Effect of Polygonum Aviculare L. on Nephrolithiasis Induced by Ethylene Glycol and Ammonium Chloride in Rats

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Purpose: Nephrolithiasis is a common urinary tract disease, in addition to the pain and treatment costs, there may be significant complications resulting from the stones. This study intended to investigate the effects of Polygonum Aviculare L. aqueous extract (PAE) on urolithiasis induced by ethylene glycol (EG) and ammonium chloride (AC) in rats.

Materials and methods: Sixty-four male Wistar rats were randomly divided into eight groups (n = 8). Rats in the normal control group (I) received no treatment. The sham groups (III and IV) were given PAE at 100 and 400 mg/kg by gavage for 28 days. The disease control group (II), the prevention groups (V and VI), and the therapeutic groups (VII and VIII), received 1% EG and .25 AC in their drinking water for 28 days. The prevention groups (from the start of EG administration), and the therapeutic groups (from the 14th day of EG administration), received PAE at 100 and 400 mg/kg by gavage. At the end of the experiment, kidneys were examined for CaOx deposits and tubulointerstitial changes.

Results: The number of CaOx crystals and tubulointerstitial changes increased significantly in group II rats compared to groups I, III, and IV (P < .001). The number of CaOx crystals (P < .001) and tubulointerstitial changes (P < .001) in the prevention groups, and the number of CaOx crystals (P < .05) and interstitial changes (P < .05) in the therapeutic groups declined significantly compared to group II.

Conclusion: Results show aqueous extract of Polygonum Aviculare L. is effective in the prevention and treatment of kidney stones.

Keywords: ammonium chloride; calcium oxalate; ethylene glycol; nephrolithiasis; Polygonum aviculare; urolithiasis.

INTRODUCTION

Nephrolithiasis is the third common disease of the urinary tract after urinary infection and pathological disorders of the prostate gland.1 In 2005, the prevalence of kidney stones was reported to be 5.7% in Iran (5.3% in females and 6.1% in males).2 Different substances in the body influence the process of stone formation, and about 80-85% of the total urinary stones are calcium stones. Urinary calcium stones usually result from increases in urine calcium, uric acid, and urinary oxalates, and from reductions in urine citrate levels.3 Recurrence of kidney stones is also highly probable. In 2005, the 1-, 5-, and 10-year recurrence rates of kidney stone were reported to be 16, 32, and 53%, respectively.2 Symptoms and signs of urinary stone include colic pain, nausea, vomiting, and hematuria. Moreover, acute urinary tract obstruction, hydronephrosis, and renal damage occur.4 Treatment of urinary stones includes use of oral drugs, removal of stones by using an ureteroscope, extracorporeal shock wave lithotripsy, removal of stones through the skin, and open surgery.5 Treatment of kidney stones by using medicinal plants has been common for a long time. Considering the risk of recurrence of urinary stones, the high costs of treatment, and the complications resulting from surgical operations, use of medicinal plants can be a suitable alternative in the prevention and treatment of kidney stones. Polygonum Aviculare L. has numerous medicinal properties. In traditional Iranian medicine, this plant is considered useful for improving urinary problems, removal of kidney stones, and is used for treating kidney, bladder, and urinary tract infections.6 Polygonum aviculare contains alkaloids, tannins, saponins,7 large quantities of phenolic and flavonoid compounds,8 and has antibacterial,9 antioxidant,10 antihypertensive, diuretic11 and anti-obesity properties.12 Since the effects of aqueous extract of Polygonum aviculare on kidney stones had not been studied yet, this research investigated the effects of this extract on the prevention and treatment of kidney stones induced by ethylene glycol and ammonium chloride in male Wistar rats.

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Table 1. Effect of Polygonum Aviculare L. on calcium oxalate deposits and tubulointerstitial changes in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Calcium Oxalate</th>
<th>Tubulointerstitial Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal Control)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>II (Disease Control)</td>
<td>10.06 ± 1.89</td>
<td>1.27 ± 1.6</td>
</tr>
<tr>
<td>III (Sham 100mg/kg)</td>
<td>11.71 ± 1.9</td>
<td>1.31 ± 1.4</td>
</tr>
<tr>
<td>IV (Sham 400mg/kg)</td>
<td>6.74 ± 1.42</td>
<td>1.31 ± 1.7</td>
</tr>
<tr>
<td>V (Preventive 100mg/kg)</td>
<td>6.98 ± 1.09</td>
<td>1.3 ± 1.4</td>
</tr>
<tr>
<td>VI (Preventive 400mg/kg)</td>
<td>11.49 ± 1.73</td>
<td>1.3 ± 1.4</td>
</tr>
<tr>
<td>VII (Curative 100mg/kg)</td>
<td>6.98 ± 1.09</td>
<td>1.31 ± 1.7</td>
</tr>
<tr>
<td>VIII (Curative 400mg/kg)</td>
<td>11.49 ± 1.73</td>
<td>1.3 ± 1.4</td>
</tr>
</tbody>
</table>

**Materials and Methods**

**Ethical statement**

The study protocol was approved by the ethics committee of Jahrom University of Medical sciences (Jums. REC. 1393.006).

**Study design**

Rats were randomized into eight groups by using a random number table.

**Experimental procedures**

The Polygonum aviculare plant was collected in spring from the Shiraz garden (Shiraz, Iran) and was identified by Amir Borjani (PhD of Plant Systematic, Jahrom Islamic Azad University (Jahrom, Iran) (Voucher number: 2357). The leaves were cleaned and dried in the shade at 25°C and powdered by mechanical grinder. The powders were soaked in distilled water. After 72 hours, the extract was filtered and then condensed by a rotary evaporator under vacuum at 50°C temperature. The powders were soaked in distilled water. After 72 hours, the extract was filtered and then condensed by a rotary evaporator under vacuum at 50°C temperature. Based on previous studies, twos of 100 and 400 mg/kg of aqueous extract of Polygonum Aviculare L. was used to see its dose depended action. The groups studied during the 28 days of the research were as follows:

- Group I (normal control group): did not receive any treatment during the study.
- Group II (disease control group): received 1% ethylene glycol and 25% ammonium chloride in their drinking water during the study.
- Sham groups (III and IV): received aqueous extract of Polygonum Aviculare L. at 100 mg/kg (group III) and 400 mg/kg (group IV) by gavage during the study.
- Prevention groups (V and VI): received 1% ethylene glycol and 25% ammonium chloride in their drinking water from the first day to the last day of the study, and were given aqueous extract of Polygonum Aviculare L. at 100 mg/kg (group V) and 400 mg/kg (group VI) by gavage for 4 weeks.
- Therapeutic groups (VII and VIII): received 1% ethylene glycol and 25% ammonium chloride in their drinking water from the first day to the last day of the study, and were given aqueous extract of Polygonum Aviculare L. at 100 mg/kg (group VII) and 400 mg/kg (group VIII) by gavage from the 14th day of the study.

**Experimental animals**

This was an animal experimental study. Male Wistar rats (200 ± 10 g) were included.

**Housing and husbandry**

Rats were kept in clean cages under standard conditions at 23 ± 2°C and 12h light/12h dark cycles at relative humidity of 50-55%, and had free access to standard food and tap water during the study.

**Sample size**

Sixty four rats were divided into eight 8-member groups. Allocation to groups:

- Group I (normal control group), Group II (disease control group), Sham groups (III and IV), Sham groups (III and IV), Prevention groups (V and VI), Therapeutic groups (VII and VIII).

**Outcomes**

At the end of the study (on the 29th day), the rats were killed by carbon dioxide inhalation, and their kidneys were quickly removed and fixed in 10% formalin buffer. After dehydration, the tissues were embedded in paraffin, and 5µm thick serial sections were prepared, stained using the H&E method, and studied under a model Olympus light microscope (at 10X magnification). Twenty slides (each containing 2 sections) from each kidney were prepared. The number of calcium oxalate crystals in 10 microscopic fields were counted and reported as mean ± standard error. Tubulointerstitial changes such as tubular necrosis, tubular dilation, and interstitial inflammation were studied using the semi-quantitative approach:

- 0 = none
- 1 = trace ( < 10%)
- 2 = mild (10-25%)
- 3 = moderate (26-50%)
- 4 = marked (> 50%) (11,12)

**Statistical Analysis**

SPSS 17 was used to analyze the data. CaOx deposits and tubulointerstitial changes were normally distributed as tested by Kolmogorov-Smirnov test. Differences between groups were assessed by one-way ANOVA, following which Tukey test was performed. The results were expressed as mean ± standard error. The differences between the groups were significant at the 5% level (P < .05).

**Results**

Pathology study was carried out to detect damages inflicted on the kidneys, and the numbers of counted calcium oxalate crystals. In group I: All tissue sections were studied under the microscope, but no CaOx crystals or tissue damage was observed in any of the sections. In group II: Calcium oxalate crystals were deposited in large numbers (19.9 ± 1.90) in kidney tissues, including the proximal tubules, the Henle’s loops, the distal tubules, and the collecting ducts. The number of calcium oxalate crystals (P < .001) and interstitial changes (P < .001), increased significantly compared to group I. The sham groups (groups III and IV) as in the case of the normal control group, neither calcium oxalate crystals, nor any tissue damage was detected. (Table 1 and Figure 1)

The prevention groups (V and VI): The number of CaOx crystals (group V, P < .001, group VI, P < .001)
Figure 1. Photomicrographs of the rat kidney stained with H&E. a, c, d, e, i, k, m, and q×(10), b, f, h, j, l, n, p, and r×(40). (a & b) Group I (normal control), (c-f) Group II (EG+AC), (g&h) Group III (Sham 100mg/kg), (i&j) (Sham 400mg/kg), (k&l) Group V (preventive 100mg/kg), (m&n) Group VI (preventive 400mg/kg), (o&p) Group VII (curative 100mg/kg), (q&r) Group VIII (curative 400mg/kg). Tubular stones (white arrows), tubulointerstitial damage (dilation, hyaline cast, tubular atrophy, interstitial inflammation and tubular cell necrosis) (black arrows).

Calcium oxalate crystals, can damage kidney epithelial cells, which causes cells to secrete materials such as free radicals. These products may promote stone formation by inducing heterogeneous crystal nucleation and agglomeration. Antioxidant administration may prevent crystal nucleation and retention. Studies have shown Polygonum Aviculare L. contains alkaloids, saponins and large quantities of phenolic and flavonoid compounds. Phenolic and flavonoid compounds have antioxidant properties. Moreover, saponins have antioxidant, antifungal, and antiviral characteristics, and are hypocholesterolemic. Research has indicated saponins have protective effects against oxidative damage and renal interstitial fibrosis, and play an important role in preventing the formation of kidney stones. Therefore, Polygonum Aviculare L. prevents heterogeneous nucleation and crystal accumulation probably because it contains saponins and has antioxidant effects.

Medicinal herbs are not only cost-effective, but also contain chemical compositions that can be served as starting points for treatment of nephrolithiasis. Despite these, medicinal herbs are not without disadvantages. Like synthetic drugs, they may have negative side effects. Besides, they may interact with other herbs or drugs. There is a limited data about safety, efficacy and compositions of extracts. Therefore, further studies need to investigate their efficacy, pharmacological qualities, safety and also drug interactions. Additionally, most of research on urolithiasis carried out in rat models because of its similarities to human in CaOx deposition, location of stones, and also cortex to medulla volume ratio. In spite of this, there are some differences between rat and human kidney including: size, weight, number of papilla and nephrons. Thus, these studies are not still applicable for treatment of urolithiasis in human. In this regard, further scientific assessment and systematic reviews are required to relate animal models to human clinical trial.

CONCLUSIONS
In this research, aqueous extract of Polygonum Aviculare L. at doses of 100 and 400 mg/kg significantly reduced accumulation of calcium oxalate crystals and kidney tissue damage in the two prevention and therapeutic groups. There were no significant differences between the different doses and prevention and therapeucutic effects against oxidative damage and renal interstitial fibrosis, and play an important role in preventing the formation of kidney stones. Therefore, Polygonum Aviculare L. prevents heterogeneous nucleation and crystal accumulation probably because it contains saponins and has antioxidant effects. Medicinal herbs are not only cost-effective, but also contain chemical compositions that can be served as starting points for treatment of nephrolithiasis. Despite these, medicinal herbs are not without disadvantages. Like synthetic drugs, they may have negative side effects. Besides, they may interact with other herbs or drugs. There is a limited data about safety, efficacy and compositions of extracts. Therefore, further studies need to investigate their efficacy, pharmacological qualities, safety and also drug interactions. Additionally, most of research on urolithiasis carried out in rat models because of its similarities to human in CaOx deposition, location of stones, and also cortex to medulla volume ratio. In spite of this, there are some differences between rat and human kidney including: size, weight, number of papilla and nephrons. Thus, these studies are not still applicable for treatment of urolithiasis in human. In this regard, further scientific assessment and systematic reviews are required to relate animal models to human clinical trial.

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apeutic groups. Therefore, it seems aqueous extract of Polygonum Aviculare L. is effective in prevention and treatment of kidney stones in rat models because it contains compounds such as saponins and phenolic and flavonoid substances, and has fat-reducing, anti-oxidant, antibacterial and diuretic effects, although more research is needed to determine the mechanisms related to these effects.

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CONFLICT OF INTEREST
The authors report that they have no conflict of interest.

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