

Preventive Effects of Aqueous Extract of *Petroselinum Sativum* on Calcium Oxalate Kidney Stones in Male Rats

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Abstract. The present study aimed to investigate the preventive effect of petroselinum sativum on calcium oxalate calculi in rats. To do this, forty-eight Wistar rats were divided into 6 groups: Group A served as normal control. Group B received 1% ethylene glycol in drinking water. Groups C, D, E, and F received 1% ethylene glycol from day 0 and were assigned as the prevention subjects. Rats in groups C and D received 200 and 600 mg/kg body weight of aerial parts aqueous extract respectively and those in groups E and F received 200 and 600 mg/kg body weight of root aqueous extract in drinking water respectively from the first day of the experiment. Serum levels of calcium decreased significantly, and that of the magnesium increased significantly. The number of calcium oxalate deposits significantly decreased in the prevention groups of C, D, E and F compared with group B on the 30th day ($P < 0.05$). The weight of the kidneys decreased significantly in prevention groups ($P < 0.05$). In conclusion *Petroselinum Sativum* had a preventive effect on CaOx stones.

Keywords: Petroselinum, kidney stone, Calcium oxalate, Parsley

1. Introduction

The annual incidence of urolithiasis in Iran in 2005 was 147.2 for men and 129.6 for women per a population of 100 000 persons [1].

The recurrence of urolithiasis represents a serious problem and thus prevention of stone formation is highly recommended. Additionally, using Extracorporeal shock-wave lithotripsy (ESWL) method may cause acute renal injury and an increase in stone recurrence [2]. Furthermore, some drugs used to prevent and treat the disease are not effective in all patients and often have adverse effects. Thus, prevention with herbal drugs has been suggested. Kidney stone formation is a complex process, which including supersaturation, nucleation, growth aggregation and retention within the renal tubules [3].

Oxalic acid is one of the toxic metabolites of ethylene glycol which precipitates as calcium oxalate monohydrate (COM) in the kidney. Studies have confirmed that oxalate or COM causes the death of kidney cell [4-6] and increases free radical production [7].

The petroselinum sativum (PS) or *parsley* is widely used in Iranian traditional medicine to prevent kidney calculi. It has been reported to be antiinflammatory, anti-edema, anti-hypertensive, antidiabetic, anti-microbial, anti-oxidant, and laxative in digestive tract [8-10] However, there is no evidence for the preventive usage of this traditional medicine.

Therefore, we decided to investigate the effect of aqueous extract of parsley on the prevention of calcium oxalate calculi in a rat model.

2. Material and Methods

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Forty-eight male Wistar rats (200 ± 10 g) were housed at 25 ± 2 °C on a standard diet and tap drinking water. They were divided randomly into 6 groups and treated for 30 days as follows. Animals of group A served as normal control group. Rats in groups B, C, D, E, and F received 1% ethylene glycol (Merck, Germany) in drinking water for 30 days. Rats in group B were assigned as ethylene glycol (EG) control group. In groups C and D, the rats received 200 and 600 mg/kg body weight of aerial parts aqueous extract respectively. Those in groups E and F also received 200 and 600 mg/kg body weight of root aqueous extract in drinking water respectively from the first day up to the end of the experiment [11]. The study was approved by the ethics committee of Mashhad University of Medical Science. The parsley was purchased from the farmers of Neyshabur, Iran. The aerial parts and roots were separated and dried under shade and powdered finely. The powders were suspended in distilled water as aqueous suspension. After removing the solvent in vacuum, the extract was dried in an oven with a temperature of 30-35 °C. Then was kept in refrigerator and added daily to the drinking water of the rats. The blood samples were collected on days 0, 14, and 30. Animals were anesthetized with diethyl ether. Blood was collected from orbital venous plexus in non-heparinized tubes and centrifuged at 3500 rpm for 15 minutes to obtain serum. Serum levels of calcium and magnesium were measured with Auto Analyzer (BT3000). At the end of the experiment (the 31st day), the kidneys were removed, weighed and prepared for histological processing. Ten slides containing five sections of each kidney were stained with Hematoxylin and Eosin, and then examined by light microscope. Aggregations of calcium oxalate deposits in the renal tubules were counted by DP2-BSW-E-B6212, V2.2 Olympus BX51 Microscope Digital Camera Software in 10 microscopic fields [11]. Each microscopic field was 141×10^9 nm². Data were analyzed with SPSS (Version 16.0) using One-way ANOVA followed by Duncan's test for multiple comparisons among all groups. P values less than 0.05 were considered statistically significant. Data were presented as mean \pm standard error.

3. Results

Serum levels of calcium decreased significantly and that of the magnesium increased significantly in the prevention groups of C, D, E and F compared with group B on the 30th day (Table 1, Figure1). In the nephron segments of group A, calcium oxalate deposits were not found, but many of them were observed in all of the segments of urinary tubules in group B (figure2). the number of calcium oxalate deposits in 10 microscopic fields of group B was significantly higher than that in group A. In the prevention groups of C, D, E and F this number decreased significantly in both doses of aqueous extract of PS compared with group B and in the different parts of the renal tubules in the prevention groups they were smaller in comparison with group B.

4. Discussion

Calcium oxalate crystals in urinary tubules can cause damages in the epithelial cells [12, 13], and consequently the cells may produce free radicals, induce heterogenous crystal nucleation and cause aggregation of crystals [14]. Aqueous extract of PS has glycoside flavonoids such as apigenin, apigenin, etc [15] and the flavonoides have antioxidant effects [16]. It can be speculated that the role of the herb extract in the prevention of calcium oxalate calculi is due to the antioxidant effects of the different compounds of the PS [17]. The calcium salts are insoluble in physiological pH [14]. The herb extract provided the optimum pH which could maintain calcium oxalate particles dispersed in the solution and thus allowed them to be eliminated easily from the kidney. Probably the mechanism of herb extract on nephrolithiasis was related to the increase of diuresis via inhibition of the Na⁺-K⁺ pump in renal epithelial cell [18]. It has been reported that calcium oxalate calculi may have a bacterial origin [19]. Antimicrobial activity of PS materials against natural microflora may be effective in this mechanism. The present results showed that the administration of EG increased the levels of serum calcium and decreased the level of serum magnesium. Karadi et al. (2006) and Celik et al. (2007) have shown that magnesium deficiency accelerates renal stone formation in rats and that the administration of magnesium results in the prevention of calcium oxalate crystallization in their kidneys [20, 21]. These findings indicate that rats supplemented with herb extract were mostly recovered from nephrolithiasis. Serum magnesium significantly diminished in EG induced urolithic rats. Magnesium complexes with oxalate, thus reduces calcium oxalate super saturation in urine [22]. Prevention with aqueous extract of PS reduced the level of serum calcium in rats. This indicates

that PS materials, mainly glycoside flavonoids, inhibiting some steps of oxalate synthesis from glycolic acid [23]. The kidney histology revealed a characteristic effect ranging from vascular congestion or haemorrhage to diffuse cortical necrosis. Whereas in all the prevention groups a moderate necrosis was also observed in the histopathological sections of the kidneys in the majority of animals, indicating a reduction of the extent of damage done at the tissue level [24]. It suggests that the effect of the extracts can be advantageous in preventing urinary stone retention by reducing renal necrosis and thus inhibiting crystal retention. Microscopic examination of kidney sections derived from group B (figure 2) showed polymorphic irregular crystal deposits inside the tubules. Prevention groups had increased the normal renal architecture. Our study showed that the aqueous extract of PS can prevent or slow down the oxidative damage in epithelial cells of kidney. Further investigation is needed to explore the exact active principles responsible for the antilithiatic activity of aqueous extract of PS and its mechanism of action.

5. Acknowledgements

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6. References

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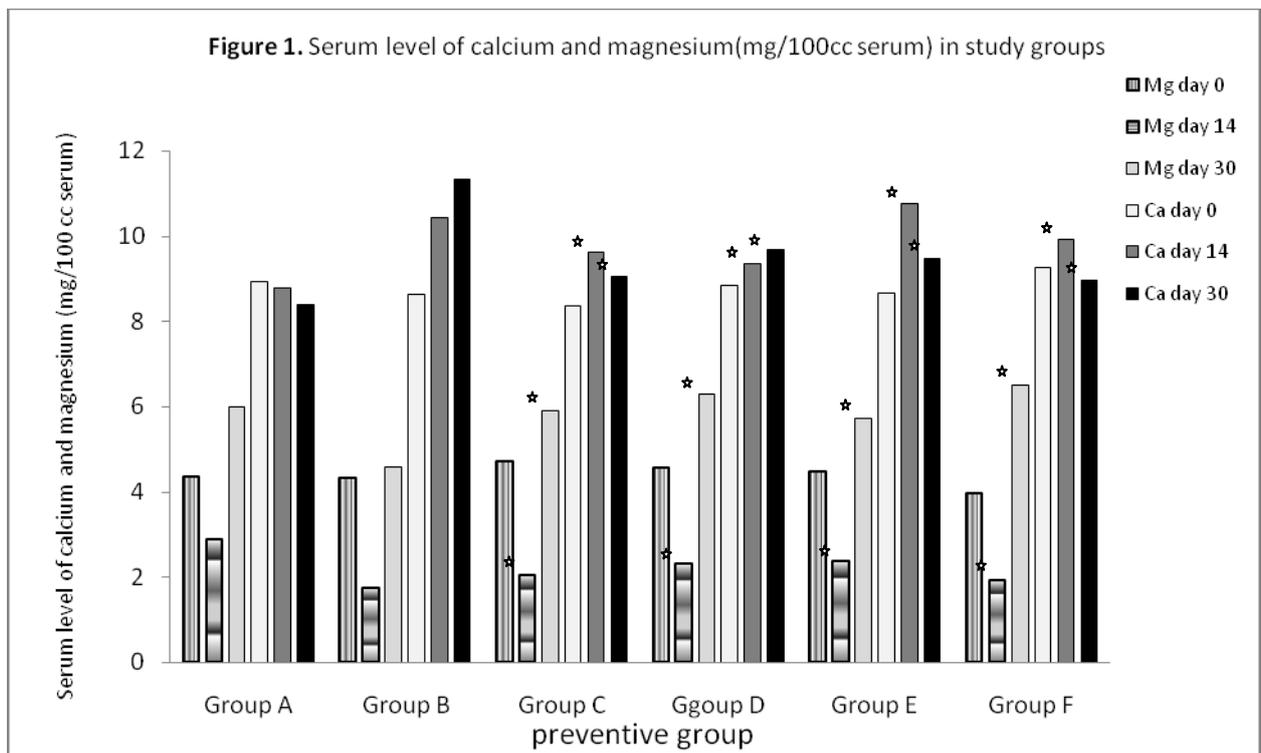
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Table 1: Concentration of serum calcium and magnesium, number of calcium oxalate crystals and weight of the kidney in control (Group A), ethylene glycol control (Group B) and preventive groups C, D, E, F. (Means±SE)

parameters	Days	Group A	Group B	Group C	Group D	Group E	Group F
Calcium (mg/100 ^{cc} serum)	0	8.95±0.15	8.65±0.13	8.37±0.14	8.86±0.21	8.68±0.31	9.27±0.08
	14	8.80±0.00	10.4±0.43 ^b	9.62±0.22 ^a	9.57±0.2 ^a	10.77±0.34 ^a	9.93±0.02 ^a
	30	8.40±0.22	11.33±0.18 ^b	9.07±0.29 ^a	9.68±0.43 ^a	9.48±0.37 ^a	8.97±0.12 ^a
Magnesium (mg/100 ^{cc} serum)	0	4.35±0.11	4.33±0.35	4.7±0.23	4.57±3.8	4.47±0.23	3.95±0.42
	14	2.87±0.17	1.75±0.12 ^b	2.05±0.1 ^a	2.32±0.17 ^a	2.38±0.12 ^a	1.93±0.03 ^a
	30	6.01±0.00	4.6±0.29 ^b	5.92±0.36 ^a	6.30±0.43 ^a	5.73±0.75 ^a	6.5±0.15 ^a
Number of Calcium oxalate crystals (in 10 microscopic fields)	30	0.00±0.00	16.72±2.22 ^b	5.89±1.36 ^a	4.87±1.02 ^a	4.92±1.17 ^a	3.16±0.39 ^a
Weight of the kidney (gr)	30	1.68±0.00	1.90±0.16 ^b	1.43±0.10 ^a	1.52±0.13 ^a	1.42±0.05 ^a	1.48±0.06 ^a

^a Indicates a significant difference between preventive groups and Group B (P<0.05)

^b Indicates a significant difference between Group B and control group (P<0.05)



* Indicates a significant difference between preventive groups and Group B (P<0.05)

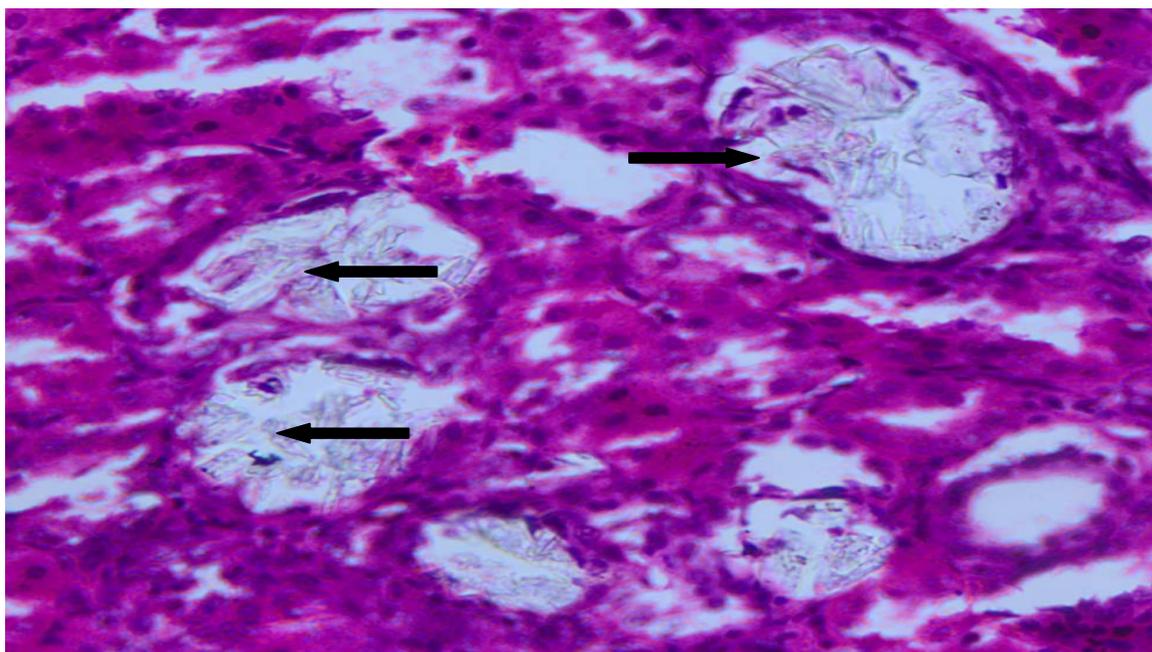


Fig. 2: Polymorphic irregular CaOx crystals inside the renal tubules (arrows). (hematoxylin-eosin, × 400)