International Journal of Veterinary Science and Medicine (2014) 2, 83–88


Cairo University

International Journal of Veterinary Science and Medicine

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Short Communication

# Electron imaging of calcium oxalate crystals in beagle dogs' urine


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Received 15 January 2014; revised 16 February 2014; accepted 17 February 2014
Available online 4 April 2014

**KEYWORDS**

Oxalate;
Crystals;
Blood;
Urine;
SEM;
Dogs

**Abstract** Calcium oxalate crystalluria appears to be a common problem in most of small animal clinics. This current study aimed at inducing a condition of oxalate crystalluria in beagles and record the primary changes in canine blood and urine on response to oxalates injection. 15 dogs were divided into two groups; those in the treatment group were injected intravenously with 0.5 M potassium oxalate and the dogs of control group were injected with physiological saline for five successive days. Urine test revealed a significant decrease in urinary creatinine and urinary urea nitrogen levels. The ultrastructural examination of urine sediment showed typical and atypical phases of calcium oxalate crystals and the X-ray defractionation of these crystals showed high content of calcium in addition to other minerals. Therefore potassium oxalate injection may provide an example of calcium oxalate crystalluria which may answer some question around the pathogenesis of this problem in dogs.

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## 1. Introduction

Urolithiasis is a recurrent clinical problem in dogs throughout the world. Although struvite uroliths have been reported as the most common uroliths worldwide [1,2], recent studies evaluated the trends in stone submissions in the period of last ten to twenty years and have revealed equalization of calcium oxalate and struvite-containing calculi, and subsequent increases in calcium oxalate-containing uroliths thereafter [3]. The main reasons of the long-term changes in mineral composition of uroliths are yet unknown [4]. However, scientists found out some predisposing factors which might incorporate in stone formation. Some of these factors might include demographic and nutritional changes, preference to certain types of breed, and complex interaction of other multiple factors [5].

The process of stone formation starts with precipitation of crystals in when the urine is oversaturated with calcium oxalate, colloquially the urine where the ion product in excess of the level at which spontaneous precipitation occurs. However, urine contains inhibitor(s) that can withhold the process of supersaturation. Most of this activity can be ascribed to macromolecules and a considerable participation by citric acid and some ions [6]. Magnesium can be considered to act as an

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Peer review under responsibility of Faculty of Veterinary Medicine, Cairo University.


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http://dx.doi.org/10.1016/j.ijvsm.2014.02.003

inhibitor of Calcium oxalate (CaOx) crystallization because of its capacity as a chelator of oxalate. Magnesium oxalate is more soluble than CaOx which enables oxalate excretion outside the urinary system. Although it has been reported that magnesium could inhibit crystal nucleation [7], growth [8] and aggregation [9], However, there is little evidence to recommend magnesium therapy in patients with urolithiasis. Oxalates are highly toxic agents to the renal tissue. They are believed to play an infamous role in calcium oxalate urinary stone formation [10] through induction of severe tubular injury, cell sloughing [11], and renal insufficiency or renal failure in some case. Moreover, oxalates may lead to changes of the whole blood picture where Robinson [12] reported a significantly decreased hemoglobin, hematocrit, erythrocytes, and neutrophilia in female rats after receiving 4% EG for 10 days. In addition, severe anemia and acute renal failure were recorded in a patient with chronic exposure to both very large doses of oxalate precursor [13].

After urinary supersaturation, the next step in stone formation process is nucleation which means formation of a solid crystal phase in a solution followed by retention of these crystals within the renal tissue to act as nucleus to simply permit further crystals deposition, growth and aggregation [14]. Urinary macromolecules prevent the nucleation step by binding within pre-formed crystals or adsorption on a crystal surface to induce degradation and dissolution of crystals faces and edges [15]. Only scanning electron microscopy (SEM) can provide these information and record the completeness of urinary inhibitors against crystallization. Furthermore SEM is supplied by additional equipment for analysis of the crystals and measure mineral and other elemental content.

In the current study we had exposed beagle dogs to potassium oxalate injection in order to induce crystalluria. The aim of this study is to assess the hematological and urinary changes accompany the process of crystalluria in canines. We also aimed at examining the ultrastructure and the chemical compositions of the biologically produced CaOx crystals after frequent injection of oxalates.

## 2. Materials and methods

### 2.1. Animals

Fifteen healthy intact adult dogs (beagles and mongrel breeds) aged between 2.5 and 4 years old were selected in this study. Seven dogs (two males and five females) were assigned to the control group, and the other eight dogs (four males and four females) were considered the treatment group. Dogs were housed in stainless net cages (1 dog per cage) with ten-day quarantine and acclimatization periods in the department of clinical veterinary medicine, Yangzhou University, Yangzhou city, China. Dry mixed food was provided for dogs at twice daily and tap-water was provided ad libitum.

### 2.2. Study design

0.5 M potassium oxalate solution ($K_2C_2O_4 \cdot H_2O$) was prepared and sterilized by passing through a 0.22-μm filter. Animals in the treatment group were given 0.5 M KOx at a dose of 0.13 ml/kg, while the same volumes of 0.9% physiological saline were given to the control ones. The Dose was chosen according to [16]. Butterfly catheters were inserted into the cephalic veins and fixed. Each group was injected with the assigned solutions three times a day for 5 consecutive days. All experiments and procedures performed on the animals were approved by the Animal Care and Use Committee of Yangzhou University.

### 2.3. Hematological picture

Blood samples were collected just prior to injection, and on days one, three and five. Five mL-blood samples were collected each time from the cephalic veins of injected dogs. Two-hundred microlitre of blood samples were added into tubes along with EDTA. The whole blood was anti-coagulated by adding EDTA and analyzed within 20 min by automatic blood analyzers (BC-2800, Mindray biomedical electronics, China). White blood cell (WBC) count, differential leukocyte, red blood cell (RBC) count, hemoglobin (HGB) hematocrit (HCT), and platelet count were measured.

### 2.4. Urine analyses

Urine samples were collected daily from day 0 till day 5 by indwelling catheters. Every urine sample was divided into two parts. One part was examined for urine pH immediately. The other part was placed in a tube and stored at −20° C for further biochemical analyses to determine the urinary levels of calcium (Ca), magnesium (Mg), urea nitrogen (UN), and creatinine (CR). The previous elements were analyzed by automatic biochemical analyser (AU 480, Backman, USA).

### 2.5. Urine sediment examination

Five millilitre of urine were centrifuged at 6000*g* for 10 min; the sediments were spread on a microscopical slide and examined by light microscopy. Sediment suspensions were dried overnight at 37° C, mounted on aluminium stubs and coated with gold 3-nm thickness using a gold sputter. The stubs were examined using an S-4800; Hitachi SEM equipped with an X-ray defractometer.

### 2.6. Statistical analyses

Mixed ANOVA using SPSS (Version 16.00) software was used for statistical analysis. Time (Day 0, Day1, Day3 and Day5) was used as a within-subject factor meanwhile the treatment was used as a between-subject factor (Saline Vs Potassium oxalate). Bonferroni's post hoc test was used to identify specific differences. A Pearson correlation test was used to determine the relationship between different urine parameters. The level of significance at which the null hypothesis was rejected was $\alpha = 0.05$. Values in the figures are means ± SE.

## 3. Results

### 3.1. Hematological findings

The hematological parameters showed non-significant differences ($P > 0.05$) between treatment and control groups.

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