Therapeutic Effects of Aqueous Extracts of *Petroselinum Sativum* on Ethylene Glycol-Induced Kidney Calculi in Rats

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Purpose: To investigate the therapeutic effects of the aqueous extract of *Petroselinum Sativum* aerial parts and roots on kidney calculi.

Materials and Methods: Thirty-six male Wistar rats were randomly assigned into 6 groups and treated for 30 days. Group A served as normal control and group B received 1% ethylene glycol in drinking water. Groups C, D, E, and F received 1% ethylene glycol from day 0 and were used as the treatment 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 14th day of the experiment.

Results: On the 14th and 30th days of the experiment, serum level of magnesium (1.71 ± 0.12 and 3.81 ± 0.25, respectively) decreased significantly while serum level of calcium (10.45 ± 0.26 and 11.33 ± 0.18, respectively) increased significantly in group B compared with the control group (14th day: magnesium = 2.87 ± 0.17 and calcium = 8.80 ± 0.00 and 30th day: magnesium = 6.01 ± 0.00 and calcium = 8.30 ± 0.22; $P < .001$). In the treatment groups of C, D, E, and F, the number of deposits decreased significantly compared with group B on the 30th day ($P < .001$). The weight of the kidneys increased significantly in group B (2.01 ± 0.17) compared with the control group (1.52 ± 0.07) and decreased significantly in treatment groups ($P < .05$).

Conclusion: *Petroselinum Sativum* has a therapeutic effect on calcium oxalate stones in rats with nephrolithiasis and reduces the number of calcium oxalate deposits.

Keywords: petroselinum, kidney calculi, ethylene glycol, calcium oxalate

INTRODUCTION

Urinary stone is the third prevalent disorder in the urinary system.[1] The annual incidence of urolithiasis in Iran in 2005 was 147.2 for men and 129.6 for women per 100 000 population. In the same year in Iran, the average cumulative recurrence rate was 16% after 1 year, 32% after 5 years, and 53% after 10 years.[2] The majority of stones, up to 80%, are mainly composed of calcium oxalate (CaOx).[3]

The recurrence of urolithiasis represents a serious problem and thus stone prevention and treatment are highly recommended. The use of extracorporeal shockwave lithotripsy (SWL) method may cause acute renal injury, a decrease in renal function, and an increase in stone recurrence.[4] Furthermore, some medications used to prevent and treat the disease are not effective in all patients and often have adverse effects.[5]

Ethylene glycol (EG) has two toxic metabolites; glycolic acid, which is responsible for the acidosis, and oxalic acid, which precipitates as calcium oxalate monohydrate (COM) in the kidney, and causes proximal tubular cell necrosis.[6] Studies have confirmed that oxalate or COM causes the kidney cell death.[7-9] The toxic effects increase free radical production and lipid peroxidation.[10]

The *petroselinum sativum (PS)* or *parsley*, which is a member of the family of Umbelliferae, is widely used in Iranian traditional medicine in the treatment of kidney calculi. It has been reported to be anti-inflammatory, anti-edema, anti-hypertensive, anti-diabetic, anti-microbial, anti-oxidant, and laxative in the digestive tract. Furthermore, it balances enzyme activities, increases glutathione in the kidney, and repairs the kidney tissue after nephrotoxicity.[11-13] However, there is no evidence for the therapeutic usage of this traditional medicine. Therefore, we aimed to evaluate the effects of aqueous extract of *parsley* on the treatment of CaOx calculi in a rat model.

MATERIALS AND METHODS

Thirty-six 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 remained untreated and served as normal control group. Rats in groups B, C, D, E, and F received 1% EG (Merck, Germany) in drinking water for 30 days. Rats in group B served as 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 14th day up to the end of the experiment.[14]

The experiment was conducted in accordance with the Guide for the Care and Use of Laboratory Animals and the study was approved by the Eth-

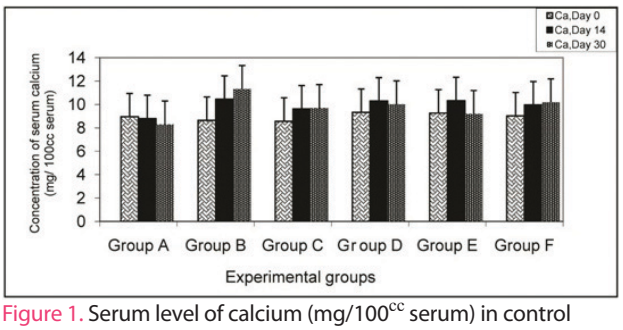


Figure 1. Serum level of calcium (mg/100^{cc} serum) in control group, ethylene glycol group, and treatment groups (C = 200 mg/kg body weight and D = 600 mg/kg body weight of aerial parts, E = 200 mg/kg body weight, and F = 600 mg/kg body weight of root aqueous extract). Data were expressed as mean ± standard error, n = 6, *P < .001.

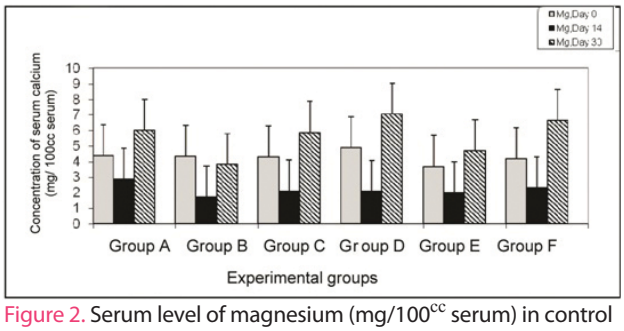


Figure 2. Serum level of magnesium (mg/100^{cc} serum) in control group, ethylene glycol group, and treatment groups (C = 200 mg/kg body weight and D = 600 mg/kg body weight of aerial parts, E = 200mg/kg body weight, and F = 600 mg/kg body weight of root aqueous extract). Data were expressed as mean ± standard error, n = 6,*P < .001.

Serum levels of calcium and magnesium, number of calcium oxalate crystals, and weight of the kidney in study groups

Parameters	Days	Group A	Group B	Group C	Group D	Group E	Group F
Serum level of calcium, mg/100 CC serum	0	8.95 ± 0.15	8.65 ± 0.13	8.57 ± 0.21	9.33 ± 0.34	9.27 ± 0.24	9.03 ± 0.23
	14	8.80 ± 0.00	10.45 ± 0.26	9.62 ± 0.24*	10.3 ± 0.21*	10.33 ± 0.23*	9.98 ± 0.26*
	30	8.30 ± 0.22	11.33 ± 0.18	9.70 ± 0.07*	10.02 ± 0.46*	9.20 ± 0.07*	10.19 ± 0.4*
Serum level of magnesium, mg/100 CC serum	0	4.35 ± 0.11	4.33 ± 0.35	4.3 ± 0.18	4.87 ± 0.35	3.95±0.26	4.18±0.26
	14	2.87 ± 0.17	1.71 ± 0.12	2.11 ± 0.06*	2.08 ± 0.35*	2.01 ± 0.05*	2.30 ± 1.60*
	30	6.01 ± 0.00	3.81 ± 0.25	5.87 ± 0.18*	7.05 ± 0.10*	4.70 ± 0.00*	6.63 ± 0.23*
Number of calcium deposits, in 10 microscopic fields	30	0.00 ± 0.00	16.72 ± 2.22	6.15 ± 1.85*	11.15 ± 0.93*	10.62 ± 0.45*	8.88 ± 0.53*
Weight of the kidney, gr	30	1.52 ± 0.07	2.01 ± 0.17	1.42 ± 0.01***	1.36 ± 0.03**	1.36 ± 0.03**	1.49 ± 0.03**

Data were expressed as mean ± standard error, n = 6,*P < .001, **P < .01, ***P < .05

ics Committee of Mashhad University of Medical Science.

The parsley was purchased from the farmers of Neyshabur, Iran and was graciously identified by the herbarium of Islamic Azad University, Neyshabur Branch (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 from the extract in vacuum, the extract was dried in an oven with a temperature of 40 °C. The extract was then 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).

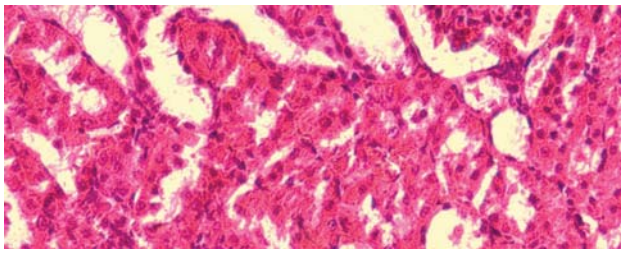
For histological examination at the end of the experiment (the 31st day), all the rats were decapitated by guillotine after they were anesthetized. Thereafter, the kidneys were removed, weighed, fixed in 10% formalin, dehydrated in a gradient of ethanol, embedded in paraffin, and then cut into 5 µ serial sections. Ten slides containing five sections from each kidney were deparaffinized, stained with Hematoxylin and Eosin, and then examined by light microscope. Aggregations of CaOx deposits in the renal tubules were counted by DP2-BSW-E-B6212, V2.2 Olympus BX51 Microscope Digital Camera Software in 10 microscopic fields.[14] Each microscopic field was 141 × 10^9 nm^2.

Data were analyzed with SPSS software (the Statistical Package for the Social Sciences, Version 16.0, SPSS Inc, Chicago, Illinois, USA) using One-way ANOVA followed by Duncan's test for multiple comparisons among all groups. P values less than .05 were considered statistically significant. Data were presented as mean ± standard error.

RESULTS

Serum levels of magnesium (P values in groups C = .989, D = .372, E = .492, and F = .885) and calcium (P values in groups C = .255, D = .132, E = .080, and F = .291) did not change significantly in all the experimental groups on day zero (for details see Table, Figures 1 and 2).

In the control group, the examination of the kidney sections showed no CaOx deposits or other abnormalities in different segments of the nephrons (Figure 3). However, CaOx deposits, composed of 3 to 4 large polygonal crystals, were found abundantly in all the segments of the urinary tubules in



EG group (Figure 4). Renal tubular dilation with epithelial damage and leukocyte reaction were also observed on pathology examination (Figure 5). The mean number of CaOx deposits in 10 microscopic fields in the kidney specimens of EG group was 16.72 ± 2.22, which was significantly higher than that in the control group ($P < .001$). In the treatment groups of C, D, E, and F, the number of deposits decreased significantly compared with EG group in both doses of aqueous extract of PS on the 30th day (C = 6.15 ± 1.85, D = 11.15 ± 0.93, E = 10.62 ± 0.45, and F = 8.88 ± 0.53; $P < .001$). Calcium oxalate crystals in different parts of the renal tubules of the treatment groups were smaller in comparison with EG group.

DISCUSSION

Our results showed that EG administration can cause a statistically significant increase and decrease in serum levels of calcium and magnesium, respectively. It has been suggested that CaOx calculi may have a bacterial origin, such as nanobacteria.[15] Antimicrobial activity of *PS* materials against natural microflora, coliforms, molds, and Staphylococcus aureus has been reported;[12] therefore, it may be effective in the treatment of CaOx calculus. The mechanisms of action of *PS* extract seem to be mediated by inhibition of the Na^+-K^+ pump, which leads to reduction in Na^+ and K^+ reabsorption, and thus results in an osmotic water flow into the lumen and dieresis.[16,17]

The decrease in serum level of calcium indicates an increase in urinary calcium and CaOx stone formation. These findings indicate that rats supplemented with aqueous extract of *PS* for 30 days mostly recovered from nephrolithiasis.

Nephrolithiasis induction by EG has been established in many researches.[14,18,19] The mechanism underlying the effect of aqueous extract of *PS* on nephrolithiasis induced by EG is apparently related to increasing diuresis and lowering urinary concentrations of stone forming constituents.[16,17]