The Beneficial Effect of *Cynodon Dactylon* Fractions on Ethylene Glycol-Induced Kidney Calculi in Rats

Abolfazl Khajavi Rad,[1] Mousa-Al-Reza Hadjzadeh,[1] Ziba Rajaei,[1]
Nema Mohammadian,[2] Saleh Valiollahi,[1] Mehdi Sonei[1]

Purpose: To assess the beneficial effect of different fractions of *Cynodon dactylon* (*C. dactylon*) on ethylene glycol-induced kidney calculi in rats.
Materials and Methods: Male Wistar rats were randomly divided into control, ethylene glycol, curative, and preventive groups. The control group received tap drinking water for 35 days. Ethylene glycol, curative, and preventive groups received 1% ethylene glycol for induction of calcium oxalate (CaOx) calculus formation. Preventive and curative subjects also received different fractions of *C. dactylon* extract in drinking water at 12.8 mg/kg, since day 0 and day 14, respectively. After 35 days, the kidneys were removed and examined for histopathological findings and counting the CaOx deposits in 50 microscopic fields.
Results: In curative protocol, treatment of rats with *C. dactylon* N-butanol fraction and N-butanol phase remnant significantly reduced the number of the kidney CaOx deposits compared to ethylene glycol group. In preventive protocol, treatment of rats with *C. dactylon* ethyl acetate fraction significantly decreased the number of CaOx deposits compared to ethylene glycol group.
Conclusion: Fractions of *C. dactylon* showed a beneficial effect on preventing and eliminating CaOx deposition in the rat kidney. These results provide a scientific rational for preventive and treatment roles of *C. dactylon* in human kidney stone disease.

Keywords: Cynodon dactylon, kidney calculi, ethylene glycol, calcium oxalate

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INTRODUCTION

Urinary calculi are the third prevalent disorder in the urinary system,[1] which may cause obstruction, hydronephrosis, infection, and hemorrhage in the urinary tract system. Extracorporeal shock wave lithotripsy, percutaneous lithotomy, transureteral lithotripsy, and even laparoscopy are widely used to remove the calculi. However, using these invasive procedures is not cost-effective and may also lead to severe complications. Therefore, it is worthwhile to replace these conventional treatments with medicinal plants or phytotherapy. Medical plants are used worldwide and there is an increasing interest in research in this area to provide a scientific basis for their beneficial effects.[2-7]

Cynodon dactylon (*C. dactylon*), a member of the family of Cynodonteae, has been used as a medicinal herb in Iranian traditional medicine for centuries. *Cynodon dactylon* has been reported to have antidiabetic,[8]

[1]*Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
[2]*Department of Pathology, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran*

Corresponding Author:
Ziba Rajaei, PhD
Department of Physiology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
Tel: +98 511 800 2224
Fax: +98 511 882 8564
E-mail: rajaeiz@mums.ac.ir

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antimicrobial,[9] antioxidant,[10] hypolipidemic, anti-inflammatory, and anti-emetic[11] properties. Its roots and rhizomes are also used in the treatment of depression, vomiting, cough, epilepsy, and hemorrhage.[12] The roots and leaves of this plant were also found to have favorable effects on dysuria, kidney stone dissolvent, bladder injuries, and inflammation.[13,14] However, the effectiveness and mechanism by which this plant works have not yet been fully understood. Therefore, the present study was designed to investigate the preventive and curative action of *C. dactylon* fractions on ethylene glycol-induced kidney calculi in the rat.

MATERIALS AND METHODS

Preparation of extract and fractions

The roots of *C. dactylon* were collected from the campus of Imam Reza garden (Mashhad, Iran) and were graciously identified by Ferdowsi University herbarium (Mashhad, Iran). The roots were dried and powdered. Thereafter, samples were boiled in distilled water (50 g in 500 mL) for 1 hour, and the liquid containing the extract was filtered and evaporated to dry. The dried extract weighted 7 g.

For the preparation of N-butanol (NB) fraction of *C. dactylon*, 3.5 g of dried extract was dissolved in 30 mL distilled water and extracted successively with 20 mL NB. The resulting extracts were evaporated to dryness and then the NB fraction (1 g) and N-butanol phase remnant (RNB) (1 g) were provided.

The same procedure was done for providing the ethyl acetate (EA) fraction of *C.dactylon*. Therefore, 3.5 g of dried extract was dissolved in 30 mL distilled water, and extracted successively with 20 mL EA. The resulting extracts were evaporated to dry and supply the EA fraction (1.2 g) and ethyl acetate phase remnant (REA) (1 g).

Experimental protocol

Curative and preventive protocols were designed to evaluate the effectiveness of *C. dactylon* fractions on male Wistar rats. 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 Mashhad University of Medical Sciences.

Male Wistar rats weighing 200 ± 10 g were housed at 25 ± 2°C on a standard diet and tap drinking water. They were randomly divided into control, ethylene glycol (EG), curative, and preventive groups, and were treated according to the experimental protocols for 35 days.

Rats in the control group (n = 7) received tap drinking water. Ethylene glycol (n = 7), curative, and preventive groups all received 1% EG (Merck, Germany) in drinking water for 35 days.[4] Curative groups, EG + NB, EG + RNB, EG + EA, and EG + REA, (n = 7 in each group) were also treated with 12.8 mg/kg of NB fraction, RNB, EA fraction, or REA of *C. dactylon* in drinking water, respectively, since 14th day through the end of the experiment.

Preventive groups, EG + NB and EG + EA (n = 7 in each group), received 1% EG along with 12.8 mg/kg of NB or EA fraction of *C. dactylon* in drinking water, respectively, since the 1st day through the end of the experiment.

Histopathologic examination

At the end of the experiment (day 35), all rats were decapitated by guillotine after anesthesia and the kidneys were removed. For histological processing, the kidneys were fixed in 10% formalin, dehydrated in a gradient of ethanol, embedded in paraffin, and then cut into 5μ serial sections. Ten slides containing five sections from each kidney were deparaffinized, stained with hematoxylin and eosin, and then examined by light microscope. Aggregations of calcium oxalate (CaOx) deposits in the renal tubules were counted in 50 microscopic fields.

Statistical analysis

Data were analyzed by SPSS software (the Statistical Package for the Social Sciences, version 14.0, SPSS Inc., Chicago, Illinois, USA) using one-way ANOVA. Data were expressed as mean ± standard error for each group. *P* values less than .05 were considered statistically significant.

RESULTS

In the control group, the examination of the kidney sections showed no CaOx deposits or other abnormalities in different segments of the nephrons (Figure 1). However, CaOx deposits were abundantly found in the nephron proximal tubules, loops of Henle, distal tubules, collecting ducts, and even the kidney calyces in EG group (Figures 2 and 3). Deposits in different segments of the renal tubules were composed of 3 to 4 large polygonal crystals. Renal tubular dilation with epithelial damage and leukocyte reaction were also observed on pathology examination (Figure 3).

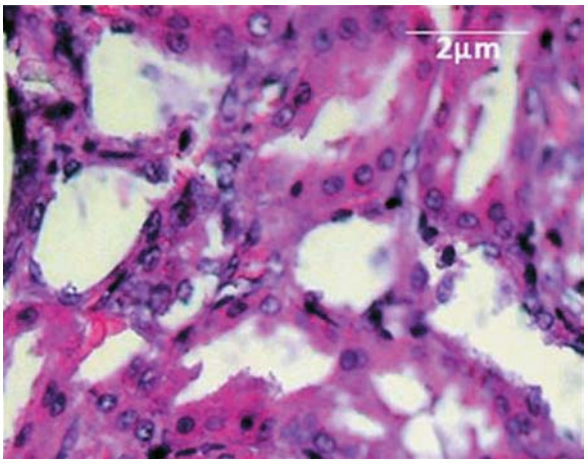

Figure 1. Normal tubules and collecting ducts (hematoxylin-eosin ×400)

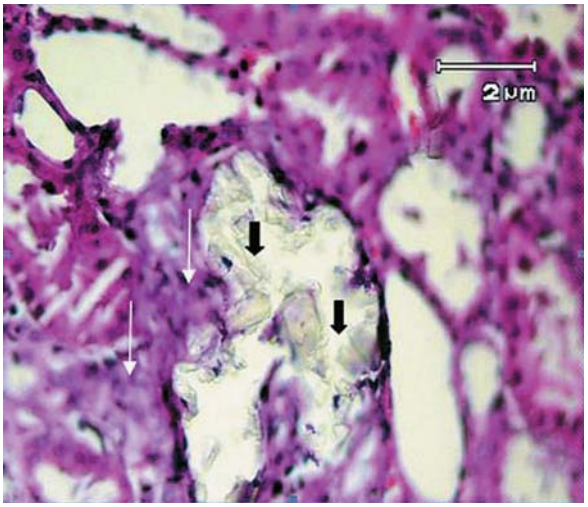

Figure 2. Multiple tubular calculi (black arrows) and inflammatory infiltration (white arrows) have been shown in ethylene glycol-treated group (hematoxylin-eosin ×400)

The mean number of CaOx deposits in 50 microscopic fields in the kidney specimens of EG group was 55.42 ± 11.13, which was significantly higher than the control group ($P < .001$; Figure 4). In curative groups, EG + NB and EG + RNB, the number of deposits was 8.14 ± 5.64 and 13.4 ± 7.09, respectively, which was significantly lower than that in EG group ($P < .001$ and $P = .01$; Figure 4). In other curative groups, EG + EA and EG + REA, the number

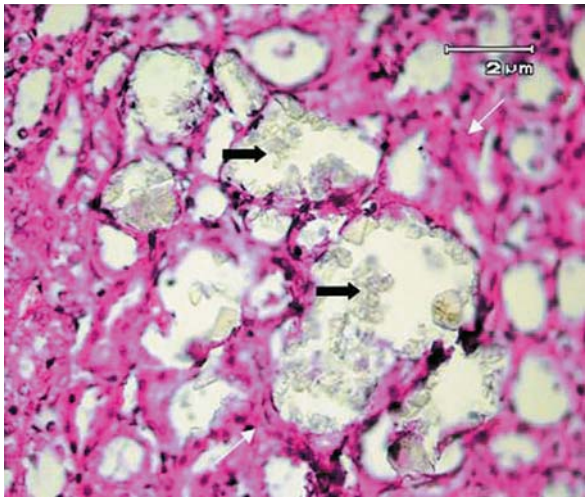

Figure 3. Renal tubular dilation and epithelial damage secondary to multiple calculi (black arrows) accompanied with inflammatory infiltration (white arrows) in peritubular space (hematoxylin-eosin ×400)

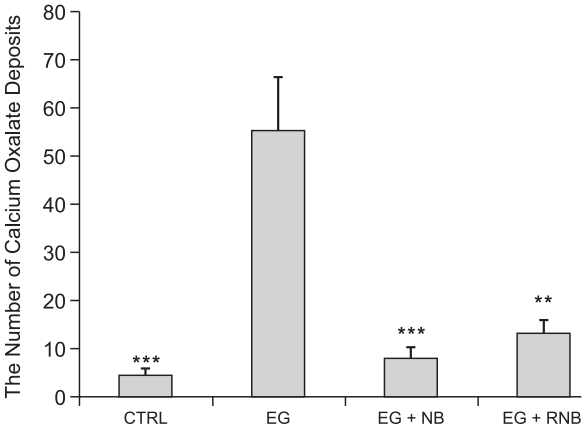

Figure 4. The number of calcium oxalate crystal deposits in 50 microscopic fields in control, ethylene glycol, and curative groups. CTRL indicates control group; EG, ethylene glycol group; EG + NB, group treated with N-butanol fraction of C.dactylon (12.8 mg/kg); and EG + RNB, group treated with N-butanol phase remnant of C.dactylon (12.8 mg/kg)/ Data were expressed as mean ± SEM, n = 7, *** $P < .001$, ** $P < .01$.

of CaOx was 46 ± 11.07 and 23.85 ± 2.21, respectively, which was lower than that in EG group (Figure 5), but the difference was insignificant.

In preventive groups, EG + NB and EG + EA, the number of deposits was 37 ± 7.59 and 22.71 ± 5.28, respectively, which was lower than that in EG group. However, this difference was only significant in EG + EA group compared with EG group ($P < .05$; Figure 6). Also CaOx crystals in different parts of the renal tubules in EG + EA group were clearly smaller in comparison with EG group.

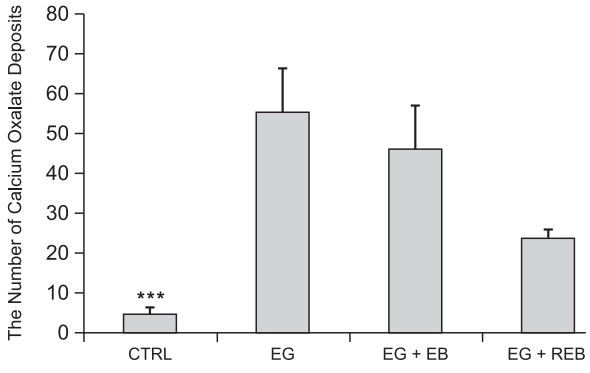

Figure 5. The number of calcium oxalate crystal deposits in 50 microscopic fields in control, ethylene glycol, and curative groups. CTRL indicates control group; EG, ethylene glycol group; EG + EA, group treated with N-butanol fraction of C.dactylon (12.8 mg/kg); and EG + REA, group treated with N-butanol phase remnant of C.dactylon (12.8 mg/kg)/ Data were expressed as mean ± SEM, n = 7, ***$P < .001$.

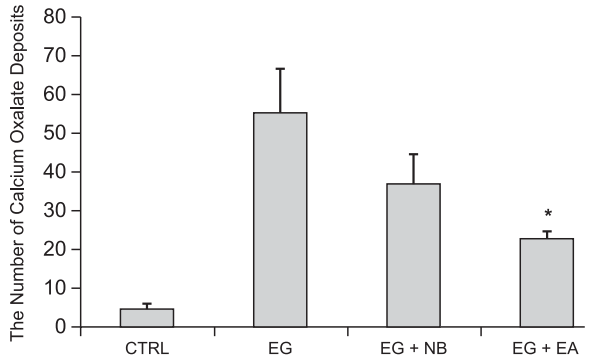

Figure 6. The number of Ca Ox crystal deposits in 50 microscopic fields in control, ethylene glycol, and curative groups. CTRL indicates control group; EG, ethylene glycol group; EG + NB, group treated with N-butanol fraction of C.dactylon (12.8 mg/kg); and EG + EA, group treated with ethyl acetate fraction of C.dactylon (12.8 mg/kg)/ Data were expressed as mean ± SEM, n = 7, *$P < .05$.

DISCUSSION

The basis of calcium stone formation is urine supersaturation with stone-forming calcium salts. A number of dietary factors and metabolic abnormalities, namely hypercalciuria, hypocitraturia, and hyperoxaluria, can change the composition or saturation of the urine and therefore enhance stone-forming propensity.[15] However, the role of other factors, such as inhibitors, infection, and matrix formation as well as urinary obstruction should not be ignored.[16]

Many in vivo models have been developed to understand the mechanisms involved in the formation of urinary calculi and to ascertain the effects of various therapeutic agents on development and progression of the disease.[17,18] Rat is the most frequently used animal to induce CaOx deposition in the kidney and mimic the etiology of the formation of stones in humans.

Accordingly, we evaluated the effectiveness of a medicinal plant, *Cynodon dactylon* fractions, which is widely used in Iranian traditional medicine in treatment of kidney stone formation, on rats rendered kidney calculi by administration of EG. This study showed that the EA fraction of *C. dactylon* had a preventive effect on CaOx calculus formation in the rat kidney. Furthermore, *C. dactylon* NB fraction and RNB decreased the number of CaOx calculi in the treated group, demonstrated a curative effect on CaOx calculi disruption formed in the kidney due to EG consumption. To the best of our knowledge, this is the first report regarding the effect of C. *dactylon* fractions on prevention and treatment of CaOx kidney calculus.

This research is an extension of our previous studies shown that the aqueous-ethanolic extract from roots and leaves of *C. dactylon* had both protective and curative effect on CaOx kidney stone induced by EG in rats (unpublished data). Atmani and coworkers have also found that the aqueous extract of *C. dactylon* at 250 mg/kg has preventive and curative effects on CaOx calculi formation.[19] These results agree with our findings in previous and present studies.

The exact mechanisms involved in the effect of *C. dactylon* fractions on CaOx calculi are

not yet clear. However, the following possible mechanisms are discussed. Calcium oxalate crystals and high levels of oxalate in the nephrons can damage the epithelial cells and consequently, the cells may produce some products as well as free radicals, which induce heterogenous crystal nucleation and cause aggregation of crystals.[20]

Phytochemical analysis of hydroalcoholic extract from *C. dactylon* rhizomes has demonstrated that the rhizomes contain sugars, flavonoids, sterols, and steroidal saponins.[21,22] Plant flavonoids are antioxidant and scavenge oxygen free radicals. It has been reported that the methanolic extract of *C. dactylon* had an antioxidant effect on COLO 320 DM cells, a colon cancer cell line, and increased the levels of antioxidant enzymes. It has been also reported that the treatment of experimental animals with methanolic extract of *C. dactylon* decreased the level of lipid peroxides.[23] Therefore, it seems that the effect of *C. dactylon* on prevention and disruption of the kidney stones may be, at least, in part due to its antioxidant effects.

Cynodon dactylon steroid saponins also have some biological and pharmacological activities, including diuretic, antibacterial, anti-inflammatory, and hypocholesteremic characteristics.[24] Therefore, it can be speculated that the role of *C. dactylon* on CaOx calculi formation and disruption, as is seen in the present study, is in part due to antioxidant and anti-inflammatory effects of *C. dactylon* compounds. These compounds may interfere with the process of epithelial cell damage induced by crystals or may exert inhibitory effect on inflammation.

It has also been reported that some kidney stones, such as struvite calculi, may have a bacterial origin like nanobacteria.[25] As *C. dactylon* has antibacterial effects,[9] this mechanism may also contribute to its curative and preventive actions on CaOx calculus formation in the kidney.

It has been suggested that the extract contains substances that coat the crystals, thereby blocking their adhesion to the cell surface.[19] Hereby, *C. dactylon* fractions may also protect renal epithelial cells at least in part by reducing cell damage via preventing crystal adhesion to the renal tubular cells.

Indeed, it has been shown in human studies that water consumption is an important factor at least with regard to the rate of recurrence in the kidney calculi formers.[26] However, the current investigation was not able to demonstrate a significant difference regarding water intake among various groups of rats. This finding was also in agreement with previous examination that has studied the effect of *C. dactylon* aqueous extract on CaOx calculi in the rat kidney.[19]

CONCLUSION

Overall, the current study data indicated that administration of *C. dactylon* N-butanol fraction, N-butanol phase remnant, and ethyl acetate fraction, at 12.8 mg/kg, showed beneficial effects on prevention and elimination of CaOx calculi in the rat kidney. Further studies are necessary to identify *C. dactylon* extract active components as well as their mechanisms involved in the treatment of kidney stones.

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CONFLICT OF INTEREST

None declared.

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