

Biochemical and Pathological Study of Hydroalcoholic Extract of *Achillea millefolium* L. on Ethylene Glycol-Induced Nephrolithiasis in Laboratory Rats

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Abstract

Background: Nephrolithiasis is of the most prevalent urinary tract disease. It seems worthwhile to replace the conventional treatments with more beneficial and safer agents, particularly herbal medicines which are receiving an increasing interest nowadays. **Aims:** In this study, we investigated the protective and curative effects of *Achillea millefolium* L. on ethylene glycol (EG)-induced nephrolithiasis in rats. **Materials and Methods:** The extract of *A. millefolium* was prepared by soxhlet method. Forty male Wistar rats were randomly divided into five groups ($N = 8$) as follows. The negative control (group A) received tap drinking water. Rats in sham (positive control group B), curative (group C and D), and preventive (group E) groups all received 1% EG in drinking water according to the experimental protocol for 30 days. In the curative groups, dosages of 200 and 400 mg/kg body weight (BW) of *A. millefolium* extract were administered orally from day 15 to the end of the experiment, group C and D, respectively. Group E received 200 mg/kg *A. millefolium* extract from the 1st day throughout the experiment. Urinary oxalate and citrate concentrations were measured by spectrophotometer on the first and 30th days. On day 31, the kidneys were removed and examined histopathologically for counting the calcium oxalate (CaOx) deposits in 50 microscopic fields. **Results:** In the curative and preventive groups, administration of *A. millefolium* extract showed significant reduction in urinary oxalate concentration ($P < 0.05$). Also, urinary citrate concentration was significantly increased in group C, D, and E. The CaOx deposits significantly decreased in group C to E compared with the group B. **Conclusions:** According to our results, *A. millefolium* extract had preventive and curative effects on EG-induced renal calculi.

Keywords: *Achillea millefolium*, Calcium oxalate, ethylene glycol, nephrolithiasis

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Introduction

Renal stones or nephrolithiasis are one of the most common urinary tract problems occurring in 1-15% of the

general population.^[1] The most important complications of kidney stones are hematuria, abdominal and flank pain, hydronephrosis, urinary tract infections, and even renal failure.^[2,3] Despite many investigations, the etiology of renal stone formation remains uncertain. Several factors have been suggested which may predispose individuals to nephrolithiasis, such as genetic predispositions, gender, age, weather conditions, water intake and its solutes, diet, and etc.^[4] Approximately 80% of renal stones are composed of calcium which has been combined with oxalate or phosphate.^[5] In Iranian traditional medicine, many plants were introduced for disposal, dissolve, and preventing the formation of

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kidney stones. On the other hand, the present therapies are to some extent effective and there is still no definite treatment for this problem.^[6] Therefore, natural and herbal medicines are believed to have many medicinal impacts as well as less adverse effects.

Achillea millefolium L., from Asteraceae family and known as yarrow, is a perennial herb which grows in Europe, Asia, and North America. In Iran, it can be found in the northern area, around the Alborz Mountain and in Azerbaijan, Lorestan, and Isfahan provinces.^[7] *A. millefolium* has been prescribed in traditional medicine as healing the wounds, to heal chapped skin, hair tonic, diaphoretic,^[8,9-12] loss of appetite, dyspepsia,^[10] flatulence, anti-dysenteric, astringent, anti-hemorrhoids, anti-inflammatory, anti-pyretic, decrease blood pressure, emmenagogue, cholagogue, anti-hemorrhagic, diuretic, urinary antiseptic, anti-spasmodic^[9-12] anti-bacterial and anti-plasmodium.^[12,13]

Currently, besides the present herbal and chemical medications, many invasive procedures such as extracorporeal shock wave lithotripsy, percutaneous lithotomy, and transureteral lithotripsy are widely used to treat nephrolithiasis. However, using these procedures is not cost-effective and may lead to serious complications.^[14-16] Hence, it seems worthwhile to replace these conventional treatments with more beneficial and safer agents, particularly herbal medicines which are receiving an increasing interest nowadays.

In this study, we aimed to evaluate the protective and curative effects of hydroalcoholic extract of *A. millefolium* L. on ethylene glycol-induced nephrolithiasis in laboratory rats.

Materials and Methods

Extract preparation

The flowers of *Achillea millefolium* L. were collected during post-flowering phases (May 2013) from the field of Medicinal Plants Institute-ACECR (Karaj, Iran) and were identified by the Pharmacognosy Department of Tehran University (herbarium code: 83001). The plants were dried in standard condition in dry shade and were powdered. Fifty grams of dried powder were soaked in 300 ml 80% ethanol for 48 hours with intermittent shaking. The plant extracts were filtered through filter paper and the filtrates were dried until a constant dry weight of extract was obtained. The extract was dissolved in distilled water to 200 and 400 mg/ml and administered to rats orally at doses of 200 and 400 mg/kg body weight (BW). The residues were stored at 4°C.

Animals

Forty male Wistar rats weighing 250-300 g were obtained from the Pasteur Institute, Karaj, Iran. The rats were housed in standard cages at 25 ± 2°C and 12:12-hours light-darkness cycle with free access to water and food *ad libitum*. All procedures used in the study were approved by the ethics committee for the use of experimental animals at Shiraz University of Medical Sciences, Shiraz, Iran.

Animals were divided randomly into five groups ($N = 8$) as follows: The control group A (control) received tap drinking water. Rats in sham (group B), curative (group C and D), and preventive (group E) groups all received 1% ethylene glycol (EG) (Mojallali Chemical Labs, Iran) in drinking water according to the experimental protocol for 30 days. In the curative groups dosages of 200 and 400 mg/kg of *A. millefolium* extract were administered orally, since day 15 to the end of the experiment, group C and D, respectively. Group E received 200 mg/kg of *A. millefolium* extract from the 1st day through the end of the experiment.

Urine analysis

For 24-hour urine collection, all rats on the first and 30th day were kept individually in metabolic cages. Urinary oxalate and citrate concentrations were measured by diagnostic kits (Darman Kaw, Tehran, Iran) with urine autoanalyzer.

Histology Examination At the end of day 31, all of the rats underwent general anesthesia and were decapitated by guillotine. Then both the kidneys were removed and kept in formalin (10%) for histological studies. Three 5 µm sections were prepared from each kidney and the slides were stained with hematoxylin-eosin (H&E) and examined by light microscope. Aggregations of calcium oxalate (CaOx) deposits in the renal tubules were counted in 10 microscopic fields.

Statistical analysis

Data were presented as the mean ± standard deviation (SD). Analysis of variance (ANOVA) test was used to determine difference among all groups. For comparison between each two groups, the Mann Whitney U test was applied. Data were analyzed by statistical software Statistical Package for the Social Sciences (SPSS) version 14.0 (SPSS Inc, Chicago, IL). Significant difference was set at $P < 0.05$.

Results

Urinary oxalate concentration in the curative and preventive groups was significantly increased in contrast

with the control group [Table 1]. In both curative groups, oxalate excretion was statistically significant at day 30 ($P = 0.04$). Also, its concentration was decreased significantly in group E in comparison with groups C and D ($P = 0.02$).

As it is shown in [Table 2], urine citrate concentration of the rats in group B was significantly lower than those of all other groups. Results showed no significant difference between group C and group B regarding urine citrate concentrations ($P = 0.06$). However, urine citrate levels in groups D and E were significantly different from those in group B ($P = 0.03$ and $P = 0.015$, respectively).

In group A, histopathological findings revealed that there was no CaOx deposit in different segments of the kidney [Figure 1]. The mean number of CaOx deposits in all groups was significantly higher than the group A [Figure 2]. The means of CaOx deposits in group C, D, and E were 20.87 ± 1.98 , 16.37 ± 1.99 , and 14.25 ± 1.98 , respectively. In groups C, D, and E, CaOx deposits were decreased significantly in comparison to the group B ($P = 0.001$, $P = 0.01$, and $P = 0.004$, respectively) [Figure 3]; however, there was no significant difference among *A. millefolium*-treated groups.

Discussion

In recent years, various herbal and synthetic medicines were produced to reduce nephrolithiasis that may be helpful in the prevention and treatment, but there is no certain medication which brings about complete

treatment and prevention of urinary stones; some patients may need surgical interventions at the end

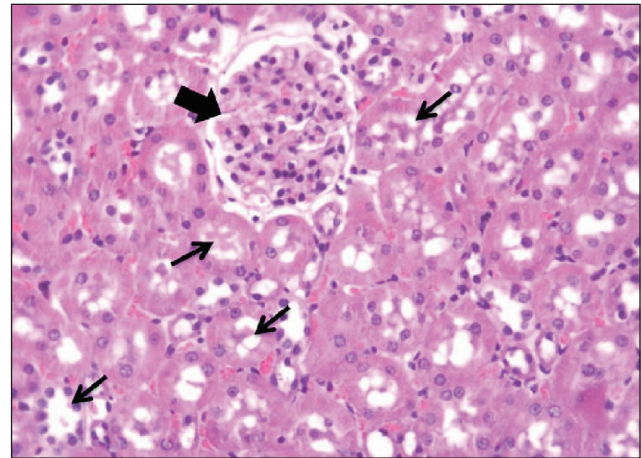


Figure 1: Histopathology (hematoxylin-eosin; original magnification, $\times 400$); Intact renal tubules (thin arrows) and glomeruli (thick arrow) are observed in group A

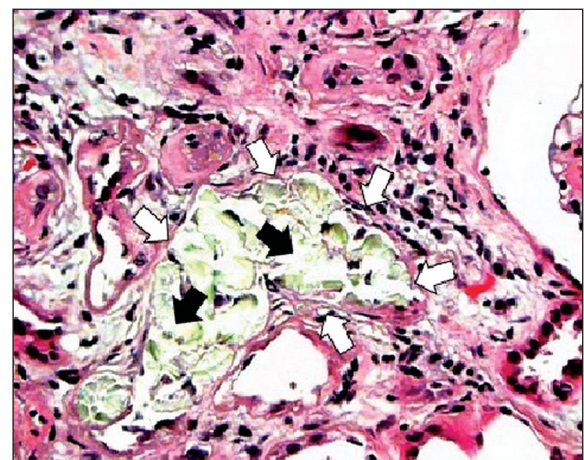


Figure 2: Histopathology (hematoxylin-eosin; original magnification, $\times 400$); Renal tubular dilation (white arrows) with calcium oxalate (CaOx) deposits (black arrows) in group B

Table 1: The 24-hour urinary oxalate concentration in all groups at the baseline and day 30 of experiment (Mean \pm standard deviation, SD)

Group	Baseline mg/dl	Day 30 mg/dl	P-value
A	0.28 \pm 0.02	0.26 \pm 0.03	0.86
B	0.27 \pm 0.03	5.84 \pm 0.03 ^a	0.014 [*]
C	0.28 \pm 0.03	1.44 \pm 0.04 ^a	0.032 [*]
D	0.26 \pm 0.01	1.26 \pm 0.03 ^a	0.034 [*]
E	0.27 \pm 0.03	1.01 \pm 0.02 ^{a, b}	0.041 [*]

*= significant, ^a $P < 0.05$ vs group A, ^b $P < 0.05$ vs groups C and D

Table 2: The 24-hour urinary citrate concentration in all groups at the baseline and day 30 of experiment (Mean \pm standard deviation, SD)

Group	Baseline mg/dl	Day 30 mg/dl	P-value
A	25.4 \pm 2.61	25.8 \pm 2.68	0.1
B	23.49 \pm 2.49	10.32 \pm 2.8 ^a	0.072
C	23.74 \pm 2.25	14.6 \pm 2.3 ^a	0.018 [*]
D	26.32 \pm 2.28	15.49 \pm 2.01 ^{a, b}	0.026 [*]
E	25.74 \pm 3.06	18.3 \pm 1.8 ^{a, b}	0.044 [*]

*= significant, ^a $P < 0.05$ vs group A, ^b $P < 0.05$ vs group B

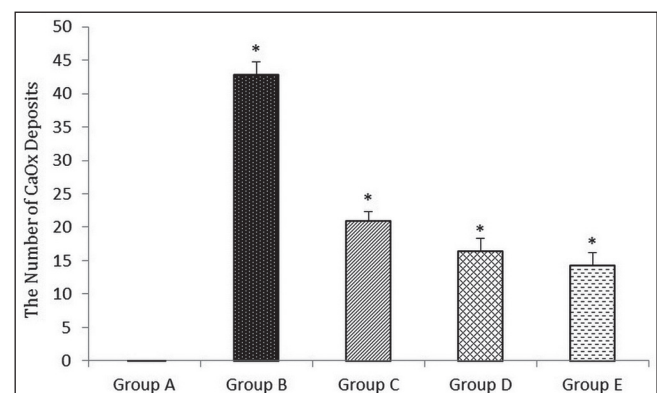


Figure 3: The effect of *A. millefolium* on the number of calcium oxalate (CaOx) deposits. All the values are expressed as mean \pm standard deviation (SD), ($n = 8$), $*P < 0.05$

due to impassable stones, those which cannot pass the tract due to their large size. Medications can lead to minimizing the stones, breaking them apart, or dilating the lumen for their passage. In this study, we investigated the protective and curative effects of *A. millefolium* on EG-induced nephrolithiasis in rats.

A high urine saturation of oxalate encourages the CaOx crystal formation in the renal tubules.^[17] Previous studies showed hyperoxaluria can damage renal epithelial cells. Free radicals which are produced by injured cell lead to induction of heterogeneous crystal nucleation and can promote calculi formation.^[14,18] According to the results of this study, EG intake led to increased urinary oxalate excretion in the rats. In both curative and preventive groups, urinary oxalate was significantly decreased. Also, its concentration was decreased by increased dosage of *A. millefolium* (200 mg/kg compared with 400 mg/kg). The exact mechanism of yarrow on EG metabolism and degradation is not known. On the other hand, anti-oxidant and anti-inflammatory abilities of *A. millefolium* have been reported.^[11,19] Therefore, one of the possible mechanisms of preventive and curative effects of *A. millefolium* can be related to these activities which may interfere with renal epithelial cells damage process and inhibit the inflammation.

It has been reported that the main risk factor of recurrent CaOx calculus is hypocitraturia.^[20] Urine citrate is able to prevent CaOx crystal formation^[20,21] due to inhibition of nucleation and growth of crystals.^[21,22] According to our results, *A. millefolium* extract increased urine citrate concentration in both preventive and curative groups in comparison to group B.

Ahmadi *et al.* showed that hydroalcoholic extract of *Alcearosea* had both preventive and curative effects on the formation of CaOx calculi apparently due to its diuretic and anti-inflammatory activities, presence of mucilaginous polysaccharides, and lowering of urinary concentrations of stone-forming constituents.^[23] Low volume of urine is one the risk factors of nephrolithiasis due to supersaturation of all solutes.^[23,24] It has been reported that *A. millefolium* has diuretic effects and increases diuresis by 25% from initial volume without changing of urine pH in the patients with calcium type of urolithiasis.^[24] Therefore, the diuretic effect of *A. millefolium* may be another possible mechanism involved in prevention of new calculi formation and dissolution of preformed stones.

Bacterial infections may play a role in the formation of some renal stones such as struvite calculi, and it has been suggested that it might be involved in calcium-based stones too.^[25] Also, Khajavi Rad *et al.* study indicated that the ethyl acetate fraction of *Cynodondactylon* had

preventive and curative effects on CaOx calculus due to its antioxidant and anti-bacterial effects.^[26] Some studies indicated that yarrow has an antimicrobial effects^[11] which may be another possible mechanism that can be involved in preventive and curative effects of *A. millefolium*.

Conclusion

The results of the present study indicated that *A. millefolium* extract had preventive and curative effects on EG-induced renal stones due to different possible mechanisms such as antioxidant, anti-inflammatory, diuretic, and antibacterial activities. However, we recommend further investigations and clinical trials for determining the effects of this plant on different aspects of nephrolithiasis treatment.

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