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

Jamileh Saremi[1] , Hossein Kargar Jahromi[2] , Mohammad Pourahmadi[1]*

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.[1] 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.[1] 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.[1] 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.[3] Polygonum aviculare contains alkaloids, tannins, saponins,[4] large quantities of phenolic and flavonoid compounds,[5] and has antibacterial,[4] antioxidant,[5] antihypertensive, diuretic,[6] and anti-obesity properties.[7] 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.

[1]Research Center for Noncommunicable Diseases, Jahrom University of Medical Sciences, Jahrom, Iran.
[2]Zoonoses Research Center, Jahrom University of Medical Sciences, Jahrom, Iran.
*Correspondence: Research Center for Noncommunicable Diseases, Jahrom University of Medical Sciences. Blvd, Jahrom, Iran. Postal Code: 7418814765.
Tel : 071 54336085. Fax: 071 54340405. Email: zahed1340@yahoo.com.
Received January 2017 & Accepted September 2017

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

	Group I (Normal Control)	Group II (Disease Control)	Group III (Sham 100mg/kg)	Group IV (Sham 400mg/kg)	Group V (Preventive 100mg/kg)	Group VI (Preventive 400mg/kg)	Group VII (Curative 100mg/kg)	Group VIII (Curative 400mg/kg)
Calcium Oxalate	0	19.9 ± 1.9 a***	0 b***	0 b***	10.06 ± 1.89 a*** b***	11.71 ±1.59 a*** b***	6.74 ± 1.42 a** b***	6.98 ± 1.09 a** b***
Tubulointerstitial Damage	0	1.91 ± .12 a***	0 b***	.02 ± .01 b***	1.27 ± .16 a*** b**	1.31 ± .14 a*** b*	1.31 ± .17 a*** b*	1.3 ± .14 a*** b*

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 Borjian (PhD of Plant Systematic, Jahrom Islamic Azad University (Jahrom, Iran) (Voucher number: 2537). The leaves were cleaned and dried in the shadow 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,[7,8] two doses 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[9] 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.[10]

Allocation to groups:

Rats were randomly divided into eight groups including: 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,[9] and studied under a model Olympus light microscope (at 10X magnification). Twenty slides (each containing 2 sections) from each kidney were prepared. The numbers 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 studies using the semi-quantitative approach:

0 = none, 1 = trace (< 10%), 2 = mild (10-25%), 3 = moderate (26-50%), and 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, $P < .001$)

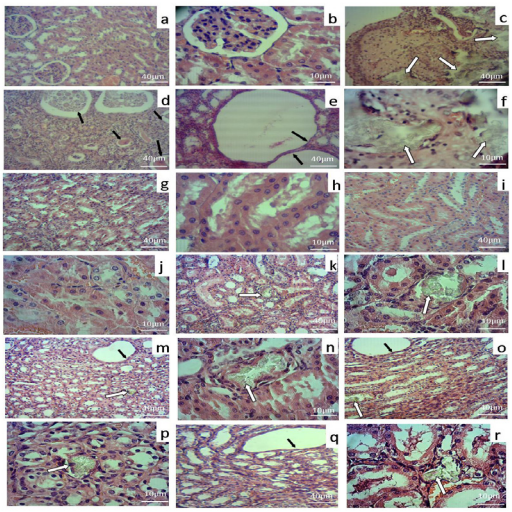

Figure 1. Photomicrographs of the rat kidney stained with H&E. a, c, d, e, g, i, k, m, o and q(x10). b, f, h, j, l, n, p and r (x40). (a & b) Group I (normal control), (c-f) Group II (EG+AC), (g&h) Group III (Sham 100mg/kg), (i&j) (Sham 400mg/kg), (k&l) Group V (preventive 100mg/kg), (m&n) Group VI (preventive 400mg/kg), (o&p) GroupVII (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).

and tissue damage (group V, $P = .007$, group VI, $P = .015$) decreased considerably compared to the disease control group. Moreover, calcium oxalate deposit and tissue damage were significantly different ($P < .001$) from those of the normal control group and sham groups, but no significant differences were observed with the therapeutic groups ($P > .05$). Furthermore, there was no significant difference between the groups V and VI($P > .05$).

The therapeutic groups (VII, VIII): CaOx crystal deposit (group VII, $P < .001$, group VIII, $P < .001$) and tubulointerstitial damage (group VII, $P = .015$, group VIII, $P = .012$) were significantly different from those of the disease control group. Moreover, the CaOx deposit and tubulointerstitial damage were significantly different from those of the normal control group ($P < .001$) and from those of the sham groups, Moreover, group VII showed no significant difference with group VIII ($P > .05$).

DISCUSSION

Results indicated that aqueous extract of Polygonum Aviculare L. at concentrations of 100 and 400 mg/kg significantly reduced the number of CaOx crystals and the extent of interstitial damage in the prevention and therapeutic groups. No studies have been carried out so far regarding the effects of Polygonum Aviculare L. on kidney stones. Therefore, it is impossible to express anything definite on how Polygonum Aviculare L. affects kidney stones and on its possible mechanisms of action. Calcium stones form in various stages, including accumulation of calcium oxalate and calcium phosphate, and crystal nucleation, growth, accumulation, and retention.[13] Low volume of urine, low pH value of urine, calcium, sodium, oxalate, and urea promote stone formation.[13]

In a study conducted on rats fed cholesterol and a high-fat diet, some metabolic disorders were observed including hyperoxaluria, hypercalciuria, nephrocalcinosis and hyperlipidemia; in other words, disorder in serum fats prepared the ground for the mentioned changes and for kidney stone formation.[14] Polygonum Aviculare L. has fat- reducing effects,[7] which may be the reason for some of its effects in preventing kidney stone formation and in removal of these stones.

Calcium stones in the kidneys may result from infections. Nanobacteria can also cause crystal nucleation and growth. They may cause damage to the epithelium of renal tubules, obstruct tubules, cause chronic infections, resulting in tissue damage and the formation of kidney stones.[15] Polygonum Aviculare L. has antibacterial properties;[4] therefore, part of its effects on curing kidney stones may be is due to its antibacterial characteristics.

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.[16] Antioxidant administration may prevent crystal nucleation and retention.[17]

Studies have shown Polygonum Aviculare L. contains alkaloids, saponins[4] and large quantities of phenolic and flavonoid compounds.[5] Phenolic and flavonoid compounds have antioxidant properties.[18] Moreover, saponins have antioxidant, antifungal, and antiviral characteristics, and are hypocholesterolemic.[19] Research has indicated saponins have protective effects against oxidative damage and renal interstitial fibrosis,[20] and play an important role in preventing the formation of kidney stones.[21] 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 of 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.[22] In spite of this, there are some differences between rat and human kidney including: size, weight, number of papilla and nephrons.[22] 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 ther-

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.

ACKNOWLEDGEMENT

We would like to express our gratitude to the Research Deputy of Jahrom University of Medical Sciences for the financial support we received to carry out this research (Grant No. 649/d/p).

CONFLICT OF INTEREST

The authors report tehat they have no conflict of interest.

REFERENCES

1. Stoller ML. Urinary stone disease. In: Tanagho EA, McAninch JW, editors. Smith's general urology. 17th ed. New York: McGraw-Hil; 2008. p.246-77..
2. Safarinejad MR. Adult urolithiasis in a population-based study in Iran: prevalence, incidence, and associated risk factors. Urol Res. 2007;35:73--82.
3. Kianmehr H. Tashkhise giyahaneh darooii [Recognition of medical herbs]. Tehran: Aiij Publications; 2008. [In Persian]
4. Salama HM, Marraiki N. Antimicrobial activity and phytochemical analyses of Polygonum aviculare L.(Polygonaceae), naturally growing in Egypt. Saudi J Biol Sci. 2010;17:57-63.
5. Hsu C-Y. Antioxidant activity of extract from Polygonum aviculare L. Biol Res. 2006;39:281-8.
6. Yin MH, Kang DG, Choi DH, Kwon TO, Lee HS. Screening of vasorelaxant activity of some medicinal plants used in Oriental medicines. J Ethnopharmacol. 2005;99:113-7.
7. Sung Y-Y, Yoon T, Yang W-K, Kim SJ, Kim D-S, Kim HK. The antiobesity effect of Polygonum aviculare L. ethanol extract in high-fat diet-induced obese mice. Evid Based Complement Alternat Med. 2013;2013.
8. Haeng PS, Sung Y, Jin NK, Kyoung KH. Anti-atherosclerotic effects of Polygonum aviculare L. ethanol extract in ApoE knock-out mice fed a Western diet mediated via the MAPK pathway. J Ethnopharmacol. 2014;151:1109.
9. Khalili M, Jalali MR, Mirzaei-Azandaryani M. Effect of hydroalcoholic extract of Hypericum perforatum L. leaves on ethylene glycol-induced kidney calculi in rats. Urol J. 2012;9:472.
10. Hadjzadeh M-A-R, Khoei A, Hadjzadeh Z, Parizady M. Ethanolic extract of nigella sativa L seeds on ethylene glycol-induced kidney calculi in rats. Urol J. 2009;4:86-90.
11. Cho HJ, Bae WJ, Kim SJ, et al. The inhibitory effect of an ethanol extract of the spores of Lygodium japonicum on ethylene glycol-induced kidney calculi in rats. Urolithiasis. 2014;42:309-15.
12. Saremi J, Kargar-Jahromi H, Pourahmadi M. Effect of Malva Neglecta Wallr on Ethylene Glycol Induced Kidney Stones. Urol J. 2015;12:2387--90.
13. Basavaraj DR, Biyani CS, Browning AJ, Cartledge JJ. The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. EAU-EBU update series. 2007;5:126-36.
14. Schmiedl A, Schwille P, Bonucci E, Erben R, Grayczyk A, Sharma V. Nephrocalcinosis and hyperlipidemia in rats fed a cholesterol-and fat-rich diet: association with hyperoxaluria, altered kidney and bone minerals, and renal tissue phospholipid–calcium interaction. Urol Res. 2000;28:404-15.
15. Kajander EO, Ciftcioglu N, Aho K, Garcia-Cuerpo E. Characteristics of nanobacteria and their possible role in stone formation. Urol Res. 2003;31:47-54.
16. Khan S, Thamilselvan S. Nephrolithiasis: a consequence of renal epithelial cell exposure to oxalate and calcium oxalate crystals. Mol Urol. 1999;4:305-12.
17. Thamilselvan S, Khan SR, Menon M. Oxalate and calcium oxalate mediated free radical toxicity in renal epithelial cells: effect of antioxidants. Urol Res. 2003;31:3-9.
18. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radic Biol Med. 1996;20:933-56.
19. Franchis G, Kerem Z, Makkar H, Becker K. The Biological Action Of Saponins In Animal Systems. Br J Nutr. 2002;88:587-605.
20. Xie X-s, Liu H-c, Yang M, Zuo C, Deng Y, Fan J-m. Ginsenoside Rb1, a panoxadiol saponin against oxidative damage and renal interstitial fibrosis in rats with unilateral ureteral obstruction. Chin J Integr Med. 2009;15:133-40.
21. Patel PK, Patel MA, Vyas BA, Shah DR, Gandhi TR. Antiurolithiatic activity of saponin rich fraction from the fruits of Solanum xanthocarpum Schrad. & Wendl.(Solanaceae) against ethylene glycol induced urolithiasis in rats. J Ethnopharmacol.. 2012;144:160-70.
22. Khan S. Animal models of kidney stone formation: an analysis. World J Urol. 1997;15:236-43.