Clinical assessment of *Elymus repens* (couch grass) as antiurolithiatic in dogs

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Abstract

The use of *Elymus repens*-based extracts as an antiurolithiatic agent in humans has been widely reported. However, in veterinary medicine, this possibility has not been explored, therefore, the objective of this study was to assess the effectiveness of a liquid formula for oral administration containing *Elymus repens* (commonly known as couch grass) as an antiurolithiatic in dogs. To carry out this assessment, we required dogs with a urolithiasis diagnosis, and they were divided into two groups: the control group (CG), which received a treatment without an active ingredient, and the treatment group (EG), which received treatment with the extract of *Elymus repens*. Both groups received the treatment for 21 days. The assessment of the treatment was performed through blood count and biochemistry. At the end of the treatment, the values observed in analytes related to urolithiasis remained unchanged in the EG group. Conversely, some analytes in CG showed changes from the beginning of the study. According to the results obtained, the treatment with the extract of *Elymus repens* demonstrated the expected antiurolithiatic effect. However, further complementary studies to confirm its therapeutic activity. Nonetheless, it can be considered as a possible alternative treatment for dogs.

Keywords: *Elymus repens*; Dogs; Nephrotoxicity; Phytopharmaceuticals; Phytochemicals.

Study contribution

Evaluation of a formulation based on *Elymus repens* to determine if the antiurolithiatic effect reported in humans can also occur in dogs and thus be considered as a treatment option, since it is already marketed for humans.

Introduction

Urinary lithiasis can be generally defined as the presence of uroliths in the urinary tract.[1] The problem of having uroliths is a multifactorial condition resulting from the combined influence of epidemiological, biochemical, and genetic factors. Generally, this is more common in males. This condition is not a disease, but a combination of several disorders, some of which can be identified and treated (such as the formation of struvite uroliths generated by infection). Others can be identified, but cannot be corrected (hyperuricosuria, present in dalmatians, because of the excretion of high levels of uric acid that form ammonium urate), while for others, the etiology is not fully known (as the formation of calcium oxalate uroliths in miniature Schnauzers).[2]

There are factors that influence the formation of these calculi, such as breed, gender, anatomical abnormalities, functional abnormalities of the urinary tract, metabolic imbalances, urinary tract infections, diet, and urine pH. Each of these factors can have different effects on different types of uroliths. For example, an alkaline pH in the urine is a risk factor for struvite uroliths, but a protective factor against cystine uroliths.[1, 2]

Uroliths are composed of crystalline or non-crystalline solid substances, and they can appear anywhere in the urinary tract. When lithogenic substances become saturated, uroliths form, and they can interfere with urine volume and voiding frequency. It’s common for uroliths to be composed of one or more types of minerals, so the formation of uroliths is not a disease, but a complication of several disorders. Some can be identified and corrected, others can be

identified but not corrected, and in other cases the underlying etiopathogenesis is unknown.[2, 3] For the diagnosis of urolithiasis, it is necessary to assess the factors involved in urolith formation, such as measuring the concentration of lithogenic substances in the urine, the influence of urine pH on crystal formation, and measuring the saturation level of the urine with crystallogenic substances.[4]

It is important to determine the causes of urolith formation. These include oversaturation of elements in the urine that lead to crystals or uroliths, due to inhibitors of crystallization and aggregation, as well as crystal growth, the crystalloid complexes, the effects of promoters of aggregation and crystal growth, as well as those of the non-crystalline matrix.[4]

The uroliths indicates problems in the lower urinary tract, such as urethral obstruction. In urolithiasis, the glomerular filtration decreases due to urinary tract obstruction caused by solid deposits in the urinary system. As a result, waste products such as the nitrogenous substances (urea, creatinine, and uric acid) may accumulate in the blood, which explains why this condition is associated with azotemia, hyperkalemia, metabolic acidosis, and dehydration.[5, 6] In general terms, the treatment of such obstruction consists of removing the blockage and to fix correcting the physiological alterations. If these obstructions are recurrent, scrotal urethrostomy may be considered in male dogs. However, these procedures are associated with a greater risk of lower urinary tract disease, as well as bacterial infections of the urinary tract.[7] Furthermore, there is a high risk of recurrence of these stones or uroliths (10–33 %), in addition to high cost. Therefore, it is essential to determine the type and composition of these uroliths.[3]

In the traditional medical systems, most remedies are derived from plants, which have demonstrated significant efficacy. Some active compounds in these plants have been

reported to effectively reduce the recurrence rate of kidney uroliths without causing adverse effects.[8] *Elymus repens* (commonly known as couch grass), a member of the Poaceae family, has been successfully used to enhance the elimination of kidney uroliths due to its mild diuretic effects. It has also been used to treat recurrent cystitis and prostate disease, as well as to reduce spasms and pain in the urinary tract, all without causing side effects.[9, 10] It has been reported that *Elymus repens* (couch grass) contains organic acids, carbohydrates, and amino acids, among which notably include tryptophan, pectins, saponins with anti-inflammatory, anti-adhesive, and diuretic effects, as well as several phenolic compounds and antioxidants.[11, 12] The available literature on *Elymus repens* highlights its therapeutic potential in humans. However, there is insufficient evidence supporting its use in veterinary medicine, therefore, the objective of this study was to assess the efficacy of a liquid formula for oral administration based on *Elymus repens* as an antiurolithiatic agent in dogs.

Material and methods

Ethical statement

All samples were collected by the veterinarians of each patient, and the samples will subsequently be provided to the researchers for processing and analysis. The owners were informed of the study's purpose and methodology upon request, and they gave written consent to participate.

Animals and treatments

A double-blind study was conducted, including 12 male dogs of different breeds, aged between 6 and 10 years, with an average weight of 9.1 kg. The inclusion criteria required that the animals had been previously diagnosed with urolithiasis based on clinical signs, as well as clinical history, imaging diagnosis (abdominal radiography), urinalysis, and biochemical profiling for creatinine, uric acid, blood urea nitrogen, and calcium levels.[13, 14] Patients with

a urinary infection or obstruction requiring surgical intervention were not included.[6, 14] Cases of urolithiasis were identified in private clinics in Mexico City. During the study, the animals remained in their home, receiving care and feeding from their owners. Their diet and food intake were not modified, and they had access to water *ad libitum*.

The animals were divided into two groups of six dogs each. The first group was considered the treatment group (EG), and they received a commercial solution containing *Elymus repens* extract, 2 mL every 12 hours (0.48 mg/kg oral route) for 21 days. The second group was considered the control group (CG), and they received a formula containing the same excipients as the treatment group, but without the *E. repens* extract, at 2 mL every 12 h, orally, for 21 days. The formula given to EG contained 0.36 g of *E. repens* extract for each 150 mL of final solution.

Sampling and processing

Blood and urine samples were collected after 12 h of fasting. Urine samples were collected individually by spontaneous urination in disposable containers on days 0, 7, 14, and 21 after treatment initiation. They were stored at 4 °C, and the analysis was performed within four hours of collection.[15, 16] A physical and chemical analysis was performed on the urine, measuring specific gravity, pH, and protein levels. Additionally, a microscopic examination to detect of leucocytes, bacteria or crystals in urine was carried out.[16, 17]

On days 0, 7, 14, and 21 after treatment, 3 to 5 mL of blood were collected from all the animals via jugular venipuncture using Vacutainer tubes without anticoagulant and a 21G needle. The serum was separated by centrifugation at 3 000 rpm for 10 min, and levels of creatinine, urea, Na, K, bicarbonate, albumin, and Ca were measured. The serum was stored at -20 °C until its analysis using diagnostic kits in a semi-automatic analyzer (DSL model DIRUI, CS-T240) at Laboratorio de Patología Clínica of the Facultad de Medicina Veterinaria

y Zootecnia of the Universidad Nacional Autónoma de México.[1] The mean and standard deviation (SD) were compared between groups using the Student's T test for independent samples, with a significance level of $P < 0.05$. The results were analyzed using IBM® SPSS Statistics 27.0.

Results

There was no statistically significant difference ($P = 0.3600–0.6140$) between the recorded density for CG and EG at days 0 and 7 after treatment. However, there was a statistical difference ($P = 0.0010–0.0300$) between CG and EG at days 14 and 21, showing a slight increase in density in CG. The pH values recorded throughout the study did not show a statistically significant difference between CG and EG at days 0 and 7 ($P = 0.4000–0.8590$). Conversely, at days 14 and 21, a statistically significant difference between CG and EG could be seen ($P = 0.0000–0.0500$). The pH and density values recorded during the study for CG and EG are in **Table 1**.

Table 1. Mean and SD of pH and density values

Day	Group	pH	Density
	CG	6.25 ± 0.17[a]	1.03 ± 0.010[a]
0	EG	6.27 ± 0.13[a]	1.02 ± 0.012[a]
	CG	6.25 ± 0.27[a]	1.02 ± 0.008[a]
7	EG	6.37 ± 0.17[a]	1.03 ± 0.010[a]
	CG	6.4 ± 0.20[a]	1.03 ± 0.005[a]
14	EG	6.68 ± 0.24[b]	1.02 ± 0.004[b]
	CG	6.28 ± 0.24[a]	1.04 ± 0.008[a]
21	EG	7.0 ± 0.11[b]	1.02 ± 0.010[b]

[a-b] Different literals indicate statistically significant differences between rows.
[1]CG = control group without *Elymus repens* (commonly known as couch grass); EG = treated group with *Elymus repens* (couch grass).

During the assessment, only traces of protein were detected in the urine samples from CG and EG. In the microscopic assessment, leukocytes or bacteria were not observed in CG and EG at different evaluation time points. From day 0 to 21, crystals with a tetrahedral shape, corresponding to dehydrated calcium oxalate, were observed in CG. These crystals were also observed in EG from day 0, and their excretion decreased throughout the study until day 21, at which point they were no longer visible in the samples from any of the animals.

Table 2, the results obtained from the blood biochemical tests across different sampling days from CG and EG. In both groups, the levels of creatinine (P = 0.5670–0.7330), urea (P = 0.8790–0.9860), sodium (P = 0.3930–0.4850), potassium (P = 0.7380–0.9360) and albumin (P = 0.7340–1.0000) did not show a statistically significant difference across the different sampling days. On the other hand, bicarbonate levels showed a statistically

significant difference (P = 0.0130) at day 21, with a mean and SD of 17.1 ± 1.4 for CG, and 21 ± 2.7 for EG. This was also observed in calcium levels, showing a statistically significant difference (P = 0.0040–0.0290) at days 14 and 21, with slightly higher calcium levels recorded in CG.

Table 2. Mean and SD of the serum components during the treatment

Day	0		7		14		21	
Group[1]	**CG**	**EG**	**CG**	**EG**	**CG**	**EG**	**CG**	**EG**
Creatinine								
μmol/L	74.6 ± 8.4[a]	77.1 ± 6.8[a]	75.8 ± 7.8[a]	77.3 ± 7[a]	75 ± 8.3[a]	76.8 ± 7.3[a]	74.5 ± 7.7[a]	77 ± 6.8[a]
Urea								
mmol/L	5.3 ± 1.5[a]	5.5 ± 1.8[a]	5.4 ± 1.32[a]	5.3 ± 1.9[a]	5.3 ± 1.3[a]	5.4 ± 1.7[a]	5.4 ± 1.5[a]	5.4 ± 1.7[a]
Sodium								
mmol/L	146 ± 1.7[a]	147 ± 1.4[a]	146 ± 1.6[a]	147 ± 1.3[a]	145 ± 2.2[a]	146 ± 1.6[a]	146 ± 2.3[a]	146 ± 1.1[a]
Potassium								
mmol/L	4.3 ± 0.4[a]	4.4 ± 0.2[a]	4.3 ± 0.4[a]	4.4 ± 0.2[a]	4.4 ± 0.4[a]	4.4 ± 0.2 [a]	4.3 ± 0.3[a]	4.4 ± 0.2[a]
Bicarbonate								
mmol/L	16.8 ± 1.1[a]	17.1 ± 1.4[a]	17.1 ± 1.7[a]	17.6 ± 1.3[a]	17.5 ± 2.5[a]	20 ± 2.3[a]	17.1 ± 1.4[a]	21 ± 2.7[b]
Albumin								
g/L	34.3 ± 3.2[a]	34.3 ± 2.4[a]	34 ± 3.0[a]	34.5 ± 1.8[a]	34.5 ± 3.0[a]	35 ± 1.7[a]	34.3 ± 3.2[a]	34.6 ± 1.5[a]
Calcium								
mmol/L	2.9 ± 0.09[a]	2.8 ± 0.18[a]	2.9 ± 0.08[a]	2.7 ± 0.1[a]	2.9 ± 0.06[a]	2.7 ± 0.09[b]	2.8 ± 0.12[a]	2.7 ± 0.08[b]

[a-b] Different literals indicate statistically significant differences between columns.

[1]CG = control group without *Elymus repens* (commonly known as couch grass); EG = treated group with *Elymus repens*.

Discussion

Typically, plant extracts have been used to treat various diseases due to their active ingredients. However, it is essential to evaluate these extracts to ensure the desired therapeutic effect, such as the antiurolithiatic effect expected with *Elymus repens* administration, It has been suggested that this effect may be due to an increase in the urine flow, which consequently enhances calcium excretion through urine and reduces the formation of calcium oxalate uroliths.[10, 12, 18]

Although crystalluria can be an important finding, the presence of crystals does not confirm uroliths but suggests the crystalline oversaturation due to lithogenic substances such as calcium oxalate. Additionally, crystalluria may be reflected as an increase in urinary density, as observed in both groups at the beginning and the end of the treatment. However, EG showed a decrease in urinary density and crystalluria compared to CG, suggesting that *Elymus repens* administration may have exhibited the antiurolithiatic effect.[2, 5, 19] Urine pH is a factor that can indicate the type of urolith present. For instance, calcium oxalate, purine, and cystine uroliths generally form in urine with a pH below 7, whereas struvite and calcium phosphate uroliths form in alkaline urine pH higher than 7.

In this study, the recorded urine pH in both groups was below 7.0, consistent with the observed calcium oxalate crystals, supporting a diagnosis of urolithiasis due to calcium oxalate crystals.[2] Urine pH is primarily influenced by the diet, which is why most dogs have acidic urine due to uric acid excretion. However, in cases of urinary tract infection, bacteria synthesize ureases, increasing the amount of urea present, which in turn raises urine pH and promotes struvite crystals formation. In this study, urine pH remained acidic, and no struvite crystals or bacteria were

observed, ruling out urinary tract infection as a contributing factor to urolithiasis in both groups.[17, 19]

In some cases of urolithiasis, hypercalcemia may be present, particularly in calcium oxalate urolithiasis. In this study, both groups showed slightly elevated blood calcium levels, which decreased by day 14 in EG, corresponding to the observed reduction in urinary density. This suggests a potential effect of the administered treatment in EG.[2]

Other blood parameters, including creatinine, albumin, Na and K, remained within reference ranges in both groups. However, it has been reported that significant alterations in these values are only observed in cases of severe kidney function loss.[19] *E. repens* has been administered for up to 31 days in patients with urinary tract disorders, with reported improvements in 44.4 % to 100 % of cases by the end of treatment. This is consistent with the improvements observed in EG at day 21 of this study.[10]

Systematic toxicity studies on *Elymus repens* have not yet been conducted. However, in this study, no alterations in blood analytes were observed that could be associated with adverse effect following its administration during 21 days.[10]

Conclusions

Based on the results, the antiurolithiatic effect of *Elymus repens* (couch grass) extract at the administered doses in patients diagnosed with urolithiasis can be considered. Additionally, no changes were observed that could be associated with adverse effects. Therefore, the administration of *E. repens* for 21 days can be considered safe.

Data availability

All relevant data are within the manuscript.

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Conflicts of interest

The authors have no conflict of interest to declare in regard to this publication

Author contributions

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