological changes in the shape of the renal calculi were recorded as well as chemical changes of the juice during a period from (0 – 24) hours. The pH value and electrical conductivity (EC) were measured for prepared watermelon juice by (radio meter, Denmark and pen conductivity meter, China), respectively every hour for 24 hours.

2.4. X-ray diffraction

The chemical properties of the calcium oxalate stones were studied before and after exposure to the watermelon juice using XRD technique (XRD -

Shimadzu 6000, Japan), X - ray emitter (Kα- Cu), λ =54.1Å, 40 KV, 30mA.

2.5. Scanning Electron Microscope Analysis

Kidney stone samples were prepared to SEM analysis by coating with a 5-nm-thick gold layer in a SPI-Module Sputter/Carbon Coater System (SPI Inc., USA). All coated specimens were examined via a dual beam Focused Ion Beam/Scanning Electron Microscope (FEI Company, USA) at 20 kV and magnification force is equal to 750X.

3. Result and Discussion

3.1. Morphological changes of renal calculi

An immediate reaction occurred between watermelon juice and submerged renal calculi, started with a rising of bubbles associated with buzz and ebullition sound. These phenomena continued about a quarter of an hour without any noticeable changes in solution clarity, after that the intensity of the reaction was reduced but the observation continued for 24 hours to record the morphological changes of the submerged calculi. Figure (1) shows the shape of calculi sample after exposure period (24) hours. They looked dissolved and fragmented with absence of solidity and rigidity beside lacking of crystallization.

Figure (1): Dissolved and fragmented of submerged renal calculi with FWJ after 24 hours.

3.2. Changes in pH value

The pH value of FWJ without any treatment was 5.6±0.02 at 25ºC. This value increased to reach up to 6.5 ±0.03 after 24 hours of renal calculi placed inside the juice container. The increasing rate about 13.84% that indicates to mix of renal calculi components with juice and causes elevation in pH value. Figure (2) shows high correlation between exposed time and pH value (R2 = 0.9834).

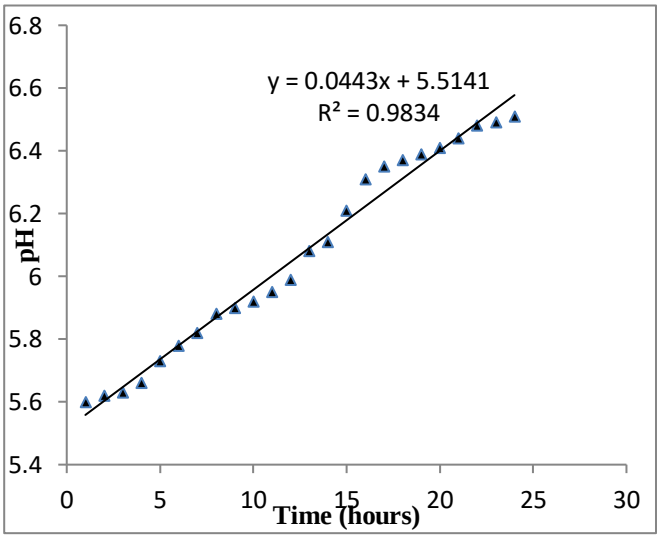

Figure (2): pH value of watermelon juice and renal calculi from 0-24 hours.

3.3. Changes in EC measurement

Electrical conductivity values are affected by the concentration of total soluble solids (TSS) in the solution, the value of the acidity and the temperature (Omigie and Agoreyo, 2014; Godwin et al, 2015). In this study. there was an increased in EC value according to exposed time due the increasing of total soluble solids and ions movement, from 0.167 S/m, EC value for FWJ to 1.95 S/m after 24 hours of exposing. Figure (3) shows a strong relationship between exposed time and total soluble solids. Increasing in EC measurement is associated with

increasing in pH value that refers to presence of linear relationship between these two factors.

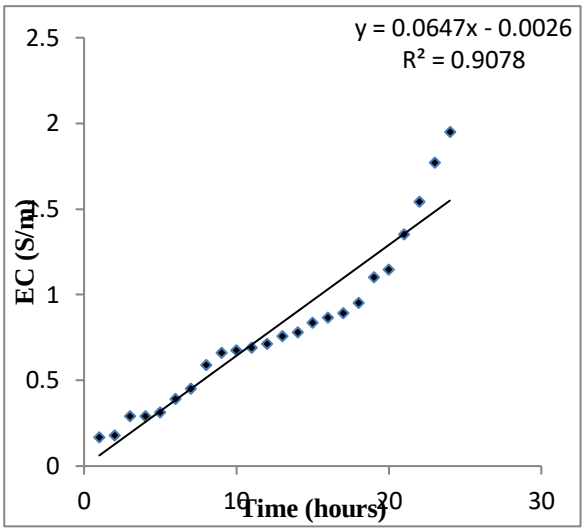

Figure (3): EC value of watermelon juice and renal calculi from 0-24 hours.

3.4. X-ray diffraction analysis

X-ray diffraction technique is a good physical method to identify the chemical nature of renal culcli cmponents and to supply semi-quantitative estimation of their ratios within a calculus.Therforeit is appropriate to utilize this technique to describe the chemical composition of renal calculi before and after treatment with fresh juice of watermelon (Ishita and Athmaselvi, 2017; Ghosh and Gangopadhyay,2004). The X-ray data (2θ versus intensity, distance versus intensity) were shown in Figure (4), before treatment (A) line all patterns were shown high degree of crystallinity 2θ=20.61º and intensity = 582.12, 2θ=39.2º and intensity = 572.11, 2θ=52.5º and intensity = 554.33 while in line (B) after 24 hours of exposure these sharp patterns were reduced 2θ=19.3º and intensity = 388.22, 2θ=30.12º and intensity = 369.5, 2θ=48.3 and intensity = 263.1 and in line (C) after 48 hours of exposure were almost disappeared sharp peaks are good indecator of the crystallinity. This means that calcium oxalate sample convert from crysal shape to amorphous shape during 48 hours of exposure to watermelon juice, with a gradual shift from calcium monohydrate (COM) to calcium dihydrate (COD).

3.5. Scanning electron microscope investigation

Scanning electron microscope images of treated and untreated renal calculi are shown in Figure (5). Actually it is easy to see the difference in the shape of the crystals that have been transformed from their regular shape as bipyramids with tetragonal crystal point group symmetry to sculpted and uncoordinated particles with the absence of a regular geometric shape of crystals no crystallization habits. This transformation is an important point to get rid of kidney stones without surgical interference after its fragmentation within the kidney and transformation from crystalline to amorphous shape. The results of XRD analysis and SEM images were correspond the practical fact to the importance of FWJ elimination of kidney stones in a relatively short time. This is may be due to its high content of water which acts as a diluted agent works on the displacement of ions or make it more soluble in water by replacing the ionic groups with another especially it is rich sources in potassium, which dissolves kidney stones and expels them outside the body or because its high content of lycopene which have potenial therapeutic effects as reduce oxidative stress which is one of the most important causes of renal calculi formation. Other important agent worth noting that treated with fresh watermelon causes calcium oxalate to be converted from COM to COD.The ability of the first type to aggregate and form renal calculi is greater. All of these reasons make the watermelon juice the optimal solution for kidney stone problems (Salama and Attia, 2008; Singh etal, 1999).

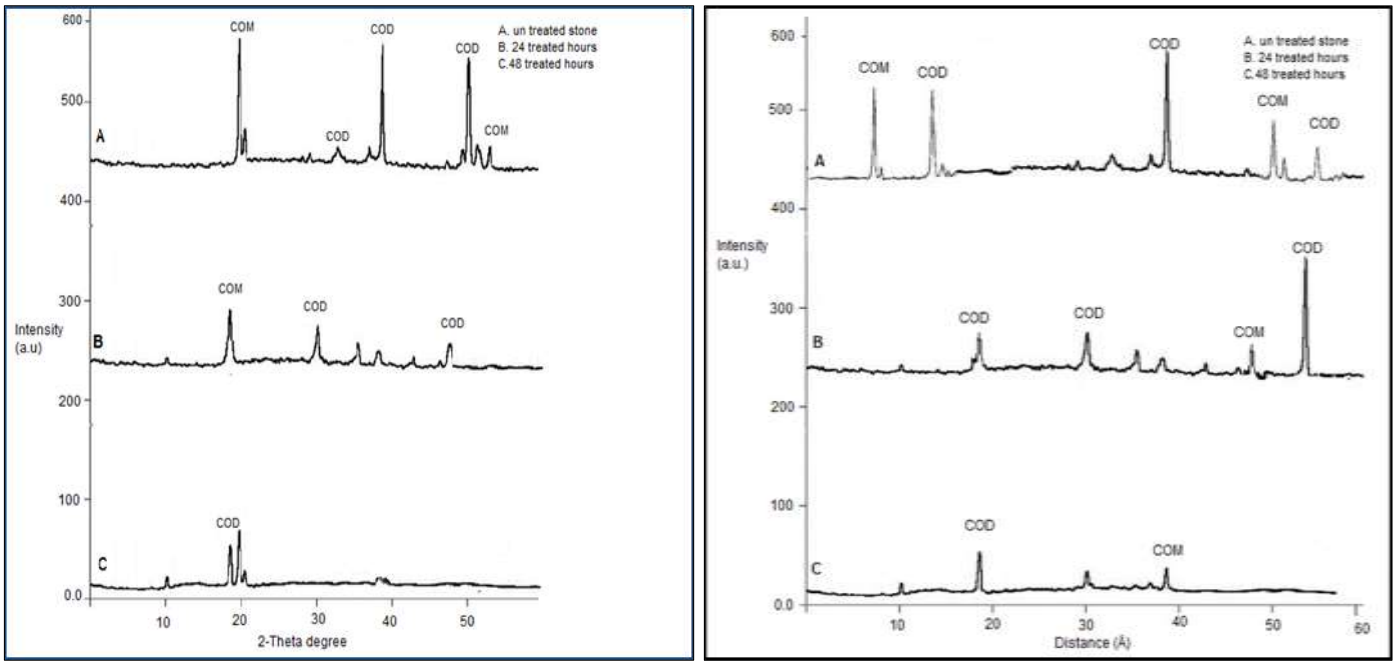

Figure (4): XRD patterns of Calcium Oxalate calculi (A) untreated (B) treated for 24 hours. (C) treated for 48 hours with FWJ

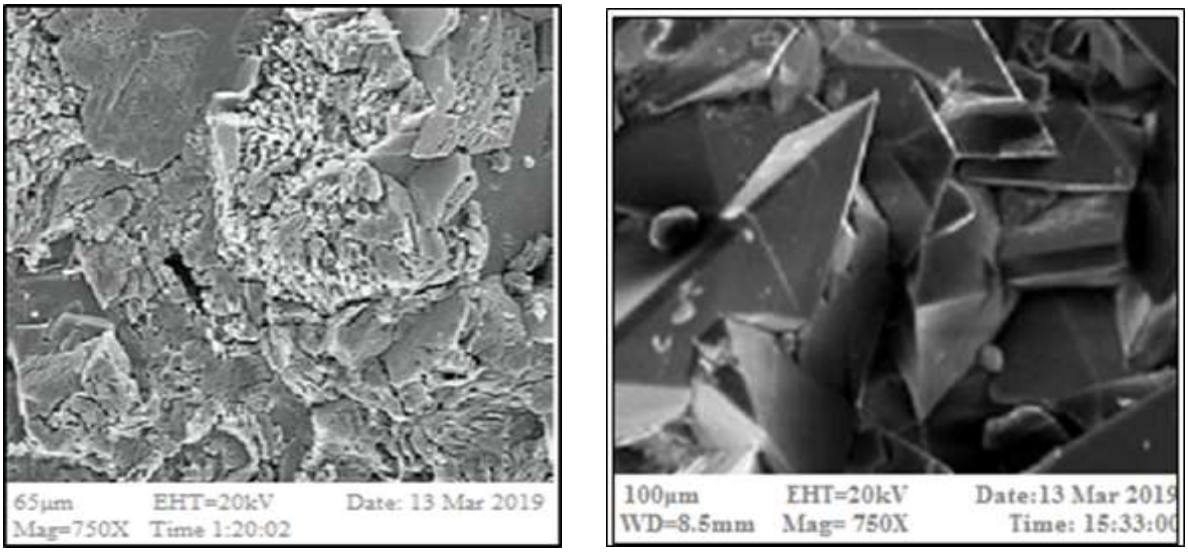

Figure (5): Scanning Electron Microscopy of (left) treated renal calculi with 24 hours. of FWJ; (right) untreated renal calculi

4. Conclusions

The antiliathetics effect of FWJ on calcium oxalate kidney stones was studied via submerging tested calculi in 25 mL of fresh juice and tracking the calculi solubility. Results showed that 24 hours of exposure was sufficient to convert calcium oxalate crystals to amorphous.

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Conflict of Interest: The authors declare that they have no conflicts of interest with the contents of this article.

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