Effect of Malva Neglecta Wallr on Ethylene Glycol Induced Kidney Stones

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Purpose: The aim of this study was to investigate the anti-urolithiasis effects of aqueous extracts of Malva neglecta Wallr on ethylene glycol and ammonium chloride induced kidney stones in a rat model.

Materials and Methods: A total number of 64 male Wistar rats were randomly divided into eight groups equally: group I (normal control), group II (disease control), groups III and IV (sham), groups V and VI (preventive groups), and groups VII and VIII (curative groups). Group I received tap drinking water, groups III and IV were given intra-peritoneal injections of 200 and 800 mg/kg aqueous extracts for 28 days. Groups II, V, VI, VII, and VIII received 1% ethylene glycol plus 0.25% ammonium chloride in drinking water for 28 days. Groups V and VI were given intra- peritoneal injections of 200 and 800 mg/kg aqueous extracts for 28 days and groups VII and VIII received intra-peritoneal injections of 200 and 800 mg/kg aqueous extracts from the 14th day of the experiment. After 28 days the kidneys were removed and observed for calcium oxalate (CaOx) deposits and tubulointerstitial changes.

Results: The extract significantly decreased CaOx deposits and tubulointerstitial damage in the preventive groups ($P < .001$). In curative groups, a low dosage of extract, reduced kidney oxalate deposits and tubulointerstitial damage ($P < .05$). In addition a significant decrease was observed in crystal deposition and tubulointerstitial damage in high dosed group ($P < .001$). However, high dosed preventive and curative groups seemed to be more effective ($P \leq .001$).

Conclusion: Malva neglecta Wallr has beneficial effects on preventing and treating CaOx deposition and decreasing tubulointerstitial damage on a dosage dependent manner. These effects may be due to the components presented in this plant such as saponins, flavonoids, mucilage, and phenolic compounds.

Keywords: animals; calcium oxalate; ethylene glycol; kidney/drug effects; kidney tubules/pathology; rats; treatment outcome; urolithiasis/chemically induced; drug therapy.

INTRODUCTION

Urolithiasis is still a worldwide problem. Despite numerous methods for its treatment, there is still no satisfactory drug to prevent and treat kidney stones. Malva neglecta Wallr commonly known as "Panirak" is extensively used in folk medicine for urolithiasis. Kidney stones are the third prevalent disorder in the urinary system.[1] In 2005, prevalence of urolithiasis in Iran was 5.7%, affecting 6.1% of men and 5.3% of women.[2] The average recurrence rate was 16% after one year, 32% after five years and 53% after 10 years.[2] About 80-85% of stones are mainly composed of calcium oxalate (CaOx). Kidney stones cause renal colic, urinary tract obstruction, hydronephrosis, infection, hematuria and loss of renal function.[1] Surgical operation, extracorporeal shock wave lithotripsy, transureteral lithotripsy and laparoscopy are widely used to remove the stones.[1] Even though these techniques have been improved in recent years, using these invasive procedures may also lead to severe complications not considering their high costs. In this regard, it seems reasonable to replace these treatments with a low side-effect herbal medication since many plants have been traditionally used in management of urolithiasis.

Malva neglecta Wallr is a plant that is used as food and medication.[3] In traditional medicine, it is used for treating kidney stones and urinary disorders.[4] It has been reported to have anti-ulcerogenic,[5] antioxidant,[6] and antibacterial[7] properties. However, no scientific data is available to validate the beneficial effect of this plant on kidney stones. This study aimed to evaluate the effect of aqueous extracts of Malva neglecta Wallr on ethylene glycol and ammonium chloride induced kidney stones in rats.

MATERIALS AND METHODS

Study Animals

This was an animal experimental study. Sixty four adult male Wistar rats (200 ± 10 g) were housed at 25 ± 2°C temperature on a standard diet and tap drinking water.[8] They maintained on 12 hours light/12 hours dark cycle. The experiments were in accordance with the guidelines

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Table. Effect of Malva neglecta Wallr on calcium oxalate deposits and tubulointerstitial changes in urolithiasis induced rat.

Variables	Group I (Normal Control)	Group II (Disease Control)	Group III (Sham 200 mg/kg)	Group IV (Sham 800 mg/kg)	Group V (Preventive 200 mg/kg)	Group VI (Preventive 800 mg/kg)	Group VII (Curative 200 mg/kg)	Group VIII (Curative 800 mg/kg)
Calcium oxalate	0 a***	19.9 ± 1.9	0 b***	0 a*** b***	9.7 ± 1.25 a* b***	4.15 ± 1.18 a*** b*	14.27 ± 1.82 a*** b***	6.75 ± 1.18
Tubulointerstitial damage	0.01 ± 0.01	1.91 ± 0.12 a***	0.01 ± 0.01 b***	0 b***	0.99 ± 0.1 a*** b***	0.4 ± 0.1 a** b***	1.35 ± 0.130. a*** b*	67 ± 0.1 a*** b***

Values are expressed as mean ± standard error (n = 8).
*** $P < .001$, ** $P < .01$, * $P < .05$.
a: comparisons made with normal control group (Group I).
b: comparisons made with disease control group (Group II).

for the care and use of laboratory animals and were approved by the ethics committee of Jahrom University of Medical sciences (Jums. REC. 1392. 042).
The Malva neglecta Wallr was collected in spring from the Zarghan garden (Shiraz, Iran) and was identified by Amir Borjian (PhD of Plant Systematic, Jahrom Islamic Azad University, Jahrom, Iran). The leaves were thoroughly washed under tap water, dried under shade, and then 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.
Urolithiasis was induced by 1% ethylene glycol and 0.25% ammonium chloride in the experimental animals. All animals were fed with a standard rat chow diet and they were randomly divided into eight groups equally:
Group I (Normal control): received tap drinking water for 28 days.
Group II: (Disease control): received drinking water containing 1% ethylene glycol and 0.25% ammonium chloride for 28 days to induce urolithiasis.
Sham groups (III and IV): received intra-peritoneal injections of 200 mg/kg (group III) and 800 mg/kg (group IV) aqueous extract for 28 days.
Preventive groups (V and VI): received drinking water containing 1% ethylene glycol and 0.25% ammonium chloride plus intra-peritoneal injections of 200 mg/kg (group V) and 800 mg/kg (group IV) aqueous extract for 28 days.
Curative group (VII and VIII): received drinking water containing 1% ethylene glycol and 0.25% ammonium chloride for 28 days plus intra-peritoneal injections of 200 mg/kg (group VII) and 800 mg/kg (group VIII) aqueous extract from the 14th day until the end of the experiment.

Histopathological Analysis of the Kidney

At the end of the experiment (the 29th day), the rats were killed by carbon dioxide inhalation. Thereafter the right and left kidneys were isolated, cleaned, and weighed. Kidneys were fixed in 10% formalin, dehydrated in a gradient of ethanol, embedded in paraffin, cut into 5 µm tick sections and stained with Hematoxylin and Eosin (H & E) and Periodic acid-Schiff (PAS). The slides were examined under a light microscope (10 × magnification). Ten slides containing five sections from each kidney were prepared. CaOx crystal deposits were counted in 10 microscopes filed.[9] Tubulointerstitial changes such as tubular cell necrosis, dilation, interstitial inflammation, hyaline cast and tubular atrophy were graded according to semiquantative system on scale of 0-4: 0 = none, 1 = trace (< 10%), 2 = mild (10-25%), 3 = moderate (26-50%) and 4 = marked (> 50%).[10]

Statistical Analysis

The results were expressed as mean ± standard error (SE). CaOx deposits and tubulointerstitial changes were normally distributed as tested by Kolmogorov-Smirnov test. The differences between groups were compared using one way analysis of variance (ANOVA) and analyzed by Statistical Package for the Social Science (SPSS Inc, Chicago, Illinois, USA) version 17. Post hoc least significant difference test was used for inter-group comparisons. *P* value less than .05 was considered significant.

RESULTS

Pathologic examination was done to detect CaOx deposits and tubulointerstitial damage in the kidney. The sediments were visible as transparent crystals in the renal tubules with an optical microscope. In group I, the examination of the kidney sections revealed no CaOx deposits or other abnormalities in different segments of the nephrons. In group II, a high number of CaOx deposits were found inside the proximal tubules, loops of henle, distal tubules and collecting ducts. Considerable tubulointerstitial changes such as tubular atrophy, dilation, hyaline cast, tubular cell necrosis and interstitial inflammation were observed in renal tissue. In sham groups (III and IV), no CaOx deposits were seen and tissue damage was nearly same as group I (**Figure, Table**).
A significant decrease was detected in the preventive group V, in crystal deposition in comparison to groups II ($P < .001$) and VII ($P = .005$). In addition, tubulointerstitial damage in group V showed a significant decrease compared to groups II ($P < .001$), VII ($P = .003$) and VIII ($P = .009$). In group VI, a few crystals of CaOx were observed when compared with groups

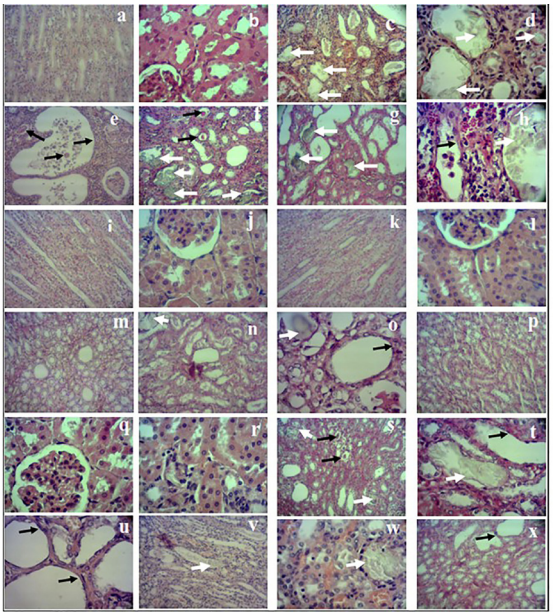

Figure. Photomicrographs of the rat kidney stained with Hematoxylin and Eosin and Periodic acid-Schiff: a (×10), c (× 10), d (× 40), e (× 10), j (× 40), l (× 40), u (× 40), and x (× 40).
Histological changes visualized by Hematoxylin and Eosin (H & E): b (× 40), f (× 10), g (× 10), h (× 40), I (× 10), k (× 10), m (× 10), n (× 10), o (× 40), p (× 10), q (× 40), r (× 40), s (× 10), t (× 40), v (× 10), and w (× 40).
Histological changes visualized by Periodic acid-Schiff (PAS): group I (a and b), group II (c-h), group III (i and j), group IV (k and l), group V (m-o), group VI (p-r), group VII (s-u), and group VIII (v-x).
Multiple tubular stones (white arrows), tubulointerstitial damage (tubular atrophy, dilation, hyaline cast, tubular cell necrosis and interstitial inflammation) (black arrows).

II ($P < .001$), V ($P = .001$) and VII ($P < .001$). Tubulointerstitial damage significantly decreased in group VI compared to groups II ($P < .001$), V ($P < .001$), VII ($P < .001$), and VIII ($P = .033$).
In the kidney specimen of curative groups, group VII showed a significant decrease in crystal deposition ($P = .033$) and tubulointerstitial damage ($P = .023$) compared to group II. The number of CaOx crystals in group VIII was significantly different from that of groups II and VII ($P < .001$). In addition, a significant decrease was found in tubulointerstitial tissue in group VIII compared with groups II and VII ($P < .001$).

DISCUSSION

Studies indicate that administration of ethylene glycol and ammonium chloride can induce renal CaOx deposition in rats[9,11] as a model to mimic the kidney stone formation in humans. Based on traditional use of Malva neglecta Wallr in kidney stones, this model was used to evaluate the effects of aqueous extracts of Malva neglecta Wallr on ethylene glycol and ammonium chloride induced kidney stones in male rats. Microscopic examination of kidney sections showed extensive renal damage and CaOx deposits in calculi induced rats.
In our study Malva neglecta Wallr had a preventive and treatment effect on CaOx calculus formation and tubulointerstitial damage with a dosage dependent manner. In prophylactic and curative treatment groups, low and high dosages of extract reduced the number of CaOx calculi and tubulointerstitial damage, although a high dosages of extract seemed to be more effective.
The exact mechanisms involved in the effect of Malva neglecta Wallr on CaOx calculi remain unclear. Studies reveal that Malva neglecta Wallr contains K, Na, P, Ca, Mg, Fe, Mn[12] alkaloids, flavonoids, saponins,[13] phenolic compounds[6] and mucilage content.[14] Phenolic compounds and flavonoids have antioxidant activities.[6] CaOx crystals and high levels of oxalate in the nephrons can damage the epithelial cells so that, the cells may produce some product as well as free radicals, which induce heterogeneous crystal nucleation and result in aggregation of crystals.[15] Therefore, it is speculated that Malva neglecta Wallr prevents the formation of CaOx calculi and tubulointerstitial damage due to its antioxidant[6] and anti-inflammatory[5] effects.
Saponins have antiviral, antifungal, antioxidant and cholesterol lowering effects.[16] Earlier studies have reported that plants rich in saponin have protective effects on the renal oxidative stress and renal interstitial fibrosis in rats induced by unilateral ureteral obstruction.[17] They plays an important role in preventing ethylene glycol induced urolithiasis.[18] Thus, anti-urolithiatic effects of Malva neglecta Wallr may be because of saponin content presented in this plant.
Nano bacteria mediate apatite nucleation and crystal growth. They may trigger renal pathology involving damage to tubular epithelium, biomineralization, and perhaps tubule obstruction and chronic infection resulting in defective tissue repair and stone formation.[19] Antibacterial activity of Malva neglecta Wallr has been reported previously.[7] Therefore, this plant may be effective for both prevention and cure of CaOx urolithiasis. The mucous lining of the urinary tract serves as a defense against CaOx crystal adherence.[20] Malva neglecta Wallr contains mucilage content that can play a role to prevent crystals adherence.

CONCLUSIONS

Aqueous extracts of Malva neglecta Wallr is effective on ethylene glycol and ammonium chloride induced kidney stones in male rats. The extract has beneficial effects on preventing and treating CaOx calculi and reducing tubulointerstitial damage in the rat kidney depending on the dosage. The anti-urolithiatic activity of this plant might be the result of its components such as saponins, flavonoids, mucilage, and phenolic compounds. Further studies are necessary to elucidate the chemical constituents of Malva neglecta Wallr responsible for anti-urolithiatic activity.

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CONFLICT OF INTEREST

None declared.

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