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The Effects of N-butanol Fraction and N-butanol Phase Remnant From 50% Aqueous-Ethanolic Extract of *Cyndon Dactylon* on Calcium Oxalate Kidney Stones in Rat

Hajzadeh Musa-Al-Reza[I]*, Behnam Rassouli Fatemeh[II], Khajavirad Abolfazl[I], Mahmoudian Alireza[III]

[I] Dept. of Physiology, Medical school, MUMS, Mashhad, Iran
[II] Dept. Biology, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
[III] Dept. of Anatomy, Medical school, MUMS, Mashhad, Iran

*** Corresponding author:** *M-AL-R Hadjzadeh Dept. of Physiology, Medical School, MUMS, Mashhad, Iran E-mail: Hajzadehmr@MUMS.ac.ir Tel: 0511-8828564-5*

ABSTRACT

Renal stones are common and its prevalence has been rising in both sexes. Treatment of patients with kidney stones in primary stages can reduce the side effects and may also prevent the surgical operations and postoperative complications. Several effects including: anti-microbial, anti-diabetic and cardioprotective effects have been reported for *Cynodon dactylon*. The aim of this study was to investigate the effects of N-butanol fractionand N-butanol phase remnant of *Cynodon dactylon* extracton calcium oxalate kidney stones in male rats.

Twenty four male Wistar rats were randomly divided into four groups. All groups were studied during 35 days of experiment according to the protocol of study. Normal control group (1) received tap drinking water. Negative control group (2) received 1% ethylene glycol in drinking water. Experimental groups (3 and 4) were treated with 1% ethylene glycol as well as 5mg/kg N-butanol fraction or 5mg/kg N-butanol phase remnant from 50% aqueous-ethanolic extract of *Cynodon dactylon* from first day of the experiment and continued during all protocol time.At the end of experiment, kidneys were removed for histopathologic study and examined for counting calcium oxalate deposits in 10 microscopic fields. Data were expressed as Mean± SEM and were analyzed by Kruskal-Wallis and subsequently by Mann-Whitney; $p<0.05$ was considered significant. The results showed that the number of calcium oxalate deposits in 10 microscopic fields in groups 3 ($p=0.008$) and 4 ($p=0.00$) *vs* group 2 were significantly decreased. The results of this study revealed that N-butanol fraction and N-butanol phase remnant from 50% aqueous-ethanolic extract of *Cynodon dactylon* can reduce calcium oxalate stones in the rat kidney by 40 and 55% respectively. Therefore, *Cynodon dactylon* has beneficial effects on kidney stone removal and might be used in human treatment.

Keywords: Calcium oxalate, *Cynodon dactylon,* Ethylene glycol, Kidney stones.

INTRODUCTION

Nephrolithiasis is a common disorder of the urinary tract that accounts for significant cost, morbidity, and loss of work (1). Although in most cases stones are source of discomfort and inconvenience without a significant risk to health, progressive loss of renal function can occur after repeated episodes of stone disease (2). There are distinct stone phenotypes. The cascade of events leading to kidney stone formation are different and depends on stone phenotype (3). Calcium oxalate (CaOx) stones are the most common stone type (60% of all stones), followed by the calcium phosphate stones (4).

Cynodon dactylon, belongs to Poaceae herbaceous, has been regarded to possess various medicinal properties. The plant posses antimicrobial, antiviral and cardioprotective

activity (5–8). The aqueous extract of plant is used as anti-diabetic, anti-inflammatory and anti-emetic agent (9–11). It also has significant application in treating dysentery, dropsy and secondary syphilis (12). The aim of this study was to investigate the effects of N-butanol fractionand N-butanol phase remnant of *Cynodon dactylon* extract on ethylene glycol induced CaOx kidney stone in rat which is compatible with the highest rate of kidney calculi in human.

MATERIALS AND METHODS

All animal procedures were carried out in according with the institute of Laboratory Animal Research guide for the care and use of laboratory animals; 24 male Wistar rats weighted 200±20g were housed at 25±2°C and 12h light/dark cycle. They were randomly divided into four groups and treated according to the experimental protocol for 35 days.

Rats in group 1 (normal control group) received tap drinking water. Group 2 (negative control group) received 1% ethylene glycol (Merk, Darmstadt, Germany) in drinking water (13, 14). Experimental groups 3 and 4 were treated with 1% ethylene glycol as well as 5mg/kg N-butanol fraction or 5mg/kg N-butanol phase remnant from 50% aqueous-ethanolic extract of *Cynodon dactylon* respectively, during all experimental protocol time.

The roots of *Cynodon dactylon* which were purchased from areas around Mashhad (Khorasan, Iran) dried and powdered. Then 80g of prepared powder was mixed with 50% ethanol and 50% water and kept in 40°C for 72h. The mixture was shacked every 6h for 15 min. The mixture was then filtered and concentrated by heating. The outcome of *Cynodon dactylon* powder was calculated as 21.7% extract.

At the end of experiment, kidneys were removed and kept in 10% foramlin for histopathologic processing. 5µm sections of both kidneys were prepared for each rat and slides were stained with hematoxylin eosin (HE). The slides were examined under light microscopy and calcium oxalate deposits determined. Aggregation of calcium oxalate deposits (tubules containing calcium oxalate deposits) were counted in 10 microscopic fields and expressed as Mean± SEM for each group. Data were analyzed by Kruskal-Wallis and subsequently by Mann-Whitney tests. P-values of less than 0.05 were considered significant.

RESULTS

As figure 1 shows the number of CaOx deposits in the kidney significantly decreased in groups 3 and 4 *vs* group 2. While there was no CaOx deposit in the kidney of normal control group (Figure 2), CaOx deposits in the proximal

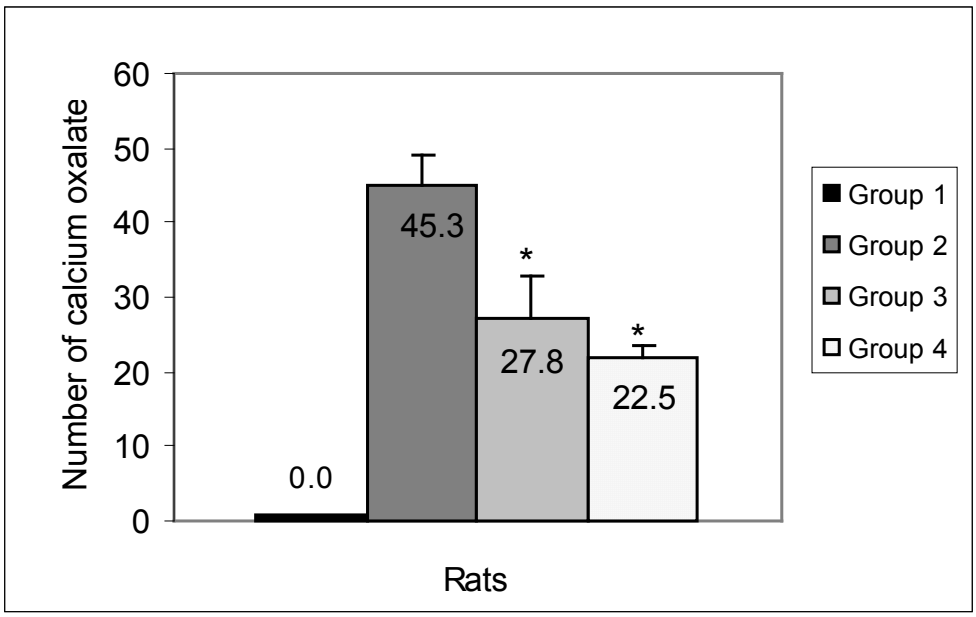

Figure 1: The number of CaOx deposits (per 10 microscopic fields) in the kidney of the rats at the end of the experiment. Data are expressed as mean ±SEM
Group 1: Normal control group
Group 2: Ethylene glycol control group
Group 3: N-butanol fraction group
Group 4: N-butanol phase remnant group

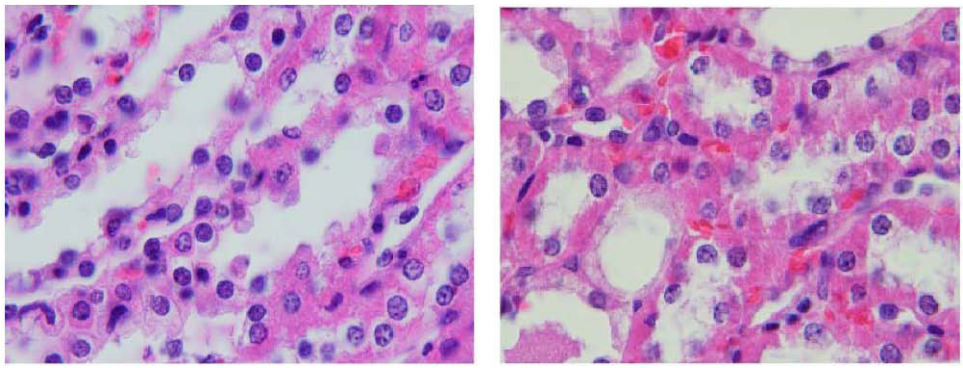

Figure 2: Normal collecting ducts in a rat's kidney; H/E (×40)

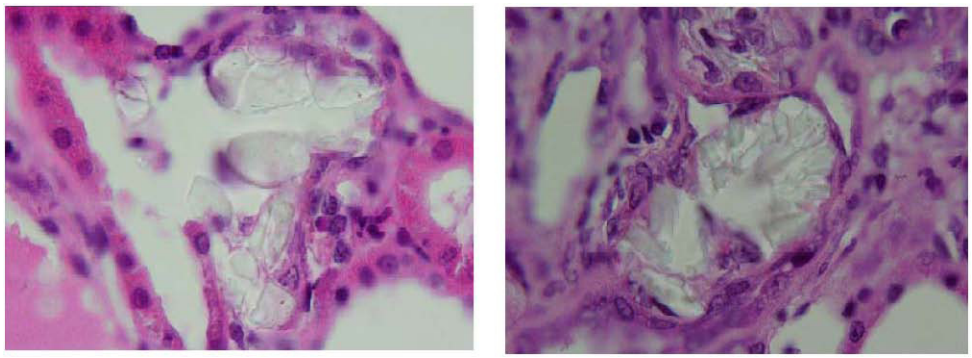

Figure 3: Tubular CaOx crystals in an EG treated rat; H/E (×40).

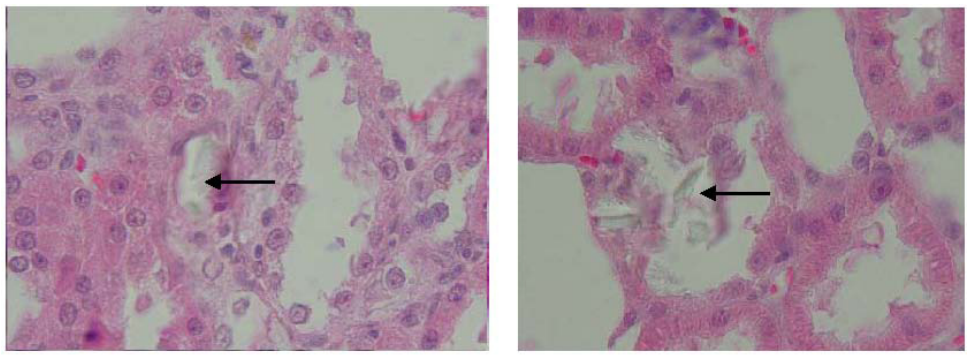

Figure 4: Few tubular CaOx crystals (arrow) in N-butanol fraction group; H/E (×40).

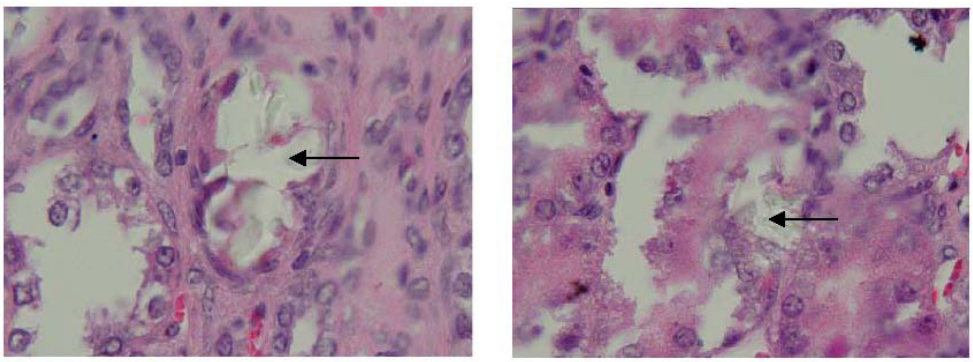

Figure 5: Tubular CaOx crystals (arrow) in N-butanol phase remnant group; H/E (×40).

and distal tubules, loop of Henle and even calyxes in group 2 (Figure 3) were plenty. In group 2 the number of calcium oxalate deposits, which were composed of 6 to 10 large polygonal crystals in different segments of the renal tube, in 10 microscopic fields in the kidney specimens was 45.3±4.27. As it is shown in Figure 1, the number of deposits in group 3 and 4 were 27.8±4.78 and 22.5±1.35 respectively, which were significantly lower than group 2 (p=0.008 and p=0.00). In comparison with group 2, not only the number of CaOx deposits in groups 3 and 4 were significantly lower but also the size of deposits in different parts of renal tubules in these groups was clearly thinner and smaller (Figures 4 and Figure 5).

The results of this study revealed that N-butanol fraction and N-butanol phase remnant from 50% aqueous-ethanolic extract of *Cynodon dactylon* were able to reduce CaOx stones in the rat kidney by 40 and 55% respectively.

DISCUSSION:

Data of the present study demonstrated that *Cynodon dactylon* had a disruptive effect on CaOx crystals formed by EG in the kidney of rat (Figure 1).Recent investigations have speculated that nanobacteria, which are gram-negative and atypical bacteria, may play a role in the formation of renal deposits by nucleating carbonate apatite on their surfaces (15). It was also demonstrated that nanobacteria were present in 70 of 72 kidney stones analyzed by scanning electron microscopy and immunofluorescent staining (16). Based on these findings, it has been hypothesized that nanobacteria colonization could damage renal tubular epithelial cells, resulting in biomineralization and subsequent stone formation (3). Since *Cynodon dactylon* has antibacterial effects, it may be effective in prevention of CaOx deposits formation (6, 7). Also, CaOx crystals in renal tubules may damage epithelial cells to produce superoxide anions and free radicals to induce "hetrogenic crystal nucleation" (17). On the other hand, *Cynodon dactylon* has anti-inflammatory effects (10). Therefore, it may be suggested that part of *Cynodon dactylon* actions on disruption of CaOx kidney calculus might be due to its anti-inflammatory effects.

We concluded that N-butanol phase remnant and N-butanol fractions from 50% aqueous-ethanolic extract of *Cynodon dactylon* significantly decreased the number and size of CaOx deposits in the rat kidney. *Cynodon dactylon* is widely used in traditional medicine in Asia; therefore it may be advised that N-butanol phase remnant and N-butanol fractions from aqueous-ethanolic extract of *Cynodon dactylon* have beneficial effect on treatment of CaOx stones in human.

REFERENCES

1 Saita A., Bonaccorsi A. and Motta M. Stone composition: where do we stand? *Urol Int.* **79**: 16–9 (2007).

2 Park S. and Pearle M.S. Pathophysiology and management of calcium stones. *Urol Clin Am.* **34**: 323–34 (2007).

3 Miller N.L., Evan A.P. and Lingeman J.E. Pathogenesis of renal calculi. *Urol Clin Am.* **34**: 295–313 (2007).

4 Mandel N.S. and Mandel G.S. Urinary tract stones disease in the united states veteran population II. Geographical analysis of variations in composition. *J Urol.* **142**: 1516–1521 (1989).

5 Dhar M.L. and Dhawan M. Melhrotra Screening of Indian plants for biological activity Part I. *Ind J Exp Biol.* **6**: 232–247 (1968).

6 Artizzu N., Bonsignore L., Cottiglia F. and Loy G. Studies of the diuretic and antimicrobial activity of *Cynodon dactylon* essential oil. *Fitoterapia.* **66**: 174–176 (1995).

7 Balasubramaning G., Sarathi M., Rajesh S. and Sahul A.S. Screening the antiviral activity of Indian medicinal plants against white spot syndrome virus in shrimp. *Aquaculture.* **263**: 15–19 (2007).

8 Najafi M., Nazemiyeh H., Garjani A., Ghavimi H. and Gharekhani A. Cardioprotective effects of *Cynodon Dactylon* against ischemia/reperfusion-induced arrhythmias. *J Mol Cell Cardiol.* **42**: 12–15 (2007).

9 Singh S.K., Kasari A.N., Gupa R.K., Jaiswal D. and Watal G. Assessment of antidiabetic potential of *Cynodon dactylon* extract in streptozotocin diabetic rats. *J Ethnopharmacol.* **114**: 174–79 (2007).

10 Singh S.K., Rai P.K., Jaiswal D. and Watal G. Evidence-based critical evaluation of glycemic potential of *Cynodon dactylon*. *Altern Med.* **17**: 1–6 (2007).

11 Ahmed S., Reza M.S. and Jabbar A. Antimicrobial activity of *Cynodon dactylon*. *Fitoterapia.* **65**: 463–64 (1994).

12 Chopra R.N. and Handa K.L. *Indigenous Drugs of India*, (Academic Publishers, India, 1982).

13 Khan S.R., Johnson J.M. and Peck A.B. Expression of osteopontin in rat kidneys: induction during ethylene glycol induced calcium oxalate nephrolithiasis. *J Urol.* **168**: 1173–1181 (2002).

14 Marengo S.R., Chen D.H. and Kaung H.L. Decreased renal expression of the putative calcium oxalate inhibitor Tamm-Horsfall protein in the ethylene glycol rat model of calcium oxalate urolithiasis. *J Urol.* **167**: 2192–2197 (2002).

15 Wood H.M. and Shoskes D.A. The role of nanobacteria in urologic disease. *World J Urol.* **24**: 51–54 (2006).

16 Ciftcioglu N., Bjorklund M. and Kuorikoski K. Nanobacteria: an infectious cause for kidney stone formation. *Kidney Int.* **56**: 1893–1898 (1999).

17 Khan S.R. and Thamilselvan S. Nephrolithiasis: a consequence of renal epithelial cell exposure to oxalate and calcium oxalate crystals. *Mol Urol.* **4**: 305–312 (2000).