

Original Article

Parsley! Mechanism as antiurolithiasis remedy

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Abstract: Parsley is a medicinal plant used widely in urolithiasis. The present study aimed to evaluate the antiurolithiatic effect of parsley and its mechanism. 24 rats divided into four groups: group A (negative control), group B (positive control), group C (cystone® group) and group D (parsley group). Group B were treated with EG and Ammonium chloride (AC). Group C were treated as B plus cystone® and group D was treated as B plus parsley. The period of experiment was 15 days. Urine samples were analysis on days 0 and 15 days. Kidneys of rats from all groups were removed, and histopathologically examined. The kidneys of parsley treated group appeared mostly to be calculi-free (less CaOx) even better than the cystone treated group. CaOx crystals was significantly lower both in histological sections and in urine samples in parsley treated group. We further investigated the mechanism of parsley by adding another 6 rats. The latter treated by parsley only after adaptation period. We found significant increase in urine volume and pH in parsley treated rats compared to negative control. We concluded that parsley acts as antiurolithiatic drug through decreasing urinary calcium excretion, increasing urinary pH, diuresis, decreasing urinary protein excretion and its nephroprotective activity. We recommended to use it in pharmaceutical forms as it is safe and effective as antiurolithiasis remedy.

Keywords: Parsley-urolithiasis-mechanism-ethylene glycol, pH, CaOx

Introduction

Nephrolithiasis (renal stones, urinary stones, urolithiasis and renal calculi) affects great number of patients worldwide [1]. Urolithiasis has afflicted humankind since antiquity and can persist with serious consequences throughout patient's lifetimes [2]. It is a recurrent disease with a relapse rate of 50% in 5-10 year and 75% in 20 year. Males are more affected than females with a higher recurrence rate (70-80% in males and 47-60% in females) [3, 4]. Calcium oxalate (CaOx) comprise 80% of renal stones, 10% struvite, 9% uric acid and the remaining 1% composed of cystine or ammonium acid urate [5].

Mechanisms involved in stone formation are: supersaturated urine, change in urinary pH, change in urine composition and nucleation [6].

Treatment of urolithiasis is different ranging from surgical removal, Extracorporeal Shock

Wave Lithotripsy (ESWL) to medical treatment using different kinds of synthetic drugs [7]. Phytotherapy is another alternative kind of treatment of urolithiasis including parsley [8].

Parsley, family *Umbelliferae*, locally known as *Baqdunis*, has been used medicinally for many European, Mediterranean and Asian countries [9]. It is a medicinal herb used world-wide because of antioxidant activity, anti-inflammatory, anti-edema, anti-hypertensive, anti-diabetic, anti-microbial, laxative in digestive tract, antioxidant, balance enzyme activities, increase glutathione in the kidney and reconstruct kidney tissue after nephrotoxicity [10, 11].

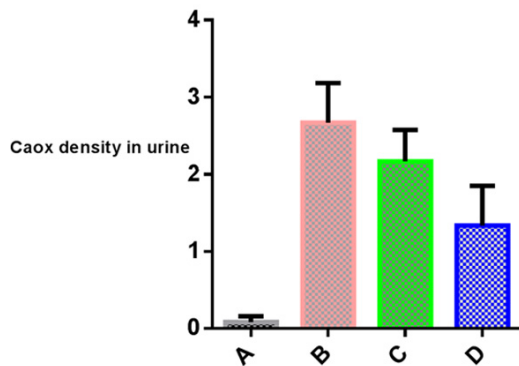
Gumaih et al. found significant decreases in serum urea, creatinine, uric acid and electrolytes and conclude the nephroprotective effect of parsley on rats treated with EG. They also found a significant decrease in urinary calcium and proteins and conclude the antiurolithiatic effect of parsley [12].

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Table 1. Calcium oxalate density in urine in different rat groups

Group	Crystal density
A	0.08±0.0
B	2.7±0.2 ^a
C	2.2±0.2 ^a
D	1.3±0.2 ^b

A= negative control, B= positive control, C= cystone treated, D= parsley treated. The values are in mean ± SEM. Significant difference between a&b (p<0.001).



Graph 1. Calcium oxalate crystal density in urine of different groups.

The present study aims to investigate other effects by which parsley combat urolithiasis to complete the full picture of its mechanisms. Cystone® (a conventional herbal drug used for urolithiasis) also used for comparison.

Materials and methods

Experimental animals

Male albino rats weighing between 150 to 200 g were used in this study. The whole experiment carried out in the same environmental conditions at room temperature. Animals stay seven days prior to experiment as adaptation period and the bedding of the animal cages changed every 48 hrs.

Extract preparation

Parsley seeds were collected from local market. The dried seeds were pulverized into fine powder using a grinder and stored in airtight container. About 750 ml of 70% ethanol was added to 100 g of powder and kept on a mechanical shaker (magnetic stirrer) at 55°C for 72 hrs, The content was filtered and

kept in an incubator at 37°C for 36 hrs. The concentrated extract was stored in dry state at -20°C in deep freeze according to others [13].

Experimental design

Stone induction: Ethylene glycol (EG) plus ammonium chloride (AC) were used to induce urolithiasis. EG (0.75% v/v) and AC (1% w/v) in drinking water *ad libitum* for 15 days according to others [14].

Dose preparation: The ethanolic extract of parsley was dissolved in distilled water (D.W) at a dose 500 mg/kg body weight of rat then shaken until completely dissolved, whereas, cystone drug was dissolved in D.W at a dose 500 mg/kg body weight of rat using a stomach tube.

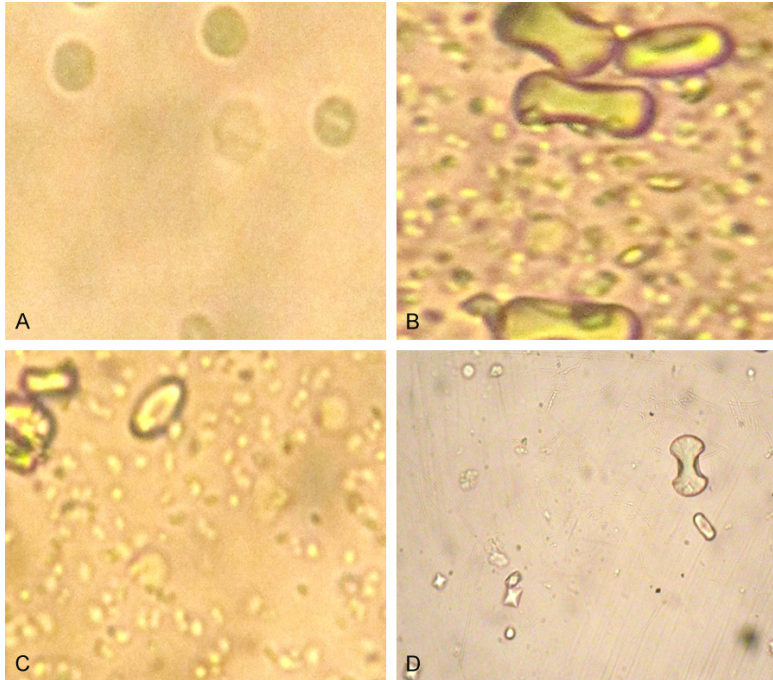
Twenty-four male rats were randomly divided into four groups, each of six rats. Group-A: negative control, fed normal diet. Group-B: took normal diet with EG (0.75%) and AC (1%) for 15 days and serve as a positive control. Group-C: took the same substances as group-B with 500 mg/kg of cystone for 15 days. Group-D: took the same substances as in group-B with 500 mg/kg of Parsley for 15 days.

Assessment of antiurolithiatic activity

Urine collection and analysis: All animals were fasted overnight then urine samples were collected from each rat only the first and last days before and after treatment respectively. These animals were kept in individual cages, and urine was collected in the morning without any preservative substances for crystalluria analysis. The type and number of the crystals were identified and counted using a light microscope (Leica Galen III, America). Crystalluria was scored as follows: "0" no crystals; "1" 1 to 5 crystals per high power field (×400); "2" 6 to 20 crystals/HPF; and "3">20 crystals/HPF.

Histopathological study: At the end of the experiment the animals were killed by cervical decapitation. The abdomen was incised and opened to remove both kidneys from each animal, cleaned off extraneous tissue by ice-cold normal saline. The kidneys fixed in formalin 10% and processed through graded alcohol series and xylene. Then the specimens were embedded in paraffin, sectioned at 5 µm, and

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Panel 1. Calcium oxalate crystals in urine in different groups. They are lesser and smaller in urine of parsley group (D); numerous and of bony type in positive control group (B) and in cysteine group (C). (A) negative control.

stained with the hematoxylin and eosin for histopathological examination under light microscope (Leica Galen III America). The number of crystal deposits were counted on 10 HPFs. The mean crystal number per field was taken for each kidney examined. Crystal density was scored as: “0” no crystal deposition; “1” 1-2 crystals/HPF; “2” 3-4 crystals/HPF; and “3” >4 crystals/HPF. Pictures were taken using digital camera (canon, 12.1 mega pixels, Japan).

Assessment of diuretic activity of parsley and its effect on urinary pH

Additional six rats were used to determine the effect of parsley on urine volume and pH.

Assessment of urine volume: The six rats were adopted for 7 days, housed individually in cages and allowed free access to food and drinking water. Then urine was collected over a 24 hrs and urine volume was measured. After this period, the same rats took ethanolic extract of parsley for another seven days through which urine collected every 24 hrs and its volume was measured. Thus every animal served as control and treated animal in the same time.

Assessment of urine pH: The fresh urine samples from the six rats was measured using standard pH paper (Cybow™, DFI Co., Ltd.

Korea). We measured urinary pH in the morning and the afternoon for each rat to know the effect of parsley on urine pH.

Statistical analysis

The data that obtained by the various parameters were statistically evaluated using SPSS software (Version 16). One-way analysis of variance (ANOVA) and Chi square were applied. The mean values \pm SEM were calculated for each parameter.

Results

Calcium oxalate in urine of different groups

Crystal density of CaOx was significantly decreased in group D compared to group B and C. No significant decrease observed in group C

compared to group B. (**Table 1; Graph 1, Panel 1**).

Calcium oxalate density in renal tissue of different groups

Calcium oxalate were plentiful in rats treated with EG/AC (group B), and larger in size. In addition, they were in tubules with obvious tubular injury and encroachment upon the interstitium (**Panel 2B**). The number and size of crystals are decreased significantly in parsley (group D) compared to either group B or C (**Table 2, Graph 2**). Also, the integrity of renal tissue is better in group (D) than positive control and cysteine groups (**Panel 2**).

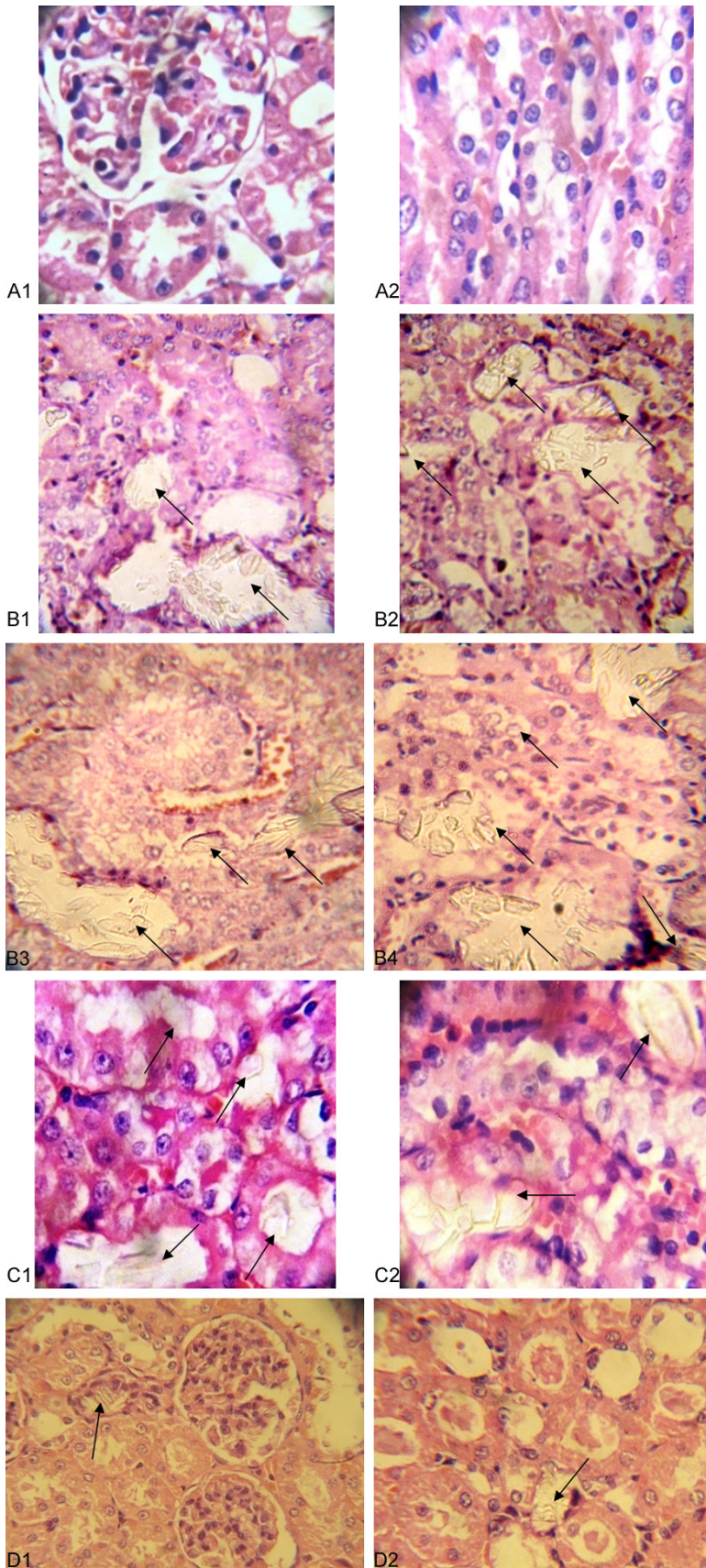
Relation between calcium oxalate density in renal tissue and in urine

Crystal density in both renal tissue and urine have direct proportion relationship and this shown in **Table 3** and illustrated in **Graph 3**. Significant decrease in crystal density in parsley group both in renal tissue and urine compared to groups B&C.

Water intake and urine volume

The mean water intake and mean urine volume are significantly increased in parsley group ($P < 0.0001$).

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Panel 2. A1, A2. No crystals seen in negative control group; B1-4. Calcium oxalate crystals (arrow) were plentiful and large in size in rats treated with EG/AC (group B). The crystals are distributed in tubules with obvious tubular injury and encroachment of the crystals upon the interstitium; C1, C2. Calcium oxalate crystals in renal tissue of cystone group; D1, D2. Few crystals in parsley group.

Urine pH in the morning and the afternoon

There was significant increase in urinary pH in parsley-taking rats compared to no-parsley group both in morning and afternoon urine samples ($P < 0.001$ & $p < 0.0003$ respectively) **Table 5, Graph 5.**

Discussion

The current study was carried out for scientific authentication of parsley used traditionally for urolithiasis. Although the role of parsley in urolithiasis is well recognized and documented, very few scientific data have been published supporting its claimed mechanism.

Calcium oxalate in urine of different groups

An increased urinary calcium concentration is a factor favoring the nucleation and precipitation of CaOx or apatite (calcium phosphate) from urine and subsequent crystal growth [15].

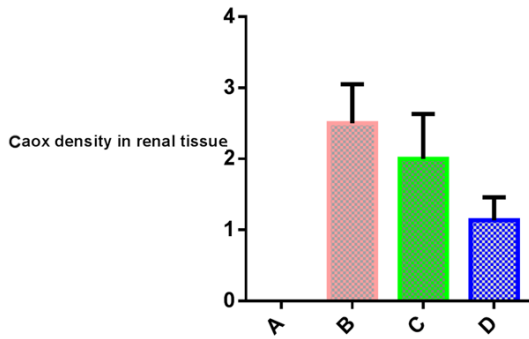
A significant increase in serum as well as urinary calcium was found in rats subjected to EG [12]. This increase may be responsible for formation of urinary CaOx noted in our study. In addi-

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Table 2. The mean crystal density in renal tissue

Group	Crystal density In renal tissue
A	0.0±0.0
B	2.5±0.2 ^a
C	2.0±0.2 ^a
D	1.1±0.1 ^b

A= negative control, B= positive control, C= cystone treated, D= parsley treated. The values are in mean ± SEM. Significant difference between a&b (p<0.001).



Graph 2. Calcium oxalate density in renal tissue.

tion, the significant decrease in CaOx in this study in parsley-treated rats is consistent with the decrease in urinary calcium in parsley treated rats found in the previous study [12].

Among calcium stone-formers, two of the most common urinary abnormalities are hypercalciuria and hypocitraturia [15]. Citrate is a potent inhibitor of stone formation by uniting with calcium making it unavailable to bind with oxalate or phosphate and prevents crystallization [17].

Whether parsley decreasing urinary calcium, hence CaOx through lowering serum Ca level [12] or through increasing urinary citrate a point needs further investigations, although there is a study denoted that no changes in urinary citrate [18].

Some studies reported that parsley has large amount of chlorophyll that may inhibit the growth of calcium oxalate dehydrate which considered to be a primary phase in calcium oxalate stone formation [19, 20].

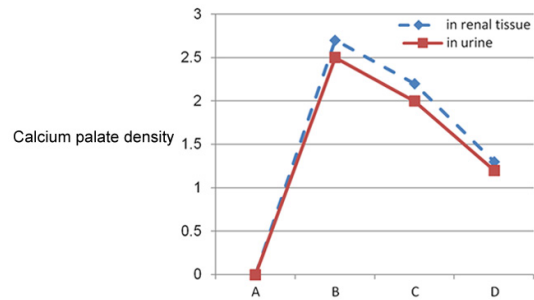
Calcium oxalate density in renal tissue of different groups

In our study CaOx crystals was seen in EG/AC rat kidneys in renal tubules with obvious tubu-

Table 3. Relation between calcium oxalate density in renal tissue and urine

Group	Crystal density	
	In urine	In renal tissue
A	0.8±0.0	0.0±0.0
B	2.7±0.2 ^a	2.5±0.2 ^a
C	2.2±0.2 ^a	2.0±0.3 ^a
D	1.3±0.2 ^b	1.1±0.1 ^b

A= negative control, B= positive control, C= cystone treated, D= parsley treated. The values are in mean ± SEM. Significant difference between a&b.



Graph 3. Calcium oxalate density in renal tissue and urine.

lar injury (cloudy swelling and irregular luminal border) and encroachment of the crystals upon the interstitium, compared to normal (negative control) kidneys. Tubular dilatation also noted but interstitial inflammation was seen focally in rare kidneys and not related to CaOx crystals. Talekar et al. stated that the presence of polymorphic irregular crystals inside the tubules causes dilatation of the proximal tubules along with interstitial inflammation [21].

We found significant decrease in CaOx density in renal tissue in rats taking parsley compared to positive control and cystone and this is consistent with others [22].

Relation between calcium oxalate density in renal tissue and in urine

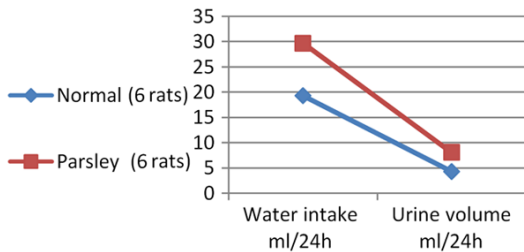
The significant decrease in density (both number and size) of renal CaOx crystals was reflected in urine crystal density. This finding denotes that parsley decreases oxalate synthesis through interacting either with EG absorption in intestine or with the enzyme responsible for oxalate synthesis from EG in the liver. Serum oxalate level should be investigated in future studies although some authors said no significant difference [18]. The latter study also

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Table 4. Water intake and urine volume in rats With and without Parsley

Group	Water intake ml/24 h	Urine volume ml/24 h
Without parsley	19.3±2.0 ^a	4.3±0.5 ^a
With Parsley	29.7±1.5 ^b	8.1±0.5 ^b

The values are in mean ± SEM. Significant difference between a&b.



Graph 4. Water intake and urine volume in rats with and without parsley.

denoted no significant difference in serum calcium both in serum and urine, findings completely different from the previous study which shows significant difference in serum calcium and urine [12].

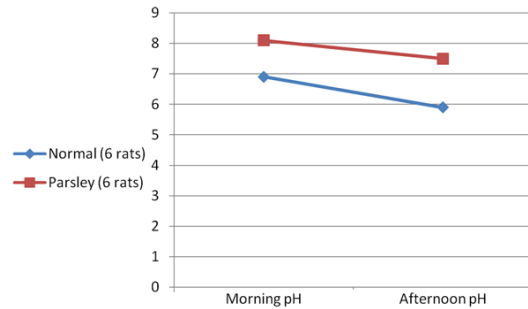
Previous studies found that the level of magnesium is slightly lowered in hyperoxaluric-rat urine, low levels of magnesium are also encountered in stone formers as well as stone forming rats. Moreover, magnesium can reduce the supersaturation of calcium oxalate by reacting with oxalate. Furthermore, magnesium has been found to decrease the growth and nucleation rates of calcium oxalate crystals [23, 24]. Similarly, parsley contains large amount of Mg so it may reduce oxalate in intestine and in urine, decreasing its availability for Ca in renal tubules preventing CaOx formation. In addition, parsley extract has the ability to reduce the toxic effects of EG because it has high amount of vitamins (A, C, riboflavin, and niacin) and minerals (Fe, Mg, P, K, Ca, Na, and Zn) [25].

Some studies suggested that a high intake of dietary calcium inhibits the absorption of oxalate through the increase in intestinal luminal calcium complexed with oxalate, thereby reducing the pool of free oxalate available for absorption, hence reducing urinary oxalate excretion and CaOx stone formation [26].

Table 5. Change in urine pH in parsley group compared to rats without parsley group

Group	Morning pH	Afternoon pH
With out parsley	6.7±0.3 ^a	5.9±0.1 ^a
With Parsley	8.1±0.2 ^b	7.5±0.3 ^b

Significant difference between a&b.



Graph 5. Change in urine pH in parsley rats and negative control.

Water intake and urine volume

Increased fluid intake correlates with a reduced saturation of calcium phosphate (brushite), calcium oxalate, and monosodium urate [27]. We found that parsley increased water intake and urine volume (**Table 4, Graph 4**). The increase in water intake may be due to its high Na content [15] hence thirst. However, serum Na decreased significantly in rats receiving concomitant EG/AC and parsley, a finding explained by parsley-induced diuresis [12] as well as by increased water intake found in present study. The direct diuretic effect of parsley was attributed to inhibition of the Na/K pump that would lead to reduction in Na⁺ and K⁺ reabsorption and osmotic diuresis [15]. Other studies attributed the diuretic effect of parsley to numerous compounds such as flavonoids, saponins or organic acids [28]. The diuretic activity of flavonoids may be due to their binding with adenosine A1 receptors [29].

The decrease in urinary output causes supersaturation of urine by calcium, oxalate and phosphate leading to formation of stones [30]. Hence, the increased water intake and urine volume noted in our study may be responsible for the decrease in urinary calcium and CaOx crystals preventing stone formation.

There are many side effects of diuretics and hypokalemia is the most dangerous one. Parsley is rich in potassium, so its diuretic effect did not lead to potassium depletion [31] making it a safe diuretic and this is consistent with a previous study that serum K didn't significantly decrease in parsley treated rats [12].

Urine pH in the morning and the afternoon

Alterations in urinary pH may be due to genetic variants or mutations in transport pathways, life style habits such as specific diets, or metabolic diseases and infections. Inappropriately acidic or alkaline urine affects the solubility of various metabolites and salts. Alkaline urine reduces solubility of calcium phosphate products, whereas acidic urine pH promotes formation of CaOx, uric acid and cystine-containing stones [32].

Urinary pH is a major determinant for kidney stone formation suggested that urine pH approximately near 6 in pH scale reduces the risk of kidney stone formation. The risk of calcium oxalate (CaOx) crystallization at different pH levels was determined in urine from recurrent CaOx-stone formers and normal subjects. The highest crystallization risk was observed between pH 4.5 and 5.5 [33]. In our study there was a significant increase in urinary pH in parsley treated group, indicating that the antiurolithiatic effect of parsley also related to elevation of urinary pH making it a good anti-CaOx remedy. However this action may enhance calcium phosphate stone formation.

Suarez and Youssef denoted that when urinary pH rises, renal tubular citrate reabsorption decreases, hence increases its excretion [34], another mechanisms by which parsley do its action.

We concluded that parsley act as antiurolithiatic drug through decreasing urinary calcium excretion, increasing urinary pH making urine unsuitable for CaOx crystallization, diuresis and increasing urine volume hence decreasing supersaturation of urine, decreasing nucleation through decreasing urinary protein excretion and its nephroprotective activity. We recommend to use it in pharmaceutical forms as it is safe and effective as antiurolithiasis remedy.

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