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Are High Proanthocyanidins Key to Cranberry Efficacy in the Prevention of Recurrent Urinary Tract Infection?

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Most research on American cranberry in the prevention of urinary tract infection (UTI) has used juices. The spectrum of components in juice is limited. This study tested whether whole cranberry fruit powder (proanthocyanidin content 0.56%) could prevent recurrent UTI in 182 women with two or more UTI episodes in the last year. Participants were randomized to a cranberry ($n = 89$) or a placebo group ($n = 93$) and received daily 500 mg of cranberry for 6 months. The number of UTI diagnoses was counted. The intent-to-treat analyses showed that in the cranberry group, the UTIs were significantly fewer [10.8% vs. 25.8%, $p = 0.04$, with an age-standardized 12-month UTI history ($p = 0.01$)]. The Kaplan–Meier survival curves showed that the cranberry group experienced a longer time to first UTI than the placebo group ($p = 0.04$). Biochemical parameters were normal, and there was no significant difference in urinary phenolics between the groups at baseline or on day180. The results show that cranberry fruit powder (peel, seeds, pulp) may reduce the risk of symptomatic UTI in women with a history of recurrent UTIs. Copyright © 2015 John Wiley & Sons, Ltd.

Keywords: Vaccinium macrocarpon; urinary tract infection; recurrent; haematology; clinical chemistry markers; urinary metabolites.

INTRODUCTION

Urinary tract infections (UTIs) are the most commonly diagnosed bacterial infection in women, with more than 50% experiencing at least one UTI during their lifetime (Foxman, 2003; Micali *et al.,* 2014). The ingestion of cranberries (*Vaccinium macrocarpon* Ait., *V. oxycoccus* L., Ericaceae) has traditionally been associated with the prevention of UTIs, which arise from colonization and subsequent infection by uropathogenic *Escherichia coli* (Guay, 2009). Major classes of constituents that may contribute to the health benefits of cranberry are phenolic acids, flavonoids, anthocyanins, proanthocyanidins and triterpenoids (Pappas and Schaich, 2009). A-type proanthocyanidins (PACs) seem to be responsible for inhibiting the adhesion of *E. coli* and other uropathogens to uroepithelial cells *in vitro* (Foo *et al.,* 2000; Howell *et al.,* 2005) and *ex vivo* (Howell *et al.,* 2010; Lavigne *et al.,* 2011). On the other hand, an *ex vivo* urine antiadhesive effect has been ascribed to high concentrations of hippuric and salicyluric acids in the urine of healthy women consuming a daily dose of 1200 mg of dried cranberry juice (Valentova *et al.,* 2007). Another possible mechanism of polyphenolic metabolite action could be the selection of less adherent bacterial strains in the gastrointestinal tract (Raz *et al.,* 2004). As food, and dietary supplements, cranberry is used in juice, juice cocktail (approximately 26% to 33% pure cranberry juice), cranberry pills/capsules containing dried juice or cranberry fruit powder (100% cranberry fruit solids). The complex mixture of bioactive components is found only in whole cranberry fruit (Grace *et al.,* 2012).

The growing concern over antibiotic resistance has stimulated interest in cranberries in the prevention of recurrent UTIs (rUTI). Over the years, a number of randomized clinical trials has been conducted to assess the efficacy of cranberry in reducing the risk of rUTIs in women (Micali *et al.,* 2014; Jepson *et al.,* 2012; Wang *et al.,* 2012). Most clinical trials in adult women with a history of rUTIs or acute bacteriuria used pure cranberry juice/cocktails or capsules containing dried juice enriched with A-type PACs. A recent randomized clinical study showed that 500/1000 mg of cranberry fruit powder (CFP) used for 90 days significantly reduced bacteriuria and symptoms of UTI in women with symptomatic UTI at baseline (Sengupta *et al.,* 2011). Interestingly, this appears to be the only study of cranberry being used in the treatment of acutely infected subjects.

One weakness of clinical trials with cranberry products has been that the cranberry preparations were not fully characterized by chemical composition. The

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efficacy of powdered whole cranberry fruit (quantified using standardized methods) in reducing the risk of rUTI in female subjects free of UTI at baseline has not yet been assessed. The aim of this 6-month randomized, double-blind and placebo-controlled trial was to evaluate whether a daily dose of 500 mg of cranberry fruit powder could prevent rUTIs in otherwise healthy women from 18 to 75 years old, with a history of UTIs.

MATERIAL AND METHODS

Cranberry material. Cranberry fruit powder (100% fruit of North American *Vaccinium macrocarpon* Aiton, Ericaceae; Batch No. 090921) was purchased from NATUREX-DBS (Sagamore, MA, USA). Declared total PACs in CFP determined by the 4-dimethylaminocinnamaldehyde (DMAC) method (Prior *et al.,* 2010) was 0.56%, and this powder was applied for the clinical trial. The results of our secondary metabolite determination of CFP by HPLC-ESI-MS/MS are shown in Table 1; PACs have been analysed according to Jungfer *et al.,* 2012 (Table 2). Each cranberry capsule contained 250 mg of CFP (1.4 mg of PACs according to DMAC). Placebo capsules contained low-density STAR-DRI® 1015A maltodextrin, canola oil, Red 40 Lake, sodium aluminium silicate and Blue 1 Lake. CFP capsules were indistinguishable in appearance from the placebo capsules.

Table 2. Content of proanthocyanidins in cranberry fruit powder

Proanthocyanidin (mg/100 g CFP)			
B-dimer B1	0.54 ± 0.01	A-trimer 3	3.06 ± 0.08
B-dimer B2	2.29 ± 0.08	A-trimer 4	2.86 ± 0.17
B-dimer B5	0.72 ± 0.04	A-trimer 7	1.92 ± 0.08
Procyanidin A2	24.30 ± 0.43	A-trimer 8	4.44 ± 0.04

Values are expressed as mean ± SD, $n = 2$. Concentrations are calculated as procyanidin A2 equivalents. Compound nomenclature is according to Jungfer *et al.,* 2012.

Design and participants. The study was conducted according to the guidelines of the Helsinki Declaration (2008 revision), and all procedures involving human subjects were approved by the Ethics Committee of the University Hospital and Faculty of Medicine and Dentistry, Palacky University, Czech Republic (reference 129/09). Written informed consent was obtained from all participants. The study was a 6-month, single-centre, randomized, double-blind and placebo-controlled trial consisting of two parallel treatment arms. It was conducted between January 2010 and April 2011 at the Clinic of Urology of the University Hospital.

The invitation to participate was through the referring physician treating the UTIs. Women aged over 18 years old and with a medical history of at least two episodes of symptomatic UTIs in the previous 12 months were eligible. Participants meeting all of the

Table 1. Content of selected secondary metabolites in cranberry fruit powder

Compound (mg/100 g CFP)			
Phenolic acids		Benzoic acid and benzaldehyde derivatives	
3,4-Dihydroxycinnamic acid	4.4 ± 0.1	Benzoic acid	167.6 ± 19.4
Chlorogenic acid	12.4 ± 0.4	3,4-Dihydroxybenzaldehyde	2.7 ± 0.2
4-Hydroxy-3-methoxycinnamic acid	2.7 ± 0.1	*p*-Hydroxybenzaldehyde	0.03 ± 0.01
3,4,5-Trihydroxybenzoic acid	5.8 ± 0.2	Vanillin	0.4 ± 0.0
4-Hydroxycinnamic acid	21.1 ± 0.5		
4-Hydroxybenzoic acid	0.3 ± 0.0	Anthocyanins/Anthocyanidins	
3,4-Dihydroxybenzoic acid	24.3 ± 0.5	Cyanidin 3-*O*-arabinoside	62.7 ± 1.0
2-Hydroxybenzoic acid	0.3 ± 0.0	Cyanidin 3-*O*-galactoside	42.4 ± 1.9
4-Hydroxy-3,5-dimethoxycinnamic acid	2.8 ± 0.1	Cyanidin	31.6 ± 1.3
4-Hydroxy-3,5-dimethoxybenzoic acid	2.8 ± 0.1	Delphinidin 3-*O*-glucoside	2.7 ± 0.2
4-Hydroxy-3-methoxybenzoic acid	5.8 ± 0.1	Delphinidin	24.4 ± 0.8
Flavonoids		Malvidin 3-*O*-galactoside	5.4 ± 0.2
Apigenin	0.4 ± 0.0	Pelargonidin 3-*O*-glucoside	7.6 ± 0.2
Catechin	2.7 ± 0.1	Peonidin 3-*O*-glucoside	82.1 ± 1.3
Epicatechin	13.4 ± 0.1	Peonidin 3-*O*-rutinoside	1.3 ± 0.2
Epigallocatechin	24.4 ± 0.2	Peonidin	13.5 ± 1.1
Hesperidin	2.6 ± 0.1		
Hyperoside	1408.0 ± 36.7	Pentacyclic triterpenoid	
Isorhamnetin	188.2 ± 22.4	Ursolic acid	921.6 ± 74.2
Kaempferol	25.2 ± 4.6		
Myricetin	482.8 ± 21.7		
Isoquercitrin	504.0 ± 14.9		
Quercetin	1138.8 ± 32.5		
Rutin	3.0 ± 0.2		

Values are expressed as mean ± SD, $n = 5$.

inclusion criteria and none of the exclusion criteria (Table 3) and consenting to study participation were randomly divided into two groups: cranberry and placebo groups (Fig. 1). The randomization plan for treatment assignment to subjects was generated using online software QuickCalcs (GraphPad Software Inc., USA). The cranberry group was given 500 mg CFP (two times 250 mg CFP capsules) to be taken once a day after breakfast for the 6-month period. The daily dose of CFP was based on the findings of McMurdo *et al.* (2009). The placebo

Table 3. Eligibility criteria

Inclusion criteria	Exclusion criteria
• Women from 18 to 75 years • A history of recurrent symptomatic UTIs (defined as a medical history of at least two symptomatic UTI episodes treated with antibiotics in the previous 12 months) • Clinical laboratory tests (haematology, clinical chemistry, urinalysis) within normal reference ranges or if outside the normal reference ranges, clinically insignificant	• Symptomatic UTI at baseline • Antibiotic treatment during the study for reasons others than UTI[a] • Pregnant and/or breast feeding women • Anatomical anomalies or other pathological findings with a possible effect on the recurrence of UTIs (stricture of urethra, nephrolithiasis, cystolithiasis, neurogenic bladder dysfunction) • Insulin-dependent diabetes mellitus • Subjects with a history of medical or surgical events that could affect the study outcome or place the subject at risk, including cardiovascular disease, gastrointestinal problems, metabolic, renal, hepatic, neurological, sexually transmitted diseases or active musculoskeletal disorders • Immunocompromised individuals or individuals receiving immunosuppressive medication • Intermittent or indwelling urinary tract catheterization • Subjects with a history of surgery within the last 6 months • Use of narcotics • Heavy episodic drinking of alcohol • Participation in a clinical research trial within 30 days prior to randomization • Simultaneous participation in another clinical trial

[a]Patients with occurrence of a symptomatic UTI during the study were treated immediately with antibiotics. At the end of antibiotics treatment, urine samples were collected to confirm the absence of bacteriuria, and patients resumed study treatment.

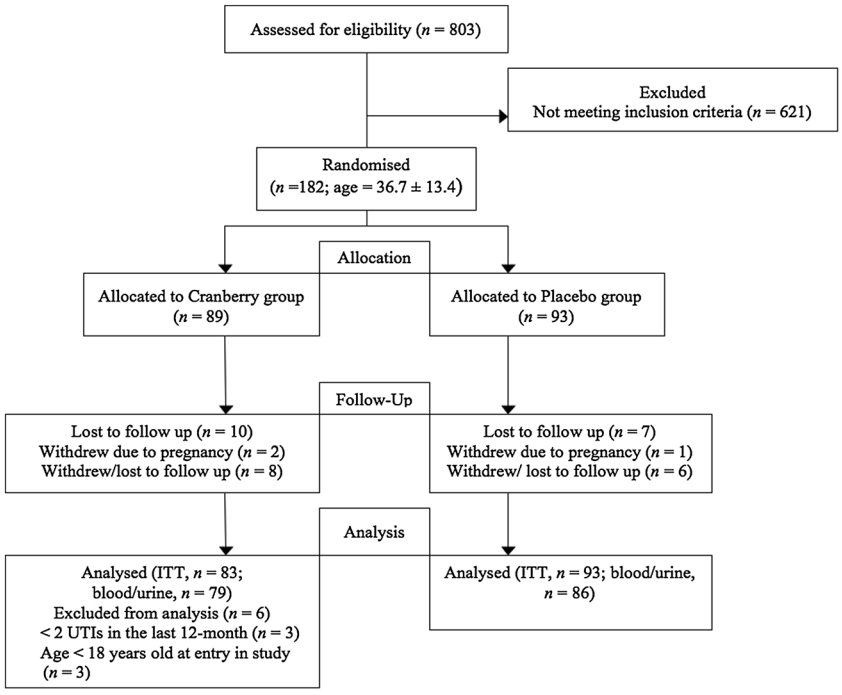

Figure 1. Flow chart of the clinical trial.

group received the same instructions as the cranberry group. Subjects were asked to refrain from consuming foods rich in phenolics, especially colour-pigment-containing fruit (berries), or vitamin supplements or to make any other dietary or lifestyle changes during the study. After randomization (baseline), the women returned to the clinic at 3 and 6 months and whenever they experienced symptoms of a UTI. The clinical report form included (i) a detailed medical history, (ii) assessment of all concurrent medication and treatment, (iii) dietary habits, (iv) kidney and bladder ultrasound and (v) complete laboratory analysis, including haematology, clinical chemistry and urinalysis. Urine samples were collected at baseline and at 3 and 6 months for analysis of the urine and urine sedimentation. If a UTI was confirmed (bacteriuria $\geq 10^5$ cfu/mL plus symptoms of a UTI; see Urinalysis, microbial examination and clinical diagnosis of UTI), the subject was treated with antibiotics, culture-directed antibiotic treatment for 1–3 days. Once the course of antibiotics was completed, urine samples were collected to confirm that the UTI had resolved, and the subject resumed taking the product. Vital signs (heart rate, systolic and diastolic blood pressure) were assessed at baseline and at 3 and 6 months.

The clinical diagnosis of a UTI was based on bacteriuria plus the manifestation of at least one of the following symptoms: pollakiuria (strong, persistent urge to urinate and passing frequent, small amounts of urine), burning sensation on micturition, hematuria, turbid or malodorous urine, subpelvic pain, pruritus, fever and dysuria.

Haematology and clinical chemistry. Blood samples were collected at baseline and at 6 months. These were drawn under aseptic conditions from the *vena cubiti*, after a several-minute rest in the half-sitting position. Serum/plasma samples were separated in a cooled centrifuge at $3000 \times g$ for 20 min. Basic haematological parameters (haemoglobin, erythrocytes, leukocytes, platelets and haematocrit) were measured in Na_2EDTA blood. Routine clinical chemistry parameters were determined in all samples: low-density lipoprotein (LDL), high-density lipoprotein (HDL) cholesterol, triacylglycerol, C-reactive protein, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, urea, creatinine, bilirubin and glucose were quantified in serum using a HITACHI Modular Evo P analyser (Hitachi, Japan).

Urinalysis, microbial examination and clinical diagnosis of UTI. The urine had to be a midstream early morning sample. The complete analysis of urine was performed on the IQ200 Automated Urinanalysis System (IRIS International, Inc., USA). The microbiological analysis was performed at the Laboratory of Clinical Microbiology, University Hospital. The laboratory diagnosis of UTI was based on a significant isolate of a single organism, and a UTI was culture-confirmed when the growth of a single bacterial strain was $\geq 10^5$ cfu/mL in a midstream urine sample. Phenolic metabolites in urine were determined at baseline and at 6 months. Analysis of free and total phenolics in urine was carried out using HPLC-ESI-ion trap MS according to our protocol (Heinrich *et al.,* 2013).

Statistical analysis. The sample size was estimated based on the assumption that at least 30% of women would experience an rUTI within 6 months in the placebo group and that the rate of UTI recurrence would be reduced to 15% in the cranberry group. The primary endpoint of this clinical trial was the 50% reduction in incidence of rUTI episodes in the cranberry group compared to the placebo group. In order to detect this effect with a power of 80% and a two-tailed alpha level of 5%, 80 women per group were needed (Kontiokari *et al.,* 2001). Thus, to account for subject attrition, a total of 182 women were recruited.

An intent-to-treat (ITT) analysis was performed. This included any individual with at least one postrandomization assessment. In order to examine the relationship between the proportion of women experiencing at least one UTI episode during the study period and assignment to the active or placebo treatment arms, a complementary log–log (CLL) binomial regression model was used. This generalized linear model (GLM) specified a binomial distribution for the random component and a complementary log–log link function. The model was fit using the GLM function in R. Age and age-adjusted history of UTI were associated with risk of UTI, and for this reason, they were included in the model. The log observation time (from randomization date to the end-of-study or dropout dates) was included as an offset term. A Kaplan–Meier estimate was used to describe the distribution of time to first UTI. In order to compare time to first UTI between the two treatment arms, the Cox proportional hazards model was fitted with treatment arm, age and age-adjusted prior 12-month history of UTI in the model. The count of UTIs found during the observation period was compared between groups using Poisson regression including age, age-adjusted prior 12-month history and an offset variable (log observation time) in the model. Continuous variables were described as means ± standard deviation or first quartile/median/third quartile and compared using a repeated-measures ANOVA at baseline and after 6 months.

RESULTS

Patient recruitment is depicted in Fig. 1. Of the 803 women who were screened for participation in the study, 621 patients did not meet the inclusion criteria or met one or more of the exclusion criteria. The remaining 182 eligible women were enrolled and were randomized to the cranberry ($n = 89$) or placebo ($n = 93$) groups. Seventeen women did not complete the study, seven (7%) in the placebo group and ten (11%) in the cranberry group. Reasons for not completing the study included loss to follow-up, voluntary withdrawal ($n = 14$) or pregnancy ($n = 3$) (Fig. 1). These 17 women were included in the ITT analysis. Six other participants, all randomized to the cranberry group, were excluded from the ITT analysis. Three of these six study participants had a UTI history of one in the previous 12 months and were excluded from the analysis because they had less than two in the previous 12 months. The other three study participants were enrolled into the trial by the Principal Investigator following parental consent but were excluded from the analysis because they were younger than 18 years. Thus, the ITT sample included a total of 176 women ($n = 83$ in the cranberry group and $n = 93$ in the placebo group). The cranberry and placebo groups were similar with regard to baseline

Table 4. Baseline parameters

	Placebo group (n = 93)	Cranberry group (n = 89)	p
UTIs in the last 12 months	3.27 ± 1.33	2.93 ± 1.22	0.08
Age (years)	38.03 ± 13.40	35.61 ± 12.97	0.23
Height (cm)	167.70 ± 6.03	166.30 ± 6.73	0.14
Weight (kg)	66.44 ± 10.79	64.18 ± 12.52	0.20
Temperature (°C)	36.32 ± 0.21	36.35 ± 0.19	0.36
Pulse (beats per minute)	69.02 ± 6.89	70.47 ± 6.32	0.15
Systolic blood pressure (mmHg)	116.90 ± 11.03	115.8 ± 10.61	0.48
Diastolic blood pressure (mmHg)	77.31 ± 7.39	76.87 ± 6.87	0.68
Urine sediment (HPF)	Negative	Negative	NA
Urine pH	5.76 ± 0.76	5.83 ± 0.67	0.64

Values are mean ± SD. UTIs, urinary tract infections.

characteristics (Table 4, Fig. 2). There was a positive quadratic association between age and prior 12-month UTI history. The residuals from this quadratic model were adopted as age-adjusted UTI history scores. Age was also found to have a quadratic association with occurrence of UTI during the intervention period and hence age, centred around 40 years, and the square were included in the following models. During the 6-month intervention, the proportion of women having at least one UTI episode was significantly lower in the cranberry group (9/83, 10.84%) than in the placebo group (24/93, 25.81%) ($p = 0.04$), with age-adjusted 12 month UTI history ($p = 0.01$), age ($p = 0.73$), and age-squared ($p = 0.05$) included in the model. This corresponds to a relative risk reduction of 58% in the cranberry group relative to the placebo group. The fitted cumulative incidence (or cumulative rate) of UTI over 6 months for a women with average duration of observation, average age, and average UTI history was 0.085 (8.5%) in the cranberry group and 0.194 (19%) in the placebo group ($p = 0.04$). The proportion of women experiencing at least one UTI episode caused specifically by *E. coli* was 7/83 women (8.43%) in the cranberry group and 22/93 women (23.66%) in the placebo group ($p = 0.03$ vs. placebo), with age-adjusted prior 12-month UTI history ($p = 0.007$), age ($p = 0.74$) and age-squared ($p = 0.12$) included in the model.

The Kaplan–Meier curves for time to first UTI are shown in Fig. 3. The time to first occurrence was different for the two groups, with a significantly longer time to first UTI observed in the cranberry group relative to the placebo group ($p = 0.04$), with age-adjusted 12-month UTI history ($p = 0.02$), age ($p = 0.69$) and age-squared ($p = 0.04$) included in the model. Of the women in the cranberry group, 10% (Kaplan–Meier estimate) experienced a UTI episode by 133 days while 10% of the participants in the placebo group experienced a UTI episode by 65 days.

During the study, there was a total of 40 UTIs that occurred in 33 women. Thirty-three of the UTIs were primary occurrences and seven were secondary occurrences (six women in the placebo group and one woman in the cranberry group experienced two episodes of UTI during the 6-month study). The average count of UTIs per subject in the study period was 0.12 (10/83) for the cranberry group and 0.32 (30/93) for the placebo group

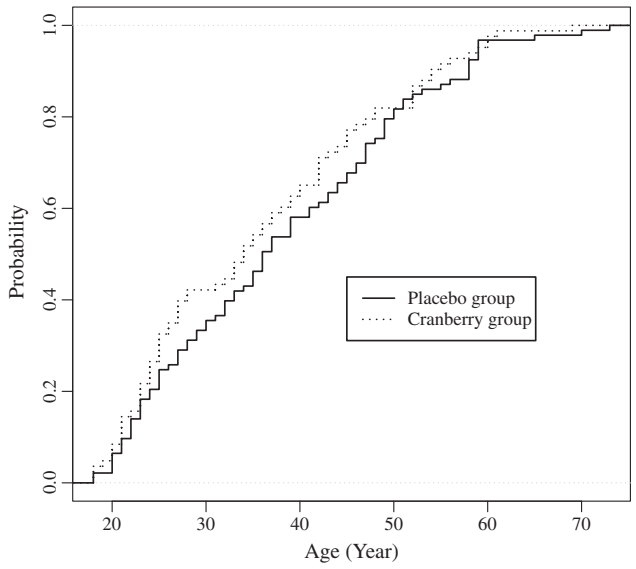

Figure 2. Differences in age distribution between subjects in the cranberry and placebo groups.

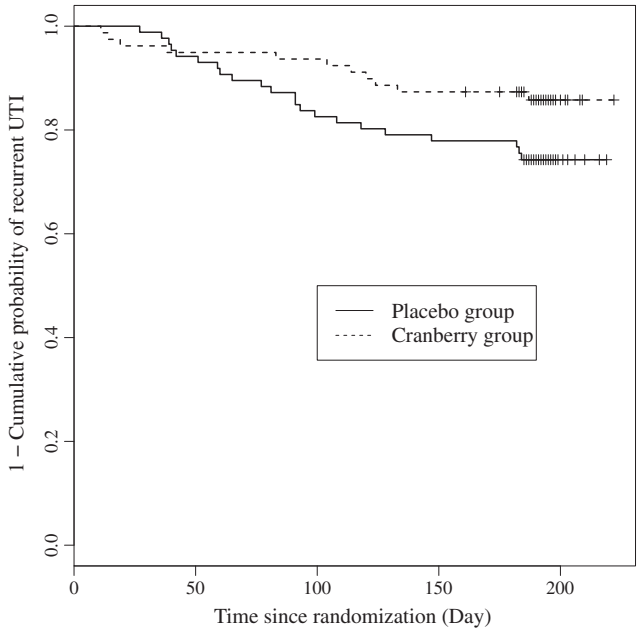

Figure 3. Kaplan–Meier curves of survival to UTI recurrence for the placebo and cranberry groups.

Table 5. Haematology and clinical biochemistry markers in placebo and cranberry groups at day 0 and day 180

	Placebo group (n = 86)		Cranberry group (n = 79)	
	Day 0	Day 180	Day 0	Day 180
Haemoglobin (g/L)	126/133/138	129/134/140[a]	126/134/140	129/134/141
Erythrocytes (10^{12}/L)	4.16/4.42/4.61	4.17/4.46/4.60	4.21/4.52/4.68	4.28/4.43/4.65
Leukocytes (10^9/L)	4.81/6.02/7.04	4.83/6.14/6.89	4.91/5.94/6.94	5.13/5.82/7.20
Haematocrit	0.37/0.39/0.41	0.37/0.40/0.42[a]	0.38/0.40/0.41	0.39/0.40/0.41
Platelets (10^9/L)	208.0/237.5/275.8	216.8/245.5/287.8	224.5/261.0/293.0	229.5/266.0/309.0[a]
Urea (mmol/L)	3.63/4.20/5.08	3.40/4.15/5.10	3.50/4.10/5.20	3.55/4.20/5.00
Creatinine (μmol/L)	60.3/66.0/73.0	61.0/64.5/71.0	61.0/68.0/73.5	61.0/66.0/72.0
Bilirubin (μmol/L)	5/8/9	6/8/11	6/8/11	5/7/11[b]
Alanine aminotransferase (μkat/L)	0.24/0.32/0.42	0.22/0.30/0.42	0.25/0.30/0.37	0.22/0.28/0.39
Aspartate aminotransferase (μkat/L)	0.34/0.40/0.48	0.34/0.39/0.46	0.36/0.40/0.45	0.34/0.37/0.42[a]
γ-Glutamyl transferase (μkat/L)	0.19/0.25/0.35	0.20/0.25/0.36	0.20/0.25/0.37	0.19/0.25/0.37
C-reactive protein (mg/L)	0.8/1.5/3.0	0.6/1.3/3.4	0.7/1.6/3.8	0.8/1.7/4.0
Total cholesterol (mmol/L)	4.35/4.87/5.44	4.45/5.11/5.87[a]	4.45/5/50.55	4.49/5.15/5.83
TAG (mmol/L)	0.78/1.11/1.49	0.78/1.07/1.39	0.86/1.12/1.45	0.88/1.15/1.41
HDL (mmol/L)	1.37/1.66/1.88	1.39/1.74/2.01[a]	1.42/1.69/1.92	1.47/1.72/1.94
Cholesterol/HDL	2.43/3.10/3.55	2.56/3.04/3.67[a]	2.59/2.96/3.65	2.53/2.89/3.83
LDL (mmol/L)	2.20/2.72/3.34	2.25/2.95/3.54[a]	2.29/2.78/3.53	2.30/2.89/3.60
Glucose (mmol/L)	4.4/4.8/5.3	4.5/4.9/5.3	4.5/4.8/5.3	4.5/4.9/5.4

[a]The value was significantly different from the value in day 0 ($p < 0.05$). The data are expressed as first quartiles, medians and third quartiles.
[b]The change-from-baseline value is significantly different from that in the placebo group ($p < 0.05$).

($p = 0.03$, after adjusting for age-adjusted 12 month history, age and age-squared). All occurrences of UTI were medically diagnosed and confirmed microbiologically. Pathogens identified among the 33 primary UTIs were *E. coli* ($n = 28$), *Klebsiella* species ($n = 3$), *Staphylococcus* species ($n = 1$) and *Streptococcus* species ($n = 1$). In the placebo group, one woman who experienced an infection with *E. coli* also had *Enterococcus* sp. identified in the urine. Pathogens identified among the seven secondary UTIs were all *E. coli*.

Changes from baseline in haematology and clinical chemistry parameters (Table 5) were similar for both groups, with the exception of bilirubin, which increased from baseline by 1.0 μmol/L in the placebo group and decreased from baseline by 0.53 μmol/L in the cranberry group ($p < 0.05$ vs. placebo). All values remained within the normal ranges. After 6 months of treatment, there were significant increases in total cholesterol (4.97 ± 0.99 to 5.18 ± 1.02 mmol/L), HDL cholesterol (1.63 ± 0.35 to 1.77 ± 0.77 mmol/L) and LDL cholesterol (2.78 ± 0.87 to 2.95 ± 0.89 mmol/L) in the placebo group. All values were within the normal ranges. There was a slight yet significant decrease in urine pH between baseline (5.83 ± 0.67) and the 6-month time point (5.64 ± 0.55; $p = 0.024$) in the cranberry group while the pH in the placebo group did not change over time.

No anthocyanins or proanthocyanidins were detected in either the plasma or urine samples of either group (data not shown). The free and total concentrations of phenolic compounds were determined in urine samples collected on days 0 and 180. There was no significant difference in phenolics between the groups (Table 6).

DISCUSSION

A number of systematic reviews and meta-analyses of human interventional clinical trials on the effects of cranberry on UTIs has been published (Micali *et al.,* 2014; Guay, 2009; Jepson *et al.,* 2012; Wang *et al.,* 2012). The results of individual studies have been largely inconsistent. These inconsistencies could be due to (i) the populations studied [history of UTI, life stage (pregnancy, menopausal status, age)], (ii) the study settings (free-living vs. institutionalized), (iii) subject compliance and/or (iv) the effectiveness of the cranberry-containing product consumption. In this regard, the prophylactic efficacy of various cranberry products (cranberry juice, dried juice, diluted juice concentrate, juice cocktails, cranberry juice powder enriched with PACs) has been tested; however, these products have been largely uncharacterized in terms of chemical composition, making it difficult to assess their true potential bioefficacy. In addition, subject compliance is generally better in studies using cranberry juice in tablet or capsule form (Stothers, 2002; McMurdo *et al.,* 2009; Beerepoot *et al.,* 2011).

In this trial, the proportion of women experiencing at least one UTI episode was significantly lower in the group using cranberry fruit powder (10.8%) relative to the placebo group (25.8%), although the recurrence rate in the placebo group was lower than the expected 30% UTI reported by Kontiokari *et al.* (2001). The difference in the proportion of women experiencing a UTI episode in the placebo and cranberry arms was significant ($p = 0.04$), likely owing to a greater magnitude of effect than was predicted in the sample size calculation. Although other trials of cranberry supplements have reported reductions in the risk of rUTIs in women with a history of UTIs, these had various limitations, including inappropriate study design, small sample size (Bailey *et al.,* 2007; Walker *et al.,* 1997), failure to fully characterize the cranberry supplement (Stothers, 2002; Beerepoot *et al.,* 2011; Walker *et al.,* 1997), lack of definition of the criteria that were used to diagnose a UTI (Bianco *et al.,* 2012) and/or a high rate of subject attrition (Bailey *et al.,* 2007; Walker *et al.,* 1997). In contrast,

Table 6. Concentration of free and total phenolics in urine of the placebo and cranberry groups on day 0 and 180

	Placebo group (n = 86)				Cranberry group (n = 79)			
	Concentration of analysed compounds (μg/g creatinine)							
	Day 0		Day 180		Day 0		Day 180	
Compound	Free	Total	Free	Total	Free	Total	Free	Total
BA	0/0/147	194/809/2552	0/0/548	276/829/2225	0/0/452	192/682/2240	0/0/497	86/472/1415
2-HBA	0/0/0	0/114/418	0/0/0	0/111/313	0/0/0	0/77/583	0/0/0	0/75/290
3-HBA	0/0/318	0/0/360	0/0/176	0/0/251	0/0/205	0/0/138	0/55/164	0/8/175
4-HBA	188/519/987	927/2049/4047	164/335/834	735/1759/3812	144/307/781	783/1572/3228	106/248/685	561/1133/2529
3,4-DHA	88/232/543	228/484/895	57/245/465	182/448/757	34/160/404	216/388/821	92/172/319	153/335/543
3,4,5-THBA	0/0/440.6	0/103/556	0/253/694	0/56/502	0/33/419	0/56/481	0/124/352	0/16/391
4-HMBA	70/249/619	620/1961/4210	61/253/564	554/1382/3281	80/172/365	495/1378/3023	36/127/426	357/1058/2262
PA	0/0/0	1052/6739/21 614	0/0/0	1145/4717/17 680	0/0/0	1025/3646/12 697	0/0/0	767/3283/14 550
2-HPA	310/732/1120	293/761/1100	281/625/1124	288/652/1020	251/577/966	288/602/881	201/576/1033	223/550/922
3-HPA	457/1115/2852	405/1199/3057	451/1004/2263	479/995/2938	407/992/2072	402/1139/3395	457/945/2008	370/875/2341
4-HPA	7077/11 913/21 062	9449/18 265/30 077	5490/11 307/16 897	8317/15 744/24 520	4790/9848/19 841	7965/16 515/27 960	4953/9276/16 407	6427/12 397/20 920
3,4-DHPA	1324/2998/6284	1702/3612/6979	1182/2547/4594	1612/3198/5797	1084/2632/4725	1251/3414/6329	1321/2479/3992	1054/1964/4512[a]
4-HMPA	2118/3575/7794	1962/3190/7136	1118/2920/5366	1419/3265/6413	1359/2845/4705	1454/3457/6273	1074/2189/4253	1435/2502/5171
2-HPPA	0/77/292	120/359/781	0/106/288	99/286/794	16/92/239	73/268/804	27/70/213	71/192/574
3-HPPA	0/0/0	0/0/803	0/0/1548	0/0/1072	0/0/1724	0/0/746	0/0/1172	0/0/1213
3,4-DHPPA	199/422/1060	385/877/1557	146/444/1038	296/780/1778	153/411/1026	339/662/1615	191/445/675	391/697/1169
4-HMPPA	0/293.8/1230	566/1282/2644	0/232/1066	480/1217/2440	0/148/702	367/1071/2250	0/141/435[a]	265/808/2103
3-HCA	0/43/122	27/99/260	0/47/180	22/115/257	0/34/126	21/79/256	0/25/87	25/82/231
4-HCA	0/24/72	20/81/155	0/19/61	31/77/179	0/0/48	36/82/228	0/21/66	18/59/135
3-HMCA	0/66/199	138/414/944	0/55/166	127/353/950	0/61/151	111/463/1009	0/43/129	126/352/834
4-HMCA	0/0/121	311/954/2109	0/19/146	215/546/1595	0/0/85	230/845/1948	0/8/90	190/630/1684
HA	105/248/466	102/243/458	109/2274/443	979/221/416	903/203/397	101/210/396	108/198/388	968/1979/353
2-HHA	62/149/285	69/131/229	53/150/385	62/144/338	40/130/262	50/130/262	57/129/259	48/129/254
QUE	0/0/0	0/0/840	0/0/0	0/96/1092	0/0/0	0/0/919	0/0/0	0/183/878

The values are expressed as expressed as first quartiles, medians and third quartiles.

BA, benzoic acid; 2-HBA, 2-hydroxybenzoic (salicylic) acid; 3-HBA, 3-hydroxybenzoic acid; 4-HBA, 4-hydroxybenzoic acid; 3,4-DHA, 3,4-dihydroxybenzoic (protocatechuic) acid; 3,4,5-THBA, 3,4,5-trihydroxybenzoic (gallic) acid; 4-HMBA, 4-hydroxy-3-methoxybenzoic (vanilic) acid; PA, phenylacetic acid; 2-HPA, 2-hydroxyphenylacetic acid; 3-HPA, 3-hydroxyphenylacetic acid; 4-HPA, 4-hydroxyphenylacetic acid; 3,4-DHPA, 3,4-dihydroxyphenylacetic acid; 4-HMPA 4-hydroxy-3-methoxyphenylacetic (homovanilic) acid; 2-HPPA, 2-hydroxyphenylpropanoic acid; 3-HPPA, 3-hydroxyphenylpropanoic acid; 3,4-DHPPA, 3,4-dihydroxyphenylpropanoic (dihydrocaffeic) acid; 4-HMPPA, 4-hydroxy-3-methoxyphenylpropanoic (dihydroferulic) acid; 3-HCA, 3-hydroxycinnamic acid; 4-HCA, 4-hydroxycinnamic (p-coumaric) acid; 3-HMCA, 3-hydroxy-4-methoxycinnamic (isoferulic) acid; 4-HMCA, 4-hydroxy-3-methoxycinnamic (ferulic) acid; HA, hippuric acid; 2-HHA, 2-hydroxyhippuric (salicyluric) acid; QUE, quercetin.

[a]The value was statistically significant at $p < 0.05$.

in the current study, the design was robust (randomized, double-blind, placebo-controlled), and the sample size was sufficient and justified. There was a relatively low rate of subject attrition, criteria appropriate for the diagnosis of a symptomatic UTI were applied and a well-characterized cranberry product was used. To the best of our knowledge, this is the first study demonstrating the efficacy of a well-characterized whole cranberry fruit in the prevention of rUTIs in women. We found no PACs in the plasma or urine samples, and there was no significant difference in the phenolic compound profile or benzoic acid derivatives in the urine samples of the women from either group on days 0 and 180. Of the phenolics determined, hippuric acid dominated.

It can be speculated that the increased urinary antiadherence and lower incidence of UTIs are connected to other cranberry constituents apart from PACs, anthocyanins, phenolic acids, flavonoids and their microbial-derived metabolites (de Llano *et al.*, 2015). The pentacyclic triterpenoids, mainly ursolic acid, may play a complementary or synergistic role together with polyphenolic constituents in the antiadhesion activity of cranberry fruit (Vasileiou *et al.*, 2013). For example, this compound caused differential gene expression in *E. coli* and inhibited biofilm formation in several bacterial species (Ren *et al.*, 2005). Ursolic acid has been shown to affect P fimbriae and the curli fibre morphology of uropathogenic *E. coli* strains and their adhesion to uroepithelial cells (Wojnicz *et al.*, 2013). Also, some metabolites are formed through the action of intestinal microflora, which is unique for each individual (Cardona *et al.*, 2013). This might explain individual sensitivity to the effects of cranberry.

CONCLUSION

In summary, results of this study showed that intake of 500 mg of cranberry fruit powder containing 2.8 mg of PACs/day for 6 months was associated with a reduction in incidence of recurrent UTIs. The compliance with the study protocol was excellent and no adverse events were recorded. From the results, it is not possible to pinpoint which compound/compounds in CFP protected the epithelium of the urinary tract against the formation of bacterial biofilm. Our data nonetheless provide encouraging evidence for the protective effect of whole cranberry (peel, seeds, pulp) in women with a medical history of rUTIs. This effect is possibly due to the synergy of all cranberry components and/or its metabolites rather than just PACs. However, additional studies are needed to determine which cranberry secondary metabolites in addition to PACs are responsible for the effects found.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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