Urinary oxalate, citrate, and gamma glutamyl transferase alterations after administration of *Cynodon dactylon* extract in cats

Janitabar, S.[1], Malmasi, A.[1*], Amin, Gh.R.[2], Bokaie, S.[3], Molazem, M.[4], Naderinezhad, F.[1], Sharifi , R.[1]

[1]*Department of Internal Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[2]*Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*

[3]*Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

[4]*Department of Surgery and Radiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran*

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Correspondence
Malmasi, A.
Department of Internal Diseases,
Faculty of Veterinary Medicine,
University of Tehran, Tehran, Iran
Tel: +98(21) 61117077
Fax: +98(21) 66438327
Email: amalmasi@ut.ac.ir

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Abstract:

BACKGROUND: Urinary oxalate and citrate are the key factors in caox urolithiasis of cats and Gamma Glutamyl Transferase is a good test for assessment of kidney damage. Favorable effects of *Cynodon dactylon* on calcium oxalate stone formation have recently been proved in rats. **OBJECTIVES:** The present study was designed to investigate the alteration of urinary oxalate, citrate, and GGT after administration of the hydroalcoholic extract of *Cynodon dactylon* to experimental hyperoxaluric cats. No scientific study has been done so far to demonstrate the beneficial effect of this plant in cats. **METHODS:** 13 mature male cats were randomly divided into 3 groups: group A received standard diet and drinking water while, group B and C also received ethylene glycol at sub-toxic dose (130 mg/kg) daily for 30 days. Group C received hydroalcoholic extract of *C.dactylon* (400 mg/kg) from day 0 to 30, as well. Urine samples were collected on days 0, 15, and 30 and were analyzed for oxalate, citrate, and GGT levels. **RESULTS:** Urine oxalate level in group B was significantly higher than group C on days 15 and 30. Urinary citrate excretion was significantly higher in group C compared to the other groups on day 15; however, it decreased during the entire experiment in groups B and C. Urinary Gamma Glutamyl Transferase level was increased in hyperoxaluric cats and decreased in the treated group during the experiment. **CONCLUSIONS:** Based on our results, *C.dactylon* extract could reduce the hyperoxaluria and has beneficial effects on preventing the renal damage in cats. Such findings provide a scientific explanation for applying *C.dactylon* in prevention and possible treatment of CaOx kidney stones in cats and humans.

Introduction

Urolithiasis has long been recognized as a cause of irritative voiding in cats. The two most common feline urinary stones are calcium oxalate (CaOx) and struvite. Their relative frequencies have changed over the years. Before the late 1980s, sterile struvite was the most common urolith; however, in 2007, CaOx was the most dominant mineral type found in cats (Palm et al., 2011; Cannon et al., 2007). This modification may be partly attributable to the change to urine-acidifying, magnesium-restricted diets that were introduced to reduce the formation of struvite (Buffington and chew, 1999).

Small increases in urinary oxalate can have a major effect on calcium oxalate crystal formation, and higher levels of urinary oxalate are a major risk factor for the formation of calcium oxalate kidney stones (Taylor and Curhan, 2007). Hyperoxaluria can occur from increased dietary intake as well as a loss or diminished activity of oxalate-degrading bacteria in the colon (*Oxalobacter formigenes*) (Palm et al., 2011). Oxalate is a metabolic end product and is excreted unchanged in the urine after absorption in the gastrointestinal tract, However, the role of dietary oxalate in the pathogenesis of calcium oxalate nephrolithiasis is unclear (Taylor and Curhan, 2007).

Citrate is an inhibitor of calcium oxalate urolith formation. Thus, Hypocitraturia may be a risk factor for CaOx stone formation in humans because citrate can become chelated to calcium, forming a more soluble salt than CaOx in the urine (Palm et al., 2011). However, Systemic metabolic derangements, such as acidosis, may decrease the urinary excretion of citrate and seem to increase the risk (Hostutler et al., 2005).

In domestic animals, Gamma Glutamyl Transferase (GGT) is mainly in the kidneys, the pancreas, and the intestine. Its liver activity is relatively high in cows, horses, sheep and goats and very low in dogs, cats and birds. Urinary GGT is a good test of kidney toxic damage. (Braun et al., 1983). So little research has been done on possible diagnostic significance of urinary GGT for renal diseases (Dierickx, 1981). Urinary GGT is an indicator of renal proximal tubular damage (Clemo, 1998).

Currently, no medical dissolution protocol is available for calcium oxalate calculi (Palm et al., 2011; Hostutler et al., 2005). Several products from medical plants have been used in treatment of urinary calculi in humans for centuries (Khajavi Rad et al., 2011a). Data from in vitro, in vivo, and clinical trials reveal that phytotherapeutic agents could be useful as either an alternative or an adjunctive therapy in the management of urolithiasis (Butterweck et al., 2009). *Cynodon dactylon* (*C.dactylon*), a member of the family of Cynodonteae, is a resilient and perennial grass native to the warm temperate and tropical regions (Khajavi Rad et al., 2011a). It also grows in Iran, locally named Margh and has been used as a medicinal herb in Iranian traditional medicine for centuries (Khajavi Rad et al., 2011b). *C.dactylon* is claimed to be diuretic and used as a remedy for urinary infections, kidney stones, and congestion (Khajavi Rad et al., 2011a). Furthermore, it has been seen that this medicinal plant has favorable effects on dysuria, kidney calcium oxalate stone dissolvent, bladder injuries and inflammation (Khajavi Rad et al., 2011b). The aqueous and hydroalcoholic extracts of root and rhizome of *C.dactylon* have had preventive and curative actions on ethylene-glycol induced kidney calculi in rats (Khajavi Rad et al., 2011a; Khajavi Rad et al., 2011b; Atmani et al., 2009; Sekkoum et al., 2010). The exact mechanism of this plant on urinary system is not clear. As mentioned above, urinary oxalate and citrate are the key factors on caox urolithiasis and GGT is a good test of kidney toxic damage. Therefore, the present study was designed to investigate the alteration of urinary oxalate, citrate, and GGT after administration of the hydroalcoholic extract of *Cynodon dactylon* rhizomes to experimentally induced hyperoxaluria in cats. We aimed to find out whether this plant could beneficially alter these factors and have the preventive effect on hyperoxaluric cats. No scientific study was undertaken so far to demonstrate the beneficial effect of the plant in cats.

Materials and Methods

Preparation of extract: Rhizomes of *C.dactylon* were bought from a local herbalist and graciously identified in department of pharmacognosy, faculty of pharmacy, Tehran University of medical sciences, Tehran, Iran. The rhizomes were powdered. Then, the powder was mixed with a sufficient volume of 70% ethanol and extracted with percolator for 3×72 hours. The solvent was removed by rotary evaporator and the extract was dried under the fume hood. The dried extract was sonicated with distilled water for 6 minutes at 20ºC in order to produce the hydroalcoholic extract with a 100 mg/ml concentration (Khajavi Rad et al., 2011a). The hydroalcoholic extract of *C.dactylon* was kept in refrigerator and fed to cats daily.

Toxicity studies: A pilot study was designed to determine a suitable dose of Ethylene glycol and the hydroalcoholic extract of *C.dactylon* for cats as it has not been developed before.

The kidney NOAEL (No Observed Adverse Effect Level) dose of Ethylene glycol for rats on the basis of previous studies (Corley et al., 2008) is 150

mg/kg. Therefore, five doses (100, 130, 150, 300, and 500 mg/kg) of Ethylene glycol were used in a 30-day preliminary study in order to find out an appropriate sub-toxic dose of Ethylene glycol in cats. Due to some adverse effects of Ethylene glycol at 500 and 300 mg/kg doses after days 8 and 10 respectively, we removed these dosages in our experiment. Ethylene glycol at 100 mg/kg did not induce CaOx monohydrate crystalluria; however, at 130 and 150 mg/kg, sufficient crystalluria were observed. Therefore, the 130 mg/kg dose was chosen in our study which showed CaOx-monohydrate (COM) crystalluria with minimum adverse effects on kidneys (Cr = 1.6 and BUN = 42.9).

Meanwhile, a preliminary study to determine the effective dose of *C.dactylon* extract was carried out. Three doses (100, 250, and 400 mg/kg) were used. The results for the dose of 100 and 250 mg/kg showed little effects on CaOx deposition in kidneys, while 400 mg/kg was effective and selected in our study.

Experimental protocol and samples collection: 13 healthy male and matured DSH (Domestic Short Hair) cats weighing 3 to 4 kg and aging between 1and 2 years old were used in this experiment.

They were randomly divided in 3 groups (A, B, C) and maintained at 25±2ºC in individual cages under 12h light/dark cycles for 30 days. The animals were given standard diet and free access to tap water ad libitum.

Group A (3 cats) served as negative control and received the standard diet and drinking water ad libitum.

Group B (5 cats) served as positive control and received a daily sub-toxic dose of Ethylene glycol (Merck, Germany) equivalent to 130 mg/kg in order to induce hyperoxaluria, the standard diet and drinking water ad libitum.

Group C (5 cats) served as treatment and received daily 130 mg/kg Ethylene glycol along with hydroalcoholic extract of *Cynodon dactylon* (100 mg/ml) at dose equivalent to 400 mg/kg, based on the preliminary study, for 30 days.

Urine samples were collected on days 0, 15, and 30 of the experiment.

Analysis of urine: Urine samples were taken by cystocentesis and analyzed for oxalate by Enzymatic/Colorimetric method (Hodgkinson and Williams, 1972) using AutoAnalyser (ZiestChem Diagnostics. Tehran, Iran). Citrate was measured by Enzymatic manual method (Petraulo et al., 1995) and GGT activity was determined by Modified IFCC method, Enzymatic Kinetic (Schumann et al., 2002) using TGHI Co. kits (LOT no. DG029202/G) by AutoAnalyser Selectra ProM (ELITech Clinical Systems).

Statistical analysis: Data were analyzed by SPSS software (version 16.0, SPSS Inc., Chicago, Illinois, USA) using Repeated Measures of ANOVA. Results are presented as means±standard errors (SE). p values less than 0.05 were considered significant.

Results

Mean urinary oxalate and citrate levels of all groups are presented in table 1. At the baseline there was no significant difference between groups in urine oxalate and citrate levels. Urine oxalate level in group B was significantly higher than group C and group A on days 15 and 30 ($p<0.001$ and $p=0.001$ respectively). Urinary oxalate excretion was increased in group B and decreased in treated group during the experiment; nonetheless, these differences were not statistically significant between days 0 and 30 of the study inside both groups.

Urinary citrate excretion was significantly higher in group C on day 15 compared to other groups ($p=0.011$); however, it decreased on day 30 ($p=0.011$). Urinary citrate excretion was decreased during the experiment inside both groups B and C, and these differences were significant ($p=0.05$ and $p<0.01$ respectively).

Urinary GGT (Gamma Glutamyl Transferase) activity was increased in hyperoxaluric cats and decreased in the treated group during the experiment (Table 2). Although these alterations were not statistically significant between days 0 and 30 of the experiment, the differences were remarkable. Urinary GGT levels in hyperoxaluric (B) and treated (C) groups are shown in figure.1 and figure.2 during the experiment, respectively.

Discussion

CaOx is the most common mineral found in cats (Palm et al., 2011; Cannon et al., 2007). There is no medical dissolution protocol available for CaOx

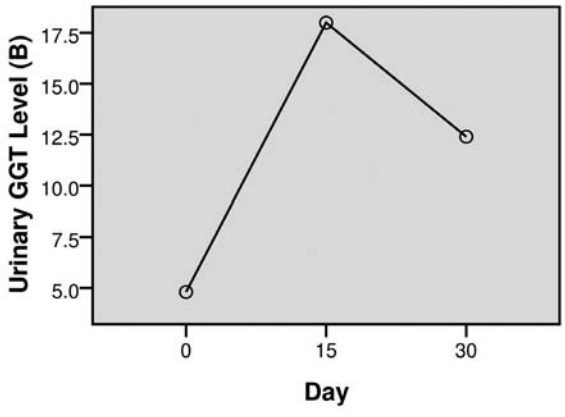

Figure 1. Urinary GGT level in hyperoxaluric group (B). GGT level was increased in hyperoxaluric cats during the experiment.

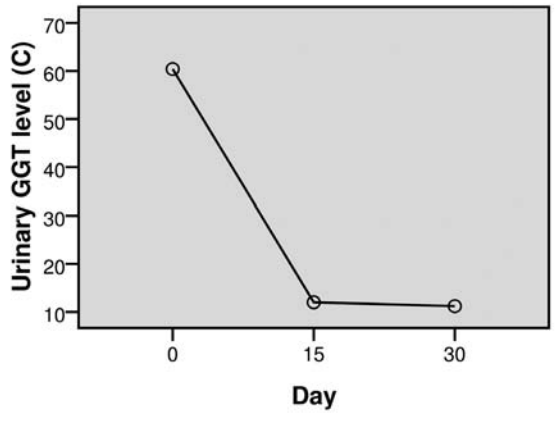

Figure 2. Urinary GGT level in treated group (C). GGT level was decreased in treated cats with *C.dactylon* during the experiment.

calculi (Palm et al., 2011; Hostutler et al., 2005), and there is no satisfactory drug to use in clinical therapy (Butterweck et al., 2009). Only conservative medical management for cats can be used that has minimal or no renal amelioration (Palm et al., 2011). Based on data from in vivo studies on rats *C.dactylon* has favorable effects on kidney CaOx stone dissolvent (Atmani et al., 2009; Khajavi Rad et al., 2011a; Khajavi Rad et al., 2011b). Since urine oxalate and citrate are the key factors on CaOx urolithiasis in cats and urinary GGT is a good test of kidney damage, the present study was designed to investigate the alterations of urinary oxalate, citrate, and GGT after administration of the hydroalcoholic extract of *Cynodon dactylon* rhizomes to experimentally induced hyperoxaluria in cats. In general, the crystallization of stone forming salts is due to an abnormal urinary composition that is either higher in crystallization promoters (e.g. calcium, oxalate, uric acid) or lower in inhibitors (e.g. citrate, glycosaminoglycans, kidney proteins such as nephrocalcin, Tamm-Horsfall mucoprotein uropontin), or both (Khajavi Rad et al., 2011a).

In our experiment, oxalate was highly excreted in the urine of cats in group B on days 15 and 30, compared to other groups, which was significant. This was in conflict with Khajavi Rad et al. and Atmani et al.'s findings who showed oxalate was highly excreted in treated versus untreated rats (Khajavi Rad et al., 2011a; Atmani et al., 2009). The urinary citrate excretion in treated cats was significantly higher than hyperoxaluric group on day 15. However, it decreased significantly during the entire experiment in both groups. Considering the current study, oxalate level was higher in the urine of calculi-induced animals (group B) that confirms the stone formation mechanism. Moreover, urine oxalate concentration decreased in hyperoxaluric cats treated with the plant extract. Since oxalate is a key risk factor in CaOx calculus formation and citrate is regarded as an effective inhibitor of CaOx stone formation (Bihl and Meyers, 2001), it is important to point out that *C.dactylon* extract used in this study seemed to have significant effect on decreasing the urinary oxalate excretion and inhibition of CaOx stone formation. Furthermore, higher citrate level in group C, compared to group B on day 15, could also explain the inhibitory effect of this medicinal plant on kidney stone formation. On the other hand, the urinary citrate excretion was decreased during the experiment inside both groups B and C. These findings are in consistent with Khajavi Rad et al.'s results which imply that citrate excretion level was decreased in treated groups. However, they declared that ethylene glycol did not have any significant effect on the urinary citrate levels which was in contrast with the current findings (Khajavi Rad et al., 2011a).

Urinary GGT (Gamma Glutamyl Transferase) level was increased in hyperoxaluric cats and decreased in treated group during the experiment. However, these alterations were not significant between days 0 and 30 inside both groups. GGT can provide information about the progression and recovery of renal damage because it could vary with the activity of renal disease (Clemo, 1998).

Table 1. Urine concentration of oxalate and citrate. A, negative control; B, positive control which received 130 mg/kg/day Ethylene glycol; C, treated group which received 400 mg/kg/day of *C.dactylon* extract. The values are expressed as mean ± S.E. Urine oxalate level in group B was significantly higher than groups C and A on days 15 and 30 ($p<0.001$ and $p=0.001$ respectively). Urinary citrate excretion was significantly higher in group C on day 15 compared to other groups ($p=0.01$); It decreased during the experiment in both groups B and C significantly ($p=0.05$ and $p<0.01$ respectively).

Groups	Oxalate (mg/dL)			Citrate(mg/dL)		
	A	B	C	A	B	C
Day 0	2.00±1.60	1.76±1.06	1.10±0.34	48.33±7.79	41.00±10.11	44.20±5.91
Day 15	2.06±0.63	5.40±0.50	0.80±0.30	22.66±6.33	10.80±2.00	35.50±6.00
Day 30	0.43±0.03	3.20±0.56	0.62±0.14	30.33±5.23	13.40±1.12	14.92±3.35

Table 2. Urinary GGT activity. A, negative control; B, positive control which received 130 mg/kg /day Ethylene glycol; C, treated group which received 400 mg/kg/day of *C.dactylon* extract. The values are expressed as mean ± S.E. Urinary GGT activity was decreased in treated group during the experiment. Despite considerable changes, however these differences were not statistically significant ($p=0.1$).

Groups	GGT (U/L)		
	A	B	C
Day 0	7.00±1.00	4.80±0.20	60.40±21.67
Day 15	9.66±3.84	18.00±6.31	12.00±7.50
Day 30	4.00±0.57	12.40±3.85	11.20±5.70

According to previous studies in dogs treated with gentamicin for 10 days, the concentration of urinary GGT slowly increased as the renal damage progressed. On the other hand, in a study where dogs were treated with a single dose of maleic acid, a decline in urinary enzyme activity was considered a measure of the rate of recovery from renal injury (Clemo, 1998). In the current study, increases in urinary GGT in hyperoxaluric cats may be associated with acute proximal tubular damage, while decline of urinary GGT level in treated group may be due to the preventive effects of *Cynodon dactylon* hydro-alcoholic extract on CaOx calculus formation. Calcium Oxalate crystals and high oxalate levels in nephrons can induce damage in epithelial cells, and consequently the cells may produce some products, as well as free radicals, inducing heterogeneous crystal nucleation and cause aggregation of crystals (Khan and Thamiselvan, 2000). It is postulated that the plant inhibits the formation of particles in kidney tubules. Thus, the plant extract may interfere directly with the inhibition of crystal adhesion to the epithelium by blocking the attachment sites located either on the cell surfaces or on the surface of the crystals themselves (Khajavi Rad et al., 2011a). In a study by Atmani et al., it has been established that C. dactylon extract has beneficial effect in preventing and eliminating CaOx deposition in the kidneys (Atmani et al., 2009). Also, the same results were reported with *Nigella sativa* seeds; its ethanolic extract reduced the number of calcium oxalate deposits in rats (Hadjzadeh et al., 2007). Phytochemical analysis of hydroalcoholic extract obtained from *C.dactylon* rhizomes has demonstrated that the rhizomes contain sugar, flavonoids, sterols, and steroidal saponins (Fazly Bazzas et al., 1997; Garjani et al., 2009; Khajavi Rad et al., 2011a). The flavonoids are antioxidant and scavenge oxygen free radicals. Therefore, it can be speculated that the role of *C.dactylon* ethanolic extract in preventing the formation of CaOx calculi is in part due to the anti-inflammatory and anti-oxidant effects of the different compounds of the *C.dactylon* (Comalada et al., 2006).

In conclusion, our findings present new data supporting that *C.dactylon* extract could prevent the hyperoxaluria and has beneficial effects on inhibiting the renal damage in cats. Such findings provide a scientific explanation for its use in prevention and possible treatment of CaOx kidney calculi and justify its use in traditional medicine for its anti-urolithiatic activity in human medicine. Further studies are necessary to clarify the exact mechanism of the preventive and curative effects of the extract in cats. Such studies are underway in our department.

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تغییرات اگزالات، سیترات و گاما گلوتامیل ترانسفراز ادرار پس از تجویز عصارهٔ *Cynodon dactylon* به گربه‌ها

سعیده جانی تبار[۱] عبدالعلی ملماسی[۱*] غلامرضا امین[۲] سعید بکایی[۳] محمد ملازم[۴] فتانه نادری نژاد[۱] رویا شریفی[۱]

۱) گروه بیماریهای داخلی، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران
۲) گروه فارماکوگنوزی، دانشکده داروسازی دانشگاه علوم پزشکی تهران، تهران، ایران
۳) گروه بهداشت مواد غذایی و کنترل کیفی، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران
۴) گروه جراحی و رادیولوژی، دانشکده دامپزشکی دانشگاه تهران، تهران، ایران

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چکیده

زمینهٔ مطالعه: اگزالات و سیترات ادرار در تشکیل سنگ ادراری اگزالات کلسیم و گاما گلوتامیل ترانسفراز در ارزیابی آسیب کلیه در گربه‌ها نقش کلیدی دارند. آثار مطلوب *Cynodon dactylon* در جلوگیری از تشکیل سنگ اگزالات کلسیم در رت‌ها به اثبات رسیده است. **هدف:** مطالعهٔ حاضر به منظور ارزیابی تغییرات مقادیر اگزالات، سیترات و GGT ادرار پس از تجویز عصارهٔ *C. dactylon* به گربه‌هایی صورت گرفت که به طور تجربی هایپر اگزالوریک شده‌اند. تاکنون هیچ تحقیقی به منظور بررسی آثار مفید این گیاه در گربه‌ها صورت نگرفته است. **روش کار:** ۱۳ گربهٔ نر بالغ به طور اتفاقی به سه گروه تقسیم شدند. گروه A جیرهٔ استاندارد و آب آشامیدنی دریافت کردند در حالی که گروه‌های B و C، روزانه اتیلن گلیکول نیز با دوز غیرسمی (۱۳۰ mg/kg) به مدت ۳۰ روز دریافت کردند. گروه C، به همراه اتیلن گلیکول عصارهٔ آبی الکلی (۴۰۰mg/kg *C. dactylon*) را نیز از روز صفر تا ۳۰ دریافت کردند. نمونه‌های ادرار در روزهای ۰، ۱۵ و ۳۰ جمع‌آوری و از نظر اگزالات، سیترات و GGT ارزیابی شد. **نتایج:** اگزالات ادرار در گروه B در روزهای ۱۵ و ۳۰، به طور معنی‌داری بیشتر از گروه C بود و سیترات ادرار در گروه C در روز ۱۵ نسبت به سایر گروه‌ها بالاتر بود. گرچه سیترات ادرار در طول مطالعه در گروه‌های B و C روند کاهشی داشته است، سطح گاما گلوتامیل ترانسفراز ادرار در طول مطالعه در گربه‌های هایپر اگزالوریک افزایش و در گروه درمانی کاهش یافته است. **نتیجه‌گیری نهایی:** بر اساس نتایج ما، عصارهٔ *C.dactylon* سبب کاهش هایپر اگزالوری می‌شود و آثار مطلوبی در پیشگیری از آسیب کلیه در گربه‌ها دارد. این یافته‌ها توضیحات علمی جهت به کار بردن *C.dactylon* در پیشگیری و درمان احتمالی سنگ کلیه در گربه‌ها و انسان‌ها را بیان می‌دارد.

واژه‌های کلیدی: گربه، سیترات، *Cynodon dactylon*، گاما گلوتامیل ترانسفراز، اگزالات

*)نویسنده مسئول: تلفن: ۶۱۱۱۷۰۷۷ (۲۱)۹۸+ نمابر: ۶۶۴۳۸۳۲۷ (۲۱)۹۸+ *Email: amalmasi@ut.ac.ir*