

THE IMPACT OF BOTTLED "PROLOM WATER" ON LITHOGENESIS OF URINARY TRACT

Dragoslav Bašić^{1,3}, Bratislav Pejić¹, Svetlana Milosavljević², Sanja Jović², Gordana Kocić³, Andrej Veljković³

Urolithiasis represents the most common urological condition nowadays, with rising trend of incidence and prevalence rates, according to geographical, climatic, ethnic, dietary and genetic factors. Prophylactic management of urolithiasis in terms of high fluid intake is of great importance in prevention of all types of urolithiasis. Prolom water has been categorized as a sodium hydro carbonic alkaline hypothermal oligomineral water.

The aim of the study was to investigate the effects of bottled Prolom water intake on serum and urinary calcium and magnesium values, as well as on urinary pH and renal microlithiasis.

A multicenter prospective trial included a total of 345 patients who daily consumed 2.5 to 3 liters had underwent of Prolom water intake, in amount of 2.5 to 3 liters/daily, for 14 days, in three follow-up in three periods.

Average values of calcium in serum (mmol/L) at on day zero, 7th and 14th were: 2.24; 2.312 and 2.242, separately respectively. Average values of calcium in urine (mmol/L) at on day zero, 7th and 14th were: 1.046; 1.582 and 1.564, separately respectively. Average values of magnesium in serum (mmol/L) at on day zero, 7th and 14th were: 0.89; 0.82 and 0.81, separately respectively. Average values of magnesium in urine (mmol/L) at on day zero, 7th and 14th were: 1.09; 1.51 and 1.61, separately respectively. Mean urinary pH values were: 6.3 at on day zero; 5.9 at on day 7th; and 6.8 at on day 14th.

Daily intake of 2.5-3 liters of bottled Prolom water has a favorable and antilithogenic effect on calcium oxalate and calcium phosphate urolithiasis.

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Key words: *bottled Prolom water, lithogenesis, urinary tract*

¹Clinic of Urology, Clinical Center Niš, Niš, Serbia

²Special Hospital for Rehabilitation Prolom Banja, Prolom Banja, Serbia

³University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Dragoslav Bašić
48 Dr Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: basicdr@gmail.com

Introduction

Urolithiasis represents the most common urological condition nowadays, with reported prevalence rates up to 20%, predominantly higher in industrialized countries (1, 2, 3). Although urolithiasis occurs in all age, sex and racial groups, it is more common in men and older patients, with more than 80% of all stone types presented by calcium oxalate (4). Renal stone disease is associated with high

recurrence rates of 50% in 5-10 years and 75% in 20 years, as well as accelerated subsequent relapse course, as reported by Trinchieri and Strauss (5, 6). The etiologic causes of urolithiasis can be classified as infectious (Magnesium ammonium phosphate; Carbonate apatite; Ammonium urate), non-infectious (calcium oxalate; calcium phosphate; uric acid), genetic (cystine; xanthine; 2.8-dihydroxyadenine) and medicamentous (7). The lifetime risk for renal stone disease ranges from 10–25% (8). Epidemiological studies have shown rising trend of incidence and prevalence rates, according to geographical, climatic, ethnic, dietary and genetic factors (7). Of these, lifestyle changes and dietary habits have been considered as the most important causes for this increase (9, 10). Among all dietary habits, fluid intake has been considered as one of the most important.

Prolom water

According to balneological classification, Prolom water has been categorized into the group of sodium-hydro-carbonic-alkaline-hypothermal-oligomineral waters. It has been taken from the depth of

200 to 600 meters. The temperature of Prolom water is 20 °C on air temperature of 20 °C, with a specific weight 1.000532 kN/m³. The pH value is 9.15 which gives an alkaline reaction. Mineralization is 215 mg/L and dry residue at 180 °C is 170 mg/L.

The chemical pattern is made of cations with predominance of sodium (Na⁺), representing 87.74 mval%, and anions with predominance of hydrocarbo-nate (HCO⁻₃), representing 79.29 mval% (11, 12) (Table 1, 2, 3).

Table 1. Physicochemical characteristics of Prolom water

Water temperature	20 °C	Electrical conductivity	170
Air temperature	20 °C	Mineralization (mg/l)	215
Colour (Pt-Co scale)	0	Dry residue 180°C (mg/l)	170
Fuzziness (NTU)	0	Total hardness (dH)	0.7
pH	9.15	Total ions of alkaline earth metals (mg/l)	5.0
Eh (mV)	-20		
rH	-	Consumption of KMnO ₄ (mg/l)	1.0

Table 2. Ionic composition of Prolom water

Kations	mg/l	mmol	mval	mval%	Anions	mg/l	mmol	mval	mval%
Na ⁺	41.9	1.882	1.882	87.74	HCO ₃ ⁻	102.0	1.669	1.669	79.29
K ⁺	0.2	0.005	0.005	0.24	CO ₃ ⁻	6.2	0.20	0.20	9.50
Li ⁺	0.003	-	-	-	OH ⁻	< 0.1	-	-	-
Nh4 ⁺	< 0.04	-	-	-	Cl ⁻	6.0	0.17	0.17	8.08
Ca ⁺⁺	4.9	0.123	0.246	11.80	Br ⁻	< 0.5	-	-	-
Mg ⁺⁺	0.05	0.002	0.004	0.19	J ⁻	< 0.5	-	-	-
Sr ⁺⁺	0.02	0.0005	0.001	0.02	F ⁻	< 0.2	-	-	-
Mn ⁺⁺	< 0.01	-	-	-	NO ₃ ⁻	1.5	0.024	0.024	1.14
Fe ⁺⁺	< 0.01	-	-	-	HPO ₄ ⁼⁼	0.04	0.0005	0.001	0.05
Al ⁺⁺⁺	< 0.04	-	-	-	SO ₄ ⁼⁼	2.0	0.021	0.042	2.00
Total	47.07	1.952	2.077	100.00	Total	117.75	2.084	2.105	100.00

Table 3. Other substances in Prolom water

Weak electrolytes		Dissolved gases		CO ₂	0
H ₂ SiO ₃	48.5	O ₂	4.0	H ₂ S total	0.08
H ₃ BO ₃	0.1	Saturation O ₂ %	44.0	H ₂ S free	0.01
Total solids (mg/l)	213.22	N ₂	8.6	HS	0.07

The aim

The aim of the study was to investigate the effects of bottled Prolom water intake on biochemical changes of serum and urinary value of calcium and magnesium cations, as well as on renal micro-lithiasis.

Materials and methods

The study was a multicenter prospective trial, jointly conducted by Prolom Spa Special Hospital for Rehabilitation, Urological Clinic of Clinical Center Niš and the Institute of Biochemistry of the Faculty of

Medicine in Niš, over the period from March 2013 to January 2018. A total of 345 patients (192 male, 153 female), mean age 46.65 years (25-82; SD = 10.69) were included in a multicenter prospective trial through the following inclusion criteria: age > 18 years; the presence of crystalluria in urine sediment (Ca-oxalate); ultrasonography finding of renal micro-lithiasis. Exclusion criteria encountered renal stone disease, anomalies of renal position, urinary tract infection, active oncological diseases, patients with urinary diversion, patients on renal replacement therapy, pregnancy, non-stable hypertension.

All patients were informed of the study protocol and gave their consent. Study protocol included:

- extensive medical history,
- laboratory blood and urine analysis (including values of magnesium and calcium) obtained from the first-morning urine and serum specimens on day zero,
- renal ultrasonography on day zero,

- serum and urinary values of magnesium and calcium obtained from the first-morning urine and serum specimens on 7th and 14th day,
- renal ultrasonography on 7th and 14th day.

According to study design, all patients were treated with daily intake of 2.5-3 liters of bottled Prolom water for 14 days. Laboratory reference ranges are listed in Table 4.

Table 4. Laboratory reference ranges

Reference range (mmol/l)	Ca	Mg
Serum	2.02 - 2.6	0.8 - 1.0
Urine	2.5 - 6.2	0.4 - 4.1

Results

Average values of calcium concentration in serum, within the examined group were: 2.24 mmol/L on day zero (SD = 0.083); 2.312 mmol/L on day 7th (SD = 0.114) and 2.242 mmol/L on day 14th (SD = 0.119) (Table 5).

Average values of calcium concentration in urine in examined patients were: 1.046 mmol/L on day zero (SD = 1.030); 1.582 mmol/L on day 7th (SD = 0.832) and 1.564 mmol/L on day 14th (SD = 1.231) (Table 6).

Table 5. Calcium concentration in serum

Ca serum (mmol/l)	0-day	7 th day	14 th day
\bar{x}	2.24	2.312	2.242
SD	0.083	0.114	0.119

Table 6. Calcium concentration in urine

Ca urine (mmol/l)	0-day	7 th day	14 th day
\bar{x}	1.046	1.582	1.564
SD	1.030	0.832	1.231

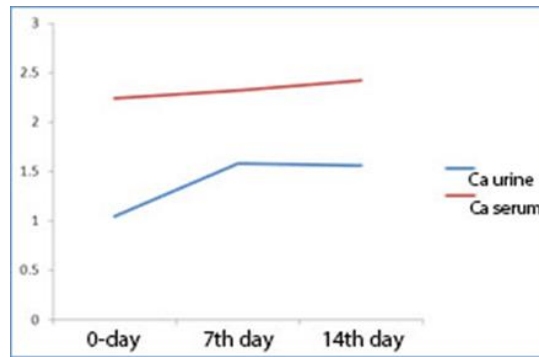
When analyzing data, it is important to notice that the values of Ca concentration of examined sample were within the reference range, both in urine and serum (Graph 1).

Average values of Mg concentration in serum of all examined patients were: 0.89 mmol/L on day zero (SD = 0.05), 0.82 mmol/L on day 7th (SD = 0.09) and 0.81 mmol/L on day 14th (SD = 0.002) (Table 7).

Average values of Mg concentration in urine in examined patients were: 1.09 mmol/L on day zero (SD = 0.849); 1.51 mmol/L on day 7th (SD =

0.821); 1.61 mmol/L on day 14th (SD = 0.479) (Table 8).

Comparing time 1 to time 3, it is noticeable that there is a relevant growth of magnesium excretion within examined periods, with statistical significance ($p < 0.05$). At the same time, there is a slight decrease in serum values of magnesium, but in lesser extent comparing to urinary excretion increase. However, both serum and urinary magnesium values were within the reference range (Graph 2).



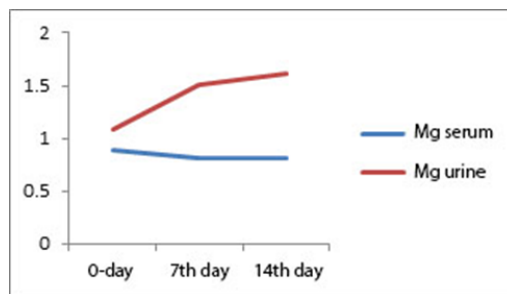
Graph 1. Calcium concentration in urine and serum

Table 7. Mg concentration in serum

Mg serum (mmol/l)	0-day	7 th day	14 th day
\bar{x}	0.89	0.82	0.81
SD	0.05	0.09	0.002

Table 8. Mg concentration in urine

Mg urine (mmol/l)	0-day	7 th day	14 th day
\bar{x}	1.09	1.51	1.61
SD	0.849	0.821	0.479



Graph 2. Magnesium concentration in urine and serum

Mean urine pH values within examined group of patients were: 6.3 on day zero (SD = 0.6), 5.9 on day 7th (SD = 0.92) and 6.8 on day 14th (SD = 0.6) (Table 9).

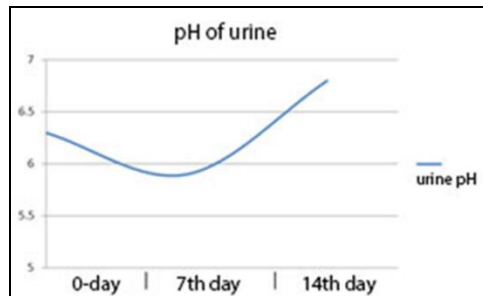
The change in urine pH value during the study period showed slight variations in the range of 0.9. During the first 7 days there was a decreasing trend in its value and moderate acidification of urine,

while in the next 7 days it increased to values higher than the initial, with moderate alkalization of the urine to almost neutral value (Graph 3).

From the zero-day onwards, renal ultrasound showed a decreasing trend of diffuse multiple hyper-echoic acoustic shadows (microlithiasis), with remarkable regression of microlithiasis at the end of the study (Table 10).

Table 9. Urinary pH

Urinary pH	0-day	7 th day	14 th day
\bar{x}	6.3	5.9	6.8
SD	0.6	0.92	0.6

**Graph 3.** Urinary pH**Table 10.** Renal ultrasonography

Day	Finding
Zero	Normal position of both kidneys, size, shape and structure, preserved corticomedullar border, with signs of diffuse multiple hyperechoic acoustic shadowing (<5mm) on both sides (microlithiasis)
7 th	Normal position of both kidneys, size, shape and structure, preserved corticomedullar border, with reduced diffusion of hyperechoic acoustic shadowing (< 5mm) on both sides (microlithiasis)
14 th	Normal positions of both kidneys, size, shape and structure, preserved corticomedullar border, with discrete signs of hyperechoic acoustic shadowing (< 5mm) on both sides (microlithiasis)

Discussion

The most common underlying conditions linked to nephrolithiasis have been described with the following prevalence rates: absorptive hypercalciuria (20-40%), renal hypercalciuria (5-8%), reabsorptive hypercalciuria (3-5%), hyperuricosuric calcium nephrolithiasis (10-40%), hypercitraturic calcium nephrolithiasis (10-50%), hyperoxaluric calcium nephrolithiasis (2-15%), hypomagnesiuric calcium nephrolithiasis (5-10%), gouty diathesis (15-30%), cystinuria (< 1%), infection stones (1-5%), low urine volume (10-50%), miscellaneous (< 3%) (13). The etiologic causes of urolithiasis can be classified as infectious (magnesium ammonium phosphate; carbonate apatite; ammonium urate), non-infectious (calcium oxalate; calcium phosphate; uric acid), genetic (cystine; xanthine; 2.8-dihydroxyadenine) and medicamentous (7).

According to epidemiological data on stone composition, there is a predominance of calcium

oxalate which accounts for more than 80% of all stone types (4). However, in terms of pathophysiology and pathogenesis, there are many open-ended questions and ambiguities that are still awaiting answers and clarifications.

Stone formation process encompasses a complex of physicochemical cellular and extracellular events which include: urine saturation, oxidative stress, cell injury and cell membrane rupture, nucleation and crystal growth, aggregation, crystal-cell interaction and retention/adhesion (14). As described by Pearle and Lotan (15), in solutions containing ions, including urine, there is a maximum level of the product of their concentration and at that level, the solution is considered saturated. In this way, the capacity of this solution is completed and the dissolution of additional quantities of crystals is not possible, as their precipitation will occur. However, by changing certain conditions in the solution, such as pH, temperature, or by adding certain substances called crystallization inhibitors, it

is possible to increase the value of the thermodynamic product of solubility, thereby preventing the formation of crystals and their precipitation. The solubility and crystallization states are determined by the thermodynamic solubility product (K_{sp}) and the formation product (K_f). Thus, depending on their values, solutions are classified as undersaturated, metastable and unstable. Of these, the metastable solution represents the most favorable and targeting area for therapeutic action, since the process of additional crystallization is not possible, although the urine has been supersaturated. Crystallization of calcium oxalate occurs after its supersaturation at the point when the concentration product goes beyond the solubility product. Circumstances promoting supersaturation include: increased concentrations of calcium, oxalates, uric acids and phosphates, separately, with a low urinary volume and low concentrations of citrate.

However, there are substances that slow down or inhibit the nucleation, growth, and aggregation of crystals. They accomplish this by acting on the surface of the crystal without affecting the concentration of crystal-forming ions. Nuclei represent precursors of crystals and their persistence in urine depends on the saturation level as well as on the nucleus stability. The last one depends on the impact of promoters and inhibitors of crystallization. In the absence of inhibitors, nucleation extends by adsorption to surrounding structures, such as epithelial cells or preexisting crystals, as described by Aleigh et al. and Umekawa et al. (14, 16).

There are organic (citrate, glycosaminoglycans, glycoproteins, lipids) and non organic physiological inhibitors (pyrophosphate, magnesium) for calcium oxalate and calcium phosphate. Among organic inhibitors, citrate, pyrophosphate and magnesium are considered as the most potent. Citrate acts at multiple levels: it inhibits Ca oxalate precipitation, nucleation and crystal aggregation; by competitive binding to Ca, it reduces ionic concentration of calcium and its capacity to form oxalates and phosphates; Anorganic pyrophosphate inhibits calcium phosphate crystallization. Magnesium acts similarly to citrate, by competitive binding to oxalates and thus decreasing their ionic concentration and potential for supersaturation (15, 17).

It has been suggested by several authors that renal urolithiasis promotes the risk to variety of diseases, including chronic kidney diseases (18), diabetes, hypertension (19), and cardiovascular diseases (20). It has also been stated that one of the most important risk factors in urinary stone formation is fluid intake, in reverse proportion (21). Therefore, prophylactic management of urolithiasis in terms of high fluid intake is of great importance in

prevention of all types of urolithiasis. It has been reported that an increase intake of water had favorable effects by reducing the recurrence rates in kidney stone formers. Hence, an increased water intake is advised commonly in all patients with renal stone disease (22-24).

According to our results, bottled Prolom water promotes urinary excretion of magnesium and calcium ions. As an inhibitor of crystallization, magnesium complexes with oxalates forming a soluble compound and therefore prevents further calcium oxalate stone formation. Additionally, by binding itself to calcium ions (70%), magnesium prevents crystallization or inhibits nucleation of calcium oxalate and calcium phosphate. Daily intake of 2.5-3 liters of bottled Prolom water achieves optimal diuresis with a specific weight of urine within the range of 1005-1015. Moreover, it changes overall pH value by decreasing it to 5.8 during the first 7 days, with a significant increase afterwards to 6.8 during the next 7 days. It represents important antilithogenic effect, since low urine pH promotes uric acid and/or calcium stone formation (25). The goal of urine pH change is to be held between 6.5 and 7.2 since these values enable better solubility of urate and cystine in the urine. It has to be emphasized that this value should not exceed 7.2 in order to avoid potential side effect of forming calcium phosphate stones. Results of renal ultrasound showed a decreasing presence of microlithiasis, with remarkable reduction at the end of the study. Although morphological, these findings are consistent with reported changes of Mg and Ca values, supporting results of moderating effects of Prolom water on urinary tract lithogenesis. Prolom water, as an independent factor has high degree of anti-lithogenicity on urolithiasis.

Conclusion

Based on the reported results, it can be concluded that daily intake of 2.5-3 liters of bottled Prolom water has a favorable and antilithogenic effect on calcium oxalate and calcium phosphate urolithiasis. These effects certainly deserve more extensive research, both in terms of pathogenetic mechanisms of action, as well as in terms of laboratory and clinical outcome.

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UTICAJ FLAŠIRANE „PROLOM VODE“ NA LITOGENEZU URINARNOG TRAKTA

Dragoslav Bašić^{1,3}, Bratislav Pejić¹, Svetlana Milosavljević², Sanja Jović²,
Gordana Kocić³, Andrej Veljković³

¹Klinika za urologiju, Klinički centar Niš, Niš, Srbija

²Specijalna bolnica za rehabilitaciju „Prolom Banja“, Prolom Banja, Srbija

³Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

Kontakt: Dragoslav Bašić
Bulevar dr Zorana Đinđića 48, 18000 Niš, Srbija
E-mail: basicdr@gmail.com

Urolitijaza predstavlja najčešće urološko oboljenje danas, sa rastućim trendom incidencije i prevalencije, u zavisnosti od geografskih, klimatskih, etničkih, dijetalnih i genetskih faktora. Profilaktičko lečenje urolitijaze, u smislu visokog unosa tečnosti, od velikog je značaja u prevenciji svih vrsta urolitijaze. Flaširana „Prolom voda“ kategorisana je kao natrijum hidrokarbonatna, alkalna, hipotermalna, oligomineralna voda. Cilj studije bio je da se ispituju efekti unosa „Prolom vode“ na serumske i urinarne vrednosti kalcijuma i magnezijuma, kao i na pH urina i bubrežnu mikrolitijazu. Multicentričnom prospektivnom studijom obuhvaćeno je ukupno 345 bolesnika, koji su tokom 14 dana oralno unosili flaširanu „Prolom vodu“ u količini od 2,5 litra do 3 litra dnevno, uz praćenje u tri perioda. Prosečne vrednosti kalcijuma u serumu (mmol/l) nultog, sedmog i četrnaestog dana bile su: 2,24; 2,312 i 2,242, ponaosob. Prosečne vrednosti kalcijuma u urinu (mmol/l) nultog, sedmom i četrnaestog dana bile su: 1,046; 1,582 i 1,564, ponaosob. Prosečne vrednosti magnezijuma u serumu (mmol/l) nultog, sedmog i četrnaestog dana bile su: 0,89; 0,82 i 0,81, ponaosob. Prosečne vrednosti magnezijuma u urinu (mmol/l) nultog, sedmog i četrnaestog dana bile su: 1,09; 1,51 i 1,61, ponaosob. Srednje vrednosti pH u urinu bile su: 6,3 nultog; 5,9 sedmog i 6,8 četrnaestog dana. Svakodnevni unos 2,5 litra do 3 litra flaširane „Prolom vode“ ima povoljan i antilitogeni uticaj na kalcijum-oksalatnu i kalcijum-fosfatnu urolitijazu.

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Ključne reči: flaširana „Prolom voda“, litogeneza, urinarni trakt