

## Therapeutic Effects of Aqueous Extracts of *Cerasus Avium* Stem on Ethylene Glycol- Induced Kidney Calculi in Rats

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**Purpose:** To investigate the therapeutic effects of the aqueous extract of *Cerasus Avium* stem on kidney calculi.

**Materials and Methods:** In this experimental study, forty-eight (48) male Wistar rats were randomly allocated into six (6) groups and were studied during a 30 day period. Group A served as normal control and Group B received 1% ethylene glycol in drinking water (EG group). C, D, E, and F Groups, received 1% ethylene glycol from day 1 and were used as prevention and treatment subjects. Rats in prevention groups of low dose (C) and high dose (D) extract, were gavaged with 200 and 400 mg/kg extract respectively from first day of the experiment and treatment groups of low dose (E) and high dose (F) extract, were gavaged with 200 and 400 mg/kg extract respectively from the 15th day of the experiment.

**Results:** On the 30th days of the experiment, serum level of magnesium and potassium decreased significantly in EG group compared with A,C,D,E and F groups ( $P < .05$ ), while serum level of calcium, creatinine, uric acid, sodium and urine level of calcium, creatinine, uric acid, increased significantly in EG group compared with A,C,D,E and F groups ( $P < .05$ ). In the prevention and treatment groups, the number of deposits decreased significantly compared with EG group on the 30th day ( $P < .05$ ).

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

**Keywords:** *Cerasus Avium* stem; ethylene glycol; kidney calculi; calcium oxalate

### INTRODUCTION

Urinary tract stones are the third important cause of urinary tract diseases<sup>(1)</sup>. The spread of urinary tract stones is increasing due to changes in peoples' diet and life style<sup>(2)</sup>. 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<sup>(3)</sup>. Kidney stone formation is a complex process that results from a succession of several physicochemical events including supersaturation, nucleation, growth aggregation and retention within renal tubules<sup>(4)</sup>. Oxalate, struvite, urate, brushite, cystine were the most commonly reported urolithiasis in man and animal species. However, epidemiological studies have shown that majority (70%) of stones commonly contain calcium oxalate<sup>(5,6)</sup>. In the present study, we successfully induced CaOx formation in the rat's kidney by adding EG to drinking water, which is in line with other studies<sup>(7-9)</sup>. Calcium oxalate stone formation is a multi-factorial process involving various etiological factors. Hyperoxaluric rat model is the most potent experimental model for preclinical evaluation of antiurolithiatic efficacy of medicinal herbs because the physiological process mimics the etiology of kidney stone formation in human and animal<sup>(10)</sup>. The hepatic enzymes metabolize EG to oxal-

ic acid by glyoxalate mechanism, which is combined with calcium ion in the renal tubular epithelium to form calcium oxalate crystals<sup>(11)</sup>. Extracorporeal shock wave lithotripsy and percutaneous nephrolithotomy techniques mainly include the surgical removal of stones. But, these techniques cause undesirable side effects such as tubular necrosis, hypertension, hemorrhage and subsequent fibrosis of the kidney leading to cell injury and recurrence of renal stone formation<sup>(12)</sup>. Nowadays, most contemporary researchers are using and focusing on homemade and natural remedies as well as their effects on the treatment of kidney stone<sup>(13)</sup>. In addition, other parts of some plants such as the stem and root are frequently used in alternative medicine<sup>(14)</sup>. *Cerasus Avium* is a tree from the family of the genus *Prunus* Rosacea and one of rare and valuable species in northern forests of Iran<sup>(15)</sup>. In recent years, *Cerasus Avium* stems have been widely used in folk medicine. After having been dried and boiled *Cerasus Avium* stem is used for treatment<sup>(14)</sup>. Its fruit stalks are sold by herbal druggists in Iran and are used as decoction to relief renal stones, edema and hypertension<sup>(16)</sup>. However, there is no evidence for the therapeutic usage of this traditional medicine. Therefore, we aimed to evaluate the effects of aqueous extract of *Cerasus Avium* stem on the treatment of CaOx calculi in a rat model.

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**Table 1.** Effect of *Cerasus avium* stem extracts on urinary and serum parameters in control and experimental animals. All values are expressed as mean  $\pm$  SEM for 8 animals in each group.

parametres	days	Agroup	B group	C group	D group	E group	F group
Serum (mg/dl)							
Calcium	15	8.8 $\pm$ 0.17	10.25 $\pm$ 0.33 a,*	10.5 $\pm$ 0.4 a,*	10.18 $\pm$ 0.25 a,*	10.28 $\pm$ 0.25 a,*	11.03 $\pm$ 0.41a,*
	30	8.92 $\pm$ 0.49	11.22 $\pm$ 0.31a,*	9.28 $\pm$ 0.18 b,*	9.32 $\pm$ 0.25 b,*	9.41 $\pm$ 0.17b,*	9.63 $\pm$ 0.42 b,*
Creatinine	15	0.62 $\pm$ 0.06	0.7 $\pm$ 0.07 a	0.59 $\pm$ 0.09 b	0.7 $\pm$ 0.07 a	0.68 $\pm$ 0.03 ab	0.75 $\pm$ 0.03 a
	30	0.55 $\pm$ 0.02	0.78 $\pm$ 0.05 a,*	0.5 $\pm$ 0.05 b,*	0.58 $\pm$ 0.03 b,*	0.65 $\pm$ 0.02 ab,*	0.64 $\pm$ 0.04 ab,*
Potassium	15	5.22 $\pm$ 0.5	4.96 $\pm$ 0.24 a	5.18 $\pm$ 0.25 a	5.63 $\pm$ 0.29 a	5.13 $\pm$ 0.26 a	5.55 $\pm$ 0.35 a
	30	5.71 $\pm$ 0.3	4.4 $\pm$ 0.36 b,**	5.65 $\pm$ 0.16a,**	5.08 $\pm$ 0.43a,**	5.51 $\pm$ 0.19 a,**	5.58 $\pm$ 0.37 a,**
Sodium	15	162.3 $\pm$ 6.27	174.2 $\pm$ 10.01ab	177 $\pm$ 2.98 ab	174 $\pm$ 3.3 ab	182 $\pm$ 4.04 a	185 $\pm$ 5 a
	30	164 $\pm$ 6.89	189.8 $\pm$ 1.15 a,*	163.1 $\pm$ 3.6 b,*	171.57 $\pm$ 4.21b,*	170.33 $\pm$ 1.76 b,*	165.66 $\pm$ 6.31 b,*
Magnesium	15	1.92 $\pm$ 0.19	1.78 $\pm$ 0.17 c,**	2.32 $\pm$ 0.12 ab,**	2.26 $\pm$ 0.18abc,**	2.11 $\pm$ 0.16abc,**	2.5 $\pm$ 0.06a,**
	30	2.07 $\pm$ 0.12	1.68 $\pm$ 0.18 c,*	2.83 $\pm$ 0.36 b,*	2.23 $\pm$ 0.18 bc,*	4.58 $\pm$ 0.21 a,*	4.61 $\pm$ 0.23 a,*
Acid Uric	15	2.05 $\pm$ 0.13	3.4 $\pm$ 0.19 a,*	3.25 $\pm$ 0.17 a,*	3.10 $\pm$ 0.22 a,*	3.63 $\pm$ 0.29 a,*	3.53 $\pm$ 0.34 a,*
	30	2.37 $\pm$ 0.30	4.14 $\pm$ 0.35 a,*	2.92 $\pm$ 0.16 bc,*	2.58 $\pm$ 0.28 bc,*	3.32 $\pm$ 0.17 b,*	3.01 $\pm$ 0.27 bc,*
Urin(mg/dl)							
Calcium	15	1.72 $\pm$ 0.21	2.33 $\pm$ 0.66 a	2 $\pm$ 0.33 a	1.98 $\pm$ 0.20 a	2.12 $\pm$ 0.31 a	2.06 $\pm$ 0.31 a
	30	1.18 $\pm$ 0.03	1.58 $\pm$ 0.09 a,**	1.21 $\pm$ 0.15 b,**	1.24 $\pm$ 0.07b,**	1.15 $\pm$ 0.07 b,**	1.39 $\pm$ 0.14 ab,**
Creatinine	15	37.37 $\pm$ 10.44	66.54 $\pm$ 25.70 a	50.52 $\pm$ 10.80 a	54.48 $\pm$ 8.74 a	60.51 $\pm$ 8.60 a	66.13 $\pm$ 13.73 a
	30	34.42 $\pm$ 7.40	81.36 $\pm$ 23.64 a**	45.88 $\pm$ 9.52 a**	45.18 $\pm$ 20.24 a**	50 $\pm$ 15.01 a**	55.20 $\pm$ 15.05 a**
Acid Uric	15	3.15 $\pm$ 0.37	6.10 $\pm$ 1.50 a	3.84 $\pm$ 0.72 a	3.93 $\pm$ 1.37 a	5.88 $\pm$ 1.70 a	5.92 $\pm$ 1.73 a
	30	3.41 $\pm$ 0.55	7.32 $\pm$ 2.45 b,**	2.95 $\pm$ 0.52 a,**	2.56 $\pm$ 0.38 a,**	3.16 $\pm$ 0.91 a,**	2.20 $\pm$ 0.10 a,**

All values are expressed as mean $\pm$ SEM for 8 animals in each group.

\*Statistically significant at  $P < .01$

\*\*Statistically significant at  $P < .05$

a Comparisons are made with Group A.

b Comparisons are made with Group B.

c Comparisons are made with Group C.

## MATERIALS AND METHODS

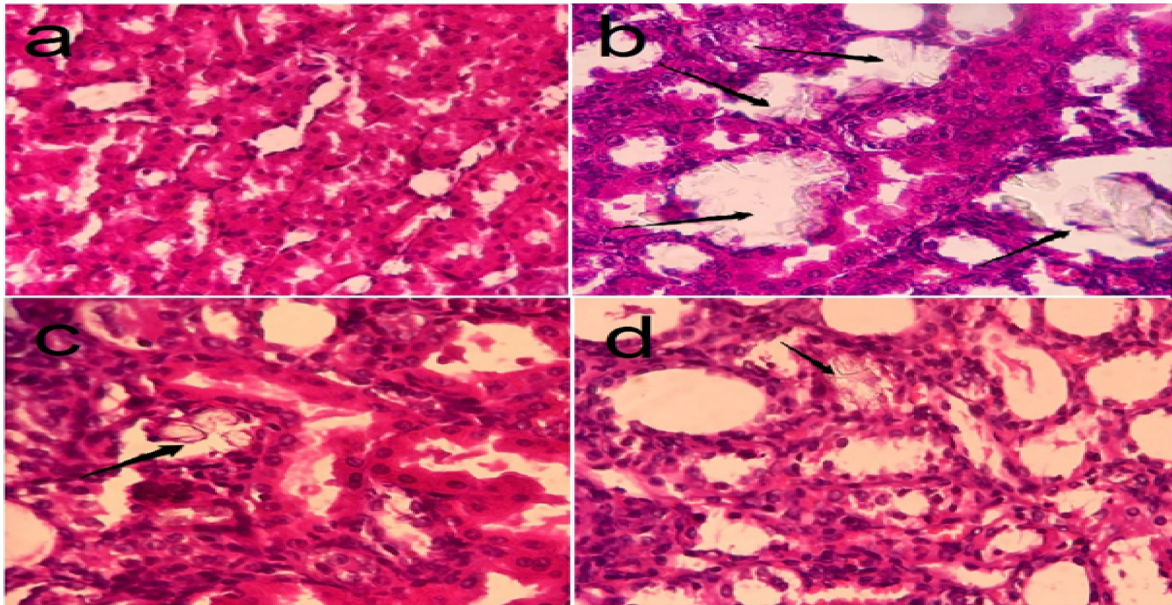
Preparation of aqueous extract of *Cerasus Avium* stem  
The *Cerasus Avium* stems were purchased from a local herb store in Birjand, Iran. They were powdered and dried. Then, 500 g of powdered herb was mixed, through the soaking method, with distilled water. After 24 hours, the extract solution was separated by filter paper. Then, the resulting solution was incubated at 40°C, until it completely dried, Then the residues were weighed (30g) and kept. Before prescribing to the an-

imal, the desired concentration of the extract was prepared in distilled water.

### Treatment of animals

The experiment was conducted in accordance with the guide for the care and use of laboratory animals and the study was approved by the Ethics committee of Birjand University of Medical Science.

Forty-eight Wistar rats with an average weight of 200  $\pm$  20 g were procured from Pasteur Institute of Iran. The animals were acclimatized to standard laboratory



**Figure 1.** Figure Representative Microscopic images of kidney sections from, (a) Control Group Shows absence of crystals deposition, (b) The large number and size of calcium oxalate crystals (arrow) in a renal tubule in ethylene glycol group (c,d) The the reduction of calculi number and size of calcium oxalate crystals in (arrow) in a renal tubule prevention and treatment groups.

conditions (temperature:  $25 \pm 2$  °C) and maintained on 12-hour light:12-hour dark cycle and they were given standard diet and had free accesses to food and water ad libitum throughout the study. They were randomly divided into six groups of 8 and treated according to the experimental protocol for 30 days. The control Group (A) only received normal water. The other five groups received 1 % ethylene glycol (Merk, Germany) during the study period. EG group (B) did not receive any other treatment during the study period. Ethylene glycol was added from the first day for thirty<sup>(30)</sup> days to the water of prevention groups of low-dose (C) and high-dose (D). From the first day of adding ethylene glycol to the water, aqueous extract of *Cerasus Avium* stem was added with 200 and 400 mg per kg of body

weight. Ethylene glycol was added from the first day for 30 days to the treatment groups of low-dose (E) and high-dose (F). From the fifteenth day of adding ethylene glycol to the water, aqueous extract was added with 200 and 400 mg per kg of body weight. In duration of experiment two rat of A and B groups died.

#### Blood samples

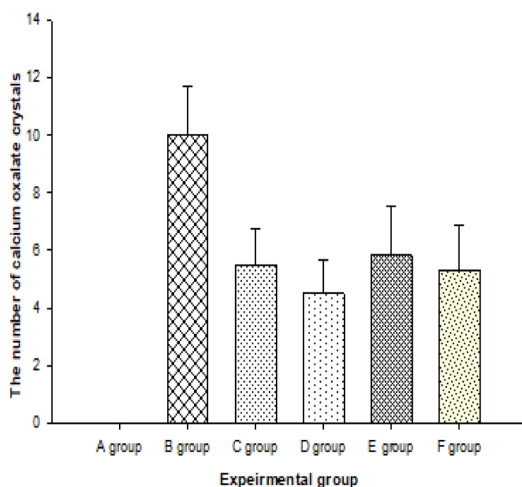
The blood samples were collected on days 15 and 30. (in the 15th day using orbital sinus and in the 30th day using cardiac puncture). Blood was collected in non-heparinized tubes and centrifuged at 3500 rpm for 15 min to obtain serum. Serum level of calcium, creatinine, uric acid, magnesium, potassium, sodium, were measured with an Auto Analyzer.(Prestige) and colorimetric method.

#### Urine samples

Twenty-four hour (24 h) urine collection of rats in each group was performed on the 15th and 30th days, individually in metabolic cages. Food and water was available during experimentation in the cages. For analysis, 1 ml of urine was taken in centrifugal tube and centrifuged at 2500 rpm for 5 min, urine level of calcium, creatinine and uric acid were measured by Prestige Auto Analyzer.

#### Histological examination

For histological examination at the end of the experiment (the 30st day), all the rats were anesthetized and the kidneys removed and fixed in 10% formaline, dehydrated in a gradient of ethanol, embedded in paraffin, and then cut in to  $5\mu$  serial sections. Then, slides containing five sections from each kidney were deparaffinized, stained with Hematoxyline and Eosine, and then examined by Olympus light microscope, in each slide 10 microscopic field with a magnification of  $10\times 40$  were selected randomly and the aggregation of CaOx deposits were counted in the aforementioned fields. Mean of oxalate crystals, number was reported.



**Figure 2.** The number of calcium oxalate crystal deposits in the kidneys of the rats at the end of the experiment. Data are expressed as mean  $\pm$  standard error.

### Data Analysis

Data were analyzed with SPSS software (Version 20.0) 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  $\pm$  standard error.

## RESULTS

### Serum parameters

The mean level of calcium, creatinine, uric acid and serum sodium of the prevention and treatment groups in the 30th day decreased significantly as compared to the EG group ( $P < .05$ ). (for details see **Table 1**). The mean concentration of magnesium and serum potassium of the prevention and treatment group in the 30th day increased significantly, as compared to EG control group ( $P < .05$ ). (for details see **Table 1**)

### Urine parameter

The calcium, creatinine and uric acid level of urine in prevention and treatment groups in the 30th day, decreased significantly compared to the EG group ( $P < .05$ ). (for details see **Table 1**)

### Pathology results

The examination of kidney sections in control group showed no calcium oxalate deposits or other abnormalities in different segments of the nephrons (**Figures 1 and 2**). But in EG group, calcium oxalate deposits, were found in different segments of the nephron (**Figures 1 and 2**). In prevention and treatment groups, the number of deposits decreased significantly compared with EG group in both doses of aqueous extract of *Cerasus Avium* stem on the 30th day. Crystals in different parts of nephrons in the kidney specimens of these groups were also thin, small, and fewer compared with those in group B. (**Figures 1 and 2**)

## DISCUSSION

Although, in recent years various chemical drugs have become available in the market that may be effective in prevention and treatment, and there is no effective drug therapy without surgery that can lead to complete treatment or prevention of urinary tract stones<sup>(2)</sup>. Several studies reported calcium oxalate crystals are injurious to renal epithelial cells by providing substrates for nucleation of crystals aggregation, and exposing sites for the attachment and retention of crystals leading to its adhesion to the epithelial cells, and consequently, the cells may produce some products as well as free radicals, inducing heterogeneous crystal nucleation and cause aggregation of crystals.<sup>(17,18)</sup> Due to the presence of substances such as caffeic acid, ferulic acid, syringic acid, ellagic acid, quercetin,  $\alpha$ -tocopherol, pyrogallol, p-hydroxybenzoic acid, vanillin, p-coumaric acid, gallic acid and ascorbic acid, the *cerasus Avium* stems have antioxidant and antiradical properties<sup>(19)</sup>. Therefore, it is speculated that *Cerasus Avium* stem, could prevent the formation of CaOx calculi and their disaggregation through its anti-inflammatory and antioxidant mechanisms. In the present study, quercetin and tocopherol reduced the mortality of tubular and level of free radicals, thereby reducing the formation of calcium oxalate crystals<sup>(20,21)</sup>. In addition, Quercetin,  $\alpha$ -tocopherol and folic acid lower cholesterol in serum<sup>(19,22,23)</sup>. According to the study, the increase of fat is one of the

causes of kidney stones<sup>(24)</sup>. In this context, oxalate has been reported to induce lipid peroxidation and cause renal tissue damage by reacting with polyunsaturated fatty acids in cell membrane<sup>(25)</sup>. It is possible that one of the reasons for the effect of *Cerasus Avium* stem on reducing calcium oxalate stones is the anti-fat roles of quercetin,  $\alpha$ -tocopherol and folic acid. Some studies suggest that calcium stones may have an infectious origin. Nano bacterium, an intracellular bacterium, is found in the kidney stones. These bacteria cause an increase in calcium stones<sup>(26,27)</sup>. Pyrogallol is a substance found in the stem of *Cerasus Avium* having anti-bacterial and anti-microbial properties<sup>(5)</sup>. Hence, it might be that the anti-bacterial property of *Cerasus Avium* stem is effective in the treatment of kidney stones in addition to afore-mentioned mechanisms in the formation of CaOx crystals. The present study showed that the major cause of kidney stone is potassium and magnesium reduction and promising results in preventing recurrence have been shown in patients treated with potassium magnesium citrate. Magnesium complexes with oxalate, reduce the supersaturation of calcium oxalate and as a consequence reduce the nucleation rate and growth of crystals<sup>(28,29,30)</sup>. The *Cerasus Avium* stem treatment increased the magnesium and potassium level of serum and thus reduced the growth of calcium oxalate crystals in drug treated animals. In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid get accumulated in blood<sup>(31)</sup>. The results of this study show that aqueous extract of *Cerasus Avium* stem reduced serum and urine creatinine and uric acid in prevention and treatment groups. It is not clear which mechanisms in the plants have effects on kidney stones. This may be as a result of its anti-inflammatory, antioxidant, anti-bacterial, and anti-fat properties. However, there is need to do more research so as to certain the effects of this plant.

## CONCLUSIONS

The aqueous extracts of *Cerasus Avium* stem significantly reduced the elevated level of calcium oxalate ions. The histopathological findings also show sign of improvement after treatment with extract. All these observation provided the basis for the conclusion that *Cerasus Avium* stem extract inhibit the stone formation induced by ethylene glycol treatment.

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