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Evaluation of Ethanolic Seed Extract of Parsley on Ethylene Glycol Induced Calcium Oxalate, Experimental Model

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Abstract: ***Urolithiasis is the third most common disorder of urinary system with high recurrence rate. The present study to evaluate the antiurolithiatic effect of parsley using experimental rats and ethylene glycol (EG) induced urolithiasis. 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. Blood and urine samples were collected from rats on days 0 and 15 days. We found significant decrease in parsley group in serum urea, creatinine, uric acid and electrolytes. Also a significant decrease in urinary calcium and proteins in this group compared to positive control. We conclude that parsley has a nephroprotective and antiurolithiatic effects.***

Keywords: Parsley, urolithiasis, calcium oxalate. ethylene glycol

1. Introduction

Kidney acts as a filter of blood from poisonous substances and helps to regulate the levels of chemicals which are important for body functions[1] . Nephrolithiasis (also known as kidney stones, renal stones, urinary stones, urolithiasis and renal calculi) affects great number of patients worldwide [2].

Urolithiasis has afflicted humankind since antiquity and can persist with serious consequences throughout patient's lifetimes[3].It is the third most common disorder of urinary system with high recurrence rate.

Urolithiasis is a recurrent disease with a relapse rate of 50% in 5-10 year and 75% in 20 year. It is estimated that 12% of world population experience renal stone disease with a recurrence rate of 70-80% in males and 47-60% in females. Moreover, the life time risk of developing urolithiasis is about 10-15% in the western world, but can be as high as 20-25% in the Middle East[4][5]. . On the other hand, approximately 80% of stones are composed of calcium oxalate (CaOx) and calcium phosphate, 10% of struvite, 9% of uric acid and the remaining 1% composed of cystine or ammonium acid urate[6].

The stone formation requires supersaturated urine. Furthermore, kidney stone formation involves three critical stages that include nucleation of CaOx crystals, growth and aggregation of crystals[7].

The present day medical management of urolithiasis is either quite expensive. Meantime, invasive treatment procedures for urolithiasis may carry serious complications along with high cost to the patient[8].

Three methods that are used in treatment of kidney stones: Drugs, Extracorporeal Shock Wave Lithotripsy (ESWL) and surgical removal[9]. But the use of ESWL method may cause acute renal injury and an increase in stone recurrence, decrease in renal function, hemorrhage and hypertension[10] , while surgical methods are invasive and require longer recovery times [11]. Although, some drugs used to prevent and treat the disease, the overuse of synthetic drugs, results in higher incidence of adverse drug reactions. These motivated humans to return to nature for safe remedies using phytotherapy[12].

Parsley, family Umbelliferae, locally known as Baqdunis, has been used medicinally for many European, Mediterranean and Asian countries[13][14]. Parsley possesses small and dark seeds with volatile oil, which found in seeds more than in stems or leaves[15]. It serves as medicinal herb and used world-wide because of antioxidant activity[16]. Furthermore, Studies reported that parsley had 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[17] [18].

The present study aims to investigate the effect of parsley seeds on renal stones compared to cystone®. The latter is a conventional herbal drug commonly used for urolithiasis.

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2. Materials and Methods

2.1 Experimental animals

Male albino rats weighing between 150 to 200g were taken 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 48hrs.

2.2 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 starrier) at 55ºC for 72 hrs, The content was filtered and kept in an incubator at 37ºC for 36 hrs. The concentrated extract was stored in dry state at -20ºC in deep freeze according to the method used in some studies [19].

2.3 Experimental design

2.3.1. 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 [20]

2.3.2. 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 shacked until completely dissolved, whereas, cystone drug was dissolved in DW 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, were fed with normal diet. Group-B: took normal diet with EG (0.75%) and AC (1%) for 15 days and serve as positive control. Group-C: took the same substances as group-B with 500 mg/kg of parsley for 15 days. Group-D: took the same substances as group-B with 500 mg/kg of cystone for 15 days.

2.4 Assessment of antiurolithiatic activity

2.4.1. 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, urine samples of 24 h were collected and drops of concentrated hydrochloric acid was added to the urine samples to analyze calcium and protein.

2.4.2 Blood collection and analysis:

Blood was collected from orbital veins in the last day of the experimental period. Then serum was separated by centrifugation at 3,000 rpm for 15 minutes to analyze uric acid, urea, creatinine and electrolytes as calcium, sodium, potassium and chloride.

3. Results

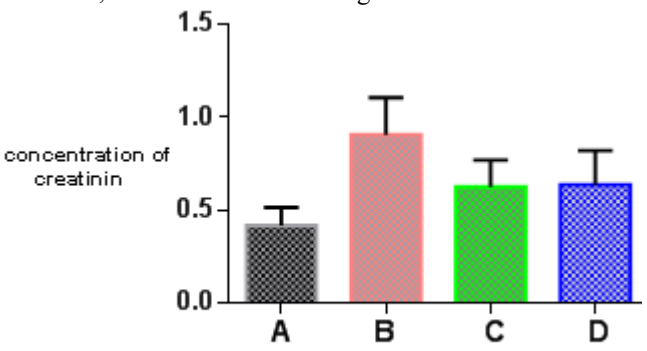
3.1 Serum creatinine, urea and uric acid in different groups

As shown In table (1) and figures (1, 2 and 3), creatinine, urea and uric acid in serum were significantly decrease in cystone and parsley groups compared with group B. No significantly differences between cystone and parsley groups compared to negative control (group A).

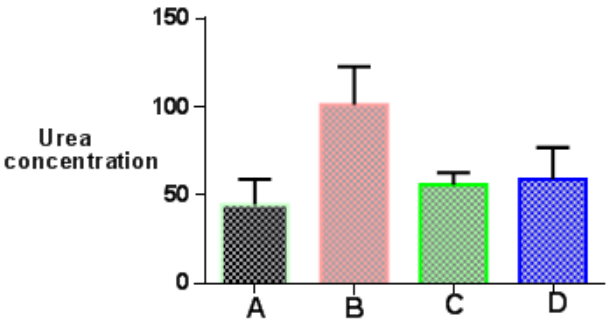
Table 1: Serum creatinine, urea and uric acids in different groups

Group	Creatinine	Urea	Uric acid
A	0.42±0.04	44.5±5.87	0.6±0.1
B	0.91±0.08[a]	101.2±8.8[a]	1.6±0.1[a]
C	0.63±0.06[b]	56.0±2.6[b]	0.6±0.1[b]
D	0.64±0.07[b]	59.3±7.4[b]	0.7±0.1[b]

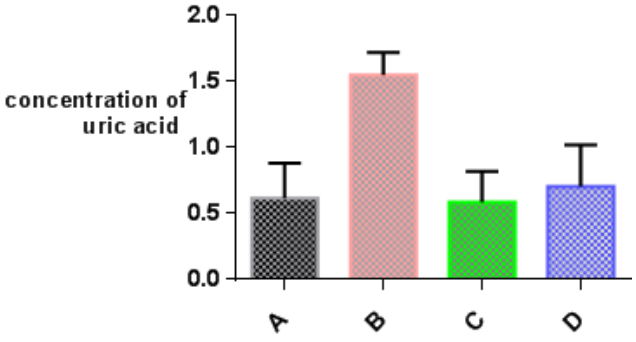
A= negative control, B= positive control, C= cystone treated, D, parsley treated. The values are in mean±SEM. Values are significant between a and both b and c ($p<0.0001$). Serum creatinine, urea and uric acid in mg/dl.



Graph 1: Creatinine in different group.



Graph 2: Urea in different groups.



Graph 3: Uric acid in different groups

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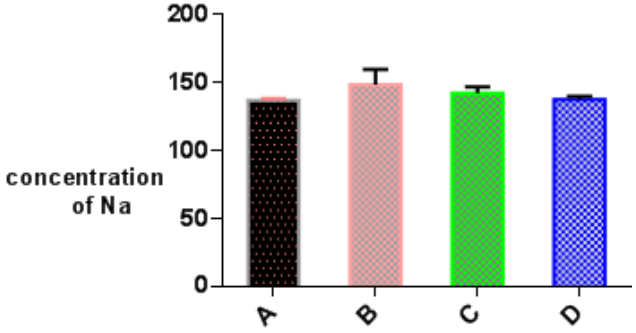
3.2 Changes in electrolytes in different groups

The serum levels of sodium, potassium, chloride and calcium were illustrated in table (2) and figures (4,5,6 and 7).

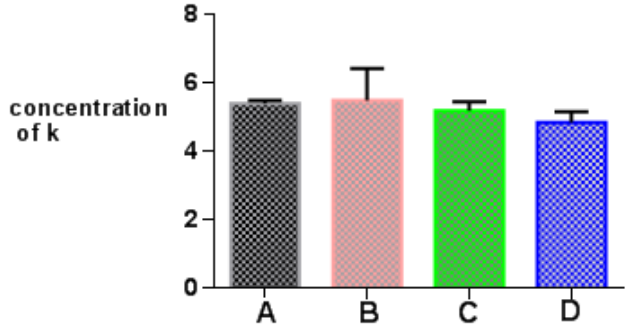
Table 2: Serum sodium, potassium, chloride and calcium in different groups

Group	Na	K	Cl	Ca
A	137.1±0.6	5.4±0.0	110.5±1.1	8.8±0.1
B	148.6±4.6[a]	5.5±0.4	120.1±4.2	11.1±0.5[a]
C	142.0±2.0	5.1±0.1	118.4±1.8	10.7±0.2[a]
D	137.8±0.8[b]	4.9±0.1	111.5±1.4	9.7±0.2 [b]

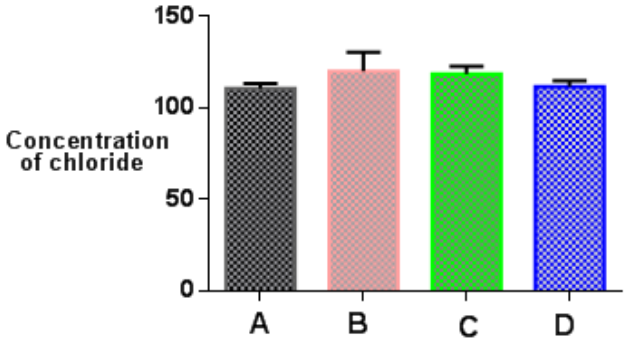
A= negative control, B= positive control, C= cystone treated, D, parsley treated. The values are in mean±SEM. Values are significant between a and b ($p<0.01$). Serum values in mmol/l.



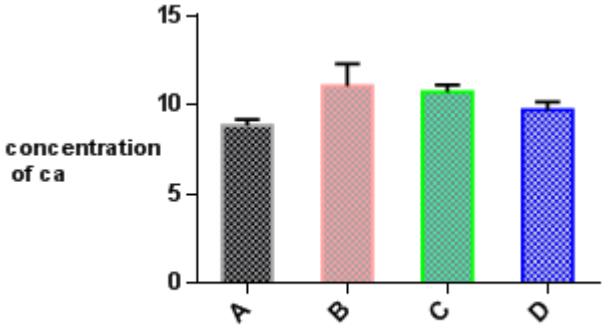
Graph 4: Sodium in different groups.



Graph 5: Potassium in different groups



Graph 6: chloride in different groups.



Graph 7: calcium in different groups.

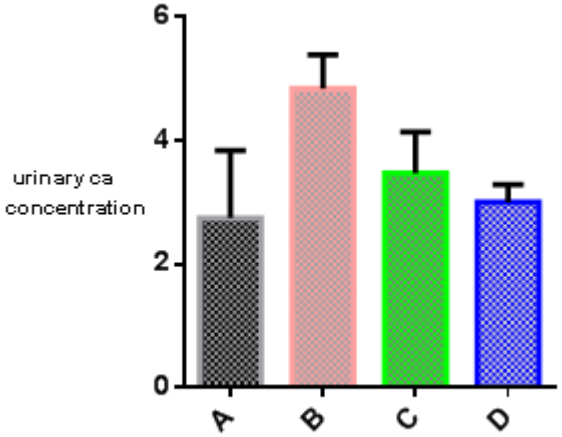
3.3. Urinary calcium and proteins in different groups

The mean values of urinary calcium and total urinary protein were illustrated in table 3 and figures (8 and 9).

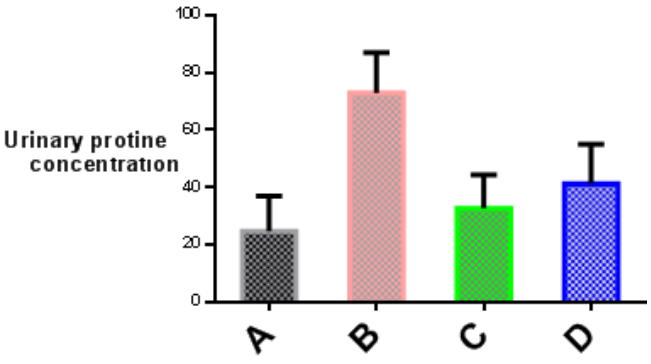
Table 3: Urinary calcium and protein in different groups

Group	Urinary calcium	Urinary protein
A	2.8 ±0.5	24.7±5.0
B	4.9±0.2[a]	72.5±5.7[a]
C	3.6±0.6[b]	32.7±4.8[b]
D	3.0±0.5[b]	41.2±5.6[b]

A= negative control, B= positive control, C= cystone treated, D, parsley treated. The values are in mean±SEM. significant difference between a&b $P<0.0005$. The values are in mg/dl.



Graph 8: Urinary calcium in different groups



Graph 9: Urinary protein in different groups

4. Discussion

This study investigated the role of parsley as one of the widely used herbs in traditional treatment of urolithiasis. We

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used 24 rats for this goal and dividing them into groups (each of six rats). We induce urolithiasis in three groups leaved the first group as negative control. Urolithiasis was induced using EG-induced hyperoxaluria model in three groups and parsley was added to one group to investigate its mechanism using different parameters. Cystone®, a conventional drug of plant origin used in one group to compare its effect with parsley.

Ethylene glycol (EG) induced lithiasis was used to assess the antilithiatic activity in albino rats[21] . The toxicity of EG results from two toxic metabolites, glycolic acid, which is responsible for the acidosis and oxalic acid which precipitates as calcium oxalate in the kidney [22]

Calcium oxalate crystals in urinary tubules can produce damages in the epithelial cells. The role of tubular epithelial cell damage and crystal retention in the nephron has been considered necessary for stone formation by CaOx crystals which can bind to renal epithelial cells (ECs) [23] [24]

EG can cause kidney toxicity via the formation of CaOx crystals in a variety of species, including humans. [22].

Hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stone than hypercalciuria[25]. Renal cell damage is also associated with lipid peroxide production indicating that cell injury was due to the production of free radicals[26].

Hyperoxaluria can provoke CaOx urolithiasis in both humans and rats. Because of oxalate metabolism is considered almost identical in rats and humans[27]. Hence the use of rat model in our study.

EG is metabolized in liver into four organic acids: glycolaldehyde, glycolic acid, glyoxalic acid and oxalic acid (oxalate) which lead to hyperoxaluria, the major initiative factor for urolithiasis[28]. The use of AC in our study aimed to promote the deposition of CaOx crystals in rat kidneys[20] .

4.1 Serum creatinine , urea and uric acid in different groups

EG poisoning can lead to acute renal failure which is characterized by proximal tubular necrosis and an accumulation of CaOx monohydrate crystals in the urine and kidney tissues[29]. Such renal injury was observed in our study by slight rise of serum creatinine, urea and uric acid in group B compared to negative control. The increased was significant but not to the level indicating renal failure which means that the doses of EG/AC used in the study were accepted and not too toxic to kidneys throughout the experiment. The slight increase in serum level of these parameters in group B in our study may be due partial renal tubule obstruction by CaOx crystals resulting in decreased glomerular filtration rate (GFR) and retention of waste products particularly nitrogenous substances such as urea, creatinine and uric acid[30].

The significant decreases in these parameters between group B and parsley treated group may be due to prevention of crystal deposition in renal tubules hence prevention of CaOx induced injury on renal tubules. These results were in agreement with the finding in some studies [31] [32] and disagreed with others [33] .

The nephroprotective effect of parsley may be due to antioxidant activity, as it has high content of essential oil, flavonoids, vitamin C and vitamin E. These substances prevent free radical damage induced by EG[19] .

Flavonoides have anti-inflammatory properties which, prevent the deleterious effects of toxic agents by modulation of inflammatory response[34] .

The nephroprotective and antiurolithatic effects of cystone was found to be due to increased citrate concentration hence reduction of crystallization of calcium oxalate[35] . We did not investigate citrate in our study.

4.2 Changes in electrolytes in different groups

We found significant decrease in serum sodium in parsley group only. This may be due to the diuretic effect of parsley which is supported by concomitant decrease in serum potassium, although such decrease was not significant. This finding is consistent with others[36], who reported that the reduction of serum potassium due to diuresis of parsley. In our study we found hypercalcemia in group B, a finding reported by others[37][36] . Calcium is an important factor in CaOx formation and we found significant decrease in serum calcium in group D compared to positive control. Although parsley is one of the calcium rich herbs[38], the mechanism by which parsley cause such decrease should be investigated.

4.3. Urinary calcium and proteins in different groups

Ethylene glycol administration increased urinary calcium level. It has been stated that hypercalciuria favors precipitation of calcium oxalate in urine[39].

We investigate the effect of parsley on urinary calcium and found a significant decrease in urinary calcium in parsley group compared to positive control and this is consistent with other studies, although such decrease in urinary calcium not explained by them [30][32][40]. In our study such decrease in parsley group may reflect merely the difference in serum calcium between the two groups (B&D) not more. If this true, the antiurolithiatic effect of parsley may be due to decreased serum calcium, hence its urinary content and prevention of urolithiasis.

Proteinuria reflects proximal tubular dysfunction[5]. It was noted that protein excretion increased in hyperoxaluric rats as well as stone formation [10] and this is consistent with our findings. The decrease in urinary protein content in group D is another evidence of nephroprotective effect as well as antiurolithiatic of parsley against EG-induced nephrotoxicity and this is consistent with others[25] [41].

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5. Conclusion

The present study found that the administration of ethanolic extract of parsley seeds effectively prevented the development of calcium oxalate through decreasing urinary calcium content and protection of renal epithelial cells. Further researches are necessary to clarify the mechanism of parsley in this regard.

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