

Full Length Research Paper

***In vitro* analysis of the dissolution rate of canine uroliths using *Moringa oleifera* root**

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Urolithiasis is a common disorder of humans and animals. The surgical intervention to correct the condition is expensive and alternative cheaper botanical treatments should be explored. Various botanicals have been shown *in vitro* not only to dissolve, but also inhibit urolith accretions. In this study the *in-vitro* efficacy, anti-urolithiatic potential and dissolution rate of aqueous and ethanol, chloroform, and ether extracts of *Moringa oleifera* roots on canine uroliths was investigated without simulation of *in vivo* experimentation. In the aqueous extract an average dissolution of calcium oxalate (CaO_x) was 77%. The rate of dissolution of the Calcium Oxalate (CaOx) increased linearly. However, ethanol and chloroform extracts increased both the rates of dissolution by weight and surface area linearly. The oral use aqueous extracts is considered a safe measure in treating various clinical conditions including urolithiasis in humans and animals. The dissolution rates of uroliths in organic solvents are probably associated with the presence of organic compounds unique to the *Moringa* family. The potential of using moringa extracts may prove to be an ethno-veterinary practice to address urolithiasis in animals.

Key words: Anti-urolithiatic, *Moringa oleifera*, dissolution rate, aqueous extract, magnesium ammonium phosphate (MAP), calcium oxalate (CaO_x)

INTRODUCTION

Urinary calculi, uroliths or kidney stones are accretions of solid mineral crystals within the urinary tract of human, goats and sheep and is a common clinical manifestation in the latter in Trinidad (Lans, 2001). Calculi are of many types including magnesium phosphate (MAP), calcium carbonate (CaO_x) including calcium oxalate which obstruct the passage of urine, in the ureter, dilating the obstructed ureter and renal pelvis. Clinical manifestations

of the condition in goats in Trinidad include restlessness, swishing of the tail, groaning, grunting, straining to urinate, protruded penis, and eventually rupture of the bladder as is found in other studies (Fazili et al., 2010). As urine is supersaturated, chemical moieties inhibiting of crystal formation in the urinary system may not be present in clinical cases of urolithiasis. The surgical treatments available to goat producers include urethral

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Figure 1. Size of canine calcium uroliths.



Figure 2. Canine magnesium (MAP) and calcium (CaOx uroliths).

process amputation, and perineal urethrostomy

Various alternative treatments have been experimented upon by several investigators who attempt to simulate in vitro conditions of uroliths formation with in vivo experimental studies (Grases et al., 1998). In vitro studies to dissolve or prevent uroliths formation of similar types as is found in humans include the use of aqueous leaf extracts of *Phyllanthus niruri* to dissolve CaO_x (Khare et al., 2014) using aqueous alcoholic rhizome extract of *Bergenia ciliata* to inhibit and prevent CaO_x formation in a synthetic urinary system (Saha and Verma, 2013) and using ethanolic and methanolic leaf extracts, respectively, of *Morus Alba L.* and of *Limnea procumbens* of ethylene glycol induced oxalate urolith formation, but ameliorated by these extract using Wistar rat models

(Maya and Pramod, 2014; Makasana et al., 2014). *Moringa oleifera* (Moringa or drumstick tree) root bark extracts have been found to reduce kidney elimination of calcium oxalate and calcium phosphate in propylene glycol induced hyper oxaluria also using a Wistar rat *in vitro* model (Karadi et al., 2006; Karadi et al., 2008). The results of these two experiments suggest that there is a possible use of *M. oleifera* root aqueous extracts in ameliorating the formation of these two types of kidney stones in humans and animals (Karadi et al., 2006; Karadi et al., 2008).

M. oleifera originated from the sub-Himalayan regions of Northwest India and is currently found ubiquitously in several African, Asian, South American, Central American and Caribbean Countries. In all parts, the plants have been used for herbal treatments probably because of the unique range of glycosidic compounds it contains (Anwar et al., 2007). Various ethno botanical concoctions from plant parts have been used for cardiac and circulatory conditions, and for medical conditions requiring interventions of antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, anti-diabetic, hepatoprotective, antibacterial and antifungal, for rural communities worldwide (Anwar et al., 2007; Sharif et al., 2016). The plant can also supply optimum nutrients for livestock productions inclusive of mineral needs, crude protein and essential amino acids (Bridgemohan et al., 2014; Sharif et al., 2016).

The purpose of this research was to investigate the dissolution of canine uroliths by *Moringha* root extracts using various aqueous and organic solvents. Because of the paucity of published work on *Moringha* effecting urolithdissolution, this preliminary study may add speculation pertaining to the treatment of uroliths in both human and animal medicine.

MATERIALS AND METHODS

Plant material

Moringa dry pod seeds were sourced from local Market vendors of Central Trinidad. Seedlings were germinated in the nursery, planted out at four weeks, and 4 m apart, on a high clayey soil at a rate of 50 seedlings per 600 per square metre. Average rainfalls at the time of harvesting of roots were 2.2 to 3.73 mm while average temperatures were between 27.7 and 28.1°C. Moringa roots were obtained from one year old plants which were grown without inputs of fertilizer or pesticides, but were irrigated manually.

Preparation of the extracts

Fresh roots (500 g) were macerated with a high speed blender, and the juice pressed out using a hydraulic press at 2500 psi and filtered (Experiment 1). The aqueous extract (AqE) was then diluted to concentrations of 40, 60, 80 and 100%, respectively. In Experiment 2, organic solvents ethanol, chloroform, and ether were used as extractants at the same concentrations.

Table 1. Effect of various aqueous dilutions aqueous of *M. oliefera* roots' dissolution rates of canine uroliths.

Concentration	Types of uroliths (% weigh loss)	
	Magnesium	Calcium
0	4.3	20
40	11.8	44
60	12.6	60
80	13.5	66
100	9.8	71

Table 2. Effect of different organic solvents on *M. oliefera* roots' dissolution rates of canine calcium uroliths.

Type of dissolution	Extractants (C)	Response	R ²
% weigh loss (% wl)	Ethanol	$y_{\%wl} = -10.2 + 0.641C$ (17.54) (0.266)	78.5
	Chloroform	$y_{\%wl} = 11.9 + 0.463C$ (6.86) (0.104)	86.7
	Ether	$y_{\%wl} = 108 - 0.93C$ (22.36) (0.405)	79.3
% reduction of surface area (% rsa)	Ethanol	$Y_{\%rsa} = 10.4 + 0.576C$ (14.53) (0.221)	79.3
	Chloroform	$Y_{\%rsa} = 21.1 + 0.55C$ (7.194) (0.109)	89.6
	Ether	$Y_{\%rsa} = 101 - 0.736C$ (22.36) (0.340)	78.9
Mean	10.4	52.2	
SD	3.67	20.66	

Anti-urolithiatic activity

Two types of canine uroliths (Figures 1 to 6), magnesium [MAP] and calcium [CaOx], were placed into the various extractants at respective concentrations. Eight (8) uroliths were used for each treatment and each test-tube placed in a shaker for 10 days after which the rates of dissolution were computed. Changes in stone-weight (g) and burden or size of concrement by surface area $\{SA = l \times W \times \pi \times 0.25\}$, and stone volume $\{SV = l \times W \times \pi \times 0.52\}$ were measured. The means and SE including a linear regression analysis was done on the rates of dissolution.

Anti-urolithiatic activity

The aqueous extract (AqE) was diluted to 40, 60, 80 and 100% concentrations (Table 1). In Experiment 2, ethanol, chloroform, and ether were used as the extractants at the same concentrations (Table 2). Changes in stone-weight (g) and burden or size of concrement by surface area $\{SA = l \times W \times \pi \times 0.25\}$, and stone volume were calculates as $\{SV = l \times W \times \pi \times 0.52\}$. Two types of uroliths used were magnesium (MAP) and calcium (CaOx). Eight (8) uroliths were used for each treatment and the test-tube placed in a shaken for 10 days after which the rates of dissolution were

measured. The mean and SE and the linear regression, wherever significant are reported.

RESULTS

In Experiment 1 (Table 1; Figures 4 and 5), the AqE did not affect the MAP uroliths (Table 2; Figure 4), but the rate of dissolution of the CaOx increased linearly (Equation 1):

$$y = 22.6 + 0.528 C. \\ [3.828] \quad [0.05825] \quad (1) \\ R^2 = 96.5\%$$

In the aqueous extract an average dissolution of calcium oxalate (CaO_x) was 77%. In Experiment 2 (Table 2), the increased concentration of ethanol and chloroform extracts increased linearly both the rates of dissolution by weight and the diminution of surface area, respectively. However, a converse response was observed with the ether extract as the rate of dissolution and diminution decreased linearly. The optimum rate of dissolution



Figure 3. Moringa roots.



Figure 4. Aqueous extraction of Moringa root.



Figure 5. Ethanol, chloroform, and ether extraction of Moringa roots.

observed for the ethanol and chloroform extracts was 70 % for weight, and 74% for surface area, respectively. The



Figure 6. Dissolution of canine uroliths in aqueous solution.

moringa root and the aqueous extractions are presented in Figures 3 and 4. The ethanol, chloroform, and ether extraction of Moringa roots and Dissolution of canine uroliths in the aqueous solution are exhibited in Figures 5 and 6, respectively.

DISCUSSION

Herbal supplements in veterinary botanical medicine is a rapidly growing and accepted intervention strategy for various clinical insults in animals (Romich, 2005). The oral use of aqueous extracts is considered a safe measure in treating various clinical conditions including urolithiasis in humans and animals.

The surgical intervention of clinical urolithiasis in sheep and goats is tube cystotomy for draining the overfilled bladder (Gazi et al., 2014). Perhaps a more innovative approach would be profusing the excised bladder with copious aqueous extracts of *Moringha* since this study suggest the latter can dissolve calcium oxalate uroliths (CaO_x) lodged within the bladder.

The dissolution rates of uroliths in organic solvents are probably associated with the presence of organic compounds unique to the *M. oliefera* plant species. These may include 4-(4'-O-acetyl- α -L-rhamnopyranosyloxy)benzyl isothiocy-anate, 4 (α Lrhamnopyranosyloxy)benzyl isothiocy-anate niazimicin pterygospermin benzyl isothiocyanate, and 4-(α -L-rhamnopyranosyloxy) benzyl glucosinolate as indicated by Sharif et al. (2016). Since we did not simulate in vitro conditions of uroliths formation with an in vivo experimental model, our findings cannot as yet be extrapolated as a preventative measure to urolithiasis. Further studies in this area may reveal its potential as an ethno-veterinary practice used in the prevention and correction of urolithiasis.

Conflict of Interests

The authors have not declared any conflict of interests.

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