High Dose Versus Low Dose Standardised Cranberry Proanthocyanidin Extract for the Prevention of Recurrent Urinary Tract Infection in Healthy Women:A Double-Blind Randomized Controlled Trial.

Mémoire

Asma Babar

Maîtrise en épidémiologie - avec mémoire

Maître ès sciences (M. Sc.)

Québec, Canada

Résumé

Introduction: Les infections urinaires (UTI) sont parmi les infections bactériennes les plus communes chez les femmes. Actuellement, les antibiotiques sont le traitement de choix pour la prévention des UTIs. Pourtant, les produits dérivés de la canneberge sont souvent utilisés avec peu d'évidence de leur efficacité. Notre objectif était d'évaluer l'efficacité d'un extrait de canneberge standardisé en proanthocyanidines de type A (PAC) sur la prévention des UTI à répétition. Méthodes: 145 femmes âgées de 18 ans et plus avec antécédents d'UTI à répétition, définie par ≥ 2 UTIs dans les derniers 6 mois ou ≥ 3 UTIs dans les derniers 12 mois, ont participé à notre essai clinique randomisé à double insu. Soixante-douze femmes ont reçu une dose optimale d'extrait de canneberge quantifié et standardisé en PACs (2 x 18,5 mg PACs par jour) et 73 ont reçu une dose contrôle (2 x 1 mg PACs par jour). L'issue principale était le nombre moyen de nouvelles UTIs symptomatiques chez les participantes durant une période d'intervention de 6 mois. Les issues secondaires étaient : 1) évaluer le nombre moyen d'UTI avec pyurie et avec confirmation microbiologique; 2) décrire les effets secondaires d'une dose quotidienne d'extrait de canneberge. Résultats: Sur la période de suivi de 6 mois, le risque d'UTIs symptomatiques n'était pas significativement différent entre les deux groupes (rapport de taux d'incidence 0,76, 95% IC 0,51-1,11 ; rapport de taux d'incidence ajusté pour l'âge 0,85, 95%IC 0,57-1,26). Parmi les participantes ayant eu moins de 5 UTIs dans l'année précédant leur participation, la prise de 2x18,5 mg était associée à une diminution des UTIs symptomatiques comparativement à une prise de 2x1 mg PACs (rapport de taux d'incidence ajusté pour l'âge 0,57,, 95%IC 0,33-0,99). Aucun effet secondaire majeur n'a été rapporté. Conclusion: La prise d'un extrait de canneberge en teneur élevée de proanthocyanidins n'a pas été associée à une réduction du taux d'incidence d'infections urinaires symptomatiques par rapport à un extrait de proanthocyanidines à faible dose. Nos résultats post-hoc suggèrent que la prise d'une dose de 2x18,5 mg PAC par jour pourrait prévenir les UTIs symptomatiques chez les femmes ayant moins de 5 UTIs par année.

Study registration: ClinicalTrials.gov; NCT02572895

Abstract

Background: Urinary tract infections (UTI) are amongst the most common bacterial infections affecting women. Although antibiotics are the treatment of choice for prevention of UTI, cranberry-derived products are often used by women to prevent UTIs, with limited evidence as to their efficacy. Our objective was to assess the efficacy of a cranberry extract capsule standardized in A-type linkage proanthocyanidins (PACs) for the prevention of recurrent UTI. Methods: 145 women aged 18 years or more with a history of recurrent UTI, defined as ≥ 2 UTIs in the past 6 months or ≥ 3 UTIs in the past 12 months were recruited in this randomized, controlled, double-blind clinical trial. Seventy-three women received an optimal dose of cranberry extract standardized in PACs (2 x 18.5 mg PACs daily) and 72 women received a control dose (2 x 1 mg PACs daily). The primary outcome for the trial was the mean number of new symptomatic UTIs in women during a 6-month intervention period. Secondary outcomes were: 1) To evaluate the mean number of new symptomatic UTIs with pyuria and with microbiological confirmation; 2) To describe the side effects of daily intake of cranberry extract. Results: No significant difference in the risk of UTI during the 24-week follow-up period was found between treatment groups (incidence rate ratio 0.75, 95%CI 0.51-1.11, age-adjusted incidence rate ratio 0.85, 95%CI 0.57-1.26). In women who experienced less than 5 UTIs in the year preceding enrolment, the daily consumption of 2x18.5 mg PACs was associated with a decrease in the risk of symptomatic UTIs reported compared to the control dose (age-adjusted incidence rate ratio 0.57, 95% confidence interval 0.33-0.99). No major side effects were reported. Conclusion: High dose twice daily proanthocyanidin extract was not associated with a reduction in the number of symptomatic urinary tract infections when compared to a low dose proanthocyanidin extract. Our post-hoc results reveal that this high dose of proanthocyanidins may have a preventive impact on symptomatic urinary tract infection recurrence in women who experienced less than 5 infections per year.

Table of contents

List of Tables and Figures

List of abbreviations

AAA = anti-adhesion activity

ATB = antibiotic

BID = Twice daily

BL-DMAC = Brunswick Lab 4-dimethylaminocinnamaldehyde

CANWARD = Canadian antimicrobial resistance alliance

CFU = Colony forming units

CUA = Canadian Urological Association

DIE = once daily

E.coli = Escherichia coli

FFQ = Food frequency questionnaire

LCCC = low calorie cranberry cocktail

MRHA = Mannose-resistant Hemagglutination assay

NS = not specified

OSC-DMAC = Ocean Spray Cranberries 4-dimethylaminocinnamaldehyde

PAC = Proanthocyanidins

RCT = randomised controlled trial

RR = relative risk

SF-36 = short-form 36

SOGC = Society of Obstetricians and Gynecologists of Canada

UPEC = Uropathogenic Escherichia coli

USD = United States dollar

UTI = define in French and English

Acknowledgements

As my participation in this project ends, I would like to thank my research director, Doctor Sylvie Dodin, for supervising and providing insight on the different phases of this clinical trial. I am especially grateful for her patience and flexibility allowing me to complete this project in harmony with my medical studies and residency. I thank my research co-director, Doctor Lynne Moore for introducing me to statistical software and fostering my ability to perform the more advanced statistical analyses needed for this research project. These incredible researchers are among my greatest role models and inspire me to continue my implication in clinical research during my medical career.

I am fortunate to have worked with an exceptional research coordinator, Vicky Leblanc, whose precision and problem-solving skills were essential for the completion of this project. I thank you for your continuous availability and suggestions throughout the past few years. I would like to acknowledge Lydia Tetreault and Iseult Grenier-Ouellet for their assistance in data collection. I thank the team of graduate students and research coordinators at the Institute on Nutrition and Functional Foods for the entertainment and lunchtime conversations. I am grateful to the one hundred and forty-five women who took the time to participate in this research project. Study visits with the participants in this project added a human dimension to my research and helped me understand the direct impacts that recurrent UTIs could have on the quality of life.

Financial support for my studies was provided through a Frederick Banting and Charles Best Canada Graduate Scholarship from the Canadian Institutes of Health Research, a research scholarship courtesy of the CHU de Quebec Foundation and a Joseph-Demers recruitment scholarship from the Fonds Joseph-Demers. I would like to extend my sincere gratitude to these organizations for defraying the costs related to my research activities.

Preface

This master's work presents the results of my master's degree research project supervised by Doctor Sylvie Dodin as my research director. My involvement with this randomized clinical trial originated through a summer research internship as a medical student at the Institute of Nutrition and Functional Foods in the summer of 2015. My interest in nutrition and women's health led to my fascination with clinical research on natural health products commonly used by women as alternative therapies. I decided that I could further my understanding of the methodology required to answer a research question through a double-blind randomized clinical trial. My collaboration included the recruitment of potential participants through the creation of posters, a radio show interview as well as social media outreach. I was equally involved in the clinical follow-up of randomized participants and with the management of UTIs in participating women. I applied the skills that I gained in my theoretical course work and performed the preliminary statistical analysis of the trials' results. Finally, as the first author, I participated in the publication of the research protocol and the writing of the research article submitted to the BMC Urology and presented in this master`s work.

The research protocol, entitled "Standardised High Dose versus Low Dose Cranberry Proanthocyanidin Extracts for the Prevention of Recurrent Urinary Tract Infection in Healthy Women [PACCANN]: a double blind randomized controlled trial protocol," was published in BMC Urology on May 2nd 2018[1]. The coauthors of this protocol article are Vicky Leblanc, Stéphanie Dudonné, Yves Desjardins, Amy Howell and Sylvie Dodin, who revised and approved the final manuscript before submission.

The results of this study are presented in chapter 3 as an article, titled "High-dose Versus Low-dose Standardized Cranberry Proanthocyanidin Extract for the Prevention Of Recurrent Urinary Tract Infection In Healthy Women: A Double-Blind Randomized Controlled Trial." This article was submitted in October 2020 to BMC Urology and a modified version as presented in this master's work was submitted in January 2021 following initial review. The roles of my collaborators are as follows: Lynne Moore contributed to the interpretation of data and statistical analyses; Vicky Leblanc contributed to the design of this study, recruitment, clinical follow up, interpretation of data and statistical analyses; Stéphanie Dudonné undertook supervision of the biomarker measures and review of the manuscript, Yves Desjardins and Simone Lemieux contributed to the original concept

and design of this study; Valérie Bochard and Denis Guyonnet represented Diana Foods in the interpretation of data, Amy Howell contributed to the original concept and design of this study. Sylvie Dodin was the major contributor to the original concept and design of this study, undertook supervision of the clinical follow-up of participants, wrote the study grant and supervised each phase of the manuscript writing. All authors read and approved the final manuscript.

Introduction

Urinary tract infection (UTI) is one of the main reasons for medical consultation. One in three women over the age of 18 will experience a UTI, and many of them will have repeated infections [2]. The majority of women with recurrent UTIs have no abnormalities in the urinary tract [3]. Sexual intercourse is the main risk factor for UTIs and there is a strong correlation between the frequency of sexual intercourse and the incidence of UTIs [4,5]. Increasing resistance of pathogens to prescribed antibiotics, both in treatment and prophylaxis, as well as the side effects of antibiotics, reinforce the demand of nutraceuticals that are effective and well tolerated [6]. These non-pharmacological approaches are more and more preferred by women [7].

Cranberry products are the most promising natural health product in the prevention of UTIs . Cranberry has been shown to inhibit the adhesion of bacteria (Escherichia coli) to uroepithelial cells lining the bladder [8]. In vitro, the proanthocyanidins (PACs), type A-linkage, mainly present in the cranberry have been clearly identified as responsible for this anti-adhesion effect [9] . A large number of clinical trials have been conducted to test the efficacy of cranberry products, mainly in the form of juices, and their results remain discordant [10]. This discrepancy is easily explained by the lack of compliance with cranberry juice intake, but especially the lack of standardized concentrations of the products used. Indeed, in the majority of clinical trials, PAC concentrations are unavailable. However, according to the most recent studies, the quantification of PACs requires standardized, reproducible methods and should be at least 37 mg / day [9]. According to our hypothesis, the efficacy of cranberry products on the prevention of repetitive urinary infections of adult women could be greatly increased by the use of an optimal dose (standardized at 37 mg / day of PACs).

The objective of this clinical trial is to test, in sexually active women with recurrent UTIs, the efficacy of an optimal dose of a standardized cranberry extract at 2x18.5 mg / day PACs compared to that of a "control" dose, quantified and standardized at 1x1 mg / day of PACs on the number of new symptomatic urinary infections during a follow-up period of 24-weeks.

Chapter 1 – Background

1.1 Recurrent Urinary Tract Infections

Definitions and Classification of Urinary Tract Infections

The presence of irritative bladder symptoms in combination with bacterial overgrowth above a threshold value in any portion of the urogenital tract signifies a UTI [11]. A UTI is classified by the site of infection, as either cystitis if the infection is limited to the lower urogenital tract, or pyelonephritis in the case of kidney involvement [11]. UTIs can further be categorized as uncomplicated or complicated. Uncomplicated UTIs occur in individuals who are healthy and who present neither anatomical nor neurological abnormalities of the urogenital tract whereas complicated UTI are associated with factors that compromise the urogenital apparatus or immune system [11]. Several subpopulations are at increased risk of complicated UTI such as those with extremes of age, pregnant women, patients with diabetes, spinal cord injuries, multiple sclerosis and/or catheters and patients with underlying urologic abnormalities [12].

Uncomplicated recurrent UTIs are defined as more than two episodes of uncomplicated UTI in the last 6 months or more than three in the prior 12 months as documented by clinical symptoms, urinalysis and/or urine culture [13]. Recurrent UTI occurs due to bacterial reinfection or bacterial persistence. Bacterial reinfection occurs when there is reoccurrence of a UTI by a different organism, the same organism 2 weeks following treatment or a sterile intervening culture while bacterial persistence denotes the persistence of causal bacteria without eradication during the urine 2 weeks following the initiation of sensitivity-adjusted antibiotic treatment [14].

Diagnosis of Urinary Tract Infection

Controversy exists regarding the optimal practical test methods for diagnosis of UTI. The gold standard for a definitive diagnosis of UTI is urine microscopy and culture of clean catch midstream urine sample. The causal bacteria is detected and identified by urine culture, which also permits the estimation of bacteriuria levels.[15]. Once the causal pathogen is identified, targeted antibiotic treatment is prescribed based on the antibiotic sensitivity profile of the bacteria [15]. The minimum level of bacteriuria indicative of UTI has not reached consensus in scientific literature or been standardized by microbiological laboratories, however most laboratories define 10^3 to 10^5 colony forming units (CFU)/mL as the

threshold depending on the types of causal bacteria [15]. A 2010 meta-analysis evaluating lower reference standards of ≥ 10^2 CFU/ml and ≥ 10^3 CFU/ml compared to ≥ 10^5 CFU/ml showed improved diagnostic accuracy when coupled with UTI symptoms [6]. Interestingly, certain women may have a negative culture in the presence of symptoms that respond to antibiotic treatment [16,17]. Others will maintain significant levels of bacteria in their urine without experiencing symptoms, a benign condition known as asymptomatic bacteriuria which does not require antibiotic treatment [18], except in certain cases, most notably during pregnancy or in catheterized patients.

Urine dipstick tests are also frequently used in clinical practice for the diagnostic testing of UTI. These dipstick tests generally detect nitrites, leukocyte esterase, protein and blood [15]. Nitrites are metabolites produced by E. coli, Klebsiella and Proteus species found in the urinary tract and their detection in urine is associated with an increased probability of UTI. According to several studies evaluating the diagnosis of UTI, the presence of nitrites is associated with a positive likelihood ratio between 2.6-10.6 [15,19-21]. Similarly, the presence of leucocyte esterase increases the probability of UTI to a lesser degree with a positive likelihood ratio between 1.0-2.6 [15,19-21].

Urine culture is the gold standard for UTI diagnosis, though it is not routinely performed due to higher costs [22] and potentially delayed treatment due to the time required to isolate the causal bacteria. A review of the accuracy of different diagnostic modalities has shown that the probability of UTI varies depending on the type of symptoms that a woman experiences when presenting to a clinician. The positive and negative likelihood ratios (LR) for symptoms of dysuria, urinary frequency, hematuria, lower abdominal pain and vaginal discharge, adapted from this review, are shown in Table 1. A combination of several symptoms, dysuria and urinary frequency without vaginal discharge or irritation assessed in one of the included studies [23] produced a significantly higher positive LR of 24.6.

Table 1. Symptoms in the Prediction of Urinary Tract Infection

Symptom	Number of studies (n)	Positive LR (95% CI)	Negative LR (95% CI)
Dysuria	8 (n=2075)	1.5 (1.2-2.0)	0.5 (0.3-0.7)
Frequency	9 (n=2159)	1.8 (1.1-3.0)	0.6 (0.4-1.0)
Hematuria	5 (n=719)	2.0 (1.3-2.9)	0.9 (0.9-1.0)
Vaginal discharge	3 (n=359)	0.3 (0.1-0.9)	3.1 (1.0-9.3)
Vaginal irritation	2 (n=1013)	0.2 (0.1-0.9)	2.7 (0.9-8.5)

Adapted from Bent et al. 2002 [24]

In the case of recurrent UTI, research shows that women can accurately self-diagnose UTI. A prospective study enrolled 172 women with a history of at least 2 UTIs in the previous year at a university-based primary health care clinic and followed them for 12 months to determine the accuracy of self-diagnosis of a culture-positive or probable (pyuria) UTI 25. During the study period, 88 women experienced a total of 172 symptomatic events and women were able to accurately diagnose 144 (84%) of microbiologically confirmed UTIs at ≥ 100 CFU/ml. This approach maximizes sensitivity as all symptomatic events are treated, however the low specificity leads to treatment for many false positives.

The Society of Obstetricians and Gynecologists of Canada (SOGC) recommends that a urinalysis and midstream urine culture and sensitivity should be performed with the first presentation of symptoms in order to establish a correct diagnosis of recurrent UTI 13. It is also suggested that urine be re-culture 1 to 2 weeks after initiating antibiotic treatment in order to evaluate bacterial persistence. The Canadian Urological Association guidelines adds that a thorough physical examination, including pelvic examination, should be performed on women in order to exclude structural or functional abnormalities 14. In line with these guidelines, a survey of Canadian primary care physicians' assessment of women with UTI symptoms, reported that physicians ordered a urine culture for 77% of the women 26. However, this survey found that 32.8% of antibiotics prescribed to women based on UTI symptoms during an office visit were unnecessary because the subsequent urine culture results were negative. Although no guidelines for urine culture exist in the United States, a cohort study using data from insurance claims in the United States identified 48 283 women with recurrent UTI between 2003 to 2011 and revealed that only 60% of women underwent urine culture to confirm their diagnosis out of 3 UTIs in 12 months 27.

Epidemiology of Recurrent Urinary Tract Infections

Urinary tract infections 28 are one of the prevalent bacterial infections worldwide 2,29. According to the 2012 National Ambulatory Medical Care Survey, UTI accounted for 40.9 million ambulatory visits and more than 10 million emergency department visits in the United States between 2006 and 2010 30. The healthcare burden of UTI is likely similar in Canada 31. UTI preferentially affects young, sexually active women with 50-60% of women reporting at least one UTI during their lifetime 2. Nearly 1 in 3 women will experience at least one episode of UTI requiring antibiotic therapy before the age of 24 years and a quarter of these women will present reoccurrence within 6 months 29. Anatomical

differences in perineum anatomy between men and women may explain why women are more susceptible to the ascension of faecal bacteria in the urinary tract due to the relative shortness of the urethra (13-20 cm long in males vs 3.8-5.1 cm long in females) 32 , the urethral meatus' proximity to the anus and a more humid surrounding environment comparatively to the male anatomy 33,34.

Physiopathology of Recurrent Urinary Tract Infections

Although the faecal-perineal-urethral route of infection is proposed as the most common aetiology of UTI, the bacteria colonizing the urinary tract do not generally cause disease because the human body can effectively remove bacteria using host defences 35. Urine's acidic pH and osmolality are intolerable by certain pathogens and the urine flow mechanically flushes out occupying bacteria regularly. The urothelial mucosa contains a mucopolysaccharide lining and secretes cytokines and chemokines that in turn decrease bacterial penetration 36. In premenopausal women, lactobacilli in the vagina produce lactic acid rendering the environment more acidic and highly unfavourable for the growth and colonization by uropathogens 37. Despite the various mechanisms, the urinary tract uses in defence against microbial infections, certain bacteria have acquired virulence factors that allow them to circumvent host defences.

The causal pathogen in 70-95% of cases of UTI is Escherichia coli (E. Coli) 38 though certain other species have been isolated such as Staphyloccocus saprophyticus, Proteus spp, Pseudomonas aeruginosa, Streptococcus agalactiae and Klebsiella spp 35. These enteric bacteria, particularly uropathogenic Escherichia coli (UPEC), acquire special capabilities that enable them to adhere to the urothelial cells in order to permit survival and colonization of the urinary tract. These virulence factors include: 1) biofilm formation and specialized adhesive pili which enable UPEC to bind and invade host cells; 2) toxins such as hemolysin and cytotoxic necrotizing factor-1 that cause an inflammatory response and inflict extensive tissue damage; 3) siderophores which deplete host iron reserves necessary for proper immune cell function 39.

Risk Factors

The first large scale prospective case-control study of women with and without a history of recurrent UTI found that the strongest risk factor for UTI in healthy women through multivariate analysis was recent sexual intercourse 40. More specifically, there is a strong association and a dose-response

relation between recent sexual intercourse and the risk of UTI in women with a history of recurrent UTI (RR for 1, 3 and 5 days with intercourse in the past week were respectively, 1.24, 1.91 and 2.96, p-value=0.002). It is proposed that sexual activity may facilitate the inoculation of the urethra and bladder by faecal flora. Similarly, frequency of sexual activity and the introduction of a new partner contribute to increased risk of UTI 41.

Additional well-established risk factors include spermicide-based barrier contraception and history of previous UTI 4,5,40,42-44. Contraceptive barrier methods such as diaphragms and condoms using spermicide with nonoxynol-9 may promote uropathogenic colonization of the vaginal introitus, an important step in the pathogenesis of UTI 45. In vitro studies have shown that nonoxynol-9 inhibits the growth of normal vaginal microbiota while failing to exhibit similar effects on UPEC 45. A prospective cohort study of 363 sexually active university women indicated that women with a history of 2 or more UTIs compared to those with only 1 or no UTI history had an increased risk of recurrence within a year (RR=5.58, 95%CI [3.24-9.63], p<0.001) 40.

Recently, genetic factors that contribute to risk of recurrent UTI were investigated in case-control study of 983 women aged 18-30 years 46. The study investigators identified a 2-4 times increased risk of recurrent UTI in women that experienced their first UTI before 15 years of age (OR=3.9; 95%CI [1.9-8.0]) and those with UTI history in the mother (OR=2.5, 95%CI 1.9-3.4) 46. The familial tendency was validated by a larger case-control study in a larger sample of women aged 18-49 with recurrent UTI 47. The researchers propose that inherited factors may be important in some women with recurrent UTI, especially those with onset before coitarche or spermicide exposure. More specifically, genetic mutations that affect the innate immune system may render certain individuals more susceptible to colonization with UPEC 47.

Several researchers have investigated additional risk factors that remain to be validated. For instance, a small cohort study showed that contraception with depot medroxyprogesterone acetate may increase risk of UTI due to the effects of progesterone on muscle tone, peristalsis of the ureter and urinary vasculature 48. The authors of another small cohort study identified antibiotic administration within the preceding two to four weeks may be additional risk factors as these medications may alter endogenous urogenital flora and predispose vaginal colonization with uropathogens 49. Alcohol consumption was

investigated in a prospective cohort study of women that experience UTI and it was shown that alcohol consumption frequency and amount correlated with sexual behaviours such as recent sexual activity, frequency of activity and the number of sexual partners, three independent risk factors for UTI [41].

Protective Factors

Expert committees recommend that women with recurrent UTI continue to consume large volumes of fluid [50] on the basis that frequent micturition may result in decreased concentration of organisms and reduce urine stagnation in the bladder [51]. Although several case-control studies have reported that recurrent UTIs are associated with lower fluid intake [52,53], few clinical studies exists that aim to determine the beneficial effects of hydration for the prevention of UTI [51]. Notably, a 2018 double blind RCT investigated the role of increased daily water intake on the prevention of UTI [54]. One hundred and forty four healthy women with recurrent cystitis, defined as ≥3 episodes in the past year, were randomly assigned to drink an additional 1.5 litres of water per day compared to no additional fluids. The primary outcome was the number of UTI episodes, defined as the presence of 1 UTI symptom (dysuria, frequency, urgency and /or suprapubic pain) plus at least 10^3 CFU/ml uropathogens in a midstream urine culture. This outcome was based on a 2013 study that compared midstream urine and urethral catheter culture and showed that presence of >10^3 CFU/ml Escherichia coli in midstream urine was highly predictive of bladder bacteriuria, with positive and negative predictive values of 96% and 84%, respectively [55]. Participant adherence was measured objectively through urine volume and osmolality. During the 12 month study, the mean number of UTIs was 1.7 in the water group compared to 3.2 in the control group (Difference in means of 1.5, 95%CI 1.2-1.8, $p<0.001$). Although the results must be interpreted with the limitations of an open label trial, this study presents an interesting alternative for women with recurrent UTI who may have low daily water intake.

Contrary to previous clinical perceptions, several case-control studies have shown that certain modifiable hygiene practices such as urination before and after sexual activity, frequency of urination or washing, the direction a women wipes after a bowel movement and the use of tampons do not impact the risk for UTI [56]. Similarly, behavioural factors such as the use of hot tubs and frequent use of pantyhose or tights are not correlated with an increased risk of UTI [46]. Unfortunately, findings from these case-control studies must be interpreted by taking into account a level of recall bias, especially since the time frame of interest was the 1-year period before the most recent episode of UTI.

Furthermore, reporting bias may have influenced the results as the potential modifiable risk factors concerned sexual experiences and hygiene. Still, discussions regarding these hygiene practices are encouraged by family physicians and nursing staff 57.

Adverse Effects, Morbidity and Health Burden

In general, uncomplicated UTIs are confined to the bladder, resolve quickly following antibiotic treatment and thus are associated with fewer severe or long-term sequelae. An observational study of 511 non-pregnant adult women with UTI presenting in primary care and treated promptly with antibiotics experienced a mean duration of 3.46 days of urinary frequency, 1.88 days of haematuria and 3.06 days of urgency 3. Interestingly, it has been shown that untreated infections can resolve in many cases without the need for pharmacological or non-pharmacological interventions. In two RCTs comparing antibiotic therapy to placebo in women with uncomplicated UTI, 50% of untreated infections resolved spontaneously within three days of initial symptoms 58,59. Furthermore, the risk that a UTI progresses to pyelonephritis is low for both infections treated with antibiotics and those that are not 60. A small placebo controlled trial reported only one case in a group receiving placebo (1 of 38 patients) 59 and a larger study reported one case in both the placebo (1 of 227 patients) and the treatment group (1 of 657 patients) 58. Although less common in healthy community dwelling women, acute pyelonephritis can result in hospitalization in 10-30% of patients, often requires intravenous antibiotic therapy and dramatically increases the costs of this infection 61.

Though viewed as a benign affliction, uncomplicated lower UTI symptoms can have considerable impacts on the patient's productivity and quality of life. In 2000, a study of university women reported that patients suffering from UTI experienced 2.4 days of restricted activity, 1.2 days of lost time and 0.4 bed bound days due to their symptoms 4. A small pilot survey also assessed the indirect effects of UTI in symptomatic women. Forty-five percentof women reported that work or school activities were affected and 29% limited their work or school activities. Thirty-six percent of women reported missing one day of work or school and 29% reported missing greater than one day. An additional 39% reported that their activities outside of school and work were affected for a mean of 3.8 days 62. Coupled with decreased productivity, symptoms can have a rapid, detrimental impact a women's perception of quality of life. Ellis et al. (2000) utilized the short form SF-36 to quantify quality of life scores between women experiencing UTI versus healthy controls Ref 63. The study reported that women with UTI maintained

significantly lower scores across all facets of the SF-36 and reported correlations between the severity of symptoms and vitality, pain, social function and role limitation caused by physical health [63]. In addition to both decreased productivity and intangible costs, an economic analysis reports that the tangible costs of treatment for each UTI are extremely high, estimated at 112-172$ USD [64]. These costs are attribute in majority to the initial visit with a medical evaluation, urine dipstick and prescription. However, repeat physician consultations due to inadequate initial antibiotic prescription can also contribute to increased health care costs.

Current Prevention and Treatment Options

Past CUA and SOGC recommendations for the treatment of uncomplicated recurrent UTI use one of three antibiotic treatment regimens: continuous antibiotic prophylaxis, post-coital antibiotic prophylaxis or self-start antibiotic therapy [14]. Optimal prophylactic therapy is not identical for all women and is therefore based on individual allergies, prior susceptibility, local resistance patterns, costs and side effects [10].

Continuous antibiotic prophylaxis consists of daily intake of low dose antibiotics over a prolonged period of time, usually 6-12 months. Antibiotic regimens suggested by the CUA and the SOGC are described in table 2. A 2004 Cochrane Database systematic review showed that this type of preventative treatment is effective compared to placebo, however it is associated with side effects including vaginal and oral candidiasis, gastrointestinal distress and dermatological lesions [65]. It is also not a curative treatment, as women that discontinue treatment revert back to their previous frequency of recurrent UTI. Another option for women that experience recurrent UTI is the possibility of post-coital antibiotics, especially if there is a connection between sexual activity and symptoms. This type of treatment entails taking antibiotics within 2 hours of intercourse and can help decrease costs and side effects associated with daily antibiotic intake. The last option suggested by the CUA is self-start antibiotic therapy. Women are prescribed a three-day treatment dose of antibiotics, which they can take at the start of symptoms. This option is based on several studies that show that women who experience recurrent UTI are able to accurately self-diagnose in 86-94% of cases [25,66,67]. A review of these different management strategies showed that daily antibiotic use is the most effective strategy for recurrent UTI prevention, yet the most expensive for the patient [68]. Although slightly less effective, symptomatic self-treatment resulted in costs saving and greatly improves quality-adjusted life-years.

Unfortunately, insufficient evidence exists for conservative measures in the prevention of recurrent UTI. A 2009 systematic review comparing antibiotics versus placebo for the treatment of dipstick or culture-confirmed UTI in healthy women presented the superiority of this treatment regimen for the cure of physical symptoms of UTI (4 RCTs, 1062 patients, OR = 4.67, 95% CI 2.34-9.35). Remarkably, in each of these studies, 20-44.4% of women presented clinical and microbiological success after 3-7 days of placebo treatment. Most notably, Ferry et al. (2004) explored the natural history of uncomplicated UTI in a RCT with 1143 women that compared three antibiotic treatments to placebo. Of the 288 women with symptomatic UTI who received placebo, 28% had neither symptoms nor bacteriuria after one week [58]. Although this study provided interesting data regarding potential auto-resolution of UTI symptoms, it is unlikely that similar studies will be performed due to ethical considerations enforced since this study was performed.

Moreover, women may receive counselling on modifiable risk factors for UTI that have been proven as contributory to their recurrent UTI, such as sexual activity and spermicide use. However, as mentioned previously, no association has been shown between pre- and post-coital patterns, frequency of urination, delayed voiding habits, douching, use of hot tubs, bubble baths, body mass index (BMI), frequent use of pantyhose or tights, use of tight clothing and bicycle riding [69,70].

Antibiotic Resistance

The empirical and preventive antibiotics for the treatment of recurrent UTI have been established as the most cost-effective way to manage these infections. However, prescribing without confirmation of diagnosis and isolation of causal bacterial contributes to the growing problem of uropathogen resistance in primary care [6]. An evaluation of microbial antibiotic susceptibility performed on 36 293 E. coli isolates cultured from the urine samples of women aged 18-65 years old in 4 hospitals in the province of Quebec from April 2010 to March 2015, examined the antibiotic resistance rates for the three most commonly prescribed antibiotics for UTI [71]. Rates of resistance for each antibiotic increased steadily per year and in 2014 the susceptibility of nitrofurantoin was the highest (95.4%) followed by ciprofloxacin (90.3%) and trimethoprim-sulfamethoxazole (81.9%). In three of the four hospital centers, the three medications remained below the threshold of 20% resistance which is cited in the literature as the maximum proportion of isolates that can be resistant to an agent without compromising empirical treatment success, based on international expert opinion and mathematical models [72]. The results are

also in congruence with an earlier nationwide study, which showed increasing TMP-SMX and ciprofloxacin resistant E.coli throughout Canada 73.

Although nitrofurantoin has been used for more than 50 years, it is proposed that nitrofurantoin resistance remains low because of its restricted use for treating UTI in Canada 31. Ciprofloxacin has been increasingly used to treat UTI, despite recommendations that it not be used as first-line therapy 74,75. The phenomenon of antibiotic resistance is seen worldwide, a meta-analysis of 54 reports similar increases in ciprofloxacin resistance in 27 countries with certain variances between developing and developed countries 76. The Canadian antimicrobial resistance alliance (CANWARD) recently released a population-based report detailing a significant increase in ciprofloxacin-resistant E. coli isolated in urine sample obtained from Canadian hospitals throughout the provinces in the five-year period between 2007-2011 77. The CANWARD report illustrated the significant increase in the percentage of ciprofloxacin-resistant E.coli isolates (primarily among urine isolate) from 20.0% in 2007 to 29.2% in 2011.

On the basis of increasing resistance patterns to commonly used antibiotics and the emergence of multi-drug resistant UPEC 77, researchers are evaluating non-antibiotic interventions that may provide adequate prophylaxis against recurrent UTI.

Alternative Preventative Measures and Treatments

Ibuprofen

On the basis of trials that assessed placebo compared with antibiotics treatment and delayed prescription of antibiotics for UTI, a 2015 double blind multicentre RCT investigated if ibuprofen could reduce the rate of antibiotic prescriptions for the treatment of acute UTI symptoms 78. Four hundred and ninety four women presenting with dysuria and/or frequency/urgency of micturition, with or without lower abdominal pain were randomized to receive either ibuprofen tablets (3x400 mg DIE for three days plus 1x1 sachet placebo granules; n=248) or fosfomycin-trometamol (1x3 g sachet plus 3x3 placebo tablets for three days; n=246). Women were advised to contact their family doctor if symptoms persisted or worsened and antibiotic treatment was initiated based on urinary culture results from the baseline visit. The primary endpoints were the number of all courses of antibiotic treatment on days 0-

28 and the burden of symptoms on days 0-7. There was a significant difference in the number of women randomized to ibuprofen who necessitated antibiotic treatment compared to those randomised to the fosfomycin group (MD -64.7, 95% CI -70.7 - -58.7, p<0.001). However, seven days following treatment, the proportion of women no longer presenting UTI symptoms was significantly higher in the fosfomycin group (82%) compared to the ibuprofen group (70%), p=0.004. Five women in the ibuprofen group developed pyelonephritis compared to one woman in the fosfomycin group (p=0.12). Interestingly, the rates of reoccurrence of symptoms 15 days following treatment was significantly higher in the fosfomycin group (11%) compared to ibuprofen (6%), p=0.049. On the basis of these results, use of ibuprofen may be discussed as a therapeutic option with patients with recurrent UTI that present mild to moderate symptoms of UTI and who wish to restrain or delay antibiotic usage.

Acupuncture

Acupuncture has been investigated for both the treatment and prevention of UTI. A 2002 RCT evaluated the effects of acupuncture treatment compared with no treatment in the prevention of uncomplicated recurrent UTI in adult women 79. One hundred women with at least 3 episodes of symptomatic UTI in the past year with at least 2 clinician diagnosed episodes were randomized to receive acupuncture treatment twice weekly for 4 weeks (n=67) or no treatment (n=27) and the number of new culture confirmed UTI (CFU≥10^5) was followed during a 6-month period. Following treatment, 27% of women in the acupuncture group experienced ≥1 culture confirmed UTI compared to 48% in the no treatment group, p=0.08. Similarly, 22% of women in the acupuncture group presented ≥ 1 episode of UTI symptoms compared to 63% of women with no treatment (RR 0.36, 95%CI 0.21-0.61, p=0.002). Although promising, few recent studies have investigated this possible prophylactic measure in order to corroborate this research teams findings.

Probiotics and Bacterial Interference

As it has been observedthat women with recurrent UTI often have alterations in their vaginal microbiota characterised by a depletion of beneficial bacteria 80, studies have investigated the role of intra-vaginal lactobacillus suppositories for the prevention of recurrent UTI 81. Most notably, a randomized, placebo-controlled phase 2 trial enrolled women with a history of ≥ 1 UTI in the past year presenting with symptomatic culture confirmed UTI (CFU≥10^2)37. One hundred women were treated for their UTI and randomized to receive Lactin-V, a probiotic H202-producing lactobacilli, or placebo 7-10 days after UTI

treatment and follow-up for 10 weeks. The rate of culture-confirmed UTI was 15% in the Lactin-V group compared to 27% in the placebo group, (RR=0.5, 95%CI 0.2-1.2, p=0.1).

Although limited to a population of patients with neurogenic bladder secondary to spinal cord injury, bacterial interference with a non-pathogenic strain of E.coli, E. coli 83972, has been investigated for the prevention of catheter associated UTI. Sunden et al. performed a small pilot trial comparing E. coli 83972 versus saline in 20 patients with neurogenic bladder on the prevention of recurrent UTI during a one-year period. The time to first UTI was longer in patients with inoculation compared to saline (median 11.3 vs 5.7 months, p=0.0129, sign test.). The number of UTI reported by the inoculated group was significantly less that in those without E. coli 83972 bacteriuria (13 vs 35 episodes, paired t-test p=0.009, 95%CI 0.31-1.89,). Although these favourable results are limited by a small sample size, this alternative prevention option may be of interest in women with recurrent UTI.

Despite the research into many alternative treatments for the prevention of recurrent UTI, the most promising, yet polarizing research lies in the use of cranberry products.

1.2 Cranberries for the Prevention of Recurrent Urinary Tract Infections

Mechanisms of Actions, In vitro and Ex vivo studies

Cranberries (*Vaccinium macrocarpon*) are native to North America and were used by indigenous populations to treat several conditions, including bladder and kidney diseases [82]. Researchers in the late 1800s postulated that the medicinal properties of cranberry were mediated through the acidification of urine, which would decrease the proliferation of bacteria in the urinary tract [83]. However, studies show that the excretion of cranberry products cause temporary changes in urine pH that last at most 10-15 minutes in humans [84]. More recent theories on the mechanism of action of cranberry have shifted towards its ability to inhibit bacteria from adhering to the uroepithelial cells lining the urethra and bladder [83]. Bacterial adhesion is mediated by fimbriae, proteinaceous fibers on the bacterial cell wall, which produce adhesins that attach to specific oligosaccharide receptors on the uroepithelial cells [85]. In particular, two virulent strains of E. coli isolated from patients with recurrent UTI and pyelonephritis are type-1 fimbriae and P-fimbriae (Pap, for pilus associated with pyelonephritis), respectively [86]. Since bacterial adhesion is the initial, crucial step in the development of UTI, the anti-adhesive activity of cranberry compounds may decrease the bacterial colonization and hence prevent infection.

As whole cranberries undergo pharmacokinetic changes after human consumption resulting in the excretion of certain metabolites, experiments have been focused on the identification of components in cranberries that responsible for their bioactive effects [87]. In vitro studies have identified two components of cranberries, fructose and PACs, that are believed to mediate anti-adhesion activity against E. coli. A 1989 experimental study assessed the action of cranberry juice cocktail against the adhesion of type 1 fimbriated (mannose-resistant) E. coli to animal and human cells [88]. In this study, the inhibition of adherence was attributed to the intrinsic fructose content in the cranberry juice cocktail. Another in vitro study isolated proanthocyanidins trimers possessing A-type interflavanoid linkages from ripe cranberry fruits and showed that these components exerted inhibitory actions against P-fimbriated as well as type 1 fimbriae E. coli strains isolated from the urine of human patients diagnosed with UTI [89]. These in vitro studies provided a basis for the development of ex vivo studies to elaborate on the antibacterial activity of metabolites of cranberry products excreted in the urine.

A first small double-blind, randomized, crossover trial involving only 8 healthy participants observed that the first morning urine collected 12 hours after consumption of 108 mg cranberry capsules (with

extraction of fructose) exhibited significant in vitro anti-adhesion activity against type 1 fimbriated and P-fimbriated E. coli isolated from urine of patients with UTI compared to urine of participants consuming placebo capsules 90. Following this study, a multicentre randomized double blind crossover trial with 32 healthy women was performed in order to assess the persistence of anti-adhesion activity in urine over time and to determine the most effective dose of cranberry derived PACs per day 9. The participants were randomized to receive three regimens distributed in random order of one dose of 0, 18, 36 or 72 mg PAC equivalents with a 1-week washout period between each regimen. Urine collection was initiated in three parts: at baseline, pooled urine between 1-6 hours and 24 hours following capsule intake. An ex vivo epithelial cell adhesion assay was performed on to evaluate the activity of collected urine against uropathogenic P-fimbriated E. coli. The results indicated a significant reduction in bacterial adhesion following the consumption of cranberry dosages containing greater than 36 mg of PACs after 1-6 hours, with a dose-dependent effect (Urinary bacterial Anti-Adhesion Activity (AAA) detected with Mannose-resistant Hemagglutination (MRHA) assay: Placebo=0%[0%] 18 mg PACs=50%[50-100%] 36 mg PACs=90.6%[50-100%] 72 mg PACs=100%[100%], $p<0.001$). Furthermore, a time-dependent effect was observed with a maximum effect at 1-6 hours compared to 24 hours ($p<0.001$), affirming that this is the most important PAC elimination period in urines as shown previously and suggesting that it may be beneficial to consume PACs in two split doses.

Since the publication of this multicenter trial, several similar studies with larger sample sizes have validated that post cranberry extract products consumption urine shows anti-adhesion effects. A 2015 randomized, double-blind, placebo-controlled trial similarly investigated an ex vivo urinary anti-adhesion activity of both a low cranberry extract beverage and a cranberry juice cocktail 91. In this trial, 59 healthy, male and female subjects without a history of recurrent UTI were randomly assigned to a cranberry extract juice beverage, a low calorie cranberry juice cocktail or placebo. Both treatment beverages were designed to contain a similar PAC content and were quantified using a modified OSC-DMAC method to > 80 mg PACs. Clean-catch urine samples were collected for 6h post-beverage consumption and pooled urine samples (0-6h) were used to assess anti-adhesion activity. Anti-adhesion activity, as measured by a hemagglutination assay, was significantly higher in the cranberry treatments than placebo (Placebo=0.49; cranberry extract juice beverage=0.85; low calorie cranberry juice cocktail=0.68, $p<0.05$).

Although these ex-vivo studies indicate that the post-cranberry PAC consumption urine is capable of exhibiting anti-adhesion activity, the exact metabolites of PACs responsible for this activity remain unknown considering that type A PACs are minimally excreted in urine 92. An alternate hypothesis of the mechanism of action of the anti-adhesion activity is an interaction between cranberry PACs and lymphoid tissue in the gastrointestinal tract. Researchers have shown that PACs are recognized and undergo active endocytosis by dendritic cells 93, which may trigger an innate immune response in the human body, including the urinary tract 94.

Foods Sources and Quantification of Proanthocyanidins

Proanthocyanidins are a type of flavanol that are categorized into two subtypes based on the type of bonds that link their flavan-3-ol units. Type-B PACs, linked through C4→C6 or C4→C8 interflavanoid bonds are found in high concentrations in fruits, vegetables, cereals and their transformed products 95. It is hypothesized that type-A PACs, linked by an additional ether bond between C2→07, are responsible for the bacterial anti-adhesion activity of cranberry products against UPEC in humans. Interestingly, it is proposed that plants produce PACs in defense against microbial infection 96.

Compared to gravimetric, chromatographic and mass spectrometric methods employed for the quantification of cranberry PACs in the past, colorimetric assays are favoured because of their rapidity and simplicity 97. The Brunswick Laboratory 4-di-methyl-amino-cinnamaldehyde method (BL-DMAC) is a colorimetric assay using a procyanidin A2 standard that has been validated with small within and inter-laboratory variability through a study in 5 different analytical laboratories 97. Succinctly, the colorimetric DMAC assay is an aromatic aldehyde that reacts with flavanol-3-ols and cranberry PACs to form a green chromophore with maximum absorbance at 640 nm, a wavelength that excludes other components in cranberries such as anthocyanidins. In contrast, the use of procyanidin A2 as a standard leads to underestimations of PAC content because it does not account for PACs components with higher molecular weight. A newer DMAC assay using a cranberry press cake was developed in order to increase the accuracy of PAC content quantification 98. This newer method, titled OSC-DMAC, results in values that are 2.2 times higher than those determined using a procyanidin A2 dimer. As BL-DMAC use is widespread in the cranberry transformation industry and past studies have used this quantification method, its use facilitates the comparison of treatment doses among clinical trials.

The concentration of PACs is variable according to the degree of transformation of food products. Fresh cranberries contain 133.5±6.0 mg total PACs and 11.2 ±2.8 mg type-A PACs / 100g fresh cranberry and 11.8±0.1 mg total PACs and 1.7±0.0 mg type-A PACs / 100 ml commercial juice cocktail, as measured by the BL-DMAC assay for total PACs and LC-MS analysis for proanthocyanidin A2 dimers 99. Type-A PACs are also found in lesser quantities in other foods such as plums, avocado, peanut skins, cinnamon and curry powder 95, although no trial to date has investigated the role of PACs derived from these foods for the prevention of recurrent UTI.

Randomized Controlled Trials

On the basis of in vitro and ex vivo studies, a considerable number of randomized clinical trials have been conducted in order to investigate the effects of cranberry derived products in the prevention of UTIs. A 2012 Cochrane systematic review and meta-analysis assessed 24 randomized trials regarding cranberry capsules for the prevention of UTI in populations including elderly men and women, patients with intermittent or indwelling catheterisation, pregnant women, children at risk of repeat UTI, patients undergoing radiation therapy for abdominal cancers and individuals with neurological disorders causing neurogenic bladder 10. The results indicate that cranberry products do not statistically decrease the number of UTI in susceptible populations. In women with recurrent UTI a non-significant risk reduction of 26% was found in women receiving a cranberry intervention compared to placebo (RR 0.74, 95%CI 0.42-1.31). The authors concluded that there was significant variability in the type of cranberry product (juice, capsules, dried fruit) and few studies quantified the PACs concentrations using standardised methods in order to ensure potency.

Conversely, a systematic review and meta-analysis published in the same year by Wang et al. (2012)(Wang, 2012 #158) excluded the trial performed by Barbosa-Cesnick et al. (2011)(Barbosa-Cesnik, 2011 #278) due to differing eligibility criteria found a RR=0.53 (95% CI 0.33-0.83). Wang et al. chose to exclude this study as their primary outcome, confirmed UTI defined as greater than 10^3 CFU/ml on urine culture and differed from other studies included in the analysis that measured clinical symptomatic UTI only. As criticized previously, 15-20% of women with recurrent UTI that present with urinary symptoms respond to antibiotic treatment despite negative culture results 101. Since these publications, which highlighted the necessity of PAC quantification in cranberry products, several RCTs have been performed in women at risk of recurrent UTI. A summary of clinical trials evaluating the

impact of cranberry products in women with recurrent UTI included in the Cochrane review and since its publication is illustrated in Table 3.

In 2012, Stapleton et al. published the results of a multicenter randomized controlled trial in premenopausal women with history of one or more clinician-diagnosed UTI in the past 12 months [102]. In this trial, 186 women with a mean number of 2 UTI in the past year were randomized to one of 3 groups of 4 oz cranberry juice, 8 oz cranberry juice or placebo daily for 6 months. The primary outcome was time to a clinical UTI event, defined as pyuria plus one or more the symptoms of dysuria, frequency, urgency, suprapubic pain or hematuria. Culture confirmed UTI and asymptomatic bacteriuria were investigated as secondary outcomes. There were no significant differences in the proportion of clinical UTI found between women in either cranberry groups (33/120) or placebo (17/56) (p=0.70). The cumulative rate of women with a clinical or culture-confirmed UTI at 6 months was similar (0.29, 95%CI 0.21-0.38 in the cranberry group and 0.37, 95%CI 0.25-0.54 in the placebo group, p=0.82). Participant adherence to treatment was between 90.3-91.8% based on self-report. Although well performed, with a rigorous double blind, RCT methodology, this study presents several limitations. Namely, the study did not reach its desired sample size with only 186 women recruited out of a planned enrolment of 350 participants. This significantly lower recruitment ensured that the study was insufficiently powered to detect an absolute reduction in the rate of symptomatic UTI recurrence. Furthermore, the authors of this study did not specify the PAC content in the standardized cranberry and placebo products used in this study, which hinder the ability to extrapolate and compare their product.

In 2015, Vostalova et al. reported the outcomes of a double blind, placebo controlled randomized trial in women aged 18 years old with a history of at least two symptomatic UTI episodes treated with antibiotics in the previous 12 months [103]. One hundred and eighty two women were enrolled and randomized to receive either cranberry fruit powder (2 x 250 mg capsules per day with a total 2.8 mg PACs per day) or placebo for 6 months. The primary end-point was a reduction in the incidence of laboratory diagnosis of UTI, defined as UTI symptoms with ≥ 100000 CFU/ml of a single organism in the urine culture. During the 6-month intervention, the proportion of women with at least one UTI was significantly lower in the cranberry group (9/83) than in the placebo group (24/93) (p=0.04). The cumulative incidence of UTI over 6 months was 8.5% in the cranberry group and 19.4% in the placebo group (p=0.04).

Table 3. Summary of Clinical Trials on Cranberry and UTI in Women with Recurrent UTI

Author, Year (Country)	Population	n	Cranberry Intervention	Daily PAC content	Control group	Duration (Mo)	Endpoint	Number of UTIs in past 12 months (mean±SD)
Barbosa-Cesnik, 2011 (U.S.A)*	Women presenting for urinalysis with ≥ 3 UTI symptoms + ≥ 10^3 CFU/ml	319	240 ml low calorie cranberry cocktail BID	224 mg **	Placebo drink	6	Culture-confirmed UTI (10^3 CFU/ml)	1.29±1.84
Kontiokari, 2001 (Finland)*	UTI caused by E.coli + urine culture ≥ 10^5 CFU/ml	150	50 ml cranberry-lingonberry juice DIE	NS	Open control Lactobacillus	12	Culture-confirmed UTI (10^5 CFU/ml)	NS
Stothers, 2002 (Canada)*	≥ 2 culture confirmed UTI in past year	150	1 capsule DIE + 250 ml placebo juice TID or 250 ml juice TID +1 placebo capsule DIE	NS	Placebo capsule and juice	12	50% decrease in culture-confirmed UTI (10^5 CF/ml) per year	2.8 (range 2-5)
Sengupta, 2011 (India)*	UTI symptoms + ≥ 10^4 CFU/ml	57	500 mg PACRAN™ capsule DIE or 1000 mg PACRAN™ capsule DIE	1.5%*** 0.4%**	Open control group	3	Culture-confirmed UTI (10^4 CFU/ml)	NS
Ledda, 2015 (Italy)	≥ 3 symptomatic UTIs in past year + ≥ 2 UTIs in six mo	44	1 capsule Anthocran™ DIE with lifestyle advice	36 mg**	Lifestyle advice only	2	Clinical UTI	NS

Table 3. Summary of Clinical Trials on Cranberry for the Prevention of UTI in Women with Recurrent UTI (continued)

Maki, 2016 (U.S.A)	≥ 2 clinician diagnosed UTI in past year + ≥ 1 UTI in previous 6 mo	373	240 ml Ocean Spray™ cranberry juice DIE	41.1 mg **	Placebo juice	6	Clinical UTI	1.65±0.11
Takahashi, 2013 (Japan)	≥ acute uncomplicated cystitis + > 1 UTI in previous 12 mo	227	125 ml UR65 cranberry juice DIE	40 mg PACs	Placebo beverage	6	Clinical UTI	NS
Vostalova, 2015 (Czech Republic)	≥2 symptomatic UTIs in the previous 12 mo	182	250 mg capsules PACRAN™ BID	2.8 mg PACs**	Placebo capsule	6	Culture-confirmed UTI (10^5 CFU/ml)	3.10±1.29
Stapleton, 2012 (U.S.A)	≥ 1 clinician diagnosed UTI in the past 12 mo	176	4 oz or 8 oz Ocean Spray™ cranberry juice	NS	Placebo juice	6	Clinical UTI	2.00±1.34

* Studies included in Jepson et al. 2012 meta-analysis ** PAC quantification by BL-DMAC *** PAC quantification using HPLC
Mo=months U.S.A=United States UTI=urinary tract infection DIE=once daily BID=twice daily NS=not specified UTI=urinary tract infection CFU=colony forming units

In 2015, Ledda et al. reported the results of a pilot registry, supplement study investigating the impact of daily consumption of 1 capsule Anthocran™ containing 36 mg PAC with lifestyle advice compared to lifestyle advice alone during 2 months in 42 women with at least 2 symptomatic UTI in the 6 months and 3 symptomatic UTIs in the year preceding enrolment(Ledda, 2015 #25). A significant reduction in frequency of UTI episodes during the 2 months study period compared with the 2 months before inclusion was found in the cranberry treatment group (73.3% vs 15.4%, p=0.012). In this pilot study, results were not adjusted for known risk factors for UTI development in the study cohort. Furthermore, the limited trial duration did not account for seasonal variations in UTI development amongst participants.

In 2016, Maki et al. published the results of a randomized, double blind, placebo-controlled, multicenter clinical trial in women aged 18 years or older with a history of ≥ 2 episodes of a UTI that were treated by a health care professional in the past year based on self report 104. In this study, 322 subjects were randomly assigned to consume a 240 ml cranberry beverage (41.1±7.1 mg PACs) per day or a placebo beverage throughout a 24-week treatment period. The primary outcome was the clinical UTI incidence density, defined as the number symptomatic UTI events in each group per unit of observation time. The results indicated a significantly decreased rate of clinical UTI in the cranberry group compared with the placebo group after adjustment for antibiotic use (incidence rate ratio: 0.61, 95%CI 0.41-0.91, p=0.016). Compliance, measured with a daily intake diary as well as the return of all unused bottles of dispensed study product was approximately 98%.

These recent studies are characterized by large sample sizes that were adequately powered to detect clinical differences between cranberry products and placebo in the prevention of UTI in women with recurrent UTI, however the results remain discordant. The eligibility criteria differ from one study to another, with certain trials evaluating women with recent UTI while others evaluate women with a history of recurrent UTI based on definitions from differing clinical guidelines. Trials have continued to use cranberry juice products102,104 regardless of the recommendations from the 2012 Cochrane meta-analysis stating that cranberry products in juice form may not be acceptable over long periods of time due to a high number of withdrawals in past studies. Furthermore, trials have continued to evaluate cranberry products without using a standardized and quantified dose of PACs or a split dose regimen.

Potential Side Effects

The consumption of cranberry extract capsules is associated with few consequences. Trials evaluating cranberry products versus placebo have reported non-significant increases in gastrointestinal side effects, such as dyspepsia, nausea, vomiting and diarrhea 10. Cranberries contain a moderately high concentration of oxalate and may aggravate the risk of nephrolithiasis in susceptible populations. A small study in 5 healthy volunteers explored the impact of 1-week administration of cranberry concentrate tablets on urine levels of oxalates and electrolytes 105. A significant increase of urine oxalate concentration and calcium oxalate supersaturation was found between baseline and 1-week 24 hour urine collections.

Product information sheet produced by the manufacturers of warfarin list an interaction with cranberry products based on case studies have presented evidence that cranberries may interact with warfarin metabolism leading to increased risk of bleeding 106. However, randomised trials using 240 ml and 2x240 ml of cranberry juice daily have shown that short-term intake of cranberry juice does not alter the pharmacodynamics of warfarin 107,108 .

Quantification of Proanthocyanidins Metabolites

The bioactive properties of cranberry derived type-A proanthocyanidins have led to an interest in their pharmacokinetic properties. In vitro studies have shown that a small fraction of procyanidin A-type dimers, trimers and tetramers are transported through human intestinal epithelial Caco-2 cell monolayers found in the small intestine 109. Proanthocyanidin A2 dimers have also been detected in human plasma and urine using liquid chromatography-mass spectrometry analysis following the ingestion of cranberry juice cocktail 110. The use of urinary proanthocyanidin A2 as a biomarker of cranberry consumption is limited, as a dose-dependent correlation has not been found between intake and urinary excretion 111. The interaction of undigested PACs with colonic microbiota may account for the lack of dose response relationship and high inter-individual variability 112.

In order to investigate the impact of cranberry consumption on the fecal microbiome, a small trial was performed in 10 healthy subjects 113. Subjects consumed 42g sweetened dried cranberries daily for two weeks. Each serving contained 26 mg PACs as measured by the OS-DMAC method. Seven

participants had increased species diversity in the fecal sample obtained after the intervention. An increase in species diversity may impact the colonisation of the gastrointestinal tract with uropathogenic E. coli, however studies have yet to investigate the impact of cranberries on fecal flora in women with recurrent UTI through the collection of fecal samples.

Chapter 2 - Rationale and Relevance

Objectives of this Study

The primary objective is to evaluate, in sexually active women who present recurrent UTIs, the effects of a standardized cranberry extract containing 37 mg type A linkage proanthocyanidins (PACs) per day (18.5 mg BID), compared to a control dose of 2 mg PACs per day (1mg BID) on the incidence rate of newly diagnosed symptomatic UTIs during a 24-week follow-up period.

Secondary objectives include:

1) To evaluate the mean number of new symptomatic UTIs with pyuria as demonstrated by a positive leucocyte esterase test.
2) To detect the mean number of new symptomatic UTIs with bacteriuria.
3) To describe the side effects of daily intake of cranberry extract containing 37 mg PACs compared to 2 mg PACs.

Additional analysis will be performed on 24-hour urine collection and stool samples obtained from certain women in order to evaluate differences in urinary anti-adhesion activity and impacts on the intestinal microbiome. These analyses will be performed by Yves Desjardins and Stéphanie Dudonné, collaborators of this study, and are not discussed in this master work.

Chapter 3 – Research Article

High Dose Versus Low Dose Standardized Cranberry Proanthocyanidin Extract for the Prevention Of Recurrent Urinary Tract Infection In Healthy Women: A Double-Blind Randomized Controlled Trial

Babar, Asma[1,2] (asma.babar.1@ulaval.ca);
Moore, Lynne[2] (lynne.moore@fmed.ulaval.ca);
Leblanc, Vicky[3] (vicky.leblanc.1@ulaval.ca);
Dudonné, Stéphanie[3] (stephanie.dudonné.1@ulaval.ca) ;
Desjardins, Yves[3] (yves.desjardins@fsaa.ulaval.ca);
Lemieux, Simone[3] (simone.lemieux@fsaa.ulaval.ca);
Bochard, Valérie[4] (vbochard@diana-food.com);
Guyonnet, Denis[5] (dguyonnet@diana-group.com);
Howell, Amy[6] (ahowell@njaes.rutgers.edu);
Dodin, Sylvie[1,2,7] (sylvie.dodin@fmed.ulaval.ca)

[1]CHU de Québec-Laval University Research Center, Québec city, Canada
[2]Department of Social and Preventive Medicine, Faculty of Medicine, Laval University, Québec city, Canada
[3]Institute of Nutrition and Functional Foods, Laval University, Québec city, Canada
[4]Diana Food, Rennes, France
[5]Diana Nova, Paris, France
[6]Marucci Center for Blueberry Cranberry Research, Rutgers, the State University of New Jersey, Chatsworth, United States of America
[7]Department of Obstetrics and Gynecology, Laval University, Québec city, Canada

Résumé

Objectifs: Notre objectif était d'évaluer l'efficacité d'un extrait de canneberge standardisé en proanthocyanidines de type A (PAC) sur la prévention des UTI à répétition.

Méthodes: Nous avons recruté 145 femmes âgées de 18 ans et plus avec antécédents d'UTI à répétition, définie par ≥ 2 UTIs dans les derniers 6 mois ou ≥ 3 UTIs dans les derniers 12 mois, dans cet essai clinique randomisé à double insu. Soixante-douze femmes ont reçu une dose optimale d'extrait de canneberge quantifié et standardisé en PACs (2 x 18.5 mg PACs par jour) et soixante-treize ont reçu une dose contrôle (2 x 1 mg PACs par jour). Durant le suivi de 24 semaines, les femmes symptomatiques ont fourni des échantillons d'urine pour la détection de la pyurie et de la bactériurie et ont reçu une prescription d'antibiotique appropriée. L'issue principale était le nombre moyen de nouvelles UTIs symptomatiques chez les participantes durant une période d'intervention de 24 semaines. Les issues secondaires étaient : 1) évaluer le nombre moyen d'UTI avec pyurie et avec confirmation microbiologique; 2) décrire les effets secondaires d'une dose quotidienne d'extrait de canneberge.

Résultats: En réponse à l'intervention, une diminution non significative de 24% du nombre d'infections symptomatiques des voies urinaires a été observée entre les groupes (rapport du taux d'incidence de 0,76, IC à 95% de 0,51 à 1,11). Selon des analyses post-hoc, chez les participantes ayant eu moins de 5 UTIs dans l'année précédant leur participation, la prise de 2x18,5 mg était associée à une diminution des UTIs symptomatiques comparativement à une prise de 2x1 mg PACs (rapport de taux d'incidence ajusté pour l'âge 0,57, IC 95% : 0,33-0,99). Aucun effet secondaire majeur n'a été rapporté.

Conclusion: La prise d'un extrait de canneberge en teneur élevée de proanthocyanidins n'a pas été associée à une réduction du taux d'incidence d'infections urinaires symptomatiques par rapport à un extrait de proanthocyanidines à faible dose. Nos résultats post-hoc suggèrent que la prise d'une dose de 2x18,5 mg PAC par jour pourrait prévenir les UTIs symptomatiques chez les femmes ayant moins de 5 UTIs par année.

Enregistrement de l'étude: ClinicalTrials.gov; NCT02572895

Abstract

Purpose: Our objective was to assess the efficacy of a high dose cranberry proanthocyanidin extract for the prevention of recurrent urinary tract infection.

Material and methods: We recruited 145 healthy, adult women with a history of recurrent urinary tract infection, defined as ≥ 2 in the past 6 months or ≥ 3 in the past 12 months in this randomized, controlled, double-blind clinical trial. Participants were randomized to receive a high dose of standardized, commercially available cranberry proanthocyanidins (2 x 18.5 mg daily, n=72) or a control low dose (2 x 1 mg daily, n=73) for a 24-week period. During follow-up, symptomatic women provided urine samples for detection of pyuria and/or bacteriuria and received an appropriate antibiotic prescription. The primary outcome for the trial was the mean number of new symptomatic urinary tract infections during a 24-week intervention period. Secondary outcomes included symptomatic urinary tract infection with pyuria or bacteriuria.

Results: In response to the intervention, a non-significant 24% decrease in the number of symptomatic urinary tract infections was observed between groups (Incidence rate ratio 0.76, 95%CI 0.51- 1.11). Post-hoc analyses indicated that among 97 women who experienced less than 5 infections in the year preceding enrolment, the high dose was associated with a significant decrease in the number of symptomatic urinary tract infections reported compared to the low dose (age-adjusted incidence rate ratio 0.57, 95%CI 0.33-0.99). No major side effects were reported.

Conclusion: High dose twice daily proanthocyanidin extract was not associated with a reduction in the number of symptomatic urinary tract infections when compared to a low dose proanthocyanidin extract. Our post-hoc results reveal that this high dose of proanthocyanidins may have a preventive impact on symptomatic urinary tract infection recurrence in women who experienced less than 5 infections per year.

Study registration : ClinicalTrials.gov; NCT02572895

Introduction

Urinary tract infection (UTI) is one of the main reasons for emergency medical consultation. One in three women over the age of 18 will experience a UTI, and many of them will have repeated infections [1]. Increasing resistance of pathogens to prescribed antibiotics, both in treatment and prophylaxis, as well as the side effects of antibiotics, reinforce the demand for alternatives that are effective and well tolerated [2].

Cranberry products are the most promising natural health alternatives for the prevention of UTIs [3]. Cranberry has been shown to inhibit the adhesion of uropathogenic Escherichia coli to uroepithelial cells [4]. In vitro, cranberry proanthocyanidins (PACs) with type A-linkages, have been identified as responsible for this anti-adhesion effect [5]. Clinical trials have been conducted to test the efficacy of cranberry products, mainly in the form of juices, but their results remain discordant [3]. This discrepancy is mainly explained by a lack of compliance, lack of statistical power and variable PAC concentrations in the tested products. Indeed, PAC concentrations are not disclosed in the majority of clinical trials. According to ex vivo clinical studies (dose-effect studies evaluating the optimal dose for urine anti-adhesion effect), the quantification of PACs requires standardized, reproducible methods and should be at least 36 mg / day [5 6]. We hypothesize that the efficacy of cranberry products on the prevention of recurrent UTIs in women could be improved with the use of an optimal PACs dose (standardized at 2x18.5 mg / day).

Materials and Methods

The Cranberry Extract for Prevention of Recurrent UTI Trial (PACCANN) was a randomized, double blind, controlled, clinical trial performed at the Institute of Nutrition and Functional Foods (INAF). Written informed consent was obtained from all study participants. The protocol and consent form of this study were reviewed and approved by the institutional ethics committee of Laval University. The protocol is available on ClinicalTrials.gov (NCT02572895) and has been published in BMC Urology 7.

Study Population

We enrolled sexually active non-pregnant women aged 18 years and over presenting with recurrent UTI as diagnosed by a physician (defined as ≥ 2 UTIs in the past 6 months or ≥ 3 UTIs in the past 12 months). Women were recruited in the Laval University community in Quebec City, Canada, through e-mail list serves and local clinician referrals as well as posters in medical clinics, social media, paid advertising and word of mouth. Eligibility of potential participants was verified by the study coordinator according to inclusion and exclusion criteria (Table 1). The risks and benefits of the study have been thoroughly discussed and the consent form was signed at the first of three visits at INAF.

Study Product

The high dose intervention consisted of twice daily intake of commercially available 120 mg Urophenol™, a purified cranberry extract from whole fruit (*Vaccinium macrocarpon Aiton*) standardized at min 15% PACs. The control dose was standardized at 1% PACs which is comparable to the majority of cranberry products approved by Health Canada 8. Cranberry PACs were manufactured by Nutra Canada (now part of Diana Food Canada) and similar in size, smell and taste. Capsules were distributed in opaque packaging in order to conceal slight colour variations from the research team. Total PAC content of each treatment was validated at INAF's analytical laboratory using the 4-dimethylaminocinnamaldehyde (BL-DMAC) method 9 (Appendix A). PACs were also characterized by normal-phase analytical HPLC coupled with fluorescence detection, as previously described 10.

Randomization

Concealed randomization was generated using computer assisted randomization by blocks of 10. Eligible women were assigned 1:1 to either high PAC (2 x 18.5 mg capsules per day) or low PAC (2 x

1 mg capsules per day) content cranberry capsules for 24 weeks. All clinical investigation, laboratory analysis, data collection and assessment were blinded to the randomization allocation.

Clinical Follow-up

Each visit (0, 12 and 24 weeks) included a short questionnaire documenting socio-demographic characteristics (T=0 only), medication and natural health product intake, quality of life (SF-12) 11, risk factors for UTIs, and a validated food frequency questionnaire 12 modified for our study to specifically include sixty-one foods containing PACs. Participants were instructed to obtain a midstream urine sample on which dipstick urinalysis and pregnancy tests were performed.

During their participation, women were asked to contact the study coordinator if they presented symptoms of UTI to schedule a visit at INAF in order to confirm the clinical diagnosis, provide a urine sample and receive an appropriate antibiotic prescription. A dipstick urinalysis using Chemstrip 9 (Roche Diagnostics USA) was used to confirm pyuria and urine samples were outsourced to the Laval University Hospital Center microbiology laboratory for culture. In line with the pragmatic aspect of this trial, women who were unable to present themselves to the research facility during a symptomatic episode were provided with an empiric antibiotic by prescription of the clinician.

Women that discontinued the intervention were asked to present themselves at the 12 and 24-week visit to complete intention to treat analysis. All participants were asked not to consume other products containing cranberry derivatives for the duration of the study.

Outcomes

The primary outcome was the number of symptomatic UTIs during the 24-week follow-up period. Symptomatic UTI was defined as acute urinary symptoms such as urine frequency, urgency, dysuria, pelvic pain, and hematuria in the absence of alternate diagnoses as assessed by study staff. The choice of symptomatic UTI was based on local 13 and international guidelines 14 as well as on realistic clinical settings in North America where empirical therapy is prescribed on the basis of clinical symptoms 14. This outcome increased our capture of UTI episodes and trial conduct as we anticipated that certain women would be unable to present themselves to the research facilities to provide a urine sample.

Secondary outcomes were symptomatic UTI with pyuria and symptomatic UTI with bacteriuria. Women who presented both symptoms and a positive leukocyte esterase dipstick result, were diagnosed as having symptomatic UTI with pyuria. Episodes were categorized as symptomatic UTI with bacteriuria in the presence of $\geq 10^3$ CFU/ml of uropathogenic bacteria. Women with antibiotic treatment for symptomatic UTI during the study period continued to take the cranberry capsules and remained in the study for 24 weeks.

Compliance and Side Effects

Participants completed a daily journal to record compliance and were asked to bring capsule bottles to each visit in order to count remaining capsules. A bi-monthly email reminder was sent to encourage participation. Side effects were evaluated at each visit and participants were asked to document symptoms in their daily journal.

Sample Size and Statistical Analysis

We estimated that 35% of patients in the control group would present at least one UTI during the 24-week follow-up period [3]. We needed to recruit 126 women to detect a clinically significant absolute difference of 25% between the 2 groups (10% of women assigned to the experimental group would experience at least 1 UTI with a power of 80%). We estimated that 15% of randomized participants would be lost to follow-up [15], therefore 148 women needed to be recruited in order for at least 126 participants to complete the 24-week intervention.

The Poisson regression model was used to compare the incidence of symptomatic UTI during the 24-week follow-up. A Kaplan Meier estimate with a log-rank test was used to compare time to first UTI between the two treatment arms. Intention-to-treat analyses were performed in all randomly assigned subjects with the observation time censored at the date that the participant abandoned or the date of last contact (either a scheduled study visit or visit for UTI treatment). All statistical analyses were performed using SAS University Edition software (SAS Institute Inc., Cary, NC, USA). *P* values reported are 2-sided ($P<0.05$).

Post-hoc and sensitivity Analysis

In the case of imbalanced groups, we generated relative rate estimates adjusted for potential confounding variables. Univariate regression analyses were performed for relevant variables collected at the baseline visit and data collected from post-hoc questions. Missing data was excluded from analyses for post-hoc questions. Variables with a p-value < 0.20 in univariate analysis were included in the multivariate regression model. Interactions with the treatment groups were tested in multivariate regression model using a backward elimination method. Interactions with a p-value < 0.05 are presented in the results by subgroup of the effect-modifying factor.

The incidence of UTI with pyuria or bacteriuria was estimated using a statistical imputation method for missing urine samples with two extreme assumptions: symptomatic UTI episodes without urine samples were classified as 1) no symptomatic UTI with pyuria or bacteriuria; and 2) symptomatic UTI with pyuria or bacteriuria.

Results

Between August 2015 and December 2016, 267 potential participants were assessed for eligibility, of which 122 were excluded mainly because they did not meet criteria for recurrent UTI (Figure 1). From August 2015 to April 2017, 145 women were recruited and randomly assigned to consume the high dose or low dose PAC capsules for a 24-week period. Reasons for discontinuing the intervention and abandoning study are shown in appendix B and C. The groups were well balanced in terms of demographic (Table 2) and clinical (Table 3) characteristics. However, women randomized to the high dose group were significantly younger (mean age 27.2±8.8 years old) than those randomized in the low-dose group (mean age 32.5±14.2 years old) (Student t-test, p=0.009).

Symptomatic UTI

A total of 45 symptomatic UTIs were diagnosed in the high dose PAC group compared to 59 in the low-dose group. The annualized incidence rate of symptomatic UTI for women receiving 2x18.5mg PACs at 24 weeks was 1.48 (95%CI 1.11-1.99) compared to 1.96 (95%CI 1.52-2.53) in women receiving 2x1mg PACs (Incidence rate ratio (IRR)=0.76, 95% CI 0.51-1.11; Table 4). UTI-free median was 24.0 weeks in the high dose group compared to 16.6 weeks in the low-dose group. The hazard ratio for the difference between the number of subjects who had experienced a first symptomatic UTI by the end of the 24-week period was 0.73 (95% CI 0.45-1.16; Figure 2).

Univariate Poisson regression analysis for total number of symptomatic UTIs and known risk factors are shown in Table 5. After adjustment for age, only the number of UTI in the 12 months preceding enrolment showed a significant interaction between groups. Among participants with less than 5 UTIs in the 12 months preceding enrolment (n=97), the age-adjusted annualized incidence rate of UTI in the high dose group was 1.32 (95%CI 0.81-2.13) compared to 2.29 (95%CI 1.66-3.16) in the low-dose group (age-adjusted IRR=0.57, 95%CI 0.33-0.99) (Table 6).

Symptomatic UTI with Pyuria

Data were obtained from women who presented themselves to the research facility in order to provide a urine sample in 70 out of 104 symptomatic UTI episodes. Eighty-one percent of the 70 urine samples obtained presented pyuria as measured by a positive leucocyte esterase dipstick test. No statistically

significant reductions in the incidence rate of symptomatic UTI with pyuria were found between treatment groups. In women with less than 5 UTIs in the 12 months prior to enrolment, the daily intake of 2x18.5 mg PAC, compared to 2x1 mg PAC, was associated with a statistically significant 46% reduction in the age-adjusted incidence rate of symptomatic UTI with pyuria (IRR=0.54, 95%CI 0.30-0.99, where symptomatic UTI without urine samples were classified as symptomatic UTI with pyuria (Table 7)).

Symptomatic UTI with Bacteriuria

Urine culture was performed in 61 of the 70 urine samples collected during symptomatic UTI. A positive culture was confirmed in 49% of the 61 urine samples analyzed during symptomatic UTI. No statistically significant reductions in the age-adjusted incidence rate of symptomatic UTI with bacteriuria were found between groups nor in sub-group analyses in women with less than 5 UTIs in the 12 months prior to enrolment (Table 8).

Compliance and Side Effects

Compliance based on number of returned capsules at 24 weeks was similar in both groups (92.9% in the high dose group vs 92.7% in the low-dose group, Student t-test p=0.9). Compliance according to daily intake journals was comparable in both groups, 87.3% in the high dose group and 88.8% in the low-dose group (Student t-test, p=0.6). No serious adverse events occurred in either of the study groups. The only reported side effect, dyspepsia, was reported by one participant in each group. Participants were asked if they were aware of which treatment group they were assigned to in order to validate the effectiveness of blinding procedures. The majority of women in both groups responded that they were unaware of group allocation as show in Appendix D.

Discussion

This randomized clinical trial is the first to evaluate the efficacy of a standardized split daily dose of 37 mg cranberry PACs in capsule form on symptomatic UTI. It is of particular interest that this dose was compared to another cranberry extract containing a low concentration of PACs, as often found on the Canadian supplement market to prevent UTI. Our results indicate that the intake of 2x18.5 mg PACs daily was associated with a non-statistically significant 24% reduction in the risk of symptomatic UTI compared to a daily dose of 2x1 mg PACs during a 24-week follow-up period, similar to the results of a recent meta-analysis 16.

The present study is distinct given that we recruited according to a guideline-based definition of recurrent UTI 17. Our cohort had a higher mean incidence of UTI prior to study enrolment (mean UTI = 2.4 / 6 months and 3.9 / 1 year) compared to similar trials such as Maki et al.18 (1.65 / 6 months), Barbosa-Cesnik et al.19 (1.13 /1 year) or Stothers et al.20 (2.8/1 year). The proportion of women in the 2 mg PAC group that experienced more than 1 UTI (53.4%) was also greater than the estimated 35% 21 used to calculate our sample size. Considering these key differences with previous reports, we explored if UTI burden at baseline could impact the treatment effect. Post hoc analysis... confirmed that number of recurrent UTI was indeed a modifying factor. In women who experienced fewer than 5 UTI (mean number=3.1) in the year prior to enrolment, symptomatic UTIs were significantly reduced by 43%. No significant effect was observed in women with higher past UTI burden.

In women experiencing higher level of UTI (>5), certain factors that were not measured in our study could explain the mitigated response to PAC intake. It is possible that in these women, UTI recurrence may be the result of complex interactions between bacterial urovirulence and a particular host susceptibility (altered gut microbiota, less efficient adaptive immune response) 22 23,24. Considering that PACs are poorly absorbed and are difficult to quantify in the urine, future studies should focus on the effect of these molecules on the gut microbiota, a natural reservoir for uropathogenic Escherichia coli 22,25. Gut microbiota composition and function largely vary between individuals which might explain differences in individual's susceptibility to UTI. This could be mediated by direct effect (e.g. growth inhibition of pathobiont 26, encroachment of uropathogenic bacteria in the gut) or indirect effect (microbial metabolism of PAC or potential bioactive urinary metabolites). In order to provide

mechanistic insight explaining different levels of recurrence, feces and urine samples are being analysed and results will be published in a future article.

Our study was based on medical practice in Quebec whereby many women receive antibiotics without delaying treatment for several days while awaiting urine culture results. Similarly, Maki et al. 18. compared daily intake of 41mg PACs from cranberry juice cocktail compared to a placebo beverage on the incidence of symptomatic UTI during a 24-week follow-up. In our study, pyuria was present in 81% and bacteriuria was confirmed in 49% of the urine samples provided by participants during symptomatic UTI. This corresponds to the similar proportion of 80% of symptomatic UTI with pyuria and 60% with bacteriuria in a total of 106 symptomatic UTI episodes presented by Maki et al. (2016).

This study had limitations that could influence our findings. The lack of placebo may explain why we were unable to find a significant reduction in the recurrence of UTI in our cohort with a high UTI burden. A study conducted by Vostavola et al. has shown that 2.8 mg PAC daily can significantly impact the incidence of recurrent UTIs compared to placebo 27. Moreover, urine cultures were incomplete for a proportion of urine samples provided by symptomatic participants. Urine culture contaminated by improper clean-catch urine technique were excluded from analyses in order to mitigate a risk of detection bias. We also experienced technical issues such as delays in delivery to the microbiology laboratory and improperly stored samples.

Conclusions

The intake of 2x18.5 mg PACs daily was associated with a non-statistically significant 24% reduction in the risk of symptomatic UTI compared to a daily dose of 2x1 mg PACs during a 24-week follow-up period. In a subset of participating women with a history of less than 5 UTIs per year, the daily consumption of 2x18.5 mg PACs resulted in a significant reduction in the rate of symptomatic UTI during the trial period compared to 2x1 mg PAC. These findings need to be tested in women with moderate burden of recurrent UTI who may benefit from a preventive treatment with a split dose of 37 mg/day of PACs from cranberry extract, with few associated side effects. Further investigations are also needed to examine dose-dependent impacts of cranberry PACs for the prevention of recurrent UTI and their effects on the microbiota.

Declarations

Ethics approval and consent to participate

The protocol and consent form of this study were reviewed and approved by the institutional ethics committee of Laval University with approval number 2015-091 A6 / 14-09-2017. The study coordinator obtained written informed consent from all study participants. This randomized clinical trial is registered in ClinicalTrials.gov, identifier: NCT02572895.

Consent for publication

Not applicable

Availability of data and materials

All data sets will be password protected and only available to project investigators. Data sets, cleaned and blinded of any identifying participant information, as well as the full protocol, will be available after the completion of the trial on request to the contacting author. Data was entered electronically and original study forms will be kept locked at the study site and maintained in storage for a period of 25 years after the completion of the study.

Competing interests

Denis Guyonnet and Valerie Bochard are employees of Diana Food Canada. All other authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

Funding

This research project was funded by the Ministry of Agriculture, Fisheries and Food of Quebec and Nutra Canada (now part of Diana Food Canada). The funders had no role in the design and conduct of this clinical trial nor the collection, management, analysis, and interpretation of data.. Diana Food scientists did have a role in the approval of the manuscript and the decision to submit the manuscript for publication. Diana Food Canada manufactured and donated the cranberry capsules used in this study.

Authors' contributions

AB performed recruitment, clinical follow-up, interpretation of data, statistical analyses and writing of the manuscript; VL contributed to the design of this study, recruitment, clinical follow-up, interpretation of data, statistical analyses and revision of the manuscript; S. Dudonne undertook supervision of the biomarker measures and reviewed of the manuscript; SL and YD contributed to the original concept and design of this study and reviewed the manuscript; DG and VB approved of the final manuscript; S. Dodin contributed to the original concept and design of this study, undertook supervision of the clinical follow-up of participants, and wrote the study grant. All authors read and approved the final manuscript.

Acknowledgements

We thank Iseult Grenier-Ouellet, Marie-Pier Bernard-Genest and Lydia Tétrault for their assistance in the clinical follow-up of participants during this clinical trial at the Institute of Nutrition and Functional Foods, Laval University. The authors are very grateful to the laboratory and clinical staff and all participants in this study.

References

1. Czaja CA, Hooton TM. Update on acute uncomplicated urinary tract infection in women. Postgraduate medicine. 2006;119(1):39-45.
2. Giesen LG, Cousins G, Dimitrov BD, van de Laar FA, Fahey T. Predicting acute uncomplicated urinary tract infection in women: a systematic review of the diagnostic accuracy of symptoms and signs. BMC family practice. 2010;11:78.
3. Jepson RG, Williams G, Craig JC. Cranberries for preventing urinary tract infections. Cochrane Database Syst Rev. 2012;10:CD001321.
4. Gupta K, Chou MY, Howell A, Wobbe C, Grady R, Stapleton AE. Cranberry products inhibit adherence of p-fimbriated Escherichia coli to primary cultured bladder and vaginal epithelial cells. The Journal of urology. 2007;177(6):2357-2360.
5. Howell AB, Botto H, Combescure C, et al. Dosage effect on uropathogenic Escherichia coli anti-adhesion activity in urine following consumption of cranberry powder standardized for proanthocyanidin content: a multicentric randomized double blind study. BMC infectious diseases. 2010;10:94.
6. Lavigne JP, Bourg G, Combescure C, Botto H, Sotto A. In-vitro and in-vivo evidence of dose-dependent decrease of uropathogenic Escherichia coli virulence after consumption of commercial Vaccinium macrocarpon (cranberry) capsules. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2008;14(4):350-355.
7. Asma B, Vicky L, Stephanie D, Yves D, Amy H, Sylvie D. Standardised high dose versus low dose cranberry Proanthocyanidin extracts for the prevention of recurrent urinary tract infection in healthy women [PACCANN]: a double blind randomised controlled trial protocol. BMC urology. 2018;18(1):29.
8. Health Canada. Licensed Natural Health Products Database. http://www.hc-sc.gc.ca/. Published 2008. Accessed January 24, 2017.
9. Prior RL, Fan E, Ji H, et al. Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. J Sci Food Agric. 2010;90(9):1473-1478.
10. Dudonné SVTV, Forato Anhê F, Dubé P, Roy D, Pilon G. Modulatory effects of a cranberry extract co-supplementation with Bacillus subtilis CU1 probiotic on phenolic compounds bioavailability and gut microbiota composition in high-fat diet-fed mice. PharmaNutrition. 2015;3:89-100.
11. Jenkinson C, Layte R, Jenkinson D, et al. A shorter form health survey: can the SF-12 replicate results from the SF-36 in longitudinal studies? J Public Health Med. 1997;19(2):179-186.
12. Goulet J, Nadeau G, Lapointe A, Lamarche B, Lemieux S. Validity and reproducibility of an interviewer-administered food frequency questionnaire for healthy French-Canadian men and women. Nutr J. 2004;3:13.
13. INESSS. Urinary Tract Infections in Adults. In: Services NIoEiHaS, ed. Quebec2017.
14. Gupta K, Hooton TM, Naber KG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. 2011;52(5):e103-120.

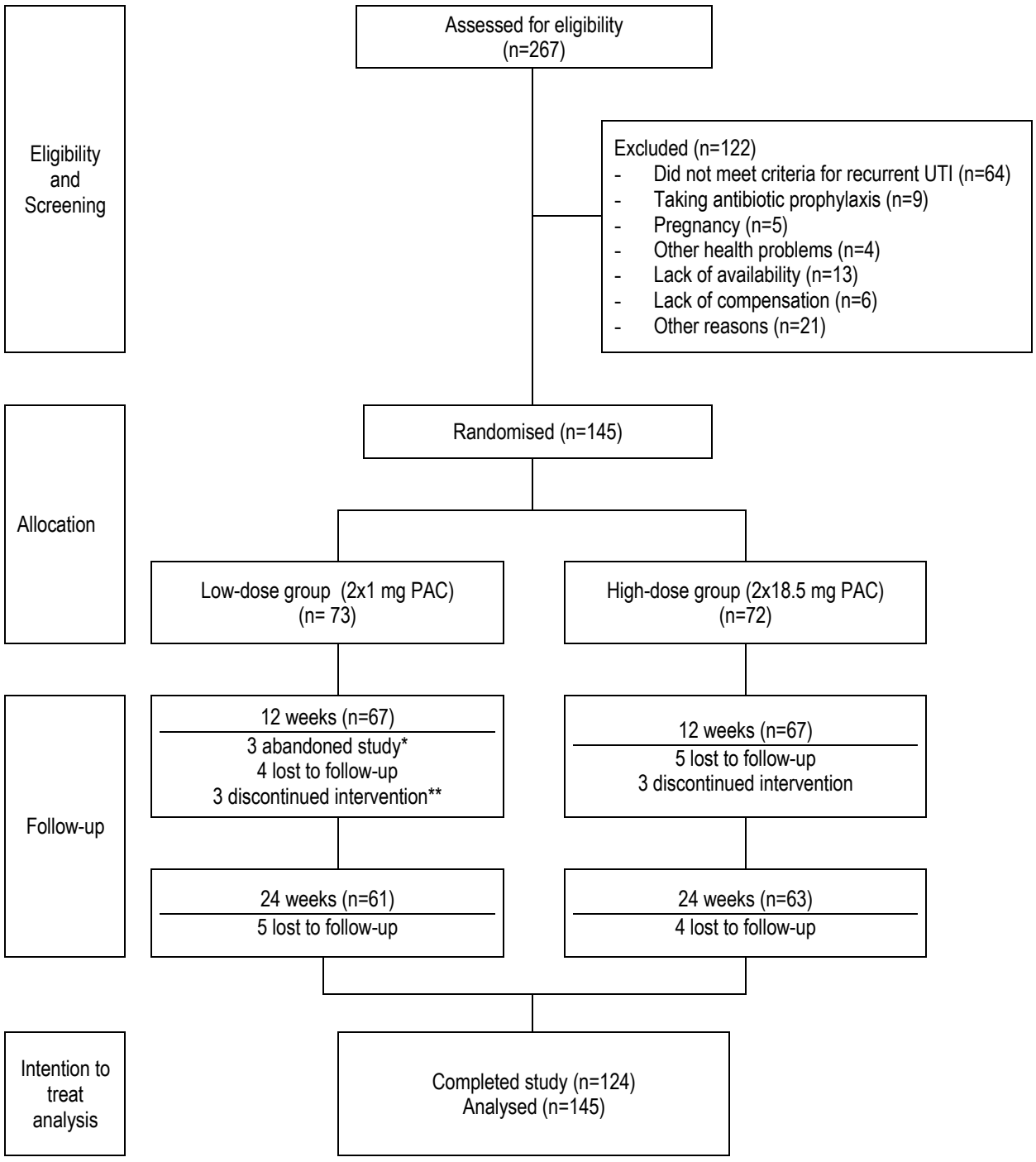
15. Mogollon JA, Bujold E, Lemieux S, et al. Blood pressure and endothelial function in healthy, pregnant women after acute and daily consumption of flavanol-rich chocolate: a pilot, randomized controlled trial. Nutr J. 2013;12:41.
16. Fu Z, Liska D, Talan D, Chung M. Cranberry Reduces the Risk of Urinary Tract Infection Recurrence in Otherwise Healthy Women: A Systematic Review and Meta-Analysis. J Nutr. 2017;147(12):2282-2288.
17. Epp A, Larochelle A, Lovatsis D, et al. Recurrent urinary tract infection. J Obstet Gynaecol Can. 2010;32(11):1082-1101.
18. Maki KC, Kaspar KL, Khoo C, Derrig LH, Schild AL, Gupta K. Consumption of a cranberry juice beverage lowered the number of clinical urinary tract infection episodes in women with a recent history of urinary tract infection. The American journal of clinical nutrition. 2016;103(6):1434-1442.
19. Barbosa-Cesnik C, Brown MB, Buxton M, Zhang L, Debusscher J, Foxman B. Cranberry juice fails to prevent recurrent urinary tract infection: Results from a randomized placebo-controlled trial. Clinical Infectious Diseases. 2011;52(1):23-30.
20. Stothers L. A randomized trial to evaluate effectiveness and cost effectiveness of naturopathic cranberry products as prophylaxis against urinary tract infection in women (Structured abstract). Canadian Journal of Urology. 2002;9(3):1558-1562. http://onlinelibrary.wiley.com/o/cochrane/cleed/articles/NHSEED-22003006156/frame.html.
21. Foxman B, Barlow R, D'Arcy H, Gillespie B, Sobel JD. Urinary tract infection: self-reported incidence and associated costs. Ann Epidemiol. 2000;10(8):509-515.
22. Foxman B. The epidemiology of urinary tract infection. Nat Rev Urol. 2010;7(12):653-660.
23. Ejrnaes K, Stegger M, Reisner A, et al. Characteristics of Escherichia coli causing persistence or relapse of urinary tract infections: phylogenetic groups, virulence factors and biofilm formation. Virulence. 2011;2(6):528-537.
24. Schreiber HLt, Conover MS, Chou WC, et al. Bacterial virulence phenotypes of Escherichia coli and host susceptibility determine risk for urinary tract infections. Science translational medicine. 2017;9(382).
25. Lewis AJ, Richards AC, Mulvey MA. Invasion of Host Cells and Tissues by Uropathogenic Bacteria. Microbiol Spectr. 2016;4(6).
26. Mena P, González de Llano D, Brindani N, et al. 5-(3',4'-Dihydroxyphenyl)-γ-valerolactone and its sulphate conjugates, representative circulating metabolites of flavan-3-ols, exhibit anti-adhesive activity against uropathogenic Escherichia coli in bladder epithelial cell. Journal of Functional Foods. 2017;29:275–280.
27. Vostalova J, Vidlar A, Simanek V, et al. Are High Proanthocyanidins Key to Cranberry Efficacy in the Prevention of Recurrent Urinary Tract Infection? Phytother Res. 2015;29(10):1559-1567.

Table 1. Admissibility Criteria

Inclusion Criteria
Sexually active healthy women
Aged 18 years and older
Recent history of recurrent urinary tract infections*
≥ 2 UTIs in the past 6 months and/or
≥ 3 UTIs in the past 12 months
No consumption of cranberry juice, polyphenol or antioxidant supplements in the last 2 weeks
Exclusion Criteria
Pregnancy
History of anatomical urogenital anomalies, urogenital tract surgery
History of acute or chronic renal failure, nephrolithiasis
History of intestinal diseases causing malabsorption
Anticoagulant medication in the last month
Known allergy or intolerance to cranberry

*UTIs diagnosed by a clinician and treated with antibiotic therapy

Figure 1. Participant Flow Diagram



*Women who abandoned the study provided a date and specific reason for their cessation of participation in the trial
**Women who ceased the intake of cranberry extract capsules, but presented themselves at study visits

Table 2. Baseline demographic characteristics of participants by study arm

Demographic characteristics	Low-dose group, 2x1mg PAC (n=73)	High-dose group, 2x18.5mg PAC (n=72)	P*
Age (mean±SD)** ***	32.5 ± 14.2	27.2 ± 8.8	0.009
Age subgroup, y, n (%)			0.028
18-24 years	30 (41.1)	37 (51.4)	
25-44 years	29 (39.7)	31 (43.1)	
>45 years	14 (19.2)	4 (5.6)	
Age subgroup, y, n (%)			0.003
18-51 years	62 (84.9)	71 (98.6)	
>51 years	11 (15.1)	1 (1.4)	
Ethnic origin			0.681
Caucasian	65 (89.0)	65 (90.3)	
Non-Caucasian	6 (8.2)	5 (6.9)	
Biracial	2 (2.7)	2 (2.8)	
Marital Status			0.230
Single	25 (34.3)	26 (36.1)	
Common law	33 (45.2)	39 (54.2)	
Married	13 (17.8)	7 (9.7)	
Divorced	2 (2.7)	0 (0)	
Has Children	21 (28.7)	12 (16.7)	0.082
Living environment			0.998
Urban	50 (68.5)	49 (68.1)	
Suburban	18 (24.7)	18 (25)	
Rural	5 (6.9)	5 (6.9)	
Education			0.814
University	39 (53.4)	42 (58.3)	
College	28 (38.4)	24 (33.3)	
Secondary school	6 (8.2)	6 (8.3)	

* Comparability of numerical and categorical baseline characteristics between groups was assessed with a student T test and ANOVA and chi-squared tests, respectively

** Numbers represent frequency (%) unless otherwise indicated

*** Significant difference between groups (Student t-test, p=0.009)

Table 3. Baseline Clinical Characteristics of participants by study arm

Clinical characteristics	Low-dose group, 2x1mg PAC (n=73)	High-dose group, 2x18.5mg PAC (n=72)	P*
Number of episodes of UTI in the past 6 months**			0.816
1	13 (17.8)	8 (11.1)	
2	34 (46.6)	37 (51.4)	
3	17 (23.3)	16 (22.2)	
4	6 (8.22)	7 (9.7)	
≥5	3 (4.11)	4 (5.6)	
Number of episodes of UTI in the past 12 months			0.249
2	13 (17.8)	7 (9.72)	
3	22 (30.1)	29 (40.3)	
4	11 (15.1)	15 (20.8)	
≥5	27 (37.0)	21 (26.17)	
Frequency of sexual intercourse (per week)			0.304
≤ 1	22 (30.1)	14 (19.4)	
2-4	34 (46.6)	41 (56.9)	
5-6	10 (13.7)	13 (18.1)	
≥ 7	7 (9.6)	4 (5.6)	
Number of sexual partners			0.256
0	5 (6.9)	1 (1.4)	
1	67 (91.8)	70 (97.2)	
2	1 (1.4)	1 (1.4)	
Stability in sexual relations			0.987
No stability	3 (4.1)	2 (1.4)	
Stable in the past month	7 (9.6)	6 (8.3)	
Stable in the past 6 months	16 (21.9)	15 (20.8)	
Stable in the past year	46 (63.0)	48 (66.7)	
Prefer not to respond	1 (1.4)	1 (1.4)	
New partner in last month	4 (5.5)	5 (6.9)	0.715
Type of Contraception			0.373
Hormonal contraception	43 (58.9)	53 (73.6)	
Spermicide	1(1.4)	0	
Non hormonal IUD	7 (9.6)	4 (5.6)	
Condom	17 (23.3)	11 (15.3)	
None	5 (6.8)	4 (5.6)	
First UTI before age 15	15 (20.6)	16 (22.2)	0.839
Maternal history of UTI	38 (52.1)	43 (59.7)	0.352
Personal history of recurrent UTI***			0.775
< 1 year	4 (8.2)	2 (4.3)	
1-2 years	9 (18.4)	12 (25.5)	
3-5 years	15 (30.6)	15 (31.9)	
6-10 years	14 (28.6)	10 (21.3)	
>10 years	7 (14.3)	8 (17.0)	
Missing data	24	25	
Hydration (average litres of water per day)***			0.147
Mean ± SD	1.62±0.75	1.81±0.63	
<1	9 (15.8)	4 (7.0)	
≥1-2	30 (52.6)	27 (47.4)	
≥2-3	13 (22.8)	23 (40.4)	
≥3	5 (8.8)	3 (7.0)	
Missing data	16	15	

Alcohol consumption (per week)***			0.140
<1	7 (12.7)	15 (25.9)	
1-3	28 (50.9)	21 (36.2)	
>4	20 (36.4)	22 (37.9)	
Missing data	18	14	
Tobacco use***			0.448
Smoker	5 (8.8)	3 (5.2)	
Non-smoker	52 (91.2)	55 (94.8)	
Missing data	16	14	

* Comparability of numerical and categorical baseline characteristics between groups was assessed with a Student T test and ANOVA and chi-squared tests, respectively

** Numbers represent frequency (%) unless otherwise indicated

*** Factors questioned during the follow-up period with missing data indicated

Table 4. Incidence of Symptomatic UTI at 24 Weeks by Study Arm

	Control group, 2x1mg PAC (n=73)	Treatment group, 2x18.5mg PAC (n=72)	Incidence rate ratio (95% CI)	p-value
Subjects reporting symptomatic UTI, episodes, n (%)				
0	34 (46.6)	41 (57.0)		
1	26 (35.6)	21 (29.2)		
2	8 (11.0)	7 (9.7)		
3	4 (5.5)	2 (2.8)		
4	0 (0.0)	1 (1.4)		
5	1 (1.4)	0 (0.0)		
≥1	39 (53.4)	31 (43.1)		
Total symptomatic UTIs, episodes	59	45		
Follow-up, days (mean±SD)	151±43	154±39		
Total person-days	10997	11088		
UTI, annualized incidence density (95% CI)	1.96 (1.52-2.53)	1.48 (1.11-1.99)	0.76 (0.51-1.11)	0.16
UTI, age-adjusted annualized incidence density (95% CI)	2.28 (1.74-2.98)	1.93 (1.36-2.72)	0.85 (0.57-1.26)	0.42

* UTI = urinary tract infection

** Incidence rate ratios and P values for the number of UTIs per woman-year of observation were determined from the generalized linear model with the Poisson distribution specified

Table 5. Univariate Poisson Regression Analysis of Total Number of Symptomatic UTIs and Baseline Risk Factors

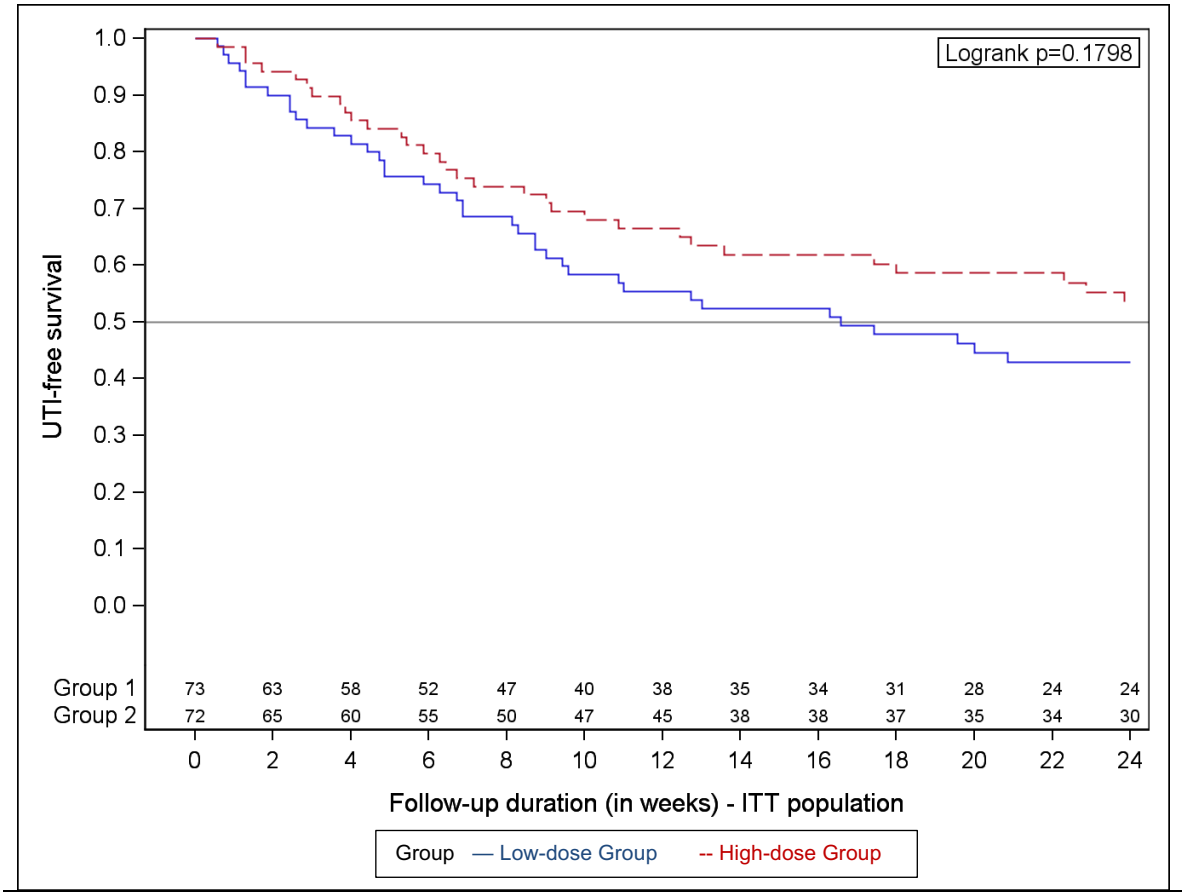
Variable	p-value Incidence rate ratio (95%CI)
Age (ref.= 18-45 years) 45 years and older	p= 0.01 1.97 (1.25- 3.11)
Number infections past 12 months (ref.<5) ≥5	p=0.02 1.62 (1.1- 2.39)
Frequency of sexual intercourse (per week) (ref.<1) 2-4 ≥5-6	p= 0.12 0.63 (0.4- 0.97) 0.78 (0.46- 1.32)
Hormonal contraception (ref.=No) Yes	p= 0.84 0.96 (0.65- 1.41)
Hydration (average number of litres of liquid per day) (ref.<1 L/day) 1-2L/day ≥2 L/day	p= 0.19 0.57 (0.32- 1.02) 0.66 (0.36- 1.18)

Table 6. Poisson Regression Sensitivity Analysis of Symptomatic UTIs by Number of UTI Prior to Enrolment

	Low-dose group, 2x1mg PAC	High-dose group, 2x18.5mg PAC	Incidence rate ratio (95% CI) p-value
Participants with less than 5 UTIs in the 12 months preceding enrolment	n=46	n=51	
Number of Symptomatic UTI	39	21	
Symptomatic UTI, Age-Adjusted Incidence Density (95% CI)*	2.29 (1.66- 3.16)	1.32 (0.81-2.13)	0.57 (0.33- 0.99) p=0.048
Participants with 5 UTIs or more in the 12 months preceding enrolment	n=27	n=21	
Number of Symptomatic UTI	20	24	
Symptomatic UTI, Age -Adjusted Incidence Density (95% CI)*	2.23 (1.37-3.61)	3.34 (1.96-5.68)	1.50 (0.81- 2.76) p= 0.195

* Incidence rate ratios and p-value for symptomatic UTIs per woman-year of observation by number of UTI in the 12 months prior to enrolment (less than 5 UTIs vs greater than 5 UTIs) and adjusted for age group (<45 years vs ≥45 year)

Figure 2. Kaplan Meier Analysis of Survival to First UTI by Treatment Group



Time to first symptomatic UTI (week 24 survival time) in subjects receiving 2x1mg PAC (Low-dose) or 2x18.5 mg PAC (High-dose). Participants who were lost-to follow-up before 12-week visit and did not report a UTI contributed the day of their baseline visit to the analysis. The HR for the difference between groups in the number of participants who had experienced a first symptomatic UTI by the end of the 24-week trial period was 0.73, 95% CI 0.45-1.16, logrank test, p=0.18.

Table 7. Incidence of Symptomatic UTI with Pyuria at 24 Weeks by Study Arm

	Low-dose group, 2x1mg PAC	High-dose group, 2x18.5mg PAC	Incidence rate ratio (95% CI) p-value
Symptomatic UTI with pyuria, episodes, n (%)*	n=73	n=72	
0	50 (68.5)	53 (73.6)	
1	17 (23.3)	14 (19.4)	
2	5 (6.8)	4 (5.6)	
3	0 (0.0)	0 (0.0)	
4	1 (1.4))	1 (1.4)	
≥1	23 (31.5)	19 (26.4)	
Total symptomatic UTIs with pyuria, episodes	31	26	
Total person-days	10997	11088	
Symptomatic UTI with pyuria, annualized incidence density (95% CI)	1.03 (0.72-1.46)	0.86 (0.58-1.26)	0.83 (0.49-1.40) P=0.49
Symptomatic UTI with pyuria, Age-adjusted annualized incidence density (95% CI)	1.24 (0.86-1.77)	1.21 (0.77-1.89)	0.98 (0.57-1.68) P=0.94
Subgroup analyses In women with < 5 UTI in past 12 months	n=46	n=51	
Symptomatic UTI with pyuria, annualized incidence density (95% CI)	1.05 (0.60-1.50)	0.55 (0.24-0.87)	0.53 (0.26-1.07) P=0.08
Symptomatic UTI with pyuria, age-adjusted annualized incidence density (95% CI)	1.27 (0.82-1.95)	0.83 (0.44-1.54)	0.65 (0.31-1.37) P=0.26
Symptomatic UTI with pyuria, episodes, n (%)**	n=73	n=72	
0	39 (53.4)	45 (62.5)	
1	23 (31.5)	18 (25.0)	
2	6 (8.2)	7 (9.7)	
3	4 (5.5)	1 (1.4)	
4	0 (0.0)	1 (1.4)	
5	1 (1.4)	0 (0.0)	
≥1	34 (46.6)	27 (37.5)	
Total symptomatic UTIs with pyuria, episodes	52	39	
Total person-days	10997	11088	
Symptomatic UTI with pyuria, annualized incidence density (95% CI)	1.73 (1.32-2.27)	1.28 (0.94-1.72)	0.74 (0.49- 1.13) p= 0.16
Symptomatic UTI with pyuria, age-adjusted annualized incidence density (95% CI)	2.05 (1.54-2.71)	1.75 (1.22-2.52)	0.86 (0.56-1.32) p=0.48
Subgroup analyses In women with < 5 UTI in past 12 months	n=46	n=51	
Symptomatic UTI with pyuria, age-adjusted annualized incidence density (95% CI)	1.75 (1.26-2.20)	0.78 (0.41-1.16)	0.45 (0.25-0.80) p=0.01
Symptomatic UTI with pyuria, age-adjusted annualized incidence density (95% CI)	2.1 (1.50-2.94)	1.14 (0.68-1.92)	0.54 (0.30-0.99) p=0.047

*Symptomatic UTI episodes without urine sample were considered as no symptomatic UTI with pyuria
**Symptomatic UTI episodes without urine sample were considered as symptomatic UTI with pyuria

Table 8. Incidence of Symptomatic UTI with Bacteriuria at 24 Weeks by Study Arm

	Low-dose group, 2x1mg PAC	High-dose group, 2x18.5mg PAC	Incidence rate ratio (95% CI) p-value
Symptomatic UTI with bacteriuria, episodes, n (%)*	n=73	n=72	
0	55 (75.3)	63 (87.5)	
1	17 (23.3)	7 (9.7)	
2	1 (1.4)	2 (2.8)	
≥1	18 (24.7)	9 (12.5)	
Total symptomatic UTIs with bacteriuria, episodes	19	11	
Total person-days	10997	11088	
Symptomatic UTI with bacteriuria, annualized incidence density (95% CI)	0.63 (0.40-0.99)	0.36 (0.20-065)	0.57 (0.27-1.21) p=0.14
Symptomatic UTI with bacteriuria, age-adjusted annualized incidence density (95% CI)	0.76 (0.48-1.21)	0.53 (0.27-1.02)	0.69 (0.32-1.49) p=0.34
Subgroup analyses In women with < 5 UTI in past 12 months	n=46	n=51	
Symptomatic UTI with bacteriuria, annualized incidence density (95% CI)	0.70 (0.42-1.18)	0.23 (0.10-0.55)	0.33 (0.12-0.91) p=0.03
Symptomatic UTI with bacteriuria, age-adjusted annualized incidence density (95% CI)	0.85 (0.50-1.43)	0.35 (0.14-0.89)	0.41 (0.14-1.19) P=0.10
Subjects reporting symptomatic UTI with bacteriuria, episodes, n %)**	n=73	n=72	
0	42 (57.5)	51 (70.8)	
1	22 (30.1)	15 (20.8)	
2	6 (8.2)	4 (5.6)	
3	3 (4.1)	1 (1.4)	
4	0 (0.0)	1 (1.4)	
≥1	31 (42.5)	21 (29.2)	
Total symptomatic UTIs with bacteriuria, episodes	43	30	
Total person-days	10997	11088	
Symptomatic UTI with bacteriuria, annualized incidence density (95% CI)	1.43 (1.06-1.93)	0.99 (0.69-1.41)	0.69 (0.43-1.10) p=0.12
Symptomatic UTI with bacteriuria, age-adjusted annualized incidence density (95% CI)	1.71 (1.26-2.33)	1.40 [0.93-2.10)	0.81 (0.50-1.32) p=0.40
Subgroup analyses In women with < 5 UTI in past 12 months	n=46	n=51	
Symptomatic UTI with bacteriuria, annualized incidence density (95% CI)	1.40 (0.97-2.03)	0.60 (0.35-1.03)	0.43 (0.22-0.83) p=0.01
Symptomatic UTI with bacteriuria, age-adjusted annualized incidence density (95% CI)	1.70 (1.17-2.46)	0.93 (0.52-1.66)	0.55 (0.28-1.08) p=0.08

*Symptomatic UTI episodes without urine sample were considered as no symptomatic UTI with uropathogenic bacteriuria > 10^3 CFU/ml
**Symptomatic UTI episodes without urine sample were considered as symptomatic UTI with uropathogenic bacteriuria > 10^3 CFU/ml

Appendix A – Cranberry Extract Composition in Polyphenols		
Compound (mg/capsule)	Low-dose group	High-dose group
PAC[1]	1.00	18.50
PAC oligomers[2]	0.35	4.15
PAC polymers[2]	0.55	6.50
Phenolic acids[3]	0.28	2.30
Anthocyanidins[3]	0.11	0.55
Flavonols[3]	0.32	1.65

[1] BL-DMAC equivalent A2
[2] HPLC normal phase fluorescence
[3] HPLC inverse phase

Appendix B – Reasons for abandoning study	
Reason	**Low-dose group (n=12)**
Moved out of province	1
Too difficult to adhere to capsule intake	1
Family disapproval of study participation	1
No explanation given	9
Reason	**High-dose group (n=9)**
Too difficult to adhere to capsule intake	3
Health problems not related to capsule intake	2
No explanation given	**4**

Appendix C – Reasons for discontinuing intervention	
Reason	**Low-dose group (n=3)**
Too difficult to adhere to capsule intake	1
Dyspepsia	1
No reason	1
Reason	**High-dose group (n=3)**
No reason	2
Dyspepsia	**1**

Perception	**Control group (n=73)**	**Intervention group (n=72)**
Intervention dose	21 (29)	17 (24)
Control dose	15 (21)	11 (15)
Don't know	22 (30)	32 (44)
Missing data	15 (21)	12 (17)

Numbers represent frequency (%)

Chapter 4 – Additional Results

Recruitment

Between August 2015 and April 2017, 375 potential participants contacted the study coordinator with an interest in study participation. A majority of these participants were solicited through email list serves (Table 1). The study coordinator contacted and assessed 267 women for eligibility, of which 122 were excluded (Table 2). The main reasons for exclusion were: (1) did not meet criteria for recurrent UTI (n=64); (2) not interested in discontinuing antibiotic prophylaxis (n=9); pregnant (n=5); lack of availability (n=13); other health problems (n=4), other reasons (n=27). Amongst the 267 women assessed for eligibility, 145 were randomised.

Table 1. Recruitment Strategies for Potential Participants

Strategy	Potential participants (n=230)	Randomized participants (n=145)
Email list serve		
Laval university	163 (70.9)	104 (71.7)
INAF list	22 (9.6)	18 (12.4)
Clinician referrals	7 (3.0)	9 (6.2)
Posters	2 (0.9)	2 (1.4)
Social media	3 (1.3)	2 (1.4)
Paid advertising	2 (0.9)	2 (1.4)
Word of mouth	4 (1.7)	8 (5.5)
Other	27 (11.7)	0

*values are presented as numbers (%)
INAF = Institute on Nutrition and Functional Foods

Table 2. Reasons for Exclusion of Potential Participants

Reason	Excluded potential participants (n=122)
Did not meet criteria for recurrent UTI	64 (52.4)
Taking antibiotic prophylaxis	9 (7.3)
Pregnancy	5 (4.1)
Other health problems	4 (3.3)
Lack of availability	13 (10.7)
Lack of compensation	6 (4.9)
Other reasons	21 (17.2)

*values are presented as numbers (%)

Compliance

Participant adherence to capsule intake as measured by number of capsules returned to the study coordinator at each visit and as noted in the daily intake journal are illustrated in Table 3. Women in both groups returned their bottles at both the 12-week and 24-week visits, excluding those who discontinued the intervention and those who abandoned or were lost to follow up. Compliance based on number of returned capsules at 24 weeks was similar in both groups (92.7% in the control group vs 92.9% in the intervention group, Student t-test p=0.9). At the end of the study, 49 women in group 1 returned their daily intake diary compared to 59 women in group 2. Compliance according to daily intake journals was comparable in both groups, 88.8% in the control group and 87.3% in the treatment group (Student t-test, p=0.6).

Table 3. Compliance

Measures of compliance	Control group	Intervention group
Number of capsules returned at 24 weeks	n=60	n=62
	92.7%	92.9%
Daily intake journal	n=49	n=59
	88.79%	87.33%

Adherence To Double-Blind Procedures

After 24 weeks of follow-up, participants were asked if they were aware of which treatment group they were assigned to in order to validate the effectiveness of blinding procedures. The majority of women in both groups responded that they were unaware of group allocation as shown in Table 4.

Table 4. End of Study Characteristics

Perception	Control group (n=73)	Intervention group (n=72)
Optimal dose	21	17
Control dose	15	11
Don't know	22	32
Missing data	15	12

Acceptability

Patient acceptability, perception of efficacy and satisfaction with participation as documented at the 24-week visit is shown in Table 5. A majority of patients in both groups thought that a twice daily intake of cranberry extract capsules was acceptable (88% in the control group, 87% in the intervention group). Likewise, a majority of participants in both group believed the capsules to be effective in reducing their incidence of UTI.

Table 5. Patient Acceptability at End of Study

	Control group (n=58)	Intervention group (n=60)
Acceptability		
Yes	51	52
No	7	8
Efficacity		
Yes	47	47
No	9	12
Satisfaction with participation in study	58	60
Perception of UTI symptoms compared to prior to study	N=59	N=64
No infection during study	29	31
Less than before intervention	9	14
Similar	20	17
Worse than before	0	1
Don't know	1	1

Chapter 5 – General Discussion

The objective of this study was to investigate the effects of cranberry PACs on the prevention of recurrent UTI in healthy women. High dose twice daily PACs extract was associated with a non-significant yet clinically important 24% reduction in the number of symptomatic UTIs when compared to a low dose proanthocyanidins extract. Our post hoc analysis suggest that the consumption of a high dose of cranberry extract capsules containing 2x18.5 mg PACs may have a beneficial effect on the recurrence of symptomatic UTI in women with 2-4 UTI per year. High rates of compliance to the intervention and few serious adverse events in the study indicate that cranberry extracts in the form of a capsule are well tolerated.

The choice of 37 mg PACs as an optimal dose was based on ex-vivo study 115 indicating that daily doses lower than 36 mg PACs per day, did not inhibit bacterial adhesion to uroepithelial cells. The choice of a split dose regimen was based on an ex-vivo study that indicated a time-dependent effect with a maximum bacterial inhibitory effect in urine at 1-6 hours compared to 24 hours proceeding PAC consumption 9. It is possible that the split dose regimen in our study did not attain sufficient concentrations in the urological tracts to exhibit an anti-adhesion effect. Moreover, the control dose of 2 mg PACs was chosen based on the Canadian context where at the time of writing the protocol of this study, only 2 mg PACs were approved for use by Health Canada 116. Still, a trial conducted by Vostolova et al. found that daily intake of 500 mg of whole cranberry fruit powder capsule containing 2.8 mg PACs, as measured by BL-DMAC method with procyanidin A2 as a standard, compared to placebo reduced the incidence of symptomatic UTI with bacteriuria > 10^5 CFU/ml in women with two or more UTI episodes in the 12 months preceding randomization 103. A significant reduction in the number of women with at least 1 UTI episode was found in the group receiving 2.8 mg PACs compared to placebo (RR 0.58, p=0.04). These results indicate that components other than PACs in the cranberry extract powder used as a control dose in our study may have influenced the risk of UTI in participating women. The comparison of an optimal dose containing 2x18.5 mg PACs and a control dose of 2x1 mg PACs per day did not allow us to evaluate the true effect of cranberry intake on UTI recurrence as cranberry components other than PACs were comparable in the treatment and control capsules.

The favorable effect of high dose PAC among only women with a low annual UTI burden could be explained though several mechanisms. It is possible that women with greater than 5 UTI per year present certain functional abnormalities such as interstitial cystitis or voiding dysfunctions. An open-label pilot study performed in women with a mean of 5.5 episodes of symptomatic UTI yearly found that 16-week treatment with pentosan polysulfate sodium significantly reduced the recurrence of UTI compared to no treatment (0% vs 64.3%, $p<0.001$ with Student's t-test)[117]. As pentosane polysufate sodium restores the integrity of the glycoaminoglycan layer in the bladder, it is possible that women with a higher yearly UTI burden presented greater damage to the urothelium and were more susceptible to bacterial colonization. Through video-urodynamic studies, a recent study found storage and voiding dysfunctions in up to 90% of a sample of 100 women with a history of recurrent UTI [118]. It is possible that women with a higher yearly UTI burden present larger post-voiding residual volumes which may interfere with the anti-adhesive properties of PACs.

Studies investigating the PACs metabolites in urine have shown high inter-individual variability raising the probability of certain high responders vs low-responders to PAC treatment [112]. It has been proposed that the anti-adhesive properties in urine after PACs consumption may not be attributable to the direct effects of PACs excreted in urine but rather the action of PACs metabolites following intestinal modification by gut bacteria. A small study in healthy men and women without history of UTI indicated that the daily intake of sweetened dried cranberries containing 26 mg PACs results in a modification of intestinal bacteria and decrease in bacteria with negative health effects [113]. However, an observational study of 20 women with recurrent UTI who consumed 42g of sweetened cranberries daily reported no difference in the heterogeneity of E.coli strains obtained from rectal swabs before and after the intervention[119]. An alternate mechanism of action for anti-adhesion activity is an interaction between cranberry PACs and lymphoid tissue in the gastrointestinal tract. Researchers have shown that PACs are recognized and undergo active endocytosis by dendritic cells [93], which may trigger an innate immune response in the human body, including the urinary tract [94]. Our study collected stool samples from certain women in order to better characterize PAC metabolites based on metabolomic differences between participants. These results will be published in a subsequent paper.

Future Research

Currently, several protocols of studies investigating the role of cranberries for the prevention of recurrent UTI are registered on clinical trials.gov. Notably, Stonehouse et al. is currently investigating the use of 500 mg Pacran®, similar to the trial by Vostalova et al.ref containing 2.8 mg PAC as measured by BL-DMAC, on the prevention of UTI in women with a history of recurrent UTI. The study features a 6-month follow-up period and a large sample size of 300 participants. Interestingly, patients that present more than five UTIs in the past 6 months will be excluded from this study. The authors do not justify this decision in the available study protocol. However, it is characterized by some of the limitations found in previous trials, such as the primary outcome being culture confirmed UTI ≥ 10^5 CFU/ml and a low dose of PACs administered once daily. Tzortzis et al.ref will recruit 160 women with recurrent UTI to evaluate the impact of oral intake of 118 mg PAC daily versus placebo on the number of UTI in a 12-month period. Based on available information, it is unclear if the trial will evaluate symptomatic UTI or culture confirmed UTI. Of note, rectal and vaginal swabs will be obtained in order to measure the effect of cranberry capsules on endogenous flora.

Clinical Perspectives

Although the comparison of a high dose versus low dose of twice daily intake of cranberry PAC does not allow us to evaluate the role of cranberry capsule intake on the prevention of recurrent UTI, it is important to note that among the women who experienced UTI during the study and responded to our post-hoc questionnaire, 33% perceived that their UTI symptoms were less than before their enrolment in the trial. In vitro studies have shown that cranberry extracts may have anti-inflammatory effects on the catalytic activity of COX-2 and other inflammatory markers 120 which may be responsible for the decreased perception of symptoms.

UTI disproportionately affects the quality of life of otherwise healthy women and constitutes a principal indication for antibiotic use. Current clinical guidelines recommend the use of prophylactic antibiotic treatments as the only means of preventing recurrent UTI, which clearly contributes to the emergence of antibiotic resistant bacteria 121. Many participants in our trial were prescribed antibiotics to be taken when presenting symptomatic UTI, without the need of a leucocyte esterase test or urine culture.

Amongst the urine samples collected during symptomatic UTI, 10% of urine samples did not exhibit pyuria or bacteriuria. This indicates that in women with recurrent UTI, there is a non-negligible risk of using antibiotic regimens when in fact women may not actually have an infection. Recently published joint guidelines from the American and Canadian Urological Associations [121] address this issue through their suggestion to obtain microbiological confirmation for each UTI episode prior to the instauration of long-term antibiotic treatment. Clinicians are also advised to obtain urine culture for each recurrent UTI episode in order to characterize the pathogen and provide an antibiogram even if the patient is on a self-start antibiotic regimen.

Conclusion

The results of this double-blind RCT indicate that twice daily intake of cranberry capsules containing 2x18.5 mg PACs per day during a 24-week period was not associated with a reduction in the incidence of recurrent UTIs when compared to the daily intake of 2x1 mg PACs per day. Post hoc sub-group analyses showed that in participating women with a history of less than 5 UTIs per year, the twice daily consumption of 2x18.5 mg PACs resulted in a 43% decrease in the rate of symptomatic UTI during the trial period compared to 2x1 mg PAC. These results indicate that select women with recurrent UTI may benefit from a preventative treatment with higher doses of cranberry PAC.

References

1. Asma B, Vicky L, Stephanie D, Yves D, Amy H, Sylvie D. Standardised high dose versus low dose cranberry Proanthocyanidin extracts for the prevention of recurrent urinary tract infection in healthy women [PACCANN]: a double blind randomised controlled trial protocol. BMC Urology 2018;18:29.

2. Czaja CA, Hooton TM. Update on acute uncomplicated urinary tract infection in women. Postgraduate medicine 2006;119:39-45.

3. Little P, Merriman R, Turner S, et al. Presentation, pattern, and natural course of severe symptoms, and role of antibiotics and antibiotic resistance among patients presenting with suspected uncomplicated urinary tract infection in primary care: observational study. BMJ 2010;340:b5633.

4. Foxman B, Frerichs RR. Epidemiology of urinary tract infection: I. Diaphragm use and sexual intercourse. Am J Public Health 1985;75:1308-13.

5. Strom BL, Collins M, West SL, Kreisberg J, Weller S. Sexual activity, contraceptive use, and other risk factors for symptomatic and asymptomatic bacteriuria. A case-control study. Annals of internal medicine 1987;107:816-23.

6. Giesen LG, Cousins G, Dimitrov BD, van de Laar FA, Fahey T. Predicting acute uncomplicated urinary tract infection in women: a systematic review of the diagnostic accuracy of symptoms and signs. BMC family practice 2010;11:78.

7. Harris PE, Cooper KL, Relton C, Thomas KJ. Prevalence of complementary and alternative medicine (CAM) use by the general population: a systematic review and update. International journal of clinical practice 2012;66:924-39.

8. Gupta K, Chou MY, Howell A, Wobbe C, Grady R, Stapleton AE. Cranberry products inhibit adherence of p-fimbriated Escherichia coli to primary cultured bladder and vaginal epithelial cells. The Journal of urology 2007;177:2357-60.

9. Howell AB, Botto H, Combescure C, et al. Dosage effect on uropathogenic Escherichia coli anti-adhesion activity in urine following consumption of cranberry powder standardized for proanthocyanidin content: a multicentric randomized double blind study. BMC infectious diseases 2010;10:94.

10. Jepson RG, Williams G, Craig JC. Cranberries for preventing urinary tract infections. Cochrane Database Syst Rev 2012;10:CD001321.

11. Foxman B. The epidemiology of urinary tract infection. Nat Rev Urol 2010;7:653-60.

12. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 2015;13:269-84.

13. Epp A, Larochelle A, Lovatsis D, et al. Recurrent urinary tract infection. J Obstet Gynaecol Can 2010;32:1082-101.

14. Dason S, Dason JT, Kapoor A. Guidelines for the diagnosis and management of recurrent urinary tract infection in women. Can Urol Assoc J 2011;5:316-22.

15. Schmiemann G, Kniehl E, Gebhardt K, Matejczyk MM, Hummers-Pradier E. The diagnosis of urinary tract infection: a systematic review. Dtsch Arztebl Int 2010;107:361-7.

16. Nicolle LE. Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. Urol Clin North Am 2008;35:1-12, v.

17. McIsaac WJ, Low DE, Biringer A, Pimlott N, Evans M, Glazier R. The impact of empirical management of acute cystitis on unnecessary antibiotic use. Arch Intern Med 2002;162:600-5.

18. Nicolle LE. Asymptomatic bacteriuria--important or not? N Engl J Med 2000;343:1037-9.

19. McIsaac WJ, Moineddin R, Ross S. Validation of a decision aid to assist physicians in reducing unnecessary antibiotic drug use for acute cystitis. Arch Intern Med 2007;167:2201-6.

20. Grude N, Tveten Y, Jenkins A, Kristiansen BE. Uncomplicated urinary tract infections. Bacterial findings and efficacy of empirical antibacterial treatment. Scandinavian journal of primary health care 2005;23:115-9.

21. Verest LF, van Esch WM, van Ree JW, Stobberingh EE. Management of acute uncomplicated urinary tract infections in general practice in the south of The Netherlands. The British journal of general practice : the journal of the Royal College of General Practitioners 2000;50:309-10.

22. Johnson JD, O'Mara HM, Durtschi HF, Kopjar B. Do urine cultures for urinary tract infections decrease follow-up visits? J Am Board Fam Med 2011;24:647-55.

23. Komaroff AL, Pass TM, McCue JD, Cohen AB, Hendricks TM, Friedland G. Management strategies for urinary and vaginal infections. Arch Intern Med 1978;138:1069-73.

24. Bent S, Nallamothu BK, Simel DL, Fihn SD, Saint S. Does this woman have an acute uncomplicated urinary tract infection? Jama 2002;287:2701-10.

25. Gupta K, Hooton TM, Roberts PL, Stamm WE. Patient-initiated treatment of uncomplicated recurrent urinary tract infections in young women. Annals of internal medicine 2001;135:9-16.

26. McIsaac WJ, Prakash P, Ross S. The management of acute uncomplicated cystitis in adult women by family physicians in Canada. Can J Infect Dis Med Microbiol 2008;19:287-93.

27. Suskind AM, Saigal CS, Hanley JM, et al. Incidence and Management of Uncomplicated Recurrent Urinary Tract Infections in a National Sample of Women in the United States. Urology 2016;90:50-5.

28. Keren R, Shaikh N, Pohl H, et al. Risk Factors for Recurrent Urinary Tract Infection and Renal Scarring. Pediatrics 2015;136:e13-21.

29. Foxman B, Barlow R, D'Arcy H, Gillespie B, Sobel JD. Urinary tract infection: self-reported incidence and associated costs. Ann Epidemiol 2000;10:509-15.

30. May L, Mullins P, Pines J. Demographic and treatment patterns for infections in ambulatory settings in the United States, 2006-2010. Academic emergency medicine : official journal of the Society for Academic Emergency Medicine 2014;21:17-24.

31. Nicolle L, Anderson, P. A. M., Conly, J., Mainprize, T. C., Meuser, J., Nickel, J. C., … Zhanel, G. G. Uncomplicated urinary tract infection in women: Current practice and the effect of antibiotic resistance on empiric treatment. Canadian Family Physician 2006;52:612-8.

32. Hickling DR, Sun TT, Wu XR. Anatomy and Physiology of the Urinary Tract: Relation to Host Defense and Microbial Infection. Microbiol Spectr 2015;3.

33. Yamamoto S, Tsukamoto T, Terai A, Kurazono H, Takeda Y, Yoshida O. Genetic evidence supporting the fecal-perineal-urethral hypothesis in cystitis caused by Escherichia coli. The Journal of urology 1997;157:1127-9.

34. Hooton TM. Pathogenesis of urinary tract infections: an update. The Journal of antimicrobial chemotherapy 2000;46 Suppl A:1-7.

35. Foxman B. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infect Dis Clin North Am 2014;28:1-13.

36. Sobel JD. Pathogenesis of urinary tract infections. Host defenses. Infect Dis Clin North Am 1987;1:751-72.

37. Stapleton AE, Au-Yeung M, Hooton TM, et al. Randomized, placebo-controlled phase 2 trial of a Lactobacillus crispatus probiotic given intravaginally for prevention of recurrent urinary tract infection. Clin Infect Dis 2011;52:1212-7.

38. Hooton TM, Stamm WE. Diagnosis and treatment of uncomplicated urinary tract infection. Infect Dis Clin North Am 1997;11:551-81.

39. Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic Escherichia coli. Exp Mol Pathol 2008;85:11-9.

40. Hooton TM, Scholes D, Hughes JP, et al. A prospective study of risk factors for symptomatic urinary tract infection in young women. N Engl J Med 1996;335:468-74.

41. Vincent CR, Thomas TL, Reyes L, White CL, Canales BK, Brown MB. Symptoms and risk factors associated with first urinary tract infection in college age women: a prospective cohort study. The Journal of urology 2013;189:904-10.

42. Remis RS, Gurwith MJ, Gurwith D, Hargrett-Bean NT, Layde PM. Risk factors for urinary tract infection. Am J Epidemiol 1987;126:685-94.

43. Fihn SD, Latham RH, Roberts P, Running K, Stamm WE. Association between diaphragm use and urinary tract infection. Jama 1985;254:240-5.

44. Fihn SD, Boyko EJ, Normand EH, et al. Association between use of spermicide-coated condoms and Escherichia coli urinary tract infection in young women. Am J Epidemiol 1996;144:512-20.

45. Hooton TM, Fennell CL, Clark AM, Stamm WE. Nonoxynol-9: differential antibacterial activity and enhancement of bacterial adherence to vaginal epithelial cells. The Journal of infectious diseases 1991;164:1216-9.

46. Scholes D, Hooton TM, Roberts PL, Stapleton AE, Gupta K, Stamm WE. Risk factors for recurrent urinary tract infection in young women. The Journal of infectious diseases 2000;182:1177-82.

47. Scholes D, Hawn TR, Roberts PL, et al. Family history and risk of recurrent cystitis and pyelonephritis in women. The Journal of urology 2010;184:564-9.

48. Ziaei S, Ninavaei M, Faghihzadeh S. Urinary tract infection in the users of depot-medroxyprogesterone acetate. Acta obstetricia et gynecologica Scandinavica 2004;83:909-11.

49. Smith HS, Hughes JP, Hooton TM, et al. Antecedent antimicrobial use increases the risk of uncomplicated cystitis in young women. Clin Infect Dis 1997;25:63-8.

50. Manz F, Wentz A. The importance of good hydration for the prevention of chronic diseases. Nutr Rev 2005;63:S2-5.

51. Beetz R. Mild dehydration: a risk factor of urinary tract infection? Eur J Clin Nutr 2003;57 Suppl 2:S52-8.

52. Stauffer CM, van der Weg B, Donadini R, Ramelli GP, Marchand S, Bianchetti MG. Family history and behavioral abnormalities in girls with recurrent urinary tract infections: a controlled study. The Journal of urology 2004;171:1663-5.

53. Mazzola BL, von Vigier RO, Marchand S, Tonz M, Bianchetti MG. Behavioral and functional abnormalities linked with recurrent urinary tract infections in girls. Journal of nephrology 2003;16:133-8.

54. Hooton TM, Vecchio M, Iroz A, et al. Effect of Increased Daily Water Intake in Premenopausal Women With Recurrent Urinary Tract Infections: A Randomized Clinical Trial. JAMA Intern Med 2018;178:1509-15.

55. Hooton TM, Roberts PL, Cox ME, Stapleton AE. Voided midstream urine culture and acute cystitis in premenopausal women. N Engl J Med 2013;369:1883-91.

56. Aydin A, Ahmed K, Zaman I, Khan MS, Dasgupta P. Recurrent urinary tract infections in women. International urogynecology journal 2015;26:795-804.

57. Davis C, Rantell A. Lower urinary tract infections in women. Br J Nurs 2017;26:S12-S9.

58. Ferry SA, Holm SE, Stenlund H, Lundholm R, Monsen TJ. The natural course of uncomplicated lower urinary tract infection in women illustrated by a randomized placebo controlled study. Scand J Infect Dis 2004;36:296-301.

59. Christiaens TC, De Meyere M, Verschraegen G, Peersman W, Heytens S, De Maeseneer JM. Randomised controlled trial of nitrofurantoin versus placebo in the treatment of uncomplicated urinary tract infection in adult women. The British journal of general practice : the journal of the Royal College of General Practitioners 2002;52:729-34.

60. Knottnerus BJ, Geerlings SE, Moll van Charante EP, ter Riet G. Women with symptoms of uncomplicated urinary tract infection are often willing to delay antibiotic treatment: a prospective cohort study. BMC family practice 2013;14:71.

61. Brown P, Ki M, Foxman B. Acute pyelonephritis among adults: cost of illness and considerations for the economic evaluation of therapy. Pharmacoeconomics 2005;23:1123-42.

62. Colgan R, Keating K, Dougouih M. Survey of symptom burden in women with uncomplicated urinary tract infections. Clinical drug investigation 2004;24:55-60.

63. Ellis AK, Verma S. Quality of life in women with urinary tract infections: is benign disease a misnomer? J Am Board Fam Pract 2000;13:392-7.

64. Rosenberg M. Pharmacoeconomics of treating uncomplicated urinary tract infections. International journal of antimicrobial agents 1999;11:247-51; discussion 61-4.

65. Albert X, Huertas I, Pereiro, II, Sanfelix J, Gosalbes V, Perrota C. Antibiotics for preventing recurrent urinary tract infection in non-pregnant women. Cochrane Database Syst Rev 2004:CD001209.

66. Schaeffer AJ, Stuppy BA. Efficacy and safety of self-start therapy in women with recurrent urinary tract infections. The Journal of urology 1999;161:207-11.

67. Wong ES, McKevitt M, Running K, Counts GW, Turck M, Stamm WE. Management of recurrent urinary tract infections with patient-administered single-dose therapy. Annals of internal medicine 1985;102:302-7.

68. Eells SJ, Bharadwa K, McKinnell JA, Miller LG. Recurrent urinary tract infections among women: comparative effectiveness of 5 prevention and management strategies using a Markov chain Monte Carlo model. Clin Infect Dis 2014;58:147-60.

69. Franco AV. Recurrent urinary tract infections. Best Pract Res Clin Obstet Gynaecol 2005;19:861-73.

70. Reid G. Potential preventive strategies and therapies in urinary tract infection. World journal of urology 1999;17:359-63.

71. Delisle G, Quach C, Domingo MC, et al. Escherichia coli antimicrobial susceptibility profile and cumulative antibiogram to guide empirical treatment of uncomplicated urinary tract infections in women in the province of Quebec, 2010-15. The Journal of antimicrobial chemotherapy 2016.

72. Gupta K, Hooton TM, Naber KG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis 2011;52:e103-20.

73. McIsaac WJ, Moineddin R, Meaney C, Mazzulli T. Antibiotic-resistant Escherichia coli in women with acute cystitis in Canada. Can J Infect Dis Med Microbiol 2013;24:143-9.

74. Mehnert-Kay SA. Diagnosis and management of uncomplicated urinary tract infections. Am Fam Physician 2005;72:451-6.

75. INESSS. Urinary Tract Infections in Adults. In: Services NIoEiHaS, ed. Quebec2017.

76. Fasugba O, Gardner A, Mitchell BG, Mnatzaganian G. Ciprofloxacin resistance in community- and hospital-acquired Escherichia coli urinary tract infections: a systematic review and meta-analysis of observational studies. BMC infectious diseases 2015;15:545.

77. Karlowsky JA, Adam HJ, Desjardins M, et al. Changes in fluoroquinolone resistance over 5 years (CANWARD 2007-11) in bacterial pathogens isolated in Canadian hospitals. The Journal of antimicrobial chemotherapy 2013;68 Suppl 1:i39-46.

78. Gagyor I, Bleidorn J, Kochen MM, Schmiemann G, Wegscheider K, Hummers-Pradier E. Ibuprofen versus fosfomycin for uncomplicated urinary tract infection in women: randomised controlled trial. BMJ (Clinical research ed) 2015;351:h6544.

79. Alraek T, Soedal LI, Fagerheim SU, Digranes A, Baerheim A. Acupuncture treatment in the prevention of uncomplicated recurrent lower urinary tract infections in adult women. Am J Public Health 2002;92:1609-11.

80. Gupta K, Stapleton AE, Hooton TM, Roberts PL, Fennell CL, Stamm WE. Inverse association of H2O2-producing lactobacilli and vaginal Escherichia coli colonization in women with recurrent urinary tract infections. The Journal of infectious diseases 1998;178:446-50.

81. Beerepoot MA. Recurrent urinary tract infections. Antibiotic resistance and non-antibiotic prophylaxis. International journal of antimicrobial agents 2013;42:S38.

82. Cranberry Institute About Cranberries. 2017. (Accessed 8 June 2017, 2017, at http://cranberryinstitute.org/.)

83. Howell AB. Cranberry proanthocyanidins and the maintenance of urinary tract health. Crit Rev Food Sci Nutr 2002;42:273-8.

84. Kahn HD, Panariello VA, Saeli J, Sampson JR, Schwartz E. Effect of cranberry juice on urine. J Am Diet Assoc 1967;51:251-4.

85. Beachey EH. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surface. The Journal of infectious diseases 1981;143:325-45.

86. Johnson JR. Virulence factors in Escherichia coli urinary tract infection. Clin Microbiol Rev 1991;4:80-128.

87. Guay DRP. Cranberry and urinary tract infections. Drugs 2009;69:775-807.

88. Zafriri D, Ofek I, Adar R, Pocino M, Sharon N. Inhibitory activity of cranberry juice on adherence of type 1 and type P fimbriated Escherichia coli to eucaryotic cells. Antimicrobial agents and chemotherapy 1989;33:92-8.

89. Foo LY, Lu Y, Howell AB, Vorsa N. A-Type proanthocyanidin trimers from cranberry that inhibit adherence of uropathogenic P-fimbriated Escherichia coli. J Nat Prod 2000;63:1225-8.

90. Lavigne JP, Bourg G, Combescure C, Botto H, Sotto A. In-vitro and in-vivo evidence of dose-dependent decrease of uropathogenic Escherichia coli virulence after consumption of commercial Vaccinium macrocarpon (cranberry) capsules. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2008;14:350-5.

91. Kaspar KL, Howell AB, Khoo C. A randomized, double-blind, placebo-controlled trial to assess the bacterial anti-adhesion effects of cranberry extract beverages. Food Funct 2015;6:1212-7.

92. Pappas E, Schaich KM. Phytochemicals of cranberries and cranberry products: characterization, potential health effects, and processing stability. Crit Rev Food Sci Nutr 2009;49:741-81.

93. Williams AR, Klaver EJ, Laan LC, et al. Co-operative suppression of inflammatory responses in human dendritic cells by plant proanthocyanidins and products from the parasitic nematode Trichuris suis. Immunology 2017;150:312-28.

94. Krueger CG, Reed JD, Feliciano RP, Howell AB. Quantifying and characterizing proanthocyanidins in cranberries in relation to urinary tract health. Anal Bioanal Chem 2013;405:4385-95.

95. Gu L, Kelm MA, Hammerstone JF, et al. Liquid chromatographic/electrospray ionization mass spectrometric studies of proanthocyanidins in foods. J Mass Spectrom 2003;38:1272-80.

96. Scalbert A, Mila I, Expert D, et al. Polyphenols, metal ion complexation and biological consequences. Basic Life Sci 1999;66:545-54.

97. Prior RL, Fan E, Ji H, et al. Multi-laboratory validation of a standard method for quantifying proanthocyanidins in cranberry powders. J Sci Food Agric 2010;90:1473-8.

98. Feliciano RP, Shea MP, Shanmuganayagam D, Krueger CG, Howell AB, Reed JD. Comparison of isolated cranberry (Vaccinium macrocarpon Ait.) proanthocyanidins to catechin and procyanidins A2 and B2 for use as standards in the 4-(dimethylamino)cinnamaldehyde assay. Journal of agricultural and food chemistry 2012;60:4578-85.

99. Grace MH, Massey AR, Mbeunkui F, Yousef GG, Lila MA. Comparison of health-relevant flavonoids in commonly consumed cranberry products. J Food Sci 2012;77:H176-83.

100. Wang CH, Fang CC, Chen NC, et al. Cranberry-containing products for prevention of urinary tract infections in susceptible populations: a systematic review and meta-analysis of randomized controlled trials. Arch Intern Med 2012;172:988-96.

101. Ferry SA, Holm SE, Stenlund H, Lundholm R, Monsen TJ. Clinical and bacteriological outcome of different doses and duration of pivmecillinam compared with placebo therapy of uncomplicated lower urinary tract infection in women: the LUTIW project. Scandinavian journal of primary health care 2007;25:49-57.

102. Stapleton AE, Dziura J, Hooton TM, et al. Recurrent urinary tract infection and urinary Escherichia coli in women ingesting cranberry juice daily: a randomized controlled trial. Mayo Clin Proc 2012;87:143-50.

103. Vostalova J, Vidlar A, Simanek V, et al. Are High Proanthocyanidins Key to Cranberry Efficacy in the Prevention of Recurrent Urinary Tract Infection? Phytother Res 2015;29:1559-67.

104. Maki KC, Kaspar KL, Khoo C, Derrig LH, Schild AL, Gupta K. Consumption of a cranberry juice beverage lowered the number of clinical urinary tract infection episodes in women with a recent history of urinary tract infection. The American journal of clinical nutrition 2016;103:1434-42.

105. Terris MK, Issa MM, Tacker JR. Dietary supplementation with cranberry concentrate tablets may increase the risk of nephrolithiasis. Urology 2001;57:26-9.

106. Suvarna R, Pirmohamed M, Henderson L. Possible interaction between warfarin and cranberry juice. BMJ (Clinical research ed) 2003;327:1454.

107. Ansell J, McDonough M, Zhao Y, Harmatz JS, Greenblatt DJ. The absence of an interaction between warfarin and cranberry juice: a randomized, double-blind trial. Journal of clinical pharmacology 2009;49:824-30.

108. Mellen CK, Ford M, Rindone JP. Effect of high-dose cranberry juice on the pharmacodynamics of warfarin in patients. Br J Clin Pharmacol 2010;70:139-42.

109. Ou K, Percival SS, Zou T, Khoo C, Gu L. Transport of cranberry A-type procyanidin dimers, trimers, and tetramers across monolayers of human intestinal epithelial Caco-2 cells. Journal of agricultural and food chemistry 2012;60:1390-6.

110. McKay DL, Chen CY, Zampariello CA, Blumberg JB. Flavonoids and phenolic acids from cranberry juice are bioavailable and bioactive in healthy older adults. Food Chem 2015;168:233-40.

111. Chen CY, Zampariello C, Blumberg J. Urinary proanthocyanidin A2 as a biomarker of consumption of cranberry juice-a pilot study. FASEB Journal 2014;28.

112. Feliciano RP, Mills CE, Istas G, Heiss C, Rodriguez-Mateos A. Absorption, Metabolism and Excretion of Cranberry (Poly)phenols in Humans: A Dose Response Study and Assessment of Inter-Individual Variability. Nutrients 2017;9.

113. Bekiares N, Krueger CG, Meudt JJ, Shanmuganayagam D, Reed JD. Effect of Sweetened Dried Cranberry Consumption on Urinary Proteome and Fecal Microbiome in Healthy Human Subjects. OMICS 2018;22:145-53.

114. Gavin JR, 3rd, Davies MJ, Davies M, Vijapurkar U, Alba M, Meininger G. The efficacy and safety of canagliflozin across racial groups in patients with type 2 diabetes mellitus. Current medical research and opinion 2015;31:1693-702.

115. Howell A, Souza D, Roller M, Fromentin E. Comparison of the Anti-Adhesion Activity of Three Different Cranberry Extracts on Uropathogenic P-fimbriated Escherichia coli: a Randomized, Double-blind, Placebo Controlled, Ex Vivo, Acute Study. Nat Prod Commun 2015;10:1215-8.

116. Licensed Natural Health Products Database. 2008. (Accessed January 24, 2017, at http://www.hc-sc.gc.ca/.)

117. Tseng CS, Chang SJ, Meng E, Chang HC, Lee YJ. The efficacy of pentosan polysulfate monotherapy for preventing recurrent urinary tract infections in women: A multicenter open-label randomized controlled trial. J Formos Med Assoc 2019.

118. Lee PJ, Kuo HC. High incidence of lower urinary tract dysfunction in women with recurrent urinary tract infections. Lower urinary tract symptoms 2019.

119. Burleigh AE, Benck SM, McAchran SE, Reed JD, Krueger CG, Hopkins WJ. Consumption of sweetened, dried cranberries may reduce urinary tract infection incidence in susceptible women - A modified observational study. Nutrition Journal 2013;12.

120. Huang Y, Nikolic D, Pendland S, Doyle BJ, Locklear TD, Mahady GB. Effects of cranberry extracts and ursolic acid derivatives on P-fimbriated Escherichia coli, COX-2 activity, pro-inflammatory cytokine release and the NF-kappabeta transcriptional response in vitro. Pharm Biol 2009;47:18-25.

121. Anger J, Lee U, Ackerman AL, et al. Recurrent Uncomplicated Urinary Tract Infections in Women: AUA/CUA/SUFU Guideline. The Journal of urology 2019;202:282-9.