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HABILITAČNÍ PRÁCE

Vaccinium macrocarpon a silymarin v profylaxi a podpůrné terapii urologických onemocnění

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Prohlášení

Prohlašuji, že jsem habilitační práci na téma „*Vaccinium macrocarpon* a silymarin v profylaxi a podpůrné terapii urologických onemocnění“ vypracoval samostatně a že jsem pravdivě uvedl veškerou literaturu a další informační zdroje, ze kterých jsem čerpal.

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Obsah

1 Souhrn

Habilitační práce shrnuje výsledky klinických studií se silymarinem, komplexním extraktem ze semen *Silybum marianum* (ostropestřece mariánského), a plodem *Vaccinium macrocarpon* (klikvy velkoplodé) realizovaných na Urologické klinice FN v Olomouci. K nejčastějším urologickým onemocněním u mužů patří benigní hyperplazie prostaty (BHP), obvykle spojená se symptomy dolních močových cest (LUTS, **L**ower **U**rinary **T**ract **S**ymptoms), a chronická nebakteriální prostatitida. U žen se pak jedná o infekce dolních močových cest (IMC), které jsou druhou nejčastěji se vyskytující bakteriální infekcí a během života se na toto onemocnění léčí více než polovina ženské populace. Ke snížení rizika vzniku těchto onemocnění jsou často, zejména staršími věkovými skupinami, užívány i přírodní látky jako např. biominerály, vitaminy a sekundární metabolity rostlin (fytoceutika) ve formě doplňků stravy. Pleiotropní účinky komplexních směsí přírodních látek na lidský organismus vedou k odlišnému pohledu na jejich aplikaci ve srovnání s individuálními látkami. Standardní léčba těchto onemocnění má široké možnosti, ale může mít také nežádoucí účinky.

V práci jsou uvedeny výsledky tří klinických zkoušek (KZ) s kombinací silymarinu (extrakt flavonolignanů ze semen *S. marianum*) se selenem nebo L-argininem a čtyř randomizovaných KZ s celým plodem *V. macrocarpon* na pacientech s historií urologického onemocnění. Cílem těchto studií bylo ověřit, jaké jsou účinky, bezpečnost a vliv na kvalitu života při dlouhodobém užívání silymarinu v kombinaci se selenem/L-argininem nebo celého plodu *V. macrocarpon* na klinicky sledovaný lidský subjekt.

V pilotní studii měla kombinace silymarinu a selenu u dosud neléčených mužů s prostatickým specifickým antigenem (PSA) ≤ 2.5 ng/ml vliv na snížení hladiny PSA, zlepšení urodynamických parametrů a skóre IPSS (**studie 1**). V placebem kontrolované studii byl prokázán vliv kombinace silymarinu a selenu na celkový zdravotní stav mužů dva měsíce po radikální prostatektomii. Došlo ke zlepšení kvality života, metabolismu lipidů a byla normalizována hladina selenu. Účinek na PSA prokázán nebyl, pravděpodobněkvůli krátkodobému užívání medikace (**studie 2**). V desetidenní intervenční klinické studii bylo zjištěno, že denní konzumace nápoje obsahujícího silymarin a L-arginin má pozitivní vliv na fyziologické a urodynamické paramentry

u zdravých mužů ve věku 38–59 let **(studie 3)**. V klinické studii bylo podáváno 1500 mg/den plodu *V. macrocarpon* po dobu šesti měsíců mužům s diagnózou LUTS a PSA ≥ 2,5 ng/ml. Byl u nich prokázán účinek komplexu látek plodu na zlepšení funkce močových cest a vliv na koncentraci volného a vázaného PSA v krvi (**studie 4**). Dvojitě slepá studie na mužích s diagnostikovanými LUTS, kterým byla podávána dávka 250 mg a 500 mg/den plodu *V. macrocarpon,* prokázala, že dávka 500 mg zlepšuje urodynamické parametry močení a kvalitu života (**studie 5**). Stejná dávka plodu *V. macrocarpon* užívaná po dobu šesti měsíců statisticky významně prodloužila dobu přežití bez infekce u žen s historií opakované IMC (**studie 6**). Práškovitý plod *V. macrocarpon* byl užíván v dávce 1500 mg pacienty po dobu 30 dní před radikální prostatektomií. Výsledky randomizované, dvojitě slepé studie ukázaly, že intaktní plod obsahuje látky regulující expresi androgen-sensitivních genů což by mohlo oddálit biochemický návrat onemocnění (**studie 7**).

Současná fytoterapie by měla být založena na vědeckých a klinicky podložených základech. V žádném případě nenahrazuje léčbu dle aktuálních urologických doporučení. Fyzikálně-chemická charakterizace podávaného přípravku a provedení klinické studie podle stanovených pravidel je základní podmínkou pro hodnocení výsledků. Naše klinické studie potvrdily, že dietní intervence zvolených komplexních extraktů měla příznivý vliv na zdraví pacienta jak v oblasti prevence tak i remise urologických onemocnění. Pozitvní je, že použité přípravky byly pacienty dobře snášeny bez vedlejších účinků. Působení testovaných přípravků hodnotíme hlavně jako podpůrné.

2 Summary

This habilitation thesis is an attempt to provide a summary of clinical studies using a complex extract of the seeds of *Silybum marianum* (milk thistle) and the fruit of *Vaccinium macrocarpon* (American cranberry) carried out at the Department of Urology.

In older men, the most common urological disorders are benign prostate hyperplasia (BPH), usually associated with lower urinary tract symptoms (LUTS, Lower Urinary Tract Symptoms), and chronic non-bacterial prostatitis. In women, lower urinary tract infection (UTI), is the second most common bacterial infection and during the course of life, a conditions treated in more than half of the female population. To reduce the risk of these conditions, especially in older age groups, the use of natural substances such as e.g. bio-minerals, vitamins and secondary plant metabolites (phytoceuticals) as dietary supplements are often used. The pleiotropic effect of complex mixtures of natural substances on the human organism leads to a different conclusion vis á vis their application than the individual substances. Standard treatments of the above conditions are widely variable and they can also have undesirable side-effects.

This thesis presents the results of 3 clinical trials (CT) conducted using a combination of silymarin (extract of flavonolignans from the seeds of *S. marianum*) with selenium or L-arginine and 4 CT using the whole fruit of *V. macrocarpon* in patients with a history of urological disease. The objective of these studies was to determine the effects, safety and impact on quality of life of the long-term use of silymarin in combination with selenium/L-arginine or whole fruit of *V. macrocarpon* through clinical monitoring of human subjects.

In a pilot study, a combination of silymarin and selenium in the treatment-naive men with prostate specific antigen (PSA) ≤2.5 ng/ml had an effect in reducing PSA levels, improving urodynamic parameters and IPSS score (**study 1**). A placebo-controlled study demonstrated the effect of a combination of silymarin and selenium on the health of men in the 2nd month after radical prostatectomy: quality of life, lipid metabolism and levels of selenium improved. No effect on PSA levels was found, probably owing to the short period of taking the supplementation (**study 2**). On the 10th day of an intervention trial, it was found that daily consumption of beverages containing

silymarin and L-arginine had a positive effect on the physiological and urodynamic parameters of healthy men aged 38-59 years (**study 3**). A clinical study involving the administration of 1500 mg per day *V. macrocarpon* fruit for sixth months in men with a LUTS diagnosis and PSA greater than 2.5 ng/ml, showed the effect of complex of fruit compounds in improving urinary tract function and levels of free and bound PSA in blood (**study 4**). A double blind study of men with diagnosed LUTS using 250 mg and 500 mg/day *V. macrocarpon* fruit demonstrated that a 500 mg dose improved the urodynamic parameters of urination and quality of life (**study 5**). The same dose of *V. macrocarpon* fruit used for six months statistically significantly prolonged survival time free of infection in women with a history of recurrent UTI (**study 6**). *V. macrocarpon* fruit powder was used in a dose of 1500 mg in patients 30 days prior to radical prostatectomy. The results of randomized, double-blind studies have shown that the intact fruit contains substance that regulate the expression of androgen-sensitive genes and this could prolong biochemical relapse (**study 7**).

Today, phytotherapy is based on a scientific and clinical evidence-based foundation. It does not replace the treatment of urological conditions according to current recommendations. The physico-chemical characterization of the administered product and conduct of the clinical trial according to the rules is a precondition for assessing the results. Our clinical studies have shown that dietary intervention using both selected complex extracts has a beneficial effect, both in the prevention and remission of urological diseases. A positive outcome was also their excellent tolerance with no side effects. The activity of the tested products is evaluated mainly as supportive.

3 Současný stav problematiky

Urologická onemocnění, benigní hyperplasie prostaty (BHP), symptomy dolních cest močových (LUTS), chronická prostatitida (ChP) a karcinom prostaty (KP) představují zdravotní a sociální problém pro stárnoucí mužskou populaci. KP je v populaci pátým a u mužů nejčastějším nádorovým onemocněním. Po plicních nádorech je druhou příčinou úmrtí na onkologickou diagnózu a jeho incidence má celosvětově rostoucí tendenci. U žen jsou v průběhu života častým problémem opakované infekce močových cest (IMC). Snížit riziko vzniku těchto onemocnění cílenou prevencí realizovanou pacientem je stále výzvou pro praktické lékaře a urology

Při léčbě nemoci lze obecně zvolit dva přístupy. Buď pasivní, což znamená léčbu po diagnostikování nemoci, nebo preventivní, tedy předcházení onemocnění, respektive snížení rizika jejího vzniku. Významnou roli má prevence u nádorových onemocnění, kde je léčba náročná pro lékaře i pacienta a často vede ke zhoršení kvality života. Možnost předcházet vzniku onemocnění je pro pacienta atraktivní.

Prevenci je možné rozdělit na tři typy. K **primární prevenci** patří ovlivnění příčiny nemoci zvýšením odolnosti organizmu podporou ochranných faktorů, zejména imunity a snížení vlivu rizikových faktorů. Hlavním cílem je zabránění či oddálení rizika vzniku onemocnění. Primární prevence závisí hlavně na samotném jedinci, jeho životním stylu, stravovacích návycích a omezení vlivu rizikových faktorů, většinou pocházejících ze životního prostředí. **Sekundární prevence** zahrnuje odhalení již přítomné poruchy či nemoci v raném stadiu umožňujícím účinnou léčbu. **Terciární prevence** jsou opatření cílená na zabránění nebo snížení dopadu již probíhajícího onemocnění nebo prodloužení doby remise.

Preventivní programy jsou součástí zdravotnické politiky všech států Evropské unie. V habilitační práci zmíněná urologická onemocnění patří k těm, kde lze snížit dlouhodobým užíváním látek obsažených v rostlinách, ovoci, zelenině či jiných potravinách riziko vzniku výše uvedených onemocnění, nebo při jejich kombinaci s léčivy je možné zlepšení průběhu terapie.

3.1 Diagnostika a léčba některých urologických onemocnění

3.1.1 Infekce dolních močových cest

Infekce močových cest (IMC) jsou u žen nejčastějším bakteriálním onemocněním, během života má více než 50 % žen aspoň jedenkrát zkušenost s IMC (Foxman, 2003; Micali *et al*., 2014). Většina IMC (95%) je způsobena různými kmeny *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabelis a Klebsiella pneumoniae* (Gupta *et al*., 2001). Mimo antibiotickou léčbu se všemi riziky (rezistence, nežádoucí účinky) má velmi důležitou roli, zvláště u recidivujících infekcí, prevence vzniku IMC. Mezi používanou prevenci patří profylaktické podávání antimikrobiálních léků a imunomodulační léčba.

3.1.2 Benigní hyperplazie prostaty, symptomy dolního močového traktu a chronická prostatitida

Benigní hyperplazie prostaty (BHP), známá také jako "zvětšená prostata", je chronické onemocnění starších mužů, obvykle spojené se symptomy dolních močových cest (LUTS) (Anderson *et al.,* 2001). Původní pohled na LUTS, kdy se u mužů předpokládaly pouze potíže v souvislosti s BHP a hyperaktivní močový měchýř (overactive bladder, OAB), byl naopak chápán jako čistě ženské onemocnění, je již přežitý (Chapple *et al*., 2006). Ukazuje se, že ne všechny LUTS u starších mužů jsou nutně způsobeny jen BHP a svou roli může hrát hyperaktivita detrusoru močového měchýře, hypoaktivita detrusoru, stejně jako strukturální nebo funkční abnormality močového traktu a ledviny (nykturie) (Oelke *et al*., 2013). Přehled nejčastějších příčin LUTS u mužů je uveden v tabulce 1.

Tabulka 1. Možné příčiny LUTS u mužů.

Druh potíží/onemocnění
benigní hyperplazie prostaty
hyperaktivita detrusoru močového měchýře
noční polyurie
hypoaktivita detrusoru
neurogenní dysfunkce močového měchýře
infekce urotraktu
cizí těleso
striktury uretry
tumor močového měchýře
iuxtavesikální ureterolithiasa

Tyto výše uvedené příčiny se u většiny pacientu kombinují. I když se jedná o nezhoubné onemocnění, jde často o onemocnění progresivní, vedoucí ke komplikacím a významnému ovlivnění kvality života, jako je akutní retence (AR) nebo nutnost operačního řešení (Djavan *et al*, 2004). Operační léčba je vyhrazena především pro muže s komplikovanou BHP, nicméně absolutních indikací k operační léčbě je nyní méně.

Farmakoterapie BHP je zaměřena převážně na zmírnění symptomů dolních cest močových (Oelke *et al*., 2013). K lékům první volby patří α-blokátory (antagonisté α-adrenoreceptorů) a inhibitory 5α-reduktázy (5ARi). Při monoterapii jsou nejčastěji používanými léky α-blokátory, které mají rychlý nástup účinku na rozdíl od 5ARi, kde úlevu od potíží můžeme očekávat nejdříve za šest měsíců od zahájení terapie. Na druhou stranu u 5ARi byl prokázán vliv na progresi benigní hyperplazie prostaty (zmenšení velikosti prostaty, snížení rizika retence moči, potřeby operačního řešení). Výsledky studií z poslední doby (Roehrborn *et al*., 2011) ukázaly, že největší benefit pro pacienty poskytuje kombinovaná léčba (α-blokátory pro rychlou úlevu od potíží a 5ARi pro dlouhodobé snížení rizika progrese či komplikací při LUTS). Stále častější je použití anticholinergik v kombinaci s α-blokátory k potlačení iritačních příznaků při LUTS a u pacientů s erektilní dysfunkcí (ED) inhibitorů fosfodiesterázy-5 (iPDE5). U α-blokátorů a inhibitorů 5α-reduktázy byly zaznamenány pro pacienta zatěžující nežádoucí účinky sumarizované v tabulce 2 (Djavan *et al.*, 1999; Wessells *et al.*, 2003).

Tabulka 2. Nežádoucí účinky α-blokátorů inhibitorů 5α-reduktázy.

α-blokátory[a]
bolesti hlavy
závratě
posturální hypotenze
astenie
ospalost
zduření nosních sliznic
retrográdní ejakulace
inhibitory 5α-reduktázy[b]
pokles libida
impotence
snížení objemu ejakulátu
vyrážka, zvětšení a citlivost prsních žláz

[a] Djavan *et al.*, 1999; [b] Wessells *et al.*, 2003

Příčina vzniku nebakteriální chronické prostatitidy není plně objasněna především pro stále nedostatečné pochopení komplexnosti tohoto onemocnění. Jedním z hlavních důvodů může být nedostatečná drenáž sekretu z duktů periferní zóny prostaty. Se zvětšováním prostaty se také objevují obstrukční příznaky a dochází k refluxu moči do prostatických duktů. Tento reflux se může objevovat u mužů se subvezikální obstrukcí, dysfunkčním močením a benigní hyperplazií prostaty a vede k chemickému dráždění a zánětu. Svou roli může hrát také životní prostředí znečištěné látkami s hormonální aktivitou (endokrinními disruptory). Vznik syndromu chronické prostatitidy může být výsledkem souhry psychologických faktorů, dysfunkcí imunitního, neurologického a endokrinního systému (Pontari *et al.*, 2004).

3.1.3 Karcinom prostaty

V současné době je pro mužskou populaci v celém světě stále větší zátěží KP (Hsing a Devesa, 2001; Hsing a Chokkalingam, 2006). V severní Americe a západní Evropě je u mužů nejčastěji diagnostikovaným nádorem a v pořadí druhou příčinou smrti spojenou s onkologickým onemocněním (Siegel *et al.*, 2015). V iniciaci rakoviny prostaty hrají klíčovou roli androgeny (testosteron) stimulující expresi a transkripční aktivitu androgenního receptoru (AR) (Huggins, 1963). Významné rozdíly v incidenci a mortalitě KP jsou mezi geografickými a etnickými populacemi. Etiologie KP není dosud zcela známa, i když se dá předpokládat, že hlavními faktory jsou biologický věk a rodinná historie onemocnění v mužské rodové linii; u žen je to pak rakovina prsu (Lichtenstein *et al*., 2000). Dalšími rizikovými faktory jsou genotoxicky/epigeneticky aktivní látky v životním prostředí (Koutros *et al*., 2015), životní styl (Kolonel *et al*., 2004; Dobosy *et al*., 2007), ale také obesita a stravovací návyky (Masko *et al*., 2013; Allot *et al*., 2013). Byla prokázána souvislost mezi chronickým zánětem a KP (Vasto *et al*., 2008). Muži, u kterých probíhá chronický zánět i jiných tkání než prostatické, mají významně zvýšené riziko agresivní formy nádorového onemocnění prostaty.

V současné době jsou inhibitory enzymu 5α-reduktázy (5ARi) finasterid a dutasterid jedinými farmakologickými preparáty, u nichž bylo prokázáno snížení prevalence karcinomu prostaty. Finasterid byl užíván v randomizované, dvojitě slepé, placebem kontrolované multicentrické studii PCPT (**P**rostate **C**ancer **P**revention **T**rial) po dobu 7 let. Studie se zúčastnilo 18 882 mužů (věk > 55 let) s normálním nálezem *per rektum* a hladinou PSA ≤ 3 ng/ml. V randomizované, dvojitě slepé, placebem kontrolované studii REDUCE (**RE**duction by **DU**tasteride of prostate **C**ancer **E**vents trial) užívalo dutasterid 8231 mužů (věk 50–75 let) po dobu čtyř let. V obou studiích bylo prokázáno snížení incidence KP: ve studii PCPT o 24,8 % a ve studii REDUCE o 22,8 %. Tyto výsledky ale byly u finasteridu i dutasteridu limitovány pro KP s Gleasonovým skórem (GS) 6 a nižším, u nádorů s GS 8–10 byla incidence vyšší u obou 5ARi (Thompson *et al.*, 2003, 2009; Andriole *et al.*, 2010).

U některých léčiv, statinů, nesteroidních antiflogistik (kyselina acetylsalicylová) a antidiabetik (metformin), byly publikovány studie o jejich inverzním vlivu na vznik KP, ale chybí jasný průkaz chemopreventivního účinku studiemi podobného designu jako s finasteridem a dutasteridem (Moyad, 2015). Inhibitory fosfodiesterázy typu 5 nemají vliv na snížení rizika vzniku KP (Jamnagerwalla *et al.,* 2016).

3.2 Přírodní látky v profylaxi a remisi urologických onemocnění

3.2.1 Účinky fytoceutik na lidský organismus

Účinky minoritních obsahových látek (sekundárních metabolitů) divoce rostoucích nebo zemědělsky pěstovaných rostlin, plodů moře a živočišných tkání na fyziologické funkce člověka jsou stále předmětem výzkumu, který splňuje požadavky medicíny založené na důkazech (**E**vidence **B**ase **M**edicine study). V indické medicíně Ayurvedě (Vaidya *et al*., 2007; Zisman *et al*., 2003), japonské bylinné medicíně Kampo (Minagawa a Ishizuka, 2015) a čínské tradiční medicíně (Chau a Wu, 2006) je potrava a látky v ní obsažené běžnou součástí prevence a zdravotní péče. V evropské medicíně bylo v minulosti také spojení mezi racionální medicínou a složením potravy mnohem „intimnější“ než v 20. století. V současné době se ambivalentní názory lékařské veřejnosti na komplementární fytoterapii mění. Klinické studie účinků obsahových látek rostlin iniciované znalostmi z tradičního léčitelství prokázaly, že lze z jejich poznatků převzít některé prvky při zachování bezpečnosti a prospěchu pacienta. Ukazuje se, že citát starověkého řeckého učence, „otce medicíny“ Hippokrata (460 – 370 p.n.l.) „Tvá potrava budiž tvým lékem a tvůj lék tvou potravou“ je stále více aktuální (Georgiou *et al*., 2011), především ve vztahu k chronickým civilizačním onemocněním. Racionální medicína třetího tisíciletí se cíleně zaměřuje na význam složení diety na riziko vzniku a průběh některých chronických onemocnění a studium preventivního a léčebného účinku fytoceutik v ní obsažených (Klein *et al*., 2012; Thomasset *et al*., 2007; Van Patten *et al*., 2008).

V dalším textu je věnována pozornost rostlinným sekundárním metabolitům. V potravinách je pro tyto látky používáno také označení nutraceutika. Patří mezi ně vitaminy, biominerály, organické kyseliny, karotenoidy, polyfenoly, terpeny a řada dalších látek. Jsou-li tyto látky jako složky komplexních extraktů nebo chemická individua komerčně dostupné ve formě přípravků, jedná se o doplňky stravy.

V trávicím ústrojí mají fytoceutika vliv na mikrofloru trávícího ústrojí, zejména dutiny ústní a tlustého střeva (probiootické a patogenní bakterie), žaludeční a střevní bariéru, oxidoredukční statut, metabolické přeměny xenobiotik a imunitní systém lymfatické tkáně tlustého střeva (**G**ut **A**ssociated **L**ymphatic **T**issue, GALT).

Ve vnitřním prostředí jsou fytoceutika prekurzory signálních molekul, případně signální molekuly, kofaktory enzymů, komponentami biomakromolekul, buněčných struktur a oxidoredukčního statutu (redox rovnováhy) organismu.

Charakterizace fytoceutika s udávaným vlivem na lidský organismus má obsahovat: (i) hodnověrné etnofarmakologické údaje o rostlině/extraktu, (ii) údaj o chemické složení, (iii) farmakologický profil, (iv) účinky potvrzené výsledky z randomizovaných klinických studií a (v) informaci o interakcích s léčivy, případně nežádoucí účinky. V Evropské unii jsou tyto údaje vyžadovány od výrobců Evropským úřadem pro bezpečné potraviny (**E**uropean **F**ood **S**afety **A**gency, EFSA). Pokud fytoceutikum (nutraceutikum) splňuje požadavky EFSA, výrobce získává oprávnění uvádět biologický účinek fytoceutika na lidský organismus jako zdravotní tvrzení. V USA se problematice fytoceutik/nutraceutik věnuje Národní centrum pro komplementární a alternativní medicínu (**N**ational **C**enter for **C**omplementary and **A**lternative **M**edicine, NCCAM), které je jedním z 27 ústavů National Instuitutes of Health (NIH).

Snaha stále větší části populace udržet si aktivní způsob života do pozdního věku a oddálit rizika spojená s řadou chronických onemocnění zvyšuje zájem o použití potravin/nutraceutik.

V dalším textu jsou uvedeny rostlinné extrakty/fytoceutika/nutraceutika, u kterých byl klinicky testován účinek na diskutovaná urologická onemocnění. U žádného z nich nebyla prokázána interakce s konvenčními léky (Izzo, 2012).

3.2.2 Fytoceutika a opakované infekce dolních močových cest

Fytoceutika a jejich metabolity s udávaným účinkem na IMC mají (i) snižovat adhezi buněčné struktury na epitel močového ústrojí nebo (ii) deaktivovat geny produkující polysacharidy a polymery rozhodující pro vznik biofilmu. Z fytoceutik/nutraceutik užívaných v komplementární terapii IMC jsou nejlépe takto charakterizované látky plodu americké brusinky (klikvy velkoplodé, *Vaccinium macrocarpon* Ait., *V. oxycoccus* L., *V. vitis-idaea* L., Ericaceae). Pravidelná konzumace šťávy nebo celého plodu je doporučována pro prevenci před opakovaným návratem IMC a při jednorázové nebo intermitentní močové katetrizaci (Jepson *et al.,* 2012; Order a Liperoti, 2016). Látky v plodu *V. macrocarpon* brání kolonizaci močových

cest zejména uropatogenními kmeny *Escherichia coli* s P-fimbriemi (Guay, 2009; podrobněji v kapitole 3.3.1.).

V čínské tradiční medicíně je ceněna pro své účinky na opakované IMC *Salvia plebeia* (Peng *et al*., 2010). Cochran metaanalýza publikovaných studií s rostlinnými přípravky čínské tradiční medicíny vedla k závěru, že žádný z klinicky zkoušených přípravků neměl významný profylaktický nebo terapeutický efekt na IMC (Flower *et al*., 2015).

Klinicky zajímavé jsou pozitivní výsledky zkoušek s přípravky obsahujícími probiotické bakterie *Lactobacillus rhamnosus a reuteri* na ženách s historií opakujících se IMC způsobené kmeny *E. coli* rezistentními vůči některým antibiotikům (Foxman a Buxton, 2013; Schwenger *et al*., 2015).

3.2.3 Fytoceutika a BHP/LUTS

Pro část mužské populace je komplementární terapie BHP/LUTS a chronického zánětu prostaty fytopřípravky atraktivní alternativou racionální medicíny. Důvodem je snadné zakoupení přípravků bez nutnosti návštěvy lékaře, vliv medií, a také že jsou vnímány jako „přírodní“ látky bez škodlivých účinků na lidský organismus. O účinnosti fytoterapie je ale k dispozici méně kvalitních a ověřených dat ze studií a její efekt může být významně ovlivněn očekáváním pacienta, např. pod vlivem reklamy (placebo efekt).

Fytoceutika s účinky na BHP a LUTS mohou (i) inhibovat aktivitu 5α-reduktázy a (ii) vazbu testosteronu na 5α-testosteronový receptor, (ii) být agonisté estradiolu pro estrogenní receptory, (iii) mít antiproliferační a (iv) protizánětlivý účinek, (v) být substráty (prebiotika) pro nepatogenní mikroflóru trávícího ústrojí (Cheetham, 2013).

Klinicky studované rostlinné extrakty na pacientech s BHP a LUTS jsou uvedeny v tabulce 3 (Cheetham, 2013; Pagano et al., 2014; Allkanjari et al., 2015). Mezi terapeuticky zajímavé patří komplexní extrakty z listů zeleného čaje (*Camellia sinensis*), pylových zrnek žita (*Secale cereale*), semen palmy plazivé (*Serenoa repens*) a ostropestřce mariánského (*Silybum marianum*), kořenů kopřivy (*Urtica diotica*) a olej ze semen tykve obecné (*Cucurbita pepo*). Extrakty byly testovány buď jednotlivě, nebo jejich různé kombinace.

K aktivním komponentám patří zejména fytosteroly, terpenoidy, polyfenoly, nenasycené mastné kyseliny, lecitiny, silice, polysacharidy. Mezi mikronutrienty s příznivým vlivem na prostatu patří vitamíny E a D, zinek a selen, lykopen a další. Obecným nedostatkem studií i přes většinou zajímavé účinky je její provedení na malém počtu subjektů, způsob randomizace a chybějící přesné složení podávaného přípravku. Účinky jsou často diskutovány na základě poznatků z mechanistických experimentů.

Tabulka 3. Komplexní extrakty rostlin s udávaným účinkem na BHP a LUTS[a].

Extrakty ze semen/plodů/kořenů/kůry
rod *Brassica* (košťáloviny)
Camellia sinensis (zelený čaj)
Cucurbita pepo (tykev obecná)
rod *Epilobium* (vrbovka)
Hypoxis rooperi (africká brambora)
Lycopersicum esculentum (rajče)
Punica granatum (granátové jablko)
Prunus africanum (slivoň africká)
Secale cereale (žito)
Serenoa repens (trpasličí palma plazivá)
Silybum marianum (ostropestřec mariánský)
Vaccinium macrocarpon (klikva velkoplodá)

[a] Cheetham, 2013; Pagano et al., 2014, Allkanjari et al., 2015

V současné době má fytoterapie své místo v léčbě BHP/LUTS a měla by být používána hlavně u pacientů s mírným stupněm LUTS (IPSS<7). Pro pacienty se středně těžkými až těžkými LUTS (IPSS ≥ 7) a větší prostatou je efektivnější farmakoterapie (α-blokátory, 5ARi) nebo chirurgická léčba (Nickel *et al*., 2010).

3.2.4 Fytoceutika a riziko onemocnění karcinomem prostaty

Pojem chemoprevence byl poprvé použit v sedmdesátých letech minulého století a je definován jako užívání přírodních či syntetických látek, které jsou schopny zablokovat nebo oddálit jeden a více kroků přirozeného vývoje nádorového onemocnění u dosud zdravých jedinců (Kelloff *et al*., 2002; Brawley, 2002). Klíčovou

chemoprotektivní aktivitou by měl být protizánětlivý účinek (Gupta *et al*., 2010). Chemoprevence může být přínosná zvláště u nádorů iniciovaných hormonálně jako je KP (Klein, Thompson, 2012), kde je doba vzniku a vývoje nádoru poměrně dlouhá (roky až desítky let). Dobrým příkladem je užívání tamoxifenu při prevenci nádoru prsu u žen (Fisher *et al*., 2005). Realita však není tak jednoduchá. Významnou roli zde hraje genetická dispozice jedince, ale zatím jasně nelze identifikovat, které genetické změny hrají hlavní roli ve vývoji nádorů.

Otázkou je, která nutraceutika mohou snížit nepříznivé účinky léčby, riziko recidivy nebo zlepšit průběh onemocnění u pacientů s již diagnostikovaným a léčeným KP. Klinických studií s tímto primárním cílem je relativně málo a nejsou dostatečně průkazné (malý počet pacientů, studované fytoceutikum/extrakt není detailněji chemicky charakterizován, nejsou stanoveny jeho koncentrace v krvi/moči, resp. metabolity). I tak je u pacientů s nádorovým onemocněním velmi rozšířené užívání fytoceutik/nutraceutik, např. 26–35 % pacientů s KP a 75–87 % pacientek s karcinomem prsu (Velicer *et al*., 2008; Paller *et al*., 2016).

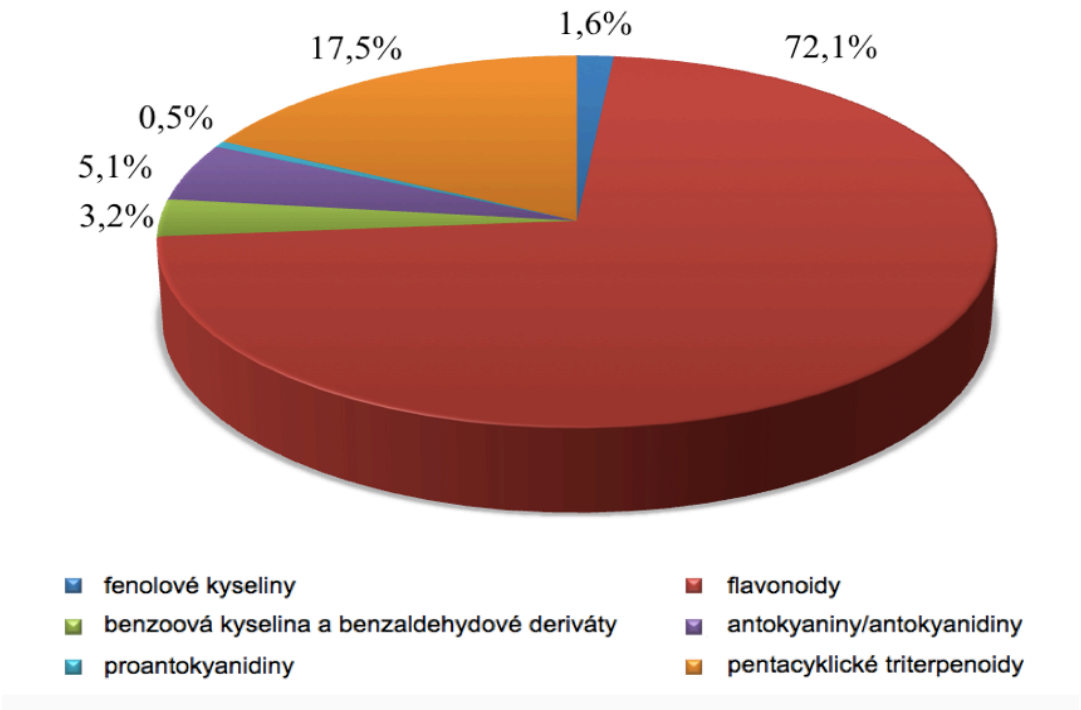
Vyhledávání přírodních látek s potenciálním chemopreventivním účinkem na KP je v posledních letech věnována stále větší pozornost (Ma *et al*., 2009; Ho et al., 2011; Mahmoud et al, 2014; Hackshaw-McGeagh et al., 2015). Nutraceutik, která mají prokazatelný protektivní účinek na progresi KP a dobu remise po radikální prostatektomii, je známo poměrně mnoho a patří mezi ně např. vitaminy D a E (alfa- a gama-tokoferol), epigalokatechingaláty čajovníku (*Camellia sinensis*), sulforafan a indol-3-karbinol (*Brassica*), isoflavony sóji genistein a daidzein (*Glycine max*), ω-3 polynenasycené mastné kyseliny lněného oleje (*Linum usitatissimum),* karotenoid lykopen, polyfenoly šťávy granátového jablka (*Punica granatum*) a kurkumin z oddenků kurkumy (*Curcuma longa*).

V literatuře existují rozporuplné názory na vztah mezi selenem a rizikem KP. V některých studiích byl prokázán vliv suplementace stravy selenem na snížení rizika vzniku KP (Yoshizawa *et al*, 1998; Ma *et al*., 2009), ale na druhou stranu byla předčasně ukončena studie SELECT (**SEL**enium and Vitamin **E** **C**ancer Prevention **T**rial) vzhledem k neprokázanému preventivnímu účineku selenu a vitamínu E na karcinom prostaty u mužů, ale i zvýšenému výskytu diabetu typu II (Lippman *et al*., 2009).

3.3 Charakterizace a biologické účinky studovaných látek

3.3.1 Klikva velkoplodá (V*accinium macrocarpon*)

Vaccinium macrocarpon Ait. (klikva velkoplodá, Ericaceae) roste v chladnějších oblastech severní polokoule s největším rozšířením v Severní Americe. Je to celoročně zelená rostlina s kulatými červenými plody, které se sklízí v říjnu. Mají trpkou chuť a v tradiční indiánské medicíně byly plody *V. macrocarpon* užívány k prevenci a léčbě infekcí dolních cest močových. Na evropském trhu jsou prodávány sušené plody, ovocné nápoje (do 20% šťávy) a doplňky stravy obsahující v práškovité formě extrakt šťávy nebo plod. V plodu klikvy vedle draslíku, hořčíku, organických kyselin, vlákniny, mono- a oligosacharidů, bílkovin, vitaminu C a n-3/n-6 polynenasycených mastných kyselin je také široké spektrum polyfenolů (fenolové kyseliny, flavonoidy, antokyaniny a proantokyanidiny typu A2 s různým stupněm polymerizace) a triterpenoidů (kyselina ursolová a její estery) (Neto, 2007; Guay, 2009). Procentové zastoupení těchto látek je na obr. 1. Biologické účinky plodu jsou v literatuře většinou připisovány antokyaninům a proantokyanidinům typu A2 (Blumberg *et al*., 2013).



Obr. 1. Obsah bioaktivních látky v plodu klikvy velkoplodé (Vidlář *et al*., 2015).

V lidském organismu jsou polyfenoly *V. macrocarpon* metabolizovány v tenkém a tlustém střevě. Biotransformace fenolových kyselin a flavonoidů se účastní enzymy 1. fáze. Do organismu jsou absorbovány nezměněné nebo jako metabolity střevní mikroflory (deriváty fenolových kyselin a aglykony flavonoidů). Antokyaniny a proantokyanidiny jsou metabolizovány intestinálními bakteriemi a do vnitřního prostředí absorbují jejich metabolity - deriváty fenolových a mastných kyselin s krátkým řetězcem (Chiou *et al*., 2014; Ou a Gu, 2014). V krvi/tělních tekutinách pak nacházíme jejich metabolity ve formě konjugátů např. s kyselinou glukuronovou nebo glycinem.

Z organismu jsou polyfenoly vylučovány jako volné nebo konjugované aromatické kyseliny a aglykony flavonoidů (Vidlar *et al*., 2015; Feliciano *et al*., 2010). Antokyanidiny a proantokyanidiny nebyly v moči detekovány. V klinické studii na zdravých mužích byla v moči stanovena návratnost 6,2 % polyfenolových látek z celkově podaného množství (Feliciano *et al*., 2016).

Komplex obsahových látek plodu/šťávy *V. macrocarpon* vykazuje v mechanistických a intervenčních klinických studiích pleiotropní účinek (Blumberg *et al*., 2013). Výsledky studií prokázaly snížení rizika vzniku kardiovaskulárních onemocnění a gastrointestinálních onemocnění způsobených *Helicobacter pylori*.

Obsahové látky plodu *V. macrocarpon* nemají výrazné bakteriostatické a baktericidní účinky. Klinicky zajímavý je jejich antiadherenční účinek. Ten lze vyvětlit buď kompetitivní inhibicí adheze fimbriálních podjednotek *E. coli* na povrch uroepitálních buněk nebo inhibicí jejich exprese komplexem obsahových látek plodu (Patel a Daniels, 2000). Howell *et al*. (2005) publikovali studii o *in vitro* účinku proantokyanidinů typu A2 na adhezi uropatogenního kmene *E. coli* s P-fimbriemi (Foo *et al*., 2000). Možným vysvětlením mechanismu antiadherenčního účinku plodu je zeslabení patogenních bakterií metabolity obsahových látek *V. macrocarpon* v tlustém střevě (Raz *et al*., 2004). Valentová *et al*. (2007) připisují *ex vivo* antiadherenční účinek moči zdravých dobrovolníků konzumaci 1,2 g/den práškovitého plodu po dobu 3 měsíců, vysoké koncentraci kyseliny hippurové ve srovnání s kontrolní skupinou. Kim *et al*. (2015) prokázali inhibiční účinek proantokyanidinů *V. macrocarpon* na tvorbu biofilmu tvořeného kariopatogením kmenem *Streptococcus mutans*.

V poslední době bylo publikováno několik prací, které prokazují, že antiadherenční účinek plodu *V. macrocarpon* na uropatogenní bakterie *E. coli* není účinkem pouze proantokyanidinů typu A2, ale také jejich metabolitů a dalších látek jako jsou fenolové kyseliny, oligosacharidy a pentacyklické triterpeny (Rafsanjany *et al*., 2015; Gonzáles de Liano *et al*., 2015; Sun *et al*., 2015; Rodriguez-Pérez *et al*., 2016).

3.3.2 Silymarin

Extrakt ze semen *Silybum marianum* (ostropestřec mariánský) je znám pod názvem silymarin a je jedním z nejlépe farmakologicky dokumentovaných rostlinných extraktů. Je používán od starověku a je součástí velkého počtu fytopreparátů a doplňků stravy především v prevenci a léčbě různých jaterních chorob (Abenavoli *et al*, 2010).

Silymarin je směs šesti flavonolignanů (silybin, isosilybin, silychristin, silydianin, silandrin a isosilandrin) a 10–30 % polyfenolové frakce (Gažák, 2007; Šimánek *et al*., 2000). Silybin (synonymum silibinin) a isosilibyn jsou směsi dvou diastereoizomerů (silybin A a B, isosilybin A a B) a tvoří hlavní aktivní komponentu silymarinu (silymarin se obvykle standardizuje na silybin). Farmakologické studie silymarinu/silybinu popisují hlavně jeho antioxidační, hypolipidemickou a cytoprotektivní aktivitu (Agarwal *et al*., 2006). Silybin je používán jako hepatoprotektivum a terapeutikum při chronické hepatitidě, cirhóze, alkoholismu a po intoxikacích. Inhibuje vstup a transport mykotoxinů *Amanita phalloides* (amanitin, falloidin) do hepatocytů a používá se jako antidotum při otravách houbami (Abenavoli *et al*, 2010).

Silybin ukázal inhibiční účinek na snížení proliferace nádorových buněk prostaty v několika *in vitro* experimentech a preklinických studiích (Cheung *et al*., 2010), proliferaci tumorózních buněk (Flaig *et al*., 2010) a angiogenezi (Singh *et al*., 2008). Angiogeneze je pravděpodobně inhibována redukcí růstového faktoru fibroblastů a vaskulárního endoteliálního růstového faktoru.

V poslední době byly popsány zajímavé účinky silybinu v oblasti regulace buněčného cyklu a jeho potenciálního kancerostatického působení. Silybin inhibuje mitogenní signalizační dráhy a působí na regulaci buněčného cyklu, což vede k inhibici růstu a smrti androgen-independentních nádorových buněk prostaty *in vitro*

(Agarwal *et al*., 2006). Po intenzivním laboratorním výzkumu a *in vivo* experimentech se ukazuje, že silybin moduluje celou škálu intracelulárních regulačních procesů. Mechanistické studie prováděné na molekulární úrovni ukázaly možnost použití silybinu v několika nových klinických indikacích, z nichž nejslibnějšími jsou chemoprevence a fotoprotekce kůže, podpůrná terapie a prevence nádorů prostaty (Deep *et al*., 2007; Singh *et al*., 2006).

Z *in vitro* studií a experimentů zaměřených na ovlivnění adenokarcinomu prostaty lze uvést stručně následující poznatky. Silybin snižuje sekreci PSA u lidských androgen-dependentních nádorových buněk (LNCaP) (Thelen *et al*., 2004). Silybin B má estrogenní aktivitu (Plíšková *et al*., 2005). Silybin moduluje signální dráhu řízenou NF–κB, což vede ke zvýšení senzitivity linií buněk karcinomu prostaty DU145 k apoptóze vyvolané tumor nekrotizujícím faktorem α (TNFα) (Dhanalakshmi *et al*., 2002). Silybin stimuluje diferenciaci, indukuje apoptózu i antiangiogenní aktivitu. Silybin při chemoterapii nesnižuje účinky doxorubicinu u linií DU145 a zvyšuje citlivost buněk k apoptóze vyvolané cisplatinou a karboplatinou (Dhanalakshmi *et al*., 2003).

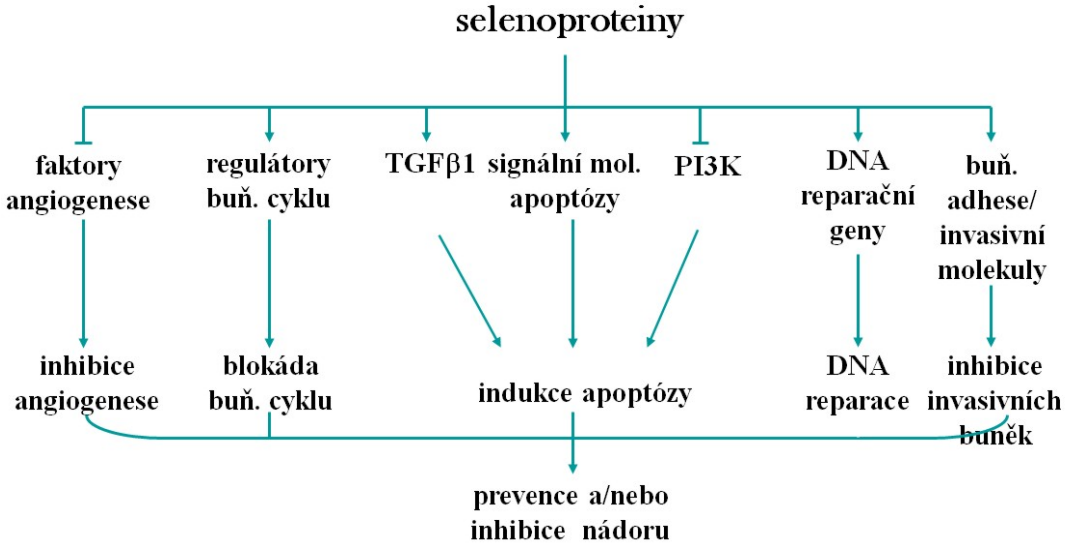
Silybin byl předmětem výzkumu při podpůrné terapii adenokarcinomu prostaty. Dvojitě slepé, zkřížené, placebem kontrolované studie se účastnilo 41 pacientů léčených pro karcinom prostaty (s nenulovým PSA po radikální prostatektomii nebo radioterapii). Účastníkům byl podáván silybin spolu s lykopenem a isoflavony sóji (Schroder *et al*., 2005). U účastníků došlo k poklesu PSA a nebyly pozorovány vedlejší účinky.

Silymarin se jeví jako bezpečný, bez nežádoucích účinků a jeho výhodou je výborná kompliace při několikaletém užívání (Wellington *et al*., 2001). Na různých myších modelech byly testovány i extrémně vysoké dávky (2 g/kg/den) bez pozorovaných toxických a vedlejších projevů (Singh *et al*., 2002; Gažák *et al*., 2007). Silybin je vzhledem ke své netoxicitě i při dlouhodobém užívání nadějný pro chemoprevenci karcinomu prostaty a jeví se jako perspektivní pro podpůrnou léčbu KP při chemoterapii či po radikální prostatektomii. Jak silymarin, tak silybin má i „vedlejší" pozitivní účinky jako hepatoprotektivum.

3.3.3 Selen

Selen patří mezi esenciální stopové prvky. Do organismu se dostává ve formě selenocysteinu, selenomethioninu a anorganických solí. Doporučená denní dávka je 55 μg a tolerovaná denní horní hranice příjmu pro dospělé je 400 μg.

Selen je znám svými antioxidačními vlastnostmi – je součástí antioxidačních enzymů a selenoproteinů). Selen ovlivňuje antiproliferaci buněk, apoptozu a moduluje hladinu androgenů a thyroidních hormonů, ovlivňuje metabolismus růstového hormonu a růstovému faktoru podobnému inzulinu 1 (insulin-like growth factor-1, IGF 1) (obr. 2). Může také způsobit dermatitidy, ztrátu nehtů či vlasů (Helzlsouer *et al*., 2000). Epidemiologické studie ukazují, že nízká hladina selenu je spojena s dysfunkcí imunitního systému a vyšším rizikem některých nádorových onemocnění včetně KP (Combs *et al*., 1997).



Obr. 2. Signální dráhy aktivované selenem.

Na vztah selenu a KP existují různé názory (Allen *et al*., 2008). Rozsáhlé studie potvrdily, že obohacení stravy selenem snižuje riziko KP, zejména u mužů s nízkou hladinou selenu v séru (Ma *et al*., 2009; Yoshizawa *et al*, 1998). Ve studii NPC (**N**utritional **P**revention of **C**ancer study) byl studován na skupině osob (1312 mužů a žen) vliv denní dávky selenu (200 μg) na snížení rizika recidivy nemelanomových kožních nádorů. Tato hypotéza potvrzena nebyla, bylo však zjištěno statisticky

významné snížení incidence KP u mužů (střední doba sledování byla více než 7 let). U mužů s hladinou PSA do 4 ng/ml byla incidence KP nižší o 65 %, zatímco u mužů s hladinou PSA nad 4 ng/ml nebyla ovlivněna (Duffield-Lillico *et al*., 2003).

Při zhodnocení výsledků studie HPFS (**H**ealth **P**rofessionals **F**ollow-Up **S**tudy), do které se zapojilo 51 529 mužů, se ukázalo, že muži s nejvyšším kvintilem hladiny selenu měli o 51 % menší výskyt pokročilého KP oproti mužům s nejnižším kvintilem hladiny selenu (Yoshizawa *et al*., 1998).

Na základě slibných výsledků se selenem a vitamínem E byla v roce 2001 zahájena prospektivní, randomizovaná, dvojitě slepá a placebem kontrolovaná multicentrická studie SELECT (**SEL**enium and Vitamin **E** **C**ancer Prevention **T**rial), do které bylo zařazeno 35 534 zdravých mužů s normálním nálezem per rektum a hladinou PSA do 4 ng/ml. Předpokládaná doba sledování účastníků byla stanovena na 7–12 let. Konec studie byl plánován v roce 2013. Muži byli náhodně rozděleni do 4 skupin podle podávané testované látky – vitamínu E (400 mg racemického α-tokoferolu), selenu (200 μg 1-selenomethioninu) nebo jejich kombinace a placeba (Lippman *et al*., 2005). Tato studie patří v oblasti chemoprevence KP nutraceutiky mezi nejvýznamnější a nejrozsáhlejší a svým designem je srovnatelná se studiemi PCPT a REDUCE. Bohužel v říjnu 2008 na základě druhé průběžné analýzy bylo doporučeno přerušení studie, protože analyzovaná data ukázala, že selen, vitamin E nebo kombinace selenu s vitaminem E nemají preventivní účinek na KP u obecně zdravé heterogenní populace mužů zařazených do studie SELECT (Lippman *et al*., 2009). Tyto závěry ovšem nevylučují, že suplementace selenem může mít pozitivní vliv na muže s rizikem vývoje pokročilého KP nebo snižovat riziko progrese probíhajícího KP. Úkolem do budoucna je identifikace mužů se specifickým genotypem nebo fenotypem tumoru, kde by užívání selenu mělo význam (Chan *et al*., 2009).

3.3.4 L-Arginin

Pro fyziologii a symptomy močového ústrojí je důležitou signální molekulou oxid dusnatý. Substrátem pro syntézu oxidu dusnatého je semiesenciální aminokyselina L-arginin (Harrison, 1997). Přirozeně se L-arginin nachází v mléčných výrobcích, hovězím, vepřovém a drůbežím mase, mořských plodech, sóji a cereáliích. Oxid dusnatý aktivuje přeměnu guanosintrifosfátu (GTP) na cyklický guanosinmonofosfát (cGMP). Enzymem zodpovědným za tvorbu oxidu dusnatého je NO-syntáza, u které jsou známy tři izoenzymy. V dolním močovém traktu má oxid dusnatý vliv na metabolismus a funkci několika druhů cílových buněk a to hladké svaloviny detrusoru, příčně pruhované svaloviny močového sfinkteru, intersticiální a epiteliální buňky močového měchýře a hladké svaloviny močové trubice (Stothers *et al*., 2003). Suplementace L-argininem prokázala zvýšení hladiny oxidu dusnatého v tkáních dolních cest močových a subjektivní zlepšení erekce a mikčních symptomů (Chen et al., 1999).

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5 Přehled vlastních výsledků

Tato část obsahuje synopse sedmi publikovaných studií, které jsou předmětem habilitační práce.

Studie 1

Vostalova J, **Vidlar A**, Ulrichova J, Vrbkova J, Simanek V, Student V. Use of selenium–silymarin mix reduces lower urinary tract symptoms and prostate specific antigen in men. Phytomedicine 2013; 21:75–81. **IF (2013) 2,877**

Studie 2

Valentová K, **Vidlář A**, Zatloukalová M, Stuchlík M, Vacek J, Šimánek V, Ulrichová J. Biosafety and antioxidant effects of a beverage containing silymarin and arginine. A pilot, human intervention cross-over trial. Food and Chemical Toxicology 2013; 56:178–183. **IF (2013) 2,61**

Studie 3

Vidlar A, Vostalova J, Ulrichova J, Student V, Krajicek M, Vrbkova J, Simanek V. The safety and afficacy of a silymarin and selenium combination in men after radical prostatectomy – Six month placebo-controlled double-blind clinical trial. Biomed Pap Med Fac Univ Palacky 2010;154(3):239–44. **IF (2010) 0,716**

Studie 4

Vostalova J, **Vidlar A**, Simanek V, Galandakova A, Kosina P, Vacek j, Vrbkova j, Zimmermann BF, Ulrichova J, Student V. Are high proanthocyanidins key to cranberry efficacy in the prevention of recurrent urinary tract infection? Phytother Res 2015; 29(10):1559-67. **IF (2015) 2,66**

Studie 5

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Studie 6

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Studie 7

Vidlar A, Vostalova J, Bouchal J, Kolar Z, Kral M, Ulrichova J, Simanek V, Student V. Cranberry Intervention in Patients with Localized Prostate Cancer prior to Radical Prostatectomy. Biomed Pap Med Fac Univ Palacky 2016;160(4):559-565. **IF (2016) 0,924**

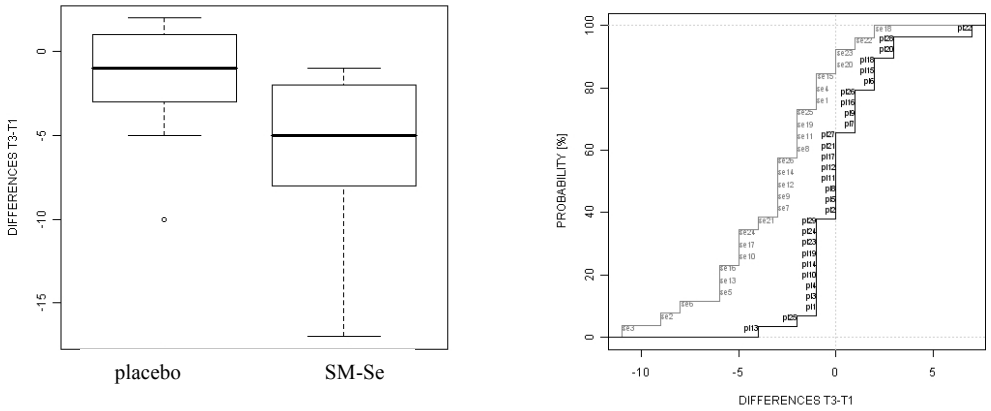
5.1 Vliv kombinace silymarinu se selenem na urologické parametry u mužů s LUTS

(studie 1)

Cílem pilotní dvojitě slepé, randomizované a placebem kontrolované studie bylo ověření vlivu šestiměsíčního užívání kombinace 570 mg silymarinu (SM) a 240 μg selenu (v formě L-selenometioninu)/den u mužů s LUTS a PSA ≤ 2.5 ng/ml. Zařazeno bylo celkem 56 mužů (29 do skupiny placebo a 26 do skupiny SM-Se).

Výsledky ukázaly statisticky významné rozdíly mezi skupinou placebo a SM-Se u následujících parametrů: snížení hladiny PSA_{tot}, zvýšení hladiny selenu, snížení IPSS skóre (obrázek 3), zlepšení kvality života a parametrů uroflowmetrie (Q_{max}, Q_{ave}, V a V_{rez}). Vliv na hladinu testosteronu nebyl prokázán. Celkově bylo užívání SM-Se velmi dobře tolerováno, bez nežádoucích účinků. Na základě těchto výsledků lze předpokládat, že zvolená kombinace silymarinu a selenu může mít příznivý vliv na zlepšení močení a zachování zdraví prostaty u mužů spolu s velmi dobrou snášenlivosti.

a) b)



Obr. 3. Vliv SM-Se na celkové IPSS skóre ve skupině placebo a SM-Se.
Hodnoty jsou vyjádřeny jako rozdíl mezi dnem 180 (T3) a dnem 0 (T1) studie.
a) boxový graf
b) graf kumulativní distribuční funkce skupin SM-Se (šedá) a placebo (černá), čísla v blízkosti linií odpovídají číslu každého účastníka
$p < 0,05$ SM-Se *vs* placebo skupina

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Use of selenium–silymarin mix reduces lower urinary tract symptoms and prostate specific antigen in men

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ABSTRACT

The aim of this double-blind, placebo controlled clinical trial was to assess the effects of a combination of selenium and silymarin in men with lower urinary tract symptoms, benign prostatic hyperplasia and a prostate specific antigen (PSA) ≤2.5 ng/ml. The volunteers were randomized to two groups: the first one ($n = 26$) received 240 μg selenium (in the form of yeast L-selenomethionine) plus 570 mg silymarin daily for 6 months and the second ($n = 29$) received placebo. Outcome measures were changes in the International Prostate Symptom Score (IPSS), bladder volume (V), urinary flow rate, ultrasound estimated postvoid residual urine volume (RV), serum PSA, testosterone and selenium levels, safety clinical biochemistry, hematology and oxidative stress parameters at baseline and on day 180. The results showed statistically significant differences ($p < 0.05$) between treatment and control groups for the following parameters: IPSS score, urodynamic parameters: maximal rate of urine flow (Q_{max}), average flow (Q_{ave}), V and RV, total PSA value and serum selenium levels. There was a significant reduction in PSA in the selenium–silymarin group but no effect on blood testosterone level. Overall the treatment was well-tolerated with no adverse effects.

Introduction

Prostate health is increasingly important in older men. Two of the most common prostate conditions affecting men older than 40 years are benign prostatic hyperplasia (BPH) and chronic prostatitis (Wang et al., 2012). They are associated with bothersome lower urinary tract symptoms (LUTS) and can lead to a number of medical complications such as untreated acute urinary retention, gross hematuria, repeated urinary tract infections, obstructive uropathy and cystolithiasis. Conventional treatments are α-andrenergic receptor blockers, 5-α-reductase inhibitors or antibiotics (Oesterling, 1995). Recent years however have seen increasing interest in natural therapies which support prostate health and show prophylactic effects on LUTS. Protective effects are generally confirmed in clinical trials for the micronutrients selenium, vitamins D and E, curcumin, resveratrol, lycopene, omega-3 polyunsaturated fatty acids (omega-3 PUFA), and phytoestrogens (genistein and daidzein). In dietary supplements, containing powdered whole plant/plant extract or seed oil, the prominent active components are from green tea (*Camellia sinesis*), saw palmetto berries (*Serenoa repens*), pumpkin seeds (*Cucurbita pepo*), flax seeds (*Linum usitatissimum*), roots of stinging nettle (*Urtica dioica*) and silymarin (*Silybum marianum*) (Demark-Wahnefried, 2008; Ma and Chapman, 2009; Syed et al., 2007). The latter is often used as a supplement by prostate cancer patients (Kren and Walterova, 2005; Vidlar et al., 2010). Silibinin, the major flavonolignan of silymarin, has demonstrated interesting preventive and anticancer properties in prostate cancer animal models (Klempner and Bubley, 2012). Recently two studies were published on the influence of selenium or omega-3 PUFA on PSA in healthy men. Zhang et al. (2011) reported that a 3-month supplementation with 200 μg selenium (in the form of glycinate) per day increased plasma and erythrocyte glutathione peroxidase (GPX) and lowered serum PSA. Twelve weeks daily consumption of omega-3 PUFA (1.12 g of eicosapentaenoic and 0.72 g docosahexaenoic acids) or coenzyme Q_{10} (100 mg) significantly reduced serum PSA level in healthy men with a PSA ≤ 2.5 ng/ml (Safarinejad et al. 2012).

The randomized, double-blind pilot trial reported here was based on the hypothesis that a 6-month daily supplementation with a selenium and silymarin combination will reduce (i) voiding symptoms, (ii) improve urodynamic parameters and (iii) lower

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values for serum PSA more than the components singly. The daily dose of selenium–yeast (240 μg) and silymarin (570 mg) was used based on Vidlar et al. (2010), the selenium dose was based on serum levels of selenium according to the U-shaped dose response curve (Dennert et al., 2011).

Materials and methods

Selenium–silymarin mix and placebo

The selenium–silymarin mix (Se–SM) and placebo tablets were supplied by FAVEA (Kopřivnice, Czech Republic). The Se–SM tablets contained a combination of 80 μg selenium as L-selenomethionine in inactivated whole cell yeast (Lalmin® Se2000, Lallemand Human Nutrition A/S, Birkerød, Denmark) and 190 mg of silymarin (SM) of the following composition (%; w/w): taxifolin 4.13, silychristin 17.00, silydianin 7.70, silibinin A 23.66, silibinin B 29.01, isosilibinin A+B 11.38, and undefined polymeric components 7.11 (silymarin; lot 040105, Teva Pharmaceuticals Industry LTD, Opava, Czech Republic), microcrystalline cellulose (250 mg), isomalt (60 mg), and hydroxypropyl cellulose (10 mg). The placebo tablets consisted of microcrystalline cellulose (250 mg), isomalt (250 mg), and hydroxypropyl cellulose (10 mg). The verum and placebo tablets were coated with hypromellose. The appearance and organoleptic characteristics of verum and placebo tablets were identical. The tablets were provided in blister packs labeled Selenium–Silymarin.

Study volunteers and inclusion/exclusion criteria

Recruitment was carried out between November 2011 and February 2012 at the Department of Urology, University Hospital in Olomouc, Czech Republic. A total of 55 non-smoking and non-alcohol dependent men aged 45–70 years were invited to participate in the study. All subjects were relatively healthy but had LUTS and BPH. At the beginning of the study the serum PSA_{tot} level was 0.18–2.53 ng/ml. None of the volunteers had food allergies, chronic liver or kidney diseases, gastrointestinal or metabolic disorder or any other chronic health condition such as diabetes mellitus identified from the findings of the interview. They were instructed not to change diet or lifestyle during the study. Exclusion criteria included clinically and/or histopathologically proven prostate cancer, histological findings of acute or chronic non-bacterial prostatitis, pathological urinary sediment and positive bacterial cultures of urine. Exclusion criteria also included: consumption of food rich in soy isoflavonoids, dietary supplements of any kind, medication with possible effects on prostate health such as antibiotics, anti-inflammatory drugs, alpha-1-adrenoreceptor antagonist and 5-alpha-reductase inhibitors.

Study design

The study was conducted according to the guidelines laid down in the Declaration of Helsinki. All procedures involving human subjects were approved by the Ethics Committee of the University Hospital and the Faculty of Medicine and Dentistry, Palacký University in Olomouc, Czech Republic. The participants signed an informed consent and were aware of the study goals from the outset. They were assigned to placebo ($n = 29$, aged 55.0 ± 10.0 years) and Se–SM ($n = 26$, aged 55.0 ± 5.8 years) groups by the simple (unrestricted) randomization. In the Se–SM group, three tablets daily were taken at approximately equal intervals throughout the day for a 6-month period. The placebo group took placebo tablets (3 tablets/day) for the same duration.

Health investigation, participant compliance and withdrawals

During the health examination on the first day, after 3-months, and on the last day of the trial the following parameters were routinely assessed: (i) detailed medical history; (ii) assessment of all concurrent medical drugs and therapies; (iii) digital rectal examination; (iv) dietary habits; (v) filling of International Prostate Symptom Score (IPSS); (vi) urine analysis; (vii) uroflowmetry with postvoidal residual urine (RV); (viii) kidney and bladder ultrasound; and (ix) a blood laboratory analysis. All blister packs were collected at the second visit and at the end of trial to check patient compliance. All subjects completed the 6 month period.

Assessment of LUTS

The volunteers completed the IPSS questionnaire. In addition to the total IPSS score, each of the seven components of the questionnaire (feeling of incomplete emptying, frequency, intermittency, urgency, weak stream, hesitancy, and nocturia) and quality of life (QoL) were used. Uroflowmetry data: maximal urinary flow rate (Q_{max}), average urinary flow rate (Q_{ave}) and bladder volume (V) were measured using FlowMic (Medkonsult, Czech Republic). RV was assessed using a BK Medical Viking 2400 with abdominal probe 3.5–5 MHz. RV was calculated using the formula for a prolate ellipsoid (width × length × height × 0.523).

Clinical biochemistry and hematology

Basic biochemical and hematological parameters were determined in all samples immediately after sampling: sodium, potassium, chloride, total cholesterol, LDL cholesterol, HDL cholesterol, triacylglycerols (TAG), apoA1, apoB, C-reactive protein (CRP), lactate dehydrogenase (LD), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GMT), alkaline phosphatase (ALP), urea, creatinine, bilirubin, and testosterone (TST) using a HITACHI Modular Evo P analyzer (Hitachi, Japan). PSA in serum was determined using an Architect type LEIA analyzer (Abbott Laboratories, Abbott Park, IL, USA). Selected parameters for evaluation of oxidative stress were determined as total antioxidant capacity (TAC) and total SH groups in plasma (T-SH), lipid peroxidation products such as malondialdehyde in plasma (PMDA) and erythrocytes (MDA), advanced oxidation protein products (AOPP) in plasma; glutathione (GSH); glutathione peroxidase (GPX); catalase (CAT); glutathione reductase (GSR); glutathione transferase (GST); superoxide dismutase (SOD) in erythrocytes as described by Vidlar et al. (2010). Selenium in plasma was determined by atomic absorption spectrometry using the AA6300 instrument (Shimadzu, Japan). Hemoglobin (Hb), hematocrit (Htc), erythrocytes (RBC), thrombocytes (PLT) and leukocytes (WBC) were measured in Na_2EDTA blood.

Urinanalysis

Urine samples were collected from a midstream clean catch and analyzed using the IQ200 Automated Urinanalysis System (IRIS International, Inc., USA).

Statistical methods

Nonparametric Wilcoxon two-sided tests (paired and unpaired) were used to determine the statistical significance between parameter values on day 0 and after 6 months and between the placebo and Se–SM groups. The level of significance was set at 5%. Values

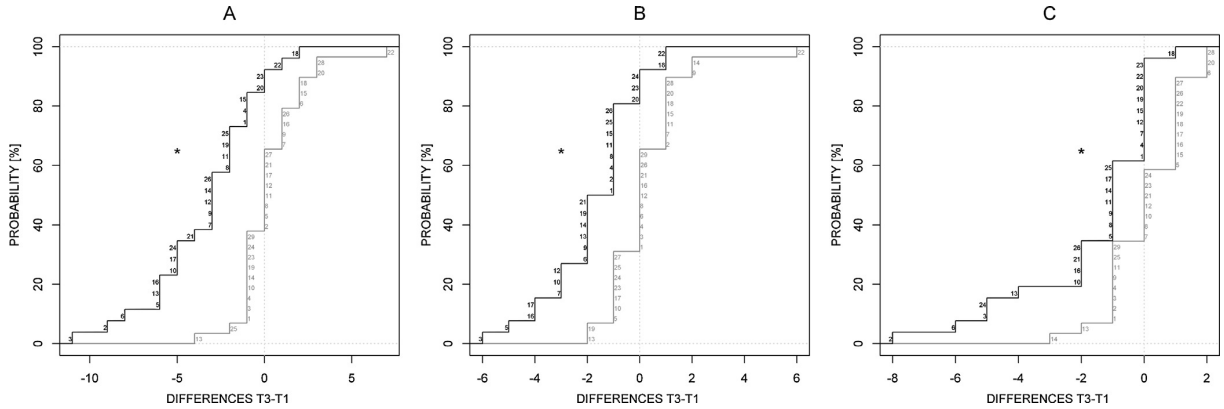

Fig. 1. Effect of selenium and silymarin mix on International Prostate Symptoms Score (Total IPSS; A), irritative (B) and obstructive (C) questions during 6 month treatment. The values are expressed as differences between day 180 and day 0 of study. Se–SM group, black line; placebo group, gray line. *$p < 0.05$ vs placebo group.

Table 1
Baseline demographics and clinical characteristics.

	Overall	Placebo	Se–SM
Age (year)	55.0 ± 8.2	55.0 ± 10.0	55 ± 5.8
BMI	27.95 ± 3.75	27.51 ± 3.55	28.45 ± 3.96
QoL	0.91 ± 0.87	0.69 ± 0.81	1.15 ± 0.88
IPSS	6.04 ± 4.71	5.69 ± 4.93	8.19 ± 5.65
PSA_{tot} (μg/l)	1.13 ± 0.85	0.99 ± 1.00	1.28 ± 0.62*
Q_{max} (ml/s)	18.49 ± 6.88	20.10 ± 7.10	16.70 ± 6.28
Q_{ave} (ml/s)	11.92 ± 4.48	13.27 ± 4.54	10.40 ± 3.95*
V (ml)	307.6 ± 148.1	326.7 ± 150.8	286.4 ± 144.9
RV (ml)	11.5 ± 19.8	5.1 ± 9.9	18.7 ± 25.2*
Selenium (μmol/l)	1.50 ± 0.58	1.54 ± 0.66	1.42 ± 0.49

The values are expressed as mean ± SD.
* $p < 0.05$ vs placebo.

are presented as 1st quartile/median/3rd quartile and means ± SD. Box plots and graphs of empirical cumulative distribution functions were used as graphic illustration of significant differences in parameters over 6 months.

Results

At baseline the groups had similar clinical and demographic characteristics except for significant differences between the PSA_{tot} value in Se–SM vs placebo group (Table 1).

The Se–SM tablets were well-tolerated with no reported adverse effects. The key LUTS were evaluated using the IPSS questionnaire (Table 2). All those who received Se–SM showed statistically significant improvement in total IPSS score, irritation and obstruction syndrome and quality of life question parts in contrast to the placebo group (Fig. 1 and Table 2). Parameters of urination (Q_{max}, Q_{ave}, V, RV) were significantly better only in the Se–SM group on day 180 (Fig. 2 and Table 3). Hematology values were unchanged with the exception of significant HTc and PLT increase in the placebo group which however were within physiological limits (Table 4). The Se–SM group started with a higher mean PSA_{tot} than the placebo group. At the end of trial PSA_{tot} significantly increased in the placebo group (+7.3%). In the Se–SM group the level of PSA_{tot} was lower (−6.3%) (Fig. 4 and Table 5). Both groups had an almost identical starting plasma selenium concentration (Table 1).

Table 2
International Prostate Symptom Score (IPSS) and quality of life in placebo and SM–Se groups.

	Difference between answer at 6 months and day 0	
	Placebo	Se–SM
Total IPSS	0.276 ± 1.980	−3.385 ± 3.073*
Irritation questions	0.207 ± 1.521	−1.769 ± 1.704*
Obstruction questions	0.069 ± 1.223	−1.615 ± 2.210*
Incomplete emptying	0.034 ± 0.499	−0.231 ± 0.652
Over the past month, how often have you had a sensation of not emptying your bladder completely after you finish urinary?		
Frequency	0.000 ± 0.756	−0.731 ± 1.002*
Over the past month, how often have you had to urinate again less than two hours after finished urinating?		
Intermittency	−0.138 ± 0.516	−0.577 ± 1.065
Over the past month, how often have you found you stopped and started again several times when you urinated?		
Urgency	−0.069 ± 0.842	−0.385 ± 0.941
Over the last month, how difficult have you found it to postpone urination?		
Weak stream	0.138 ± 0.743	−0.538 ± 1.174*
Over the past month, how often have you had a weak urinary stream?		
Straining	0.034 ± 0.680	−0.269 ± 0.604
Over the past month, how often have you had to push or strain to being urination?		
Nocturia	0.276 ± 0.702	−0.654 ± 0.846*
Over the past month, how many times did you most typically get up to urinate from the time you went to bed until the time you got up in the morning?		
Quality of life	0.069 ± 0.593	−0.731 ± 0.724*

The values are expressed as mean ± SD.
* $p < 0.05$ vs placebo.

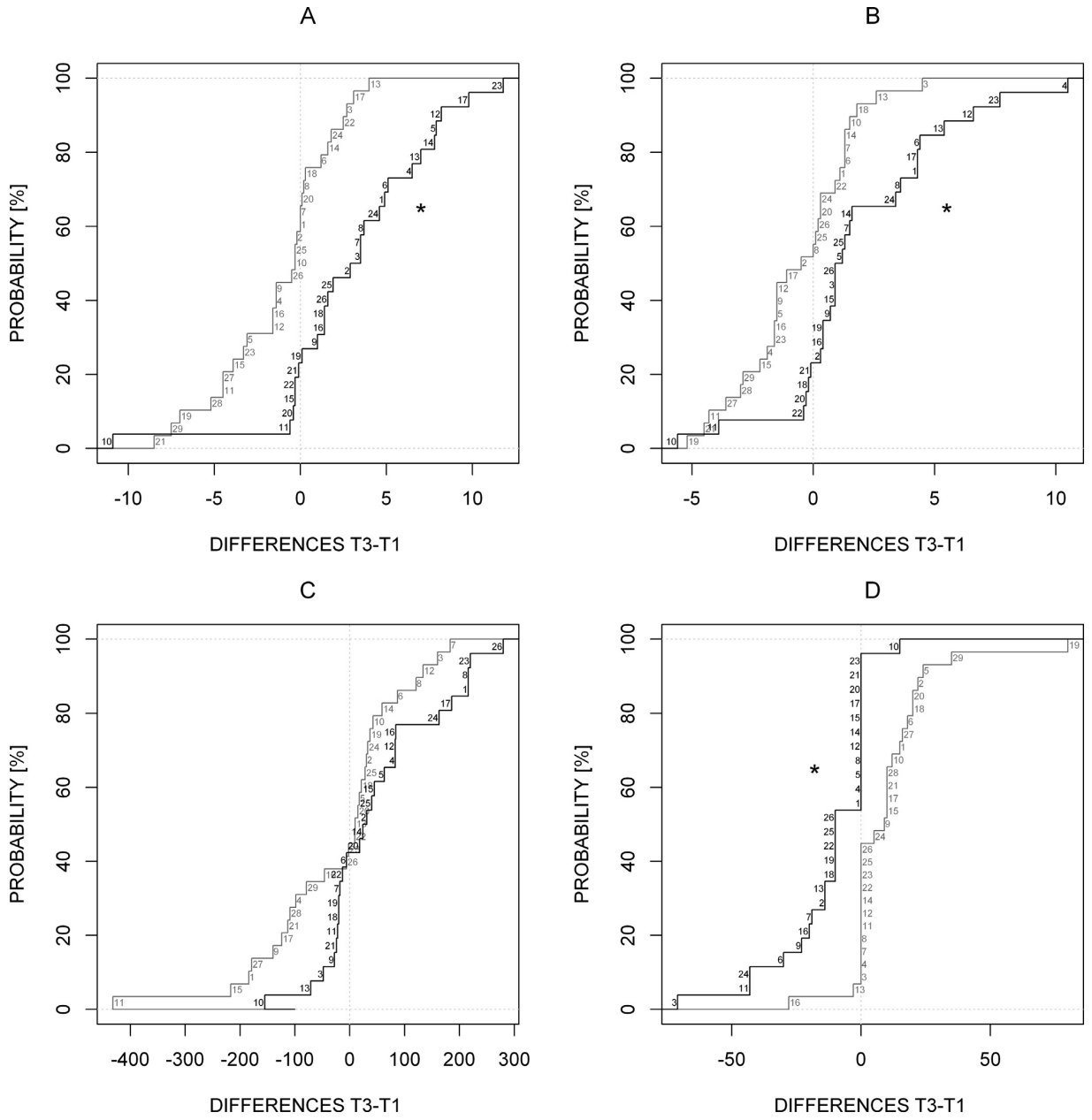

Fig. 2. Effect of selenium and silymarin mix on uroflowmetry parameter maximal urinary flow rate (Q_{max}; A), average urinary flow rate (Q_{ave}; B), bladder volume (V; C) and postvoid residual urine (RV; D) volumes during 6 month treatment. The values are expressed as differences between day 180 and day 0 of study. Se–SM group, black line; placebo group, gray line. *$p < 0.05$ vs placebo group.

Table 3
Values of uroflowmetry in placebo and Se–SM groups.

Parameter	Placebo		Se–SM	
	Day 0	6th month	Day 0	6th month
Q_{max} (ml/s)	15.3/18.7/25.6	13.5/17.7/24.2	14.2/16.6/20.2	15.8/20.4/24.8*
Q_{ave} (ml/s)	8.9/13.3/17.2	9.5/12.3/15.8	7.7/10.3/11.4	9.8/12.7/14.5*
V (ml)	210.0/323.0/412.2	184.0/257.1/380.0	177.3/287.5/364.0	245.3/305.5/411.5*
RV (ml)	0.0/0.0/6.0	0.0/10.0/20.0*	0.0/10.0/28.5	0.0/0.0/7.5*

The values are expressed as 1st quartile/median/3rd quartile
* $p < 0.05$ vs day 0.

Table 4
Parameters of hematology in placebo and Se–SM groups.

Parameter	Placebo		Se–SM	
	Day 0	6th month	Day 0	6th month
Hb (g/l)	149/153/157	147/154/157	147/158/166	148/157/163
RBC (10^{12}/l)	4.89/5.03/5.29	4.79/4.97/5.17	4.86/5.00/5.31	4.91/5.06/5.18
WBC (10^9/l)	4.75/5.60/6.53	4.94/5.65/6.50	5.21/6.35/6.92	4.82/5.76/7.04
Htc	0.43/0.44/0.46	0.42/0.44/0.45*	0.43/0.45/0.47	0.43/0.44/0.47
PLT (10^9/l)	178/192/255	178/200/264*	196/217/245	193/233/247

Values are expressed as 1st quartile/median/3rd quartile.
* $p < 0.05$ vs day 0.

Table 5
Markers of clinical chemistry in placebo and Se–SM groups.

Parameter	Placebo		Se–SM	
	Day 0	6th month	Day 0	6th month
Na (mmol/l)	138/139/140	138/139/141	139/140/141	139/141/142
K (mmol/l)	4.14/4.25/4.55	4.04/4.19/4.37*	4.17/4.36/4.60	4.12/4.31/4.48
Cl (mmol/l)	101/103/104	101/104/107	102/104/106	103/105/106
Urea (mmol/l)	4.5/4.9/5.3	4.5/5.2/5.8	4.23/4.80/5.40	4.53/5.30/5.97*
Creatinine (μmol/l)	74/78/84	75/82/88*	76/80/88	79/87/91
Bilirubin (μmol/l)	6/8/10	6/9/12	6.0/7.5/13.0	7.3/10.0/15.3
ALT (μkat/l)	0.39/0.51/0.62	0.40/0.49/0.71	0.48/0.56/0.66	0.45/0.56/0.70
AST (μkat/l)	0.41/0.50/0.58	0.44/0.50/0.61	0.42/0.49/0.59	0.42/0.47/0.57
ALP (μkat/l)	1.31/1.60/1.83	1.24/1.49/1.71	1.11/1.32/1.90	1.12/1.36/1.75
GMT (μkat/l)	0.40/0.54/0.90	0.35/0.52/0.77	0.35/0.58/0.78	0.33/0.64/0.91
LD (μkat/l)	2.47/2.64/2.91	2.51/2.67/2.90	2.69/2.84/3.09	2.52/2.82/3.05
CRP (mg/l)	1/1/2	1/1/1.25*	1/1/2	1/1/2
Cholesterol (mmol/l)	4.52/5.27/6.00	4.53/5.00/5.50*	4.67/5.03/5.56	4.48/5.08/5.95
TAG (mmol/l)	1.13/1.36/2.26	1.27/1.74/2.20	1.12/1.73/2.31	1.17/1.67/2.23
HDL (mmol/l)	1.12/1.29/1.46	1.01/1.21/1.35*	1.15/1.33/1.51	1.15/1.31/1.47
LDL (mmol/l)	2.49/3.22/3.96	2.40/3.08/3.37	2.58/2.91/3.48	2.58/2.99/3.65
ApoA1 (g/l)	1.38/1.49/1.59	1.30/1.38/1.46*	1.38/1.52/1.73	1.38/1.50/1.66
ApoB (g/l)	0.68/0.82/1.08	0.82/0.95/1.08*	0.73/0.84/0.98	0.78/0.94/1.09*
PSA_{tot} (μg/l)	0.45/0.71/1.17	0.54/0.71/1.21*	0.76/1.19/1.65	0.72/1.15/1.70
PSA_f (μg/l)	0.32/0.34/0.96	0.40/0.69/1.21	0.31/0.34/0.51	0.34/0.42/0.48
PSA_f/PSA_{tot}	0.22/0.24/0.26	0.26/0.27/0.30	0.18/0.26/0.29	0.21/0.28/0.32
TST (nmol/l)	15.6/17.5/19.3	14.1/16.8/19.6	14.0/18.6/24.7	14.1/19.5/22.9
Selenium (μmol/l)	1.05/1.42/1.88	0.80/0.91/1.26*	1.17/1.33/1.62	1.18/1.71/2.05

The values are expressed as 1st quartile/median/3rd quartile.
* $p < 0.05$ vs day 0.

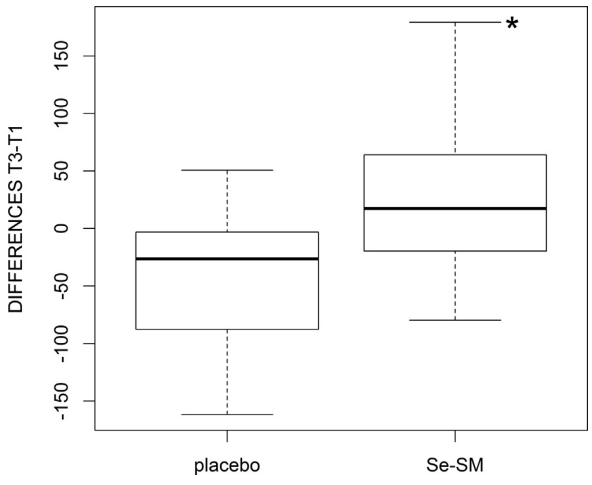

Fig. 3. Effect of selenium and silymarin mix on plasma selenium level during 6 month treatment. The values are expressed as differences between day 180 and day 0 of study. The box graphs show the median as the middle line. The box extends from the 25th to the 75th percentile. *Median was significantly different from that of the placebo group ($p < 0.05$).

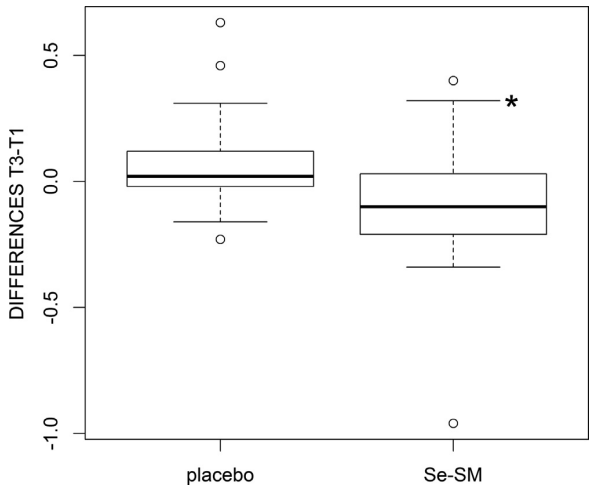

Fig. 4. Effect of selenium and silymarin mix on plasma PSA level during 6 month treatment. The values are expressed as differences between day 180 and day 0 of study. The box graphs show the median as the middle line. The box extends from the 25th to the 75th percentile. *Median was significantly different from that of the placebo group ($p < 0.05$).

Table 6
Markers of oxidative stress in placebo and Se–SM groups.

Parameter	Placebo		Se–SM	
	Day 0	6th month	Day 0	6th month
PMDA (nmol/g)[a]	59.13/67.56/75.98	52.14/60.89/73.64	49.98/61.86/76.97	47.19/55.36/78.07
AOPP (μmol/l)	125.5/166.0/221.9	140.6/165.4/203.3	130.5/164.0/205.5	129.5/164.1/208.2
TAC (nA)	5.20/5.94/6.41	5.06/5.74/6.73	4.49/5.90/6.71	4.74/5.74/6.71
T-SH (μmol/g)[a]	3.48/4.01/4.34	3.07/3.65/4.68	2.78/3.69/4.57	2.59/3.66/4.30
MDA (nmol/g)[b]	0.32/0.38/0.45	0.37/0.46/0.54*	0.24/0.42/0.49	0.24/0.42/0.60
GSH (μmol/g)[b]	10.10/11.33/12.71	10.54/11.41/13.01	9.00/10.48/12.18	9.42/10.93/12.17
SOD (U/g)[b]	2.41/2.53/2.85	2.40/2.75/2.96	2.17/2.56/3.00	2.33/2.54/2.92
GPX (μmol/min/g)[b]	21.77/26.25/33.54	23.09/26.83/32.29	19.21/22.71/26.14	19.75/24.91/30.12
CAT (μmol/min/g)[b]	38.24/123.1/152.56	39.51/135.92/166.16*	42.13/132.62/149.29	48.71/132.45/153.47*
GST (μmol/min/g)[b]	9.53/15.50/33.92	8.53/13.14/33.70	8.54/11.21/27.97	8.37/11.42/26.85
GSR (μmol/min/g)[b]	3.81/5.66/7.89	4.71/6.61/7.96	4.27/5.74/6.68	5.35/5.92/7.09

The values are expressed as 1st quartile/median/3rd quartile.
[a] The value is expressed on g of protein.
[b] The value is expressed on g of hemoglobin.
* $p < 0.05$ vs day 0.

On day 180 the plasma selenium level was significantly lower in the placebo group. In the Se–SM group the end selenium values were statistically significant increased (Fig. 3 and Table 5).

In the placebo group, serum levels of cholesterol, HDL, and apoA1 were significantly decreased while levels of apoB increased in both groups. The positive effect of selenium–silymarin supplementation on cholesterol metabolism can be traced back to the stabilization of HDL and apoA1 levels in the Se–SM group (Table 5).

Although changes in the values of several physiological markers were significantly different after 6 months for both groups, the fluctuation was within normal physiological limits (Table 5). In neither group did oxidative stress markers reach statistical significance (Table 6). We found only non-significant higher amounts of lipid peroxidation products in the placebo and catalase activity in both groups. From this point of view, the Se–SM group was better protected against the oxidative stress even though the difference was not significant.

Discussion

Benign prostatic hyperplasia (BPH) is an age-related, non-malignant enlargement of the prostate gland and decreased caliber and force of urination are its classic signs. Poor stream, hesitancy, terminal dribbling, incomplete voiding and frequent urination, especially at night are common. These symptoms are collectively called lower urinary tract symptoms, LUTS (Sausville and Naslund, 2010). PSA is a serine protease with a role in semen liquefaction (Hernandez and Thompson, 2004). Starting in the late 1980s, serum PSA determination gained prominence as a means of screening for prostate cancer but it has low specificity. It remains, however, that PSA is a clinically useful maker in intervention studies with dietary supplements and it helps us to evaluate the effects of tested substances on prostate health (van Weerden and Schröder, 2008).

The plasma selenium concentration found to be associated with reduction in total prostate cancer risk is 135 ng/ml and recent publications caution against selenium over-supplementation (Dennert et al., 2011). Running clinical trials with selenium (e.g. high-selenium yeast and L-selenomethionine) have been reviewed by Parnes (2013).

Silymarin is a standardized extract of polyphenolics from the seeds of *Silybum marianum* (L.) Gaertn. (*Carduus marianus* L., Asteraceae; milk thistle) and is recommended mostly for liver protection (Kren and Walterova, 2005). SM is also a respected antioxidant with cytoprotective and hypocholesterolemic effects (Skottova and Krecman, 1998; Vidlar et al., 2010). It is well tolerated and no unpleasant effects or interactions of silymarin with commonly used drugs are known (Jancova et al., 2007). *In vitro* and in human xenografts, prostate anticancer activity of SM and silibinin has been demonstrated (Gazak et al., 2007; Klempner and Bubley, 2012). Selenium plays an important role in prostate health and prostate cancer prevention (Hurst et al., 2012).

This study tested the hypothesis that a 6-month daily supplementation of 240 μg selenium–yeast and 570 mg SM would reduce urination symptoms associated with BPH. The clinical trial confirmed that a daily dose of a selenium (in the form of Se yeast) and SM significantly reduced LUTS. A placebo treatment, however, did not. In participants taking the Se–SM mix for 6 months, there was a statistically decrease in the IPSS score (Fig. 1 and Table 2), in addition a marked improvement in all urodynamic parameters (Fig. 2 and Table 3). A study on rats showed that the effect of SM on urinary excretion was similar to potassium-sparing diuretics (On Alarc de la Lastra et al., 2006). This was confirmed in this trial as the potassium level was unchanged in the Se–SM group throughout the experiment. In the placebo group there was a statistically significant decrease in blood potassium (Table 5). These results indicate that SM had an effect on urinary excretion. Use of the Se–SM mix also affected serum PSA (Fig. 4 and Table 5). In the Se–SM group, there was a reduction in the PSA value (Fig. 4) without an affect on C-reactive protein or testosterone levels (Table 5). Seidlova-Wuttke et al. (2003) demonstrated that SM binds is exclusively to the estrogen receptor beta. Recently, we found that SM modulates endocrine functions by disruption of the estrogen receptor (Pliskova et al., 2005). This finding could explain the beneficial effect of silymarin in synergy with selenium on PSA. The decrease in PSA in the Se–SM group shows that prophylaxis due to the Se–SM combination may be as effective as 5-alpha-reductase inhibitor treatment (Tindall and Rittmaster, 2008). The use of selective 5-alpha-reductase inhibitors has often been linked to hormone changes associated with unpleasant sexual side effects, in particular, erectile dysfunction and decreased libido (Giuliano, 2006). Contrary to Zhang et al. (2011), six-month supplementation with Se–SM mix did not increase erythrocyte GPX activity (Table 6) even though in the Se–SM group plasma selenium increased after 3 months from 112.5 ± 38.41 ng/ml to 137.0 ± 42.2 ng/ml. Then it was stable until the end of the trial. Our results show that the trial with the Se–SM mix was safe and not associated with unphysiological levels of serum selenium (136.9 ± 48.4 ng/ml on day 180), which was stable around values recommended for prostate cancer prevention and redox status (Ghiselli et al., 2000).

Conclusions

This trial is the first, to the best of our knowledge, to evaluate the effects of a Se–SM combination on LUTS specifically in men with

BPH. It does not provoke unpleasant side effect. The results show the tested combination was very effective in reducing both voiding dysfunction and PSA. In the Se–SM group, no associations were found between Se–SM consumption and seleno-enzyme activities. Unlike currently used medication for LUTS, Se–SM mix has no adverse effects. Our findings may assist men suffering from LUTS and also their clinicians, to decide for a treatment that is both inexpensive and natural and without side effects. The limitation of this trial is the small number of subjects.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgment

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5.2 Vliv kombinace silymarinu s argininem na urologické parametry u zdravých mužů

(studie 2)

Cílem intervenční, zkřížené a placebem kontrolované klinické studie bylo ověřit účinky 10. denní konzumace nealkoholického piva obsahujícího kombinaci silymarinu a L-argininu na urodynamické a fyziologické paramentry u 22 zdravých mužů ve věku 38–59 let. Dle výsledků byla denní dávka 400 mg silymarinu a 295 mg L-argininu v 500 ml experimentálního nápoje dobře tolerována a bez vlivu na vybrané markery laboratorní medicíny. Ve srovnání s placebem nebyl nalezen statisticky významný vliv experimentálního nápoje na IPSS skóre a parametry uroflowmetrie (tab. 4). Klinicky zajímavým výsledkem se ukázalo statisticky významné zvýšení hladiny redukovaného glutationu v erytrocytech. Bylo experimentálně prokázáno, že za ochranu erytrocytů vůči oxidačnímu stresu odpovídají flavonolignany silymarinového komplexu.

	den 0	**placebo, 10. den**	**exp. nápoj, 10. den**
IPSS	2,0 (1,0-5,3)	3,0 (1,0-6,3)	3,0 (1,0-4,5)
Uroflowmetrie			
Qmax (ml/s)	26,8 (16,0-32,5)	23,0 (15,5-31,5)	24,8 (18,7-37,5)
Qave (ml/s)	16,9 (10,1-20,7)	15 (10,0-19,0)	15,4 (11,6-20,6)
Vol (ml)	349 (219-460)	270 (194-397)	335 (215-461)
Res (ml)	18,0 (0,0-27,0)	11,5 (0,8-29,0)	17,0 (0-35,0)

Tab. 4. Vliv 10. denní konzumace placeba/experimentálního nápoje na IPSS skóre a parametry uroflowmetrie.

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Biosafety and antioxidant effects of a beverage containing silymarin and arginine. A pilot, human intervention cross-over trial

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ABSTRACT

The study objective was to investigate the potential of a beverage containing silymarin and L-arginine to alter basic physiological and urodynamic parameters in 22 normal healthy men aged 38–59 years. The volunteers drank 500 ml/day beverage without silymarin and L-arginine for 10 days followed, after a 7-day washout period, by the beverage with 400 mg silymarin and 295 mg L-arginine for 10 days. Blood and urine samples were collected on days 0, 10 and 27. The beverages were well-tolerated with no adverse effects. Most of the biochemical, hematological and urodynamic parameters remained unchanged. Total antioxidant capacity, total level of antioxidants, lipoperoxidation products (malondialdehyde), advanced oxidation products of proteins in plasma and glutathione, glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase levels in erythrocytes were not influenced. Serum γ-glutamyl transferase, malondialdehyde level and activity of glutathione *S*-transferase in erythrocytes were lowered at day 27 and the concentration of total plasma SH-groups was higher on day 10. Using an *ex vivo* system, we found that silymarin/silybin at 10–100 μM is able to adsorb onto human erythrocytes and the complexes displayed antioxidant properties as studied using *ex situ* square-wave voltammetry. The trial showed that silymarin *in vivo* may protect erythrocytes against oxidative damage.

1. Introduction

Silymarin, an extract from the seeds of *Silybum marianum* (L.) Gaertn. (*Carduus marianus* L., Asteraceae; milk thistle), was originally known for its anti-phalloidin activity, and is used in the treatment of various liver disorders (Abenavoli et al., 2010; Wellington and Jarvis, 2001). The main component of silymarin, silybin (a mixture of two diastereomers A and B in approximately 1:1 proportion, in the literature also denoted as *silibinin*) and its congeners such as 2,3-dehydrosilybin, isosilybin, silydianin and silychristin, are respected antioxidants with cytoprotective and hypocholesterolemic effects (Gažák et al., 2007; Kroll et al., 2007; Křen and Walterová, 2005; Šimánek et al., 2000). More recently, silybin derivatives have attracted attention because of their anticancer activity (Deep et al., 2012, 2008a, 2008b; Gažák et al., 2011; Křen and Walterová, 2005) and due to specific interactions with cell signalling pathways (Gažák et al., 2007). Silymarin is available on the market in the form of dietary supplements or phytopharmaceuticals. Various silymarin preparations are usually standardized based on silybin content, their major constituent, causing an unfortunate muddling of the terms silybin, which is a defined chemical substance, and silymarin, which is a complex mixture (Kroll et al., 2007; Šimánek et al., 2000). Silymarin containing preparations are recommended mostly for liver protection against hepatotoxic substances. The recent literature describes the anticancer and chemoprotective effects of silymarin and its components in prostate cancer treatment (Deep et al., 2012; Flaig et al., 2010; Gažák et al., 2007; Vidlář et al., 2010). No side effects or interactions of silymarin with commonly used drugs are known (Jančová et al., 2007). Its effects in the organism are limited owing to low water solubility and poor bioavailability. Efforts to increase the solubility of silymarin and its components include preparation of derivatives, e.g. glycosides (Kosina et al., 2002), gallates (Gažák et al., 2011), silybin dihydrogen disuccinate disodium

Abbreviations: ALT, alanine aminotransferase; AOPPs, advanced oxidation protein products; AST, aspartate aminotransferase; GGT, γ-glutamyl transferase; GPX, glutathione peroxidase; GSR, glutathione reductase; GST, glutathione *S*-transferase; HBSS, Hank's balanced salt solution; HDL, high density lipoprotein cholesterol; IPSS, International Prostate Symptom Score; MDA, malondialdehyde in red blood cells; NO, nitric oxide; PGE, pyrolytic graphite electrode; PMDA, malondialdehyde in plasma; PSA, prostate specific antigen; RBC, red blood cells; SOD, superoxide dismutase; SWV, square-wave voltammetry; TAC, total antioxidant capacity; TAOs, total level of antioxidants; TSH, total SH groups in plasma.

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salt for parenteral application (Mengs et al., 2012) and complexes with phosphatidylcholine (lecithin, Flaig et al., 2010; Kidd and Head, 2005; Morazzoni et al., 1993) or β-cyclodextrine (Voinovich et al., 2009). In clinical trials, silymarin is usually applied in various forms, including pills, capsules (El-Kamary et al., 2009; Valentová et al., 2008; Vidlář et al., 2010) and powdered form mixed with applesauce (Flaig et al., 2010).

Recently, one of us developed water-soluble combinations of silymarin with basic amino-acids (L-lysine, L-histidine, L-arginine and L-ornithine, Stuchlík and Kopenec, 2008b) usable in formulations of beer and beer-based non-alcoholic beverages (Stuchlík and Kopenec, 2008a). Of these L-arginine (2-amino-5-guanidinopentanoic acid) is an important, versatile and conditionally essential amino acid. Besides serving as a building block for tissue proteins, arginine plays a critical role in ammonia detoxification, nitric oxide (NO) and creatine production. It is recommended as an immune, vitality and performance enhancer (Bescos et al., 2012). In the lower urinary tract, NO targets various cells, including detrusor smooth muscle cells, striated muscles involved in the urinary sphincter, interstitial and epithelial cells in the bladder, and vascular smooth muscle cells in the urethra (Stothers et al., 2003). This double-blind cross-over intervention trial aimed to investigate the effect of a non-alcoholic beverage containing silymarin and L-arginine on metabolic and urological parameters in healthy men.

2. Materials and methods

2.1. Chemicals

Unless otherwise indicated, all reagents and materials used in this work were obtained from Sigma–Aldrich (St. Louis, MO, USA). Silybin (CAS No. 22888-70-6; 88%) and silymarin (lot 040105, of the following composition (%; w/w): taxifolin 4.13, silychristin 17.00, silydianin 7.70, silybin A 23.66, silybin B 29.01, isosilybin A + B 11.38 (total 60% of flavonolignans) and undefined polymeric components 7.11% were from TEVA Pharmaceuticals CR, Ltd. (Opava, Czech Republic).

2.2. Characterization of the tested and control beverage

Five hundred milliliters of the tested beverage contained a mixture of silymarin (400 mg) with 295 mg of 98% L-arginine (molar ratio 1:2) in an alcohol-free beer-based beverage. The mean dose of silymarin and L-arginine was 4.3 and 3.1 mg/kg of body weight. The chemical composition of the tested and control beverages was determined by the Research Institute of Brewing and Malting in Prague and by the Institute of Microbiology of the Academy of Sciences of the Czech Republic and is given in Table 1.

2.3. Study design

Recruitment and data collection were performed between January and March 2012 at the Department of Urology, University Hospital in Olomouc, Czech Republic. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee of the University Hospital and the Faculty of Medicine and Dentistry, Palacký University in Olomouc, Czech Republic. All participants signed an informed consent and they were aware of the study goals before any study procedures were initiated. The subjects drank the control beverage (500 ml/day) for the first 10 days. After a washing period of 7 days, they consumed the tested beverage with silymarin and L-arginine (500 ml/day) for 10 consecutive days (Fig. 1). Venous blood and midstream urine samples were collected on days 0, 10 and 27, about 20 h after the last drink. Plasma, red blood cells and urine samples were stored at −80 °C prior to analysis of oxidative stress parameters (Table 3) and metabolites.

2.4. Study subjects and inclusion/exclusion criteria

Twenty-two healthy non-smoking and non-alcohol dependent men aged 38–59 years (49.9 ± 6.0 years, BMI 26.2 ± 3.9, prostate specific antigen (PSA) value 0.92 ± 0.44 ng/ml) were recruited. Exclusion criteria were age (⩽35 or ⩾60), disease including diabetes mellitus, and all kinds of dietary supplements, hepatoprotective drugs and medication that might interfere with lipid metabolism, one week before the beginning and throughout the trial.

Table 1
Chemical composition of the tested and control beverage.

	Tested	Control
Original extract (% w/w)[a]	2.73	2.73
Final attenuation (%)[a]	22.2	22.2
Bitterness (JH)[a]	24	24
Head retention (S/30 mm)[a]	255	253
Si (mg/l)[a]	46	48
Alcohol (%)[a]	0.49	0.49
Energetic value (kJ/l)[a]	88.55	78.52
L-arginine (mg/l)	590	–
Phenolics (mg/l)[a]	**890**[c]	**90**[c]
Gallic acid[a]	0.04	0.04
Protocatechuic acid[a]	0.03	0.03
Gentisic acid[a]	0.02	0.02
4-Hydroxybenzoic acid[a]	1.86	1.86
Aesculin[a]	2.12	2.12
4-Hydroxyphenylaceticacid[a]	0.25	0.25
Catechin[a]	0.22	0.22
Vanilic acid[a]	0.23	0.23
Chlorogenic acid[a]	0.14	0.14
Caffeic acid[a]	0.05	0.5
Syringic acid[a]	0.08	0.08
Vanillin[a]	0.01	0.01
p-Coumaric acid[a]	0.15	0.15
Ferulic acid[a]	0.75	0.75
Sinapic acid[a]	0.18	0.18
Rutin[a]	0.32	0.32
4-Hydroxycoumarin[a]	28.3	28.3
Naringin[a]	2.32	2.32
Myricetin	0.005	0.005
Quercetin[a]	0.01	0.01
Apigenin[a]	0.05	0.05
Biochanin A[a]	2.08	2.08
Umbelliferone[a]	0.04	0.04
Scopoletin[a]	0.16	0.16
Silymarin:	800	–
Silychristin[b]	183.2	–
Silydianin[b]	47.2	–
Silybin A[b]	186.4	–
Silybin B[b]	300	–
Isosilybin A[b]	65.6	–
Isosilybin B[b]	17.6	–

[a] Determined using standard operation protocols of the Research Institute of Brewing and Malting in Prague, Czech Republic.
[b] Determined by HPLC-DAD (column Chromolith Performance RP C18 (100 × 3 mm), mobile phase ($CH_3CN/CH_3OH/H_2O$/HCOOH, 2/37/61/0.05, 1 ml/min, 25 °C and detection at 285 nm) at the Institute of Microbiology of the Academy of Sciences of the Czech Republic.
[c] Total content of phenolics including all compounds below.

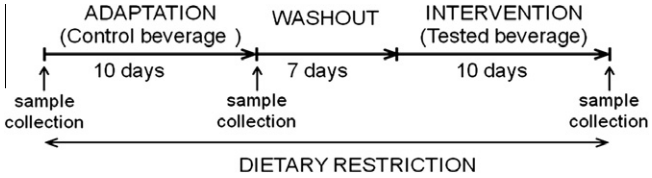

Fig. 1. Design of the intervention study.

2.5. Health investigation

During the health examination on the days 0, 10 and 27, the following parameters were routinely assessed: (i) detailed medical history; (ii) assessment of any concurrent medical drug or treatment; (iii) dietary habits; (iv) quality of life score (QoL); (v) urinanalysis; (vi) routine blood analysis. Kidney and bladder ultrasound was performed on day 0.

2.6. Clinical biochemistry and hematology

Basic biochemical and hematological parameters were determined in all samples immediately after sampling: total cholesterol, low-density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triacylglycerols, C-reactive protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST),

γ-glutamyl transferase (GGT), urea, creatinine, and bilirubin, using a HITACHI Modular Evo P analyzer (Hitachi, Japan). PSA in serum was determined using an Architect type LEIA analyzer (Abbott Laboratories, Abbott Park, IL, USA). Selected parameters for evaluation of oxidative stress were determined as total level of antioxidants (TAOs), total antioxidant capacity (TAC) and SH groups (TSH) in plasma, lipid peroxidation products such as malondialdehyde in plasma (PMDA) and red blood cells (MDA), advanced oxidation protein products (AOPPs) in plasma; glutathione; glutathione peroxidase (GPX); catalase; glutathione reductase (GSR); glutathione *S*-transferase (GST); superoxide dismutase (SOD) in red blood cells as described previously (Vidlář et al., 2010). Hemoglobin, hematocrit, red blood cells (RBC), platelets and white blood cells were measured in K_2EDTA blood.

2.7. Urinanalysis

Urine samples were collected from a midstream clean catch and analyzed using the IQ200 Automated Urinanalysis System (IRIS International, USA). Urination parameters including the International Prostate Symptom Score (IPSS) and voiding parameters (rate of urine flow, average flow, total volume and post void residual volume) were measured as well.

2.8. HPLC determination of silybin and its conjugates in blood plasma

HPLC determination of free and total (free and conjugated) silybin as the main silymarin component was carried out in blood plasma of volunteers before and after intervention as described previously (Křen et al., 2000; Wen et al., 2008).

2.9. Binding of flavonolignans to red blood cells

Freshly collected non-fasting human blood in EDTA was obtained by consent from one of the healthy volunteers. The samples of whole blood with silymarin (silybin) were prepared as described (Koren et al., 2010). Briefly, aliquots of whole blood were incubated for 10 min at room temperature with silybin, silymarin (10 and 100 μM, dissolved in DMSO) and/or L-arginine (20 and 200 μM). The red blood cells were then collected by repeated centrifugation at 500*g* (up to 12 times) in Hank's balanced salt solution (HBSS). Aliquots of the red blood cell suspension, (50 μl of red blood cells + 50 μl HBSS), and acquired supernatants were analyzed separately using *ex situ* square-wave voltammetry.

2.10. Ex situ square-wave voltammetry (SWV)

Pyrolytic graphite electrode (PGE, working electrode) was first dipped into 5-μl aliquot of the studied sample (red blood cell suspension and/or supernatant, see above). After an accumulation period of 60 s, PGE was washed by deionized water and placed in an electrochemical cell containing supporting electrolyte: Britton–Robinson buffer (pH 7.4). SWV parameters: initial (0 V) and end (+1.5 V) potential, frequency 200 Hz, step potential 5 mV, amplitude 25 mV. SWV was performed at room temperature with a μAutolab III analyzer (EcoChemie, NL) in a three-electrode setup (Ag/AgCl/3M KCl electrode as a reference and platinum wire as an auxiliary electrode). For other details (see Zatloukalová et al., 2011).

2.11. Statistics

Data were analyzed using the SPSS statistical software version 15 (SPSS Inc., Chicago, USA). Normality of data was assessed using Shapiro–Wilk test. Student paired *t*-tests with Benferroni correction of significance were used for normative data. The Friedman test and Wilcoxon paired test with Benferroni correction were used for non normative data. Differences were evaluated between values of parameters on days 0, 10 and 27. The level of significance was set at 5%. Values are presented as 25th percentile/median/75th percentile.

3. Results and discussion

3.1. Safety evaluation

A total of 22 participants were recruited for this double-blind controlled cross-over study. All the participants fulfilled the inclusion criteria, their physical and ultrasound examination revealed no chronic or acute disease and their biochemical, hematological and urodynamic parameters were within the normal range (Table 2). The study design is shown in Fig. 1; the drop-out rate was zero.

The drink was well-tolerated with no reported adverse effects and the sensory properties were acceptable. Most of the biochemical, hematological and urodynamic parameters remained unchanged, as well as the international prostate symptom score ($p < 0.05$, Table 2). The results were in accord with previous studies with silymarin on human and animal subjects (El-Kamary et al., 2009; Krecman et al., 1998; Skottova and Krecman, 1998; Škottová et al., 2004; Tedesco et al., 2004; Valentová et al., 2008; Vidlář et al., 2010). In the case of L-arginine, our dose of 295 mg/day was lower than the typical regular dietary intake of about 4–5 g and doses as high as 14.2 g/day that have been safely used in long-term intervention studies (180 days, Bescos et al., 2012). In addition, no adverse effects of the beverage on the lower urinary tract were found in the present trial.

Beverages supplemented with silymarin therefore seem to be an interesting alternative way to supply medium doses of this phytopharmaceutical.

3.2. Antioxidant status

3.2.1. Serum/plasma

The activity of serum γ-glutamyl transferase (GGT) was reduced ($p = 0.004$) on day 27 vs. day 10 (Table 2, Fig. 2A). This enzyme catalyzes the transfer of the γ-glutamyl moiety of glutathione to an acceptor and is found in many tissues, especially in the liver, and usually serves as a marker of liver damage (Whitfield, 2001). GGT plays a key role in the γ-glutamyl cycle, a pathway for the synthesis and degradation of glutathione (Whitfield, 2001) but it can also have prooxidant activity and membrane-bound GGT is now recognized as a source of low levels of reactive oxygen species (Dominici et al., 2005; Pompella et al., 2007). GGT is considered a sensitive biomarker of hepatic dysfunction and alcohol abuse. Recently, GGT levels were shown to be positively associated with the risk of the metabolic syndrome independently of alcohol consumption (Liu et al., 2012). In contrast, decrease in GGT activity is not linked with any human pathology and we focused our attention on selected markers of oxidative stress in order to evaluate the significance of this decrease.

Total antioxidant capacity, total level of antioxidants, lipoperoxidation products (malondialdehyde) and advanced oxidation products of proteins in blood plasma were not increased during the trial (Table 3) and neither free nor conjugated silybin was detected in the plasma samples (not shown). Silymarin components are *in vivo* quickly metabolized by phase I and particularly phase II enzymes (Jančová et al., 2007) and it was thus impossible to find detectable levels hours after the last dose of the tested beverage. We found a significant increase in total plasma SH-groups on day 10 compared to day 0 ($p = 0.012$, Table 3, Fig. 2B), i.e. after consumption of the control drink. This may be attributable to other components of the beverage, particularly polyphenols originating in hops and malt (Arranz et al., 2012) or to improved water intake.

3.2.2. Red blood cells

In red blood cells, glutathione level, glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase activities were not altered by the trial. In contrast, the activity of glutathione *S*-transferase (GST) at the end of the trial was lowered vs. day 0 ($p = 0.012$, Table 3, Fig. 2C). Some flavonoids have been shown to have inhibitory effects on GST (Boušová et al., 2012; Boušová and Skálová, 2012). GST plays a crucial role in the detoxification of xenobiotics and GST inhibition/induction may affect their metabolism. This should be considered in interactions with drugs and toxins (e.g., acetaminophen, simvastatin, cyclophosphamide, cisplatin, polycyclic aromatic hydrocarbons, chlorpyrifos, acrylamide, and isocyanates), which are GST substrates (Boušová and Skálová, 2012).

Finally, the level of lipoperoxidation products was decreased by a factor of 1.5 on day 27 vs. both days 10 and 0 ($p = 0.005$ and 0.0004, Table 3, Fig. 2D). This finding is in good agreement with the discovered protective effect of various polyphenols including

Table 2
Parameters of uroflowmetry, hematology, and clinical biochemistry before (day 0) and after (day 10) adaptation and after intervention (day 27).

	Day 0	Day 10	Day 27
Uroflowmetry			
IPSS	2.0 (1.0–5.3)	3.0 (1.0–6.3)	3.0 (1.0–4.5)
Q_{max} (ml/s)	26.8 (16.0–32.5)	23. (15.5–31.5)	24.8 (18.7–37.3)
Q_{ave} (ml/s)	16.9 (10.1–20.7)	15.0 (10.0–19.0)	15.4 (11.6–20.6)
Urine volume (ml)	349 (219–460)	270 (194–397)	335 (215–461)
Residual volume (ml)	18.0 (0.0–27.0)	11.5 (0.8–29.0)	17.0 (0.0–35.0)
Hematology			
Hemoglobin (g/l)	153 (148–160)	152 (146–158)	150 (146–157)
Red blood cells (10^{12}/l)	4.87 (4.69–5.20)	4.94 (4.63–5.10)	4.86 (4.63–5.11)
White blood cells (10^9/l)	6.01 (4.59–7.28)	6.47 (4.93–7.26)	6.32 (4.46–7.18)
Platelets (10^9/l)	241 (205–275)	235 (197–264)	238 (218–274)
Hematocrite	0.44 (0.43–0.46)	0.44 (0.42–0.46)	0.43 (0.42–0.45)
Clinical biochemistry			
Creatinine (μM)	77.5 (68.0–86.3)	76.0 (68.8–85.0)	78.0 (71.8–85.0)
Urea (mM)	5.00 (4.43–5.53)	4.80 (4.50–5.68)	4.85 (4.30–5.90)
Bilirubin (μmol/l)	10.5 (7.0–14.3)	10.0 (8.8–12.0)	10.0 (6.8–12.3)
ALT (μkat/l)	0.53 (0.44–0.76)	0.57 (0.43–0.72)	0.51 (0.47–0.65)
AST (μkat/l)	0.43 (0.37–0.58)	0.44 (0.38–0.63)	0.48 (0.38–0.52)
GGT (μkat/l)	0.68 (0.43–0.92)	0.71 (0.39–1.14)	**0.66** (0.38–0.91)[a]
C-reactive protein	1.50 (0.65–2.95)	1.75 (0.62–2.63)	1.53 (0.65–3.40)
Cholesterol (mmol/l)	5.62 (4.54–6.12)	5.70 (4.61–6.16)	5.54 (4.54–6.24)
Triacylglycerols (mmol/l)	1.49 (0.99–2.44)	1.54 (1.18–1.89)	1.65 (1.00–2.37)
HDL (mmol/l)	1.33 (1.14–1.50)	1.33 (1.14–1.59)	1.33 (1.14–1.52)
LDL (mmol/l)	3.47 (2.63–4.02)	3.57 (2.17–3.94)	3.41 (2.62–4.02)
Glucose (mmol/l)	5.40 (4.88–6.10)	5.40 (5.20–5.83)	5.25 (4.78–6.20)

Data are expressed as medians (interquartile range).
Bold values were significantly different.
[a] $p = 0.004$ vs. day 10.

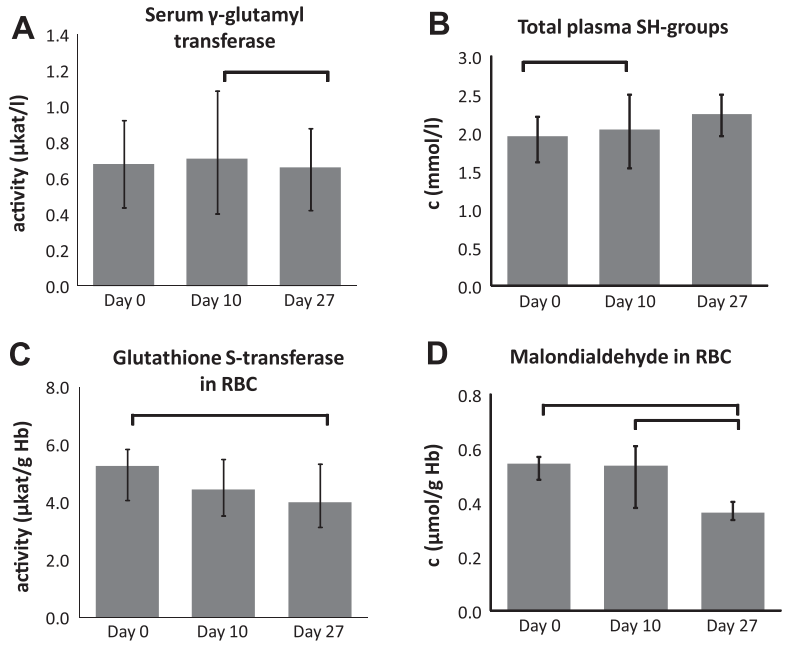

Fig. 2. Effect of the intervention on serum γ-glutamyl transferase (A), total plasma SH-groups (B), glutathione *S*-transferase (C) and malondialdehyde (D) in red blood cells (RBCs); $p < 0.05$.

silybin against red blood cell lipid peroxidation *in vitro* (Alvarez-Suarez et al., 2012; Duchnowicz et al., 2012; Kosina et al., 2002) and the protective effects of silymarin against high-sucrose induced oxidative stress in rats (Škottová et al., 2004). In human subjects, this protective effect is described here for the first time.

As the main effect of the trial was protection of red blood cells against oxidative damage (Table 3), we investigated the interactions of flavonolignans with erythrocytes *ex vivo*. It has been shown recently that erythrocytes can form complexes with polyphenols and thus participate in protection against cell damage induced by reactive oxygen species (Ginsburg et al., 2011; Koren et al., 2010). Here, we found that silymarin and its main component silybin bind to erythrocytes and the complex displayed antioxidant properties as studied using *ex situ* square wave voltammetry (SWV). For evaluation of silymarin binding to erythrocytes, we observed the oxidation peak 1 of silybin (E_1 = +0.5 V) after adsorption of purified silymarin–erythrocyte complex (see Section 2.9) onto working electrode, i.e. PGE (Fig. 3). The peak 1

Table 3
Parameters of oxidative stress in plasma and red blood cells before (day 0) and after (day 10) adaptation and after intervention (day 27).

	Day 0	Day 10	Day 27
Plasma			
TAC (mA × 10^6)	3.02 (2.39–3.51)	3.01 (2.53–3.58)	2.67 (2.24–3.61)
TAOs (mmol/l)	1.77 (1.69–1.88)	1.68 (1.62–1.81)	1.63 (1.63–1.75)
TSH (mmol/g)	1.95 (1.59–2.27)	**2.04** (1.50–2.53)[a]	2.25 (1.90–2.51)
PMDA (nmol/g)	20.8 (17.1–24.9)	22.9 (18.3–25.5)	22.4 (18.8–32.5)
AOPP (mmol/g)	201 (158–299)	191 (155–325)	213 (184–258)
Red blood cells (RBC)			
MDA (μmol/g)	0.54 (0.48–0.57)	0.54 (0.37–0.62)	**0.36** (0.33–0.42)[b]
Glutathione (μmol/g)	9.07 (8.43–9.97)	8.88 (8.58–9.47)	8.87 (8.22–9.55)
SOD (U/g)	3.00 (2.86–3.10)	2.93 (2.86–3.20)	3.02 (2.82–3.15)
GPX (U/g)	29.3 (23.7–34.8)	33.8 (24.5–36.5)	32.8 (23.3–39.6)
Catalase (U/g)	7.96 (7.33–8.52)	7.62 (7.29–8.18)	7.68 (7.22–8.44)
GST(μkat/g)	5.24 (4.00–5.88)	4.44 (3.46–5.56)	**3.99** (3.13–5.38)[c]
GSR (μkat/g)	3.94 (2.85–4.75)	3.83 (2.67–4.63)	3.99 (2.81–4.72)

Data are expressed as medians (interquartile range).
Bold values were significantly different.
[a] $p = 0.012$ vs. day 0.
[b] $p = 0.0004$ vs. day 0 and 0.005 vs. day 10.
[c] $p = 0.012$ vs. day 0.

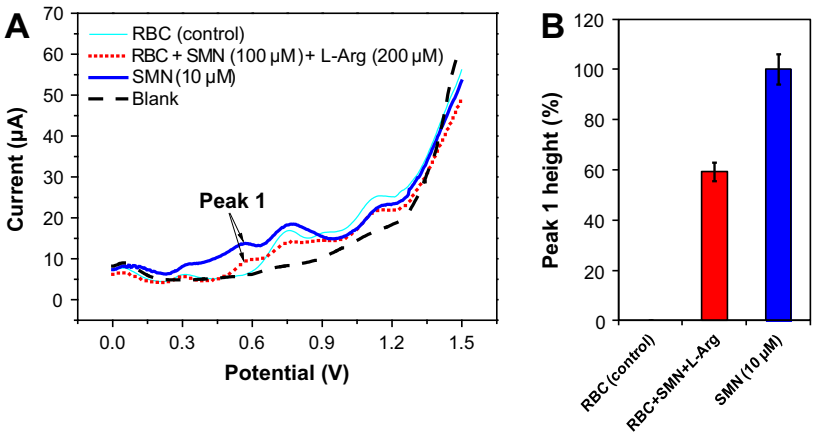

Fig. 3. (A) Square-wave voltammograms of human red blood cells (RBC) associated with silymarin (SMN) components under *ex vivo* conditions. (B) The percentage representation of silybin oxidation peak 1 height in analyzed samples, $n = 6$. For other details see Section 2.10.

of silybin is attributed to oxidation of its hydroxyl group at C20 (Zatloukalová et al., 2011). The immobilization of erythrocytes, eventually their complexes with silymarin, onto PGE surface is a spontaneous adsorption process. This statement is in good agreement with other findings showing the ability of erythrocytes to bind to several artificial surfaces (see Absolom et al., 1985). The silybin peak 1 was observed after silymarin binding to erythrocytes, which indicates that silymarin components may be subject to oxidation, and thus act as antioxidants, not only free in solution but also after binding to erythrocyte membranes (Fig. 3A). Nevertheless, the complex formed was unstable and subsequently dissociated. Following prolonged washing procedure (see Section 2.9), a decrease in silybin peak 1 could be observed, which is consistent with the reversible (quasi-reversible) binding of flavonoids to cell membranes and proteins (Cao et al., 2012; Hendrich, 2006). Non-treated erythrocytes (control) and/or erythrocytes incubated only with L-arginine did not provide the peak 1 under the same experimental conditions (Fig. 3B). In addition to the silybin peak 1, many other peaks can be observed in SW voltammograms of silymarin, which most probably indicates the oxidation of other silymarin components such as flavonols around +0.2 V (Zatloukalová et al., 2011). Several oxidation peaks were also observed for non-treated erythrocytes which corresponds to the oxidation of the integral components of the cell membrane and a broad spectrum of compounds attached to the erythrocyte membranes in a native state (Fig. 3A). On the basis of the results, we assume that the flavonolignans in the silymarin complex may interact with the erythrocyte membrane and provide protective effects there. Modulation of oxidative stress via flavonolignan binding to cell surface receptors cannot be excluded as well (Gažák et al., 2007). In this trial, the above described interaction was probably responsible for lowering the MDA and glutathione *S*-transferase in red blood cells.

4. Conclusion

A daily dose of 400 mg silymarin and 295 mg L-arginine in a non-alcoholic beer-based beverage for 10 days was well tolerated and had no adverse effects. Such beverage can therefore serve as an alternative way for silymarin supplementation. We found that the beverage had a protective effect on red blood cells. This was confirmed by the *ex vivo* ability of silymarin to bind on red blood cell membranes. These results imply that silymarin in the organism acts to protect against oxidative erythrocyte damage.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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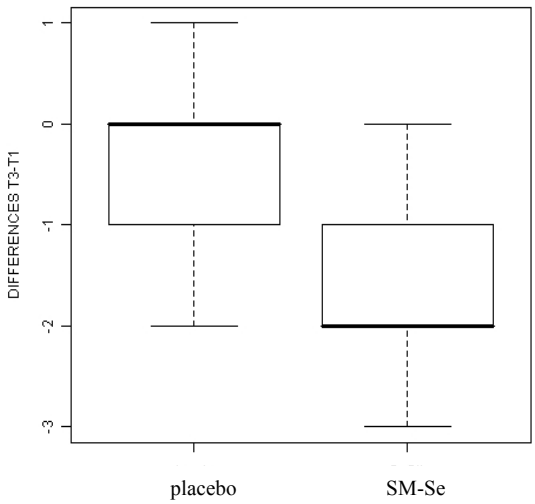
5.3 Vliv kombinace silymarinu se selenem na muže po radikální prostatektomii

(studie 3)

V dvojitě slepé, randomizované a placebem kontrolované studii byl ověřován účinek kombinace 570 mg silymarinu a 240 μg selenu (v formě L-selenometioninu)/den na muže 2. měsíce po radikální prostatektomii. Do studie bylo zařazeno celkem 37 mužů, kteří byli randomizováni do dvou skupin (placebo a SM-Se). Studie trvala 180 dní.

Pozitivní efekt kombinace SM-Se byl pozorován na kvalitu života (obrázek 4), lipidový metabolismus a zvýšení nebo normalizaci hladiny selenu. Studie neprokázala v literatuře popsaný účinek na snížení hladiny PSA (viz Schroder *et al*., 2005). Výsledky studie prokázaly, že dlouhodobé užívání kombinace SM-Se může být prospěšné u mužů po radikální prostatektomii s rizikem biochemického návratu karcinomu prostaty.



Obr. 4. Vliv SM-Se na hodnoty kvality života (QoL) ve skupině placebo a SM-Se.
Hodnoty jsou vyjádřeny jako rozdíl mezi dnem 180 (T3) a dnem 0 (T1) studie.
$p < 0,05$ SM-Se *vs.* placebo skupina

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THE SAFETY AND EFFICACY OF A SILYMARIN AND SELENIUM COMBINATION IN MEN AFTER RADICAL PROSTATECTOMY – A SIX MONTH PLACEBO-CONTROLLED DOUBLE-BLIND CLINICAL TRIAL

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Key words: Silymarin/Selenium/Prostate cancer/Radical prostatectomy/Clinical chemistry/Plasma lipoproteins/ Blood cholesterol

Background. Silymarin, a milk thistle flavonolignan mixture, has anti-proliferative and anti-angiogenic activities in xenografts of human prostate cancer (PCa). Low dietary selenium on the other hand has been associated with increased incidence of PCa. The purpose of the current trial was to determine whether a daily administration of a silymarin and selenium (SM-Se) combination for 6 months would alter basic clinical chemistry and oxidative stress markers, and improve the quality of life score (QoL) in men after radical prostatectomy (RP).

Methods. Thirty seven participants, 2–3 months after RP, were randomly assigned to receive 570 mg of silymarin and 240 μg of selenium as selenomethionine (n = 19, SM-Se group) or placebo (n = 18, Placebo group) daily for six months. Both groups had similar clinical and demographic characteristics. Physical examination, QoL score, haematology, basic clinical chemistry and oxidative stress markers, selenium and testosterone levels, antioxidant status were evaluated at baseline, at 3 and 6 months.

Results. The six months administration of silymarin and selenium improved the QoL score, decreased low density lipoproteins (LDL) and total cholesterol and, increased serum selenium levels. The combination had no effect on blood antioxidant status and no influence on testosterone level. No adverse events were recorded. No improvement was found in the placebo group.

Conclusions. The selected combination of silymarin and selenium significantly reduced two markers of lipid metabolism known to be associated with PCa progression, LDL and total cholesterol in the blood of men after RP. This suggests that this combination may be effective in reducing PCa progression.

INTRODUCTION

Prostate cancer (PCa) is the third leading cause of cancer death after colon/rectum and lung cancer in Europe[1]. The pathogenesis of PCa reflects hereditary, environmental and dietary components[2]. The latter are mainly dietary fats and calcium which may contribute to prostate cancer risk[3,4]. In recent years there has been an increasing interest in the potential chemopreventive properties of dietary components that may protect against prostate cancer[4]. Dietary factors proposed to decrease the risk of PCa are isothiocyanate sulforaphane and indole-3-carbinol in cruciferous vegetables, green tea polyphenols, particularly (-)-epigallocatechine-3-gallate, isoflavons genistein and daidzein found mainly in soybeans, long-chain n-3 polyunsaturated fatty acids, mixed tocotrienols and lycopene, the major carotenoid in tomatoes. In the literature, there are various opinions on the relation between the essential trace nutrient selenium acquired from normal dietary intake and the risk of PCa[5]. However, large studies have confirmed that selenium supplementation reduces the risk of PCa, particularly in men with low serum selenium levels[4,6]. The above-mentioned components are all nutraceuticals. Of phytoceuticals, only the extract from the seeds of milk thistle (*Silybum marianum*) silymarin and its major constituent, silibinin have shown efficacy in arresting human prostate carcinoma proliferation in a number of *in vitro* and *in vivo* preclinical models[7]. Silibinin blocks tumor cell proliferation[8] and angiogenesis. The latter is inhibited possibly by silibinin reduction of basic fibroblast growth factor and vascular endothelial growth factor[9].

Radical prostatectomy (RP) offers the best chance of curing prostate cancer. Unfortunately however, some patients have elevated levels of prostate-specific antigen (PSA) even after surgery. For this reason we hypothesized that a combination of silymarin and selenium (SM-Se) might be effective in reducing PCa progression. The aim of the present study was to evaluate the safety and tolerability of a 6 months daily consumption of 570 mg

silymarin and 240 μg selenium as a potential dietary supplement to be used in the tertiary prevention in patients after RP.

MATERIALS AND METHODS

Characteristics of study drug and placebo. The study preparations were manufactured according to Good Manufacturing Practise (GMP) by FAVEA (Kopřivnice, Czech Republic). The SM-Se tablet contained 190 mg silymarin (lot 040105, TEVA Pharmaceutical Company, Opava, Czech Republic) of the following composition (%; w/w): taxifolin 4.13, silychristin 17.00, silydianin 7.70, silibinin A 23.66, silibinin B 29.01, isosilibinin A+B 11.38, and undefined components 7.11; 80 μg selenium as selenomethionine (Lalmin® Se2000, Lallemand Human Nutrition A/S, Birkerød, Denmark); microcrystalline cellulose (250 mg), isomalt (60 mg), and hydroxypropyl cellulose (10 mg). The placebo tablet consisted of microcrystalline cellulose (250 mg), isomalt (250 mg), and hydroxypropyl cellulose (10 mg). The SM-Se and placebo tablets were coated with hypromellose, hydroxypropyl cellulose, talc, titanium dioxide, and caramel.

Study Design. A 6 months double blind placebo controlled trial was designed to assess the effect of a silymarin and selenium combination on patients after RP. The study protocol was approved by the Ethics Committee of the University Hospital and the Faculty of Medicine and Dentistry, Palacky University in Olomouc, Czech Republic. All of the participants signed an informed consent before any study procedures were initiated. The study took place from August 2007 to September 2008 at the Department of Urology, University Hospital in Olomouc, Czech Republic.

Study Subjects. Thirty seven men after RP, 51 to 72 years old, were invited to participate in the study. All subjects entering the study were 2 or 3 months after RP. Exclusion criteria included no nutrients, vitamins and minerals such as lycopene, vitamin E, selenium or herbal products with possible effects on prostate health, antibiotics, anti-inflammatory drugs, α-blockers and 5α-reductase inhibitors, intake of a vegetarian diet rich in isoflavonoids, history of food allergies, chronic liver or kidney diseases, gastrointestinal, or metabolic disorder or any other chronic health condition such as diabetes identified from the findings of the interview. Participants were randomly divided into two groups: Placebo (n = 18, aged 65.0 ± 3.9 years) and SM-Se (n = 19, aged 62.4 ± 6.4 years). They were instructed not to consume food rich in phenolics or make dietary or lifestyle changes during the study. In the SM-Se group, three tablets daily were given at approximately equal intervals throughout the day for a 6-month period. The placebo group received placebo tablets (3 tablets/day).

Health Investigation. During the health examination on the first day, after 3-months, and on the last day of study the following parameters were routinely assessed: (i) detailed medical history; (ii) assessment of all concurrent medical drug and therapies; (iii) dietary habits; (iv) quality of life score (QoL); (v) urinanalysis; (vi) kidney and bladder ultrasound; and (vii) a routine blood analysis.

Clinical Biochemistry and Hematology. Basic biochemical and hematological parameters were determined in all samples – sodium, potassium, chlorides, total cholesterol, low-density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triacylglycerols (TAG), apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), C-reactive protein (CRP), lactate dehydrogenase (LD), alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GMT), alkaline phosphatase (ALP), urea, creatinine, bilirubin, and testosterone (TST) using a HITACHI Modular Evo P analyzer (Hitachi, Japan). Prostate specific antigen (PSA) in serum was determined using an Architect type LEIA analyzer (Abbott Laboratories, Abbott Park, IL, USA). Selected parameters for evaluation of oxidative stress were determined as total antioxidant capacity (TAC) and SH groups in plasma (SHG_{tot}), lipid peroxidation products such as malondialdehyde in plasma (PMDA) and erythrocytes (MDA), advanced oxidation protein products (AOPP) in plasma; glutathione (GSH); glutathione peroxidase (GPX); catalase (CAT); glutathione reductase (GSR); glutathione transferase (GST); superoxide dismutase (SOD) in erythrocytes as described by Vidlar et al.[10] Selenium in plasma was determined by atomic absorption spectrometry using the AA6300 instrument (Shimadzu, Japan). Hemoglobin (Hb), hematocrit (Htc), erythrocytes (RBC), thrombocytes (PLT) and leukocytes (WBC) were measured in Na_2EDTA blood. *Urinanalysis.* Urine samples were collected from a midstream clean catch and analyzed using the IQ200 Automated Urinanalysis System (IRIS International, USA).

Statistics. Data were analyzed using software R. Nonparametric Wilcoxon two-tailed tests (paired-sample and two-sample) were used to determine any statistically significant differences between values of parameters on day 0 and after 6 months for the two groups. The level of significance was set at 5%. Values are presented as 1st quartile/median/3rd quartile or mean ± standard deviation. Box plots and graphs of empirical cumulative distribution functions are used as graphic illustration of significant differences in progression during 6 months between the Placebo and SM-Se groups.

RESULTS

Patients were recruited from August 2007 to March 2008, 37 men with a history of PCa and 2–3 months after radical prostatectomy participated in this randomized double-blind, placebo-controlled study. The baseline clinical and demographic characteristics are shown in Table 1 for all subjects and two randomization groups. The daily dose of silymarin and selenium was 570 mg and 240 μg respectively or placebo. Patients who received the combination of both components for 6 months (SM-Se group) had a better QoL score (Fig. 1) than those in the Placebo group. Haematology values were unchanged with the ex-

Table 1. Baseline demographics and clinical characteristics.

	Overall (*n* 37)	Placebo group (*n* 18)	SM-Se group (*n* 19)
Age (year)	63.8 ± 5.3	65.0 ± 3.9	62.4 ± 6.4
BMI	28.14 ± 2.48	27.9 ± 2.68	28.37 ± 2.33
QoL	2.19 ± 1.13	1.89 ± 0.96	2.47 ± 1.22
Selenium (μmol/l)	1.35 ± 0.48	1.24 ± 0.33	1.45 ± 0.58

Mean values and standard deviations.

Table 2. Markers of haematology in Placebo and SM-Se groups.

Parameter	Placebo group		SM-Se group	
	Day 0	Day 180	Day 0	Day 180
Hb (g/l)	145.5/150.5/155.5	142.0/152.5/158.8	135.0/145.0/148.5	**144/151/153***
RBC (10^{12}/l)	5.00/5.25/5.40	4.90/5.05/5.31	4.70/4.99/5.24	**4.86/5.12/5.33***
WBC (10^9/l)	5.89/7.27/8.68	6.01/6.68/7.67	5.53/7.01/7.92	5.42/6.59/7.45
Htc	0.42/0.45/0.47	0.42/0.44/0.47	0.41/0.42/0.44	0.42/0.44/0.45
PLT (10^9/l)	187/230/261	191/203/243	192/221/252	181/221/241

The values were expressed as 1st quartile/median/3rd quartile. The values are expressed as mean ± S.D. *$p < 0.05$ *vs* placebo.

Table 3. Markers of clinical chemistry in Placebo and SM-Se groups.

Parameter	Placebo group		SM-Se group	
	Day 0	Day 180	Day 0	Day 180
Na (mmol/l)	139/140/141	140/141/142	140/141/143	140/141/143
K (mmol/l)	4.25/4.43/4.58	4.18/4.44/4.62	4.12/4.30/4.47	4.17/4.35/4.56
Cl (mmol/l)	103/104/106	104/105/107	102/104/107	103/105/106
Urea (mmol/l)	4.95/5.80/6.18	5.60/6.05/6.47	4.70/5.30/6.70	5.10/5.80/6.75
Creatinine (μmol/l)	71.5/77.5/81.8	73.8/83.0/88.8	75.0/83.0/96.0	74.0/85.0/93.5
Bilirubin (μmol/l)	5.0/6.5/8.8	6.0/7.0/9.0	5.0/5.0/8.5	**5.0/7.0/11.0***
ALT (μkat/l)	0.34/0.40/0.50	0.38/0.46/0.66	0.39/0.44/0.71	0.43/0.53/0.67
AST (μkat/l)	0.42/0.46/0.50	0.39/0.44/0.56	0.40/0.47/0.56	0.46/0.53/0.57
ALP (μkat/l)	1.45/1.75/1.94	1.31/1.87/2.08	1.24/1.65/1.92	1.21/1.71/1.92
GMT (μkat/l)	0.38/0.45/0.75	**0.42/0.61/0.98***	0.29/0.48/0.54	0.33/0.45/0.53
LD (μkat/l)	2.44/2.72/3.07	2.62/2.74/3.05	2.71/2.82/3.04	2.67/2.85/3.05
CRP (mg/l)	1.0/1.0/2.0	1.0/1.5/2.8	1.0/1.0/2.5	1.0/1.0/2.0
Cholesterol (mmol/l)	4.86/5.23/6.03	4.53/4.93/5.87	5.06/5.55/7.08	**4.57/5.31/5.70***
TAG (mmol/l)	1.25/1.53/2.24	1.48/1.77/2.65	1.23/1.62/2.39	1.09/1.63/2.38
HDL (mmol/l)	1.12/1.31/1.58	**1.01/1.23/1.36***	1.10/1.19/1.64	1.12/1.23/1.44
LDL (mmol/l)	2.84/3.08/3.61	2.45/2.93/3.55	2.74/3.09/4.55	**2.51/2.98/3.70***
ApoA1 (g/l)	1.23/1.43/1.62	1.22/1.46/1.57	1.27/1.41/1.51	1.33/1.50/1.58
ApoB (g/l)	0.80/0.96/1.08	0.75/1.00/1.24	0.84/1.03/1.24	0.82/1.05/1.12
PSA_{tot} (μg/l)	0.00/0.00/0.02	0.00/0.01/0.10	0.00/0.00/0.01	0.00/0.00/0.04
Testosterone (nmol/l)	13.32/15.15/18.78	14.35/17.05/21.30	10.25/12.50/17.30	11.15/13.70/17.35
Selenium (μmol/l)	1.03/1.29/1.43	**0.68/0.86/1.13***	1.02/1.34/1.84	1.79/2.21/1.84*

The values are expressed as 1st quartile/median/3rd quartile. * $p < 0.05$ *vs* day 0.

Table 4. Markers of oxidative stress in Placebo and SM-Se Groups.

parameter	Placebo group		SM-Se group	
	Day 0	Day 180	Day 0	Day 180
PMDA (nmol/g)[a]	35.2/54.6/65.9	38.3/57.8/68.7	38.6/58.8/72.2	39.61/59.46/78.59
SHG_{tot} (μmol/g)[a]	2.37/3.16/3.81	2.44/3.14/3.49	2.37/3.19/4.14	2.16/3.40/4.08
AOPP (μmol/l)	123.4/159.8/268.1	140.0/168.6/221.5	159.0/214.9/240.5	152.0/168.8/213.9
TAC (nA)	5.49/6.82/7.17	5.4/7.0/7.7	5.61/6.24/7.45	5.63/6.58/7.27
MDA (nmol/g)[b]	0.34/0.39/0.43	0.36/0.40/0.49	0.39/0.44/0.46	0.38/0.43/0.48
GSH (μmol/g)[b]	12.16/12.96/14.15	**11.23/11.98/13.15***	10.62/11.78/13.57	10.60/11.04/12.41
SOD (U/g)[b]	2.33/2.57/3.05	**2.04/2.49/2.82***	2.34/2.73/2.99	2.19/2.55/3.08
GPX (μmol/min/g)[b]	17.59/22.16/31.52	**16.8/19.6/26.9***	20.56/27.31/35.54	18.75/26.95/37.56
CAT (μmol/min/g)[b]	117.3/129.3/153.7	119.9/132.1/151.5	101.9/117.2/151.3	99.62/124.83/154.28
GST (μmol/min/g)[b]	9.04/14.43/42.31	8.71/12.8/43.1	10.4/15.5/40.5	8.8/13.2/39.3
GSR (μmol/min/g)[b]	3.81/4.89/5.72	3.62/4.21/5.94	4.06/5.75/13.78	4.96/5.84/13.20

The values are expressed as 1st quartile/median/3rd quartile. * $p < 0.05$ *vs* day 0. [a]The value is expressed on g of protein. [b] The value is expressed on g of hemoglobin.

ception of a significant increase in erythrocyte count and hemoglobin in the SM-Se group, but the fluctuation was within normal physiological limits (Table 2). The effect of the SM-Se combination was further evaluated by monitoring liver function (ALT, AST, and GMT), lipid metabolism (cholesterol, HDL-cholesterol, LDL-cholesterol, TAG, ApoA1, and ApoB) and other laboratory parameters used for assessment of the safety and efficacy of the administrated preparation. In the SM-Se group, significant positive effects were found on plasma cholesterol and LDL-cholesterol levels (Table 3). On the other hand, a significant decrease in HDL-cholesterol level was found in the Placebo group. Long term consumption of the SM-Se combination led to raised levels of serum selenium (Fig. 2). Although change in bilirubin concentration was significantly different after six months for the SM-Se group, the fluctuation was within normal physiological limits. The oxidative stress markers are shown in Table 4. In the SM-Se group the silymarin and selenium consumption did not alter blood antioxidant status or other markers of oxidative stress. The antioxidant potential of the blood, measured as reduced glutathione and activity of antioxidant enzymes, superoxide dismutase and glutathione peroxidase differed significantly from baseline values in the Placebo group. After six months, PSA and testosterone levels were unchanged in both groups (Table 3). All participants finished the study and no subjective adverse effects were reported.

DISCUSSION

Factors that increase the risk of prostate cancer are (i) genetic disposition, (ii) age, (iii) ethnic background, (iv) chronic prostatitis, and (v) environmental factors including diet[2]. Finasteride and dutasteride, inhibitors of 5α-reductase, are recommended for the prevention of benign prostatic hyperplasia connected with chemoprotective effects on PCa[11]. The role of diet in prostate cancer development or PCa post-diagnosis is not fully understood[4]. Two studies on gene-enviroment interaction in relation to PCa showed the importance of dietary composition[12,13]. Of nutraceuticals, phytoceuticals and complex plant extracts or a combination of them, lycopene[14], soy flavonoids[15], silibinin[16] and preparations of soy and a combination of other supplements including vitamin E, selenium, lycopene and silymarin have been tested in human clinical trials (reviewed in ref.[4]). The data from these studies suggest that the phytochemicals tested retard the PSA double time increase in prostate cancer patients with relapsing disease and may delay progression of both hormone-refractory and hormone-sensitive PCa[17]. Silymarin is a complex of seven non-nutritive flavonolignans and one flavonoid[18]. It is one of the best pharmacologically characterized plant extracts. In mechanistic studies, silymarin displays antioxidant, anti-inflammatory, anti-proliferative, anti-fibrotic, anti-viral, and immunomodulatory activities[19]. It has clinical applications in alcoholic liver diseases, liver cirrhosis, *Amanita phalloides* poisoning, vi-

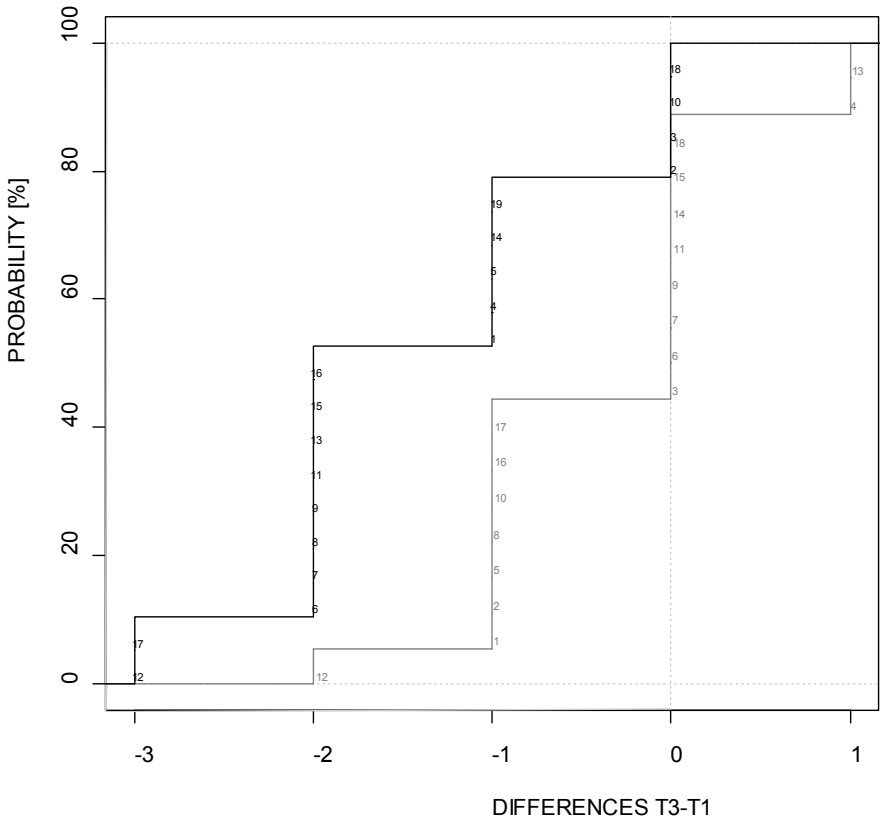

Fig. 1. Effect of silymarin and selenium combination on quality of life score during 6 month treatment. The values are expressed as differences value on day 180 and day 0 of study. SM-Se group, black line; Placebo group, grey line. *P < 0.05 vs Placebo group.

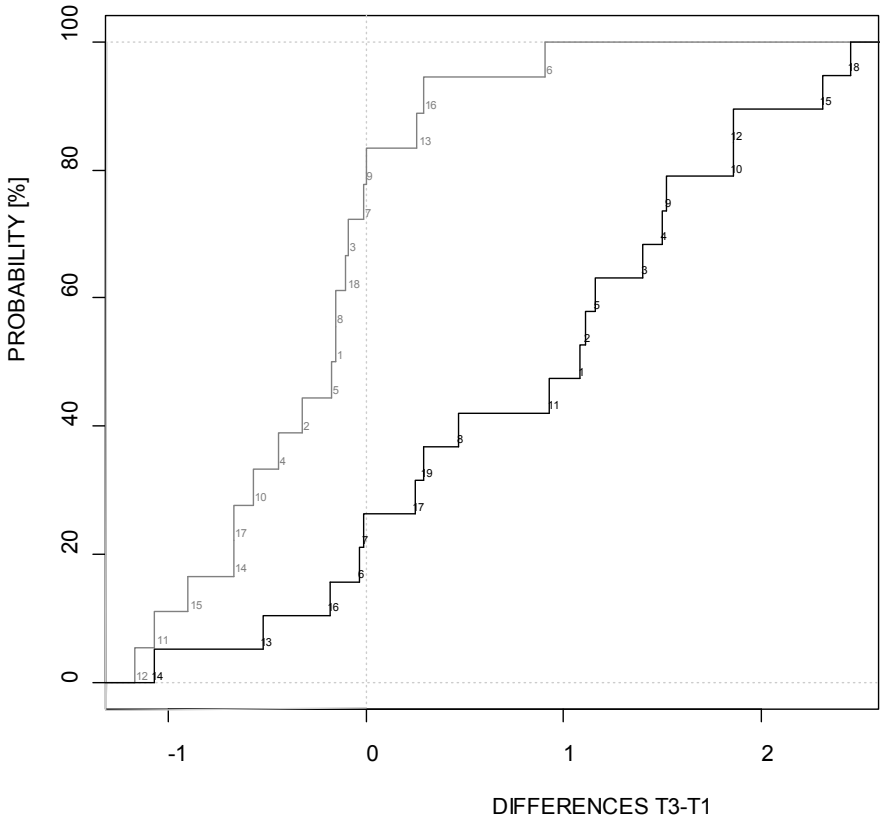

Fig. 2. Effect of silymarin and selenium combination on plasma selenium level during 6 month treatment. The values are expressed as differences value on day 180 and day 0 of study. SM-Se group, black line; Placebo group, grey line. *P < 0.05 vs Placebo group.

ral hepatitis C, and toxic and drug induced liver diseases[20]. Recently we reported that silymarin has a direct effect on LDL-cholesterol and total cholesterol in the blood of human volunteers[21,22]. In the study presented here, we selected patients after radical prostatectomy. For our pilot study a daily dose of 570 mg silymarin was chosen according to results from earlier study on healthy volunteers[21]. Our choice of 240 μg/day of selenium in the form of selenomethionine was based on the low level of selenium in our area. The 6 months administration of silymarin and selenium improved the QoL, decreased low density lipoproteins (LDL) and total cholesterol levels and increased the concentration of selenium in the blood. The combination had no effect on blood antioxidant status and had no influence on testosterone level. No adverse events were recorded. No improvement was found in the placebo group.

In summary, our study demonstrated that the orally administered silymarin and selenium had a positive effect on the organism of men after RP by (i) improving significantly the lipid parameters and (ii) increasing the blood selenium level. These findings suggest that a dietary intervention with a SM-Se combination could benefit patients after radical prostatectomy and who are at the risk of PCa progression.

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All authors declare having no conflict of interest.

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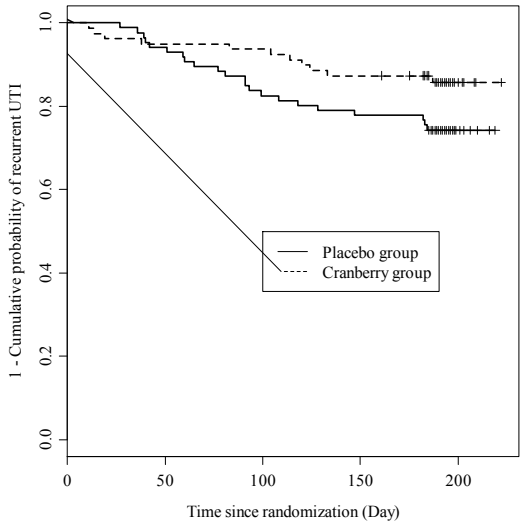
5.4 Vliv *V. macrocarpon* na recidivující infekce močových cest u žen (studie 4)

U 182 žen s anamnézou dvou a více IMC v průběhu předchozích 12 měsíců byl ověřen efekt celého lyofilizovaného plodu klikvy velkoplodé na snížení počtu recidiv IMC**.** Po randomizaci účastnic do skupiny placebo nebo klikva ženy užívaly po dobu šesti měsíců placebo nebo 500 mg práškovitého plodu klikvy/den. Ve skupině klikvy v porovnání se skupinou placebo (vyhodnoceno ITT metodou) byl prokázán nižší výskyt IMC (10,8% vs. 25,8%, p=0,04). Doba "přežití bez infekce" byla ve skupině klikvy významně delší oproti skupině placebo (p = 0,04, obr. 5). Výsledky ukázaly, že lyofilizovaný plod klikvy velkoplodé snižuje výskyt IMC u žen s anamnézou recidivujících IMC. Účinek plodu klikvy je dán komplexním účinkem obsahových látek plodu, ne pouze pronatokyanidiny, jak je uváděno ve většině publikovaných klinických studií (Maki *et al*., 2016).



Obr. 5. Vliv klikvy velkoplodé dobu na výskyt IMC ve skupině placebo a klikva (Kaplan-Meierova křivka).

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

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

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

INTRODUCTION

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

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

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

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

MATERIAL AND METHODS

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

Table 2. Content of proanthocyanidins in cranberry fruit powder

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

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

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

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

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

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

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

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

Table 3. Eligibility criteria

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

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

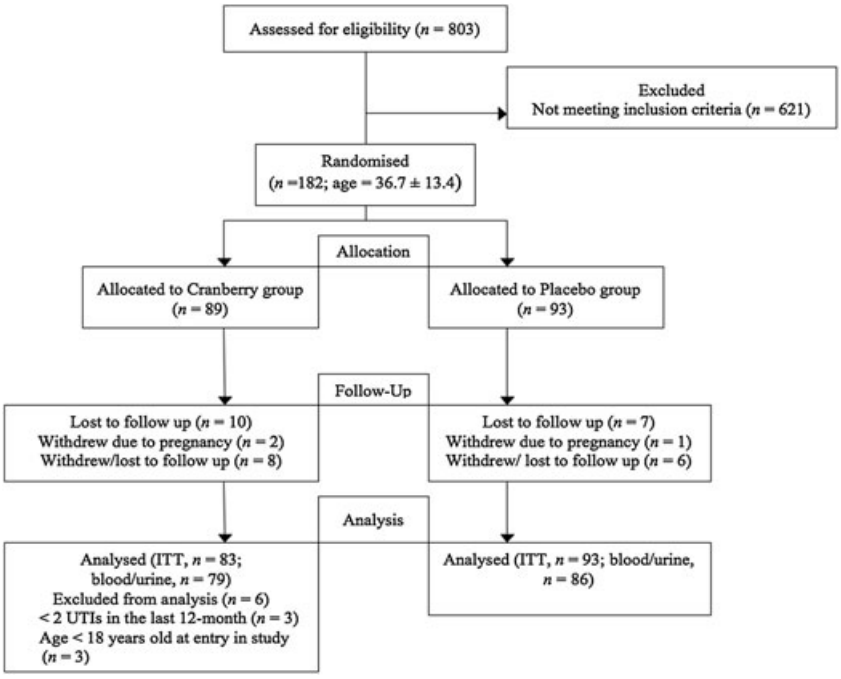

Figure 1. Flow chart of the clinical trial.

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

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

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

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

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

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

RESULTS

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

Table 4. Baseline parameters

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

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

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

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

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

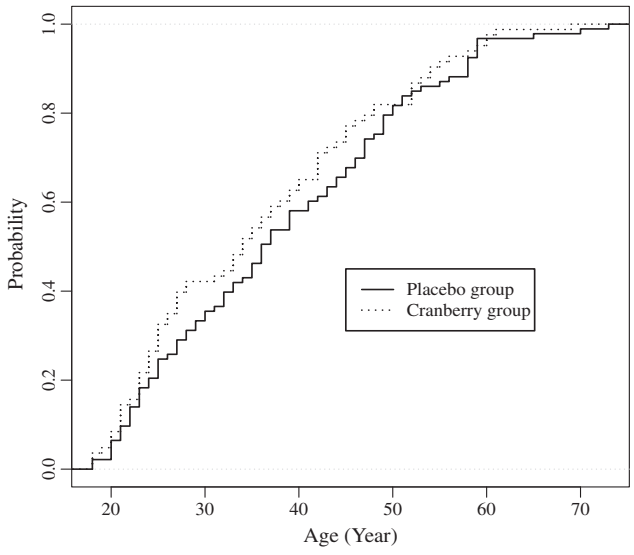

Figure 2. Differences in age distribution between subjects in the cranberry and placebo groups.

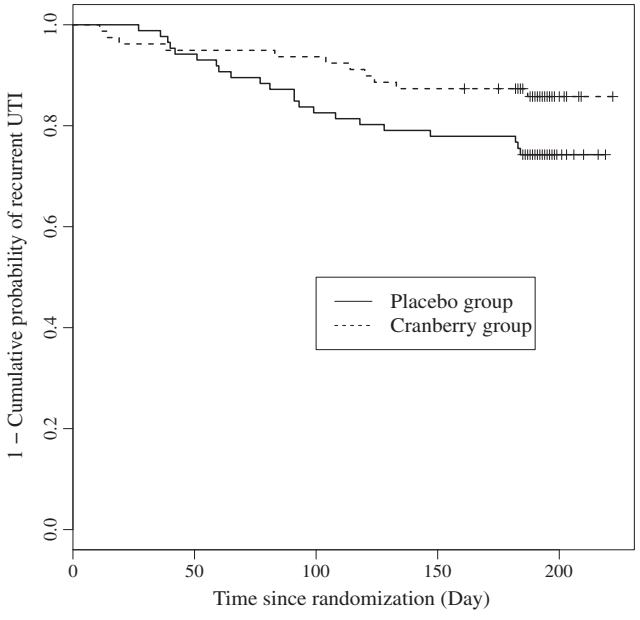

Figure 3. Kaplan–Meier curves of survival to UTI recurrence for the placebo and cranberry groups.

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

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

CONCLUSION

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

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Conflict of Interest

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

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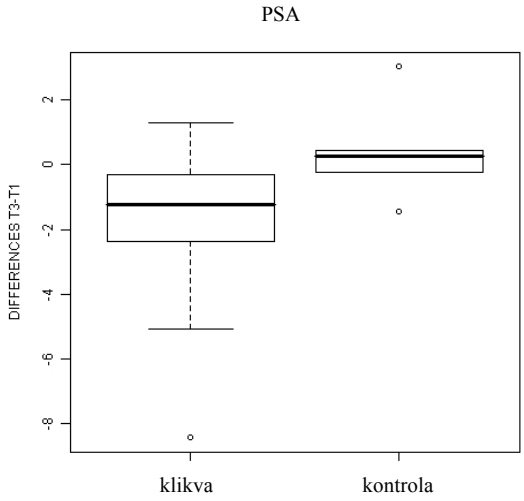
5.5 Vliv *V. macrocarpon* na urologické parametry mužů s LUTS a chronickou nebakteriální prostatitidou (studie 5)

Účinek plodu klikvy velkoplodé byl studován u mužů s LUTS, zvýšenou hladinou PSA a biopticky prokázanou nebakteriální prostatitidou. Celkem 42 mužů bylo rordělen do dvou skupin (klikva a kontrola). Ve skupině klikva účastníci užívali 1500 mg lyofilizovaného plodu klikvy velkoplodé/den po dobu šesti měsíců. V kontrolní skupině účastníci byli bez konzumace klikvy a byly sledovány stejné parametry jako v experimentální skupině.

Získané výsledky prokázaly, že lyofilizovaný celý plod klikvy velkoplodé může velmi efektivně ovlivnit zdraví prostaty zlepšením LUTS, včetně modulace hladiny PSA (obr. 6). Důležitým faktem je, že užívání lyofilizovaného plodu klikvy velkoplodé je na rozdíl od léků používaných při léčbě prostatitidy i LUTS bez nežádoucích účinků a nevyvolává riziko vzniku bakteriální rezistence. Naše výsledky mohou pomoci mužům s LUTS, ale i jejich lékařům, ke zvolení levné a „přírodní“ léčby bez vedlejších účinků. Také ukazují na novou oblast využití klikvy velkoplodé u mužů trpících potížemi ve smyslu „prostatitidy“.



Obr. 6. Vliv klikvy velkoplodé na hodnoty PSA_{tot} ve skupině kontrola a klikva.
Hodnoty jsou vyjádřeny jako rozdíl mezi dnem 180 (T3) a dnem 0 (T1) studie.
$p < 0,05$ skupina klikva *vs*. kontrola

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The effectiveness of dried cranberries (*Vaccinium macrocarpon*) in men with lower urinary tract symptoms

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Lower urinary tract symptoms (LUTS) are a common condition in older men. The objective of the present study was to evaluate the efficacy and tolerability of cranberry (*Vaccinium macrocarpon*) powder in men at risk of prostate disease with LUTS, elevated prostate-specific antigen (PSA), negative prostate biopsy and clinically confirmed chronic non-bacterial prostatitis. Forty-two participants received either 1500 mg of the dried powdered cranberries per d for 6 months (cranberry group; *n* 21) or no cranberry treatment (control group; *n* 21). Physical examination, International Prostate Symptom Score, quality of life (QoL), five-item version of the International Index of Erectile Function (IIEF-5), basic clinical chemistry parameters, haematology, Se, testosterone, PSA (free and total), C-reactive protein (CRP), antioxidant status, transrectal ultrasound prostate volume, urinary flow rate, ultrasound-estimated post-void residual urine volume at baseline, and at 3 and 6 months, and urine *ex vivo* anti-adherence activity were determined in all subjects. In contrast to the control group, patients in the cranberry group had statistically significant improvement in International Prostate Symptom Score, QoL, urination parameters including voiding parameters (rate of urine flow, average flow, total volume and post-void residual urine volume), and lower total PSA level on day 180 of the study. There was no influence on blood testosterone or serum CRP levels. There was no statistically significant improvement in the control group. The results of the present trial are the first firm evidence that cranberries may ameliorate LUTS, independent of benign prostatic hyperplasia or C-reactive protein level.

***Vaccinium macrocarpon*: Cranberries: Urinary tract disorders: Prostatitis: Prostate-specific antigen**

Prostate diseases are a major health concern for the male population throughout the Western world. Benign prostatic hyperplasia (BHP) and chronic prostatitis (CP), two of the most common medical conditions affecting older men (aged over 40 years), are associated with lower urinary tract symptoms (LUTS) which can have a negative impact on the quality of life (QoL). LUTS are divided into irritative and obstructive symptoms. The former include frequency, urgency and nocturia. The latter consist of slow urine stream and incomplete bladder emptying. Recently, a significant association between the serum levels of C-reactive protein (CRP) and irritative LUTS in both men and women was found[1,2]. On the other hand, CRP levels were not significantly associated with obstructive LUTS, or prostate-specific antigen (PSA) levels[3]. Untreated BHP and CP can lead to a number of medical complications, such as acute urinary retention, gross haematuria, repeated urinary tract infections, obstructive uropathy and cystolithiasis. The current standard of preventive care for men at risk of BHP and/or CP is treatment with α-adrenergic receptor blockers, 5-α-reductase inhibitors or antibiotics[4]. In recent years, there has been increasing interest in dietary supplements in the prevention of prostate diseases[5–8]. The proposed active components of these preparations include Se, vitamin E, vitamin D, lycopene, plant oils, *n*-3 fatty acids, phytosterols, terpenoids, lectins, polysaccharides, flavonolignans, flavonols and isoflavones. Some important dietary supplements for prostate health are complex extracts from green tea leaf

Abbreviations: BHP, benign prostatic hyperplasia; CFP, cranberry fruit powder; CP, chronic prostatitis; CRP, C-reactive protein; IIEF-5, five-item version of the International Index of Erectile Function; IPSS, International Prostate Symptom Score; LUTS, lower urinary tract symptoms; PSA, prostate-specific antigen; PSA_{free}, free prostate-specific antigen; PSA_{tot}, total prostate-specific antigen; Q_{max}, maximal urinary flow rate; QoL, quality of life.

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Table 1. Baseline demographics and clinical characteristics
(Mean values and standard deviations)

	Overall (*n* 42)		Control group (*n* 21)		Cranberry group (*n* 21)	
Variable	Mean	SD	Mean	SD	Mean	SD
Age (years)	63·0	5·5	64·0	5·4	62·0	5·4
BMI (kg/m^2)	26·46	3·04	24·91	2·09	28·00*	3·09
QoL	1·36	0·91	1·29	0·72	1·43	1·08
IPSS	10·48	5·95	10·86	5·88	10·10	6·14
PSA_{tot} (μg/l)	6·82	4·61	5·80	4·18	7·85	4·89
PSA_{free}:PSA_{tot}	0·22	0·04	0·18	0·02	0·21	0·07
Q_{max} (ml/s)	14·26	5·04	16·56	4·38	11·97*	4·68
Se (μmol/l)	1·12	0·67	0·97	0·53	1·27	0·77

QoL, quality of life questionnaire; IPSS, International Prostate Symptom Score; PSA_{tot}, total prostate-specific antigen; PSA_{free}, free prostate-specific antigen; Q_{max}, maximal urinary flow rate.
* Mean value was significantly different from that of the control group ($P<0·05$).

(*Camellia sinensis*), saw palmetto berry (*Serenoa repens*), milk thistle seed (*Silybum marianum*), pumpkin seed (*Cucurbita pepo*) and stinging nettle root (*Urtica dioica*). Cranberry (*Vaccinium macrocarpon*) is a source of organic and phenolic acids, flavonoids, flavonoid glycosides, anthocyanins, proanthocyanidins and triterpenoids of the ursane type with beneficial effects on the urinary tract[9,10]. Cranberry preparations are used as natural treatments for urinary tract infections, may reduce the ability of *Helicobacter pylori* to cause gastrointestinal ulcers and display anti-plaque activity[11–13]. The medicinal effectiveness and safety of intact cranberry fruits, juice and extracts have been critically evaluated recently[14]. Among recently reported effects of cranberry are its anti-inflammatory action through reduced cyclo-oxygenase-2 expression, suppression of IκBα degradation in human colon cancer cells[15] and inhibition of the growth and proliferation of several types of tumour cells including prostate[16]. However, to date there has been no published clinical study assessing whether cranberry reduces LUTS in men at risk of developing prostate diseases.

The aim of the present study was to evaluate the effect on urinary tract function of a 6-month daily consumption of 1500 mg cranberry fruit powder (CFP) in men with LUTS based on the International Prostate Symptom Score (IPSS), elevated PSA, BHP and histopathologically confirmed non-bacterial CP.

Table 2. International Prostate Symptom Score (IPSS), quality of life (QoL) score and International Index of Erectile Function (IIEF-5) in control and cranberry groups
(Mean values and standard deviations)

	Difference between answer							
	(Day 90 − day 0)				(Day 180 − day 0)			
	Control group		Cranberry group		Control group		Cranberry group	
Score	Mean	SD	Mean	SD	Mean	SD	Mean	SD
IPSS	0·00	2·39	−2·95*	2·48	1·43	4·09	−4·48*	3·74
Irritation questions	0·14	1·15	−1·14*	1·53	0·86	2·15	−1·62*	1·86
Obstruction questions	−0·14	1·68	−1·81*	1·94	0·57	2·38	−2·86*	2·52
Incomplete emptying: Over the past month how often have you had a sensation of not emptying your bladder completely after you had finished urinating?	0·14	1·28	−0·24	1·04	0·29	1·52	−0·38	0·92
Frequency: Over the past month how often have you had to urinate again less than 2 h after you finished urinating?	−0·14	0·65	−0·57	1·03	0·29	1·06	−0·76*	1·18
Intermittency: Over the past month how often have you found you stopped and started again several times when you urinated?	0·00	0·77	−0·38	1·12	0·14	0·85	−0·76*	1·22
Urgency: Over the last month how difficult have you found it to postpone urination?	0·29	0·72	−0·33*	0·73	0·29	1·06	−0·48*	1·03
Weak stream: Over the past month how often have you had a weak urinary stream?	−0·14	0·65	−0·67*	1·24	0·14	1·15	−1·19*	1·40
Straining: Over the past month how often have you had to push or strain to urinate?	−0·14	0·36	−0·52	0·75	0·00	0·52	−0·52*	0·93
Nocturia: Over the past month how many times did you most typically get up to urinate from the time you went to bed until the time you got up in the morning?	0·00	0·55	−0·24	0·70	0·29	0·72	−0·38*	0·86
QoL	−0·14	0·65	−0·52	0·68	0·00	0·77	−0·81*	0·75
IIEF-5	0·57	1·33	0·48	2·79	−0·71	2·17	0·05	1·88

* Mean value was significantly different from that of the control group ($P<0·05$).

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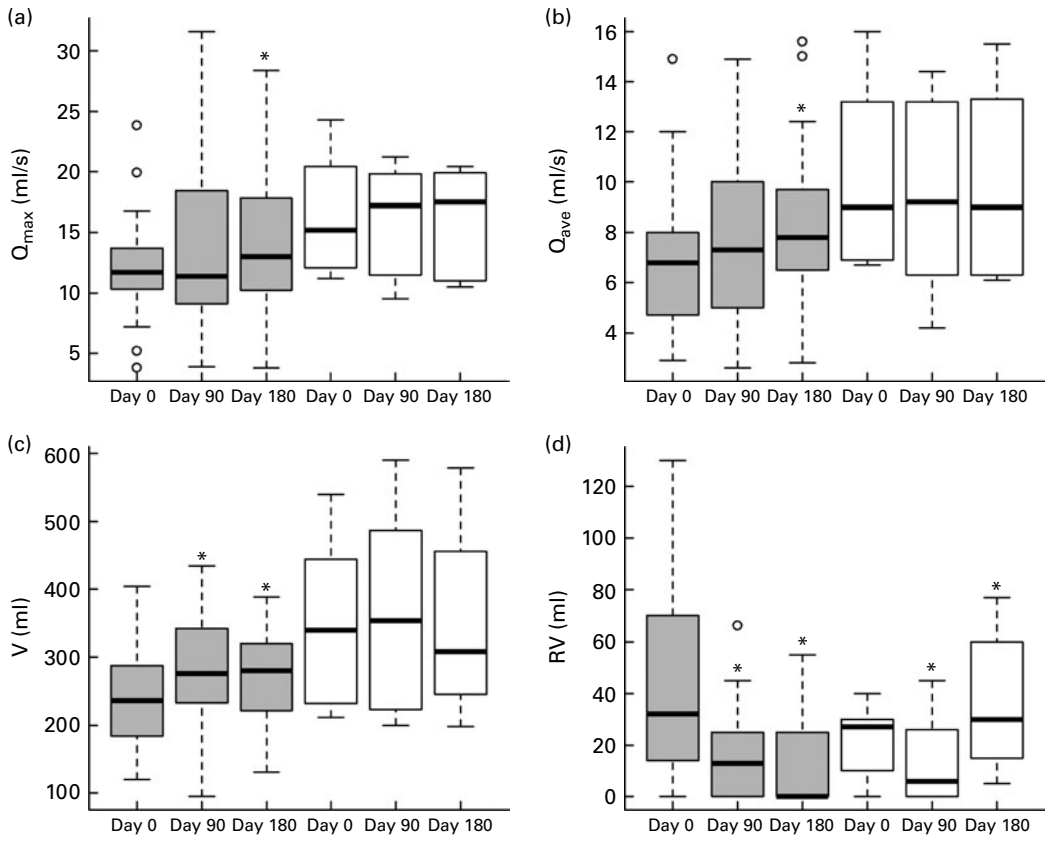

Fig. 1. Effect of cranberry (*Vaccinium macrocarpon*) on uroflowmetry parameters of maximal urinary flow rate (Q_{max}; a), average urinary flow rate (Q_{ave}; b), prostate bladder voiding volume (V; c) and post-void residual urine volume (RV; d) during 6 months of treatment. (▩), Cranberry group; (□), control group. The box-and-whisker graphs show the median as the middle line. The box extends from the 25th to the 75th percentile and the whiskers extend from the lowest value to the highest. ○, Outside values. * Median was significantly different from that of the control group ($P<0{\cdot}05$).

Materials and methods

Cranberry fruit powder characterisation

CFP (lot 070306-B/07-0659 supplied by Decas Botanical Synergies, LLC, Carver, MA, USA), containing 14·85 % (w/w) organic acids, 15·5 % sugars, 0·11 % anthocyanins, 1·95 % condensed tannins, 3·49 % total phenolics, was used for the clinical part of the study. One gelatine capsule contained 500 mg CFP.

Study subjects and data collection

The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the Ethics Committee of the University Hospital and Faculty of Medicine and Dentistry, Palacky University in Olomouc, Czech Republic. Written informed consent was obtained from all participants. A 6-month randomised controlled trial was conducted from October 2008 to November 2009 at the Department of Urology of the University Hospital.

Study subjects

We invited forty-two men, aged 45 to 70 years (mean age 63 (SD 5·5) years), to participate in the study. All subjects entering the study had LUTS, elevated PSA and/or BHP. Other inclusion criteria were histological findings of acute or chronic non-bacterial prostatitis, normal urinary sediment and negative bacterial cultivation of urine. The diagnosis was asymptomatic inflammatory prostatitis category IV according to the National Institute of Health classification system[17]. Exclusion criteria were no supplements such as Se, vitamins E and D, lycopene or herbal products with possible effects on prostate health, a diet rich in isoflavones, antibiotics, anti-inflammatory drugs, α-blockers or 5α-reductase inhibitors, food allergies, chronic liver or kidney diseases, gastrointestinal or metabolic disorder or any other chronic health condition such as diabetes, all identified from interview. Participants were randomly divided into two groups: control (*n* 21; mean age 64·0 (SD 5·4) years) and cranberry (*n* 21; mean age 62·0 (SD 5·4) years). In the cranberry group, three capsules (1500 mg CFP per d) were taken at approximately equal intervals daily throughout the day for the 6-month period. The size of the daily dose was based on our double-blind study in young women[18]. They were instructed not to consume food rich in phenolics, especially anthocyanin-containing fruits, and to make no other dietary or lifestyle changes during the study. The control group received the same instructions as the cranberry group but no cranberry supplementation.

Data collection

Each case report form included: (i) a detailed medical history; (ii) assessment of all concurrent medical drugs and therapies;

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(iii) digital rectal examination; (iv) dietary habits; (v) IPSS, QoL and the abridged five-item version of the International Index of Erectile Function (IIEF-5)[19]; (vi) urinanalysis; (vii) uroflowmetry with post-voidal residual urine; (viii) kidney and bladder ultrasound; (ix) transrectal ultrasound prostate volume; (x) a complete blood laboratory analysis. The following data were also collected at baseline and at 3 and 6 months in all subjects: Se; testosterone; free PSA (PSA_{free}); total PSA (PSA_{tot}); CRP; antioxidant status; urine *ex vivo* anti-adherence activity.

Lower urinary tract symptoms

All participants completed the IPSS including each of the seven areas (feeling of incomplete emptying, frequency, intermittency, urgency, weak stream, hesitancy and nocturia), QoL and five-item version of the International Index of Erectile Function (IIEF-5) questionnaires. Uroflowmetry data – maximal urinary flow rate (Q_{max}) and average urinary flow rate (Q_{ave}) – were measured using the FlowMic (Medkonsult, Olomouc, Czech Republic). Prostate bladder voiding volume (V) and post-void residual urine volume (RV) were assessed using the BK Medical Viking 2400 (BK Medical World Headquarters, Herlev, Denmark) with abdominal probe 3–7 MHz. V and RV were calculated using the formula for a prolate ellipsoid (width × length × height × 0·523). Histopathological examination of prostate tissue was done using ultrasound-guided prostate biopsy (BK Medical Viking 2400, transrectal probe 5–12 MHz; BK Medical World Headquarters) in all subjects.

Clinical biochemistry and haematology

Basic biochemical and haematological parameters were determined in all samples: Na, K, chlorides, total cholesterol, LDL, HDL, TAG, apoA, apoB, CRP, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase, lactate dehydrogenase, urea, creatinine, and testosterone were quantified in serum using a Hitachi Modular Evo P Analyzer (Hitachi, Tokyo, Japan). PSA (PSA_{tot} and PSA_{free}) in serum was determined using an Architect type LEIA Analyzer (Abbott Laboratories, Abbott Park, IL, USA). Analysis of selected parameters, i.e. total antioxidant capacity and total thiol (SH) groups in plasma, lipid peroxidation products such as malondialdehyde in plasma and erythrocytes, advanced oxidation protein products in serum, glutathione, glutathione peroxidase, catalase, glutathione reductase, glutathione transferase, and superoxide dismutase in erythrocytes was carried out as described by Psotova *et al.*[20]. Se in plasma was determined by atomic absorption spectrometry using the AA6300 instrument (Shimadzu, Kyoto, Japan). Hb, packed cell volume, erythrocytes, thrombocytes and leucocytes were measured in Na_2EDTA blood.

Urinanalysis

Urine samples were collected from a midstream clean catch and analysed by the IQ200 Automated Urinanalysis System (IRIS International, Inc., Chatsworth, CA, USA).

Table 3. Values of uroflowmetry in control and cranberry groups
(First quartiles, medians and third quartiles)

	Cranberry group								Control group							
	Day 0			Day 90			Day 180		Day 0			Day 90			Day 180	
Parameter	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile
Q_{max} (ml/s)	10·3	11·7	13·7	9·1	11·4	18·4	10·2	13*	17·8	12·1	15·2	20·4	11·5	17·2	19·8	11	17·5	19·9
Q_{ave} (ml/s)	4·7	6·8	8	5	7·3	10	6·5	7·8*	9·7	6·9	9	13·2	6·3	9·2	13·2	6·3	9	13·3
V (ml)	184	236	288	233	276*	342	221	280*	320	232	340	444	223	354	487	245	308	456
RV (ml)	14	32	70	0	13*	25	0	0*	25	10	27	30	0	6*	26	15	30*	60

Q_{max}, maximal urinary flow rate; Q_{ave}, average urinary flow rate; V, prostate bladder voiding volume; RV, post-void residual urine volume.
*Median was significantly different from that at day 0 ($P<0·05$).

Table 4. Markers of haematology and clinical chemistry in control and cranberry groups
(First quartiles, medians and third quartiles)

	Cranberry group									Control group								
	Day 0			Day 90			Day 180			Day 0			Day 90			Day 180		
Parameter	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile
Hb (g/l)	143	149	159	142	152	156	142	152	159	143	152	160	141	149	156	140	150	155
Erythrocytes (10^{12}/litre)	4·84	4·97	5·22	4·92	5·09	5·28	4·90	5·06*	5·31	4·41	4·94	5·30	4·48	4·95	5·14	4·45	4·94	5·25
Leucocytes (10^{9}/litre)	6·10	6·98	7·68	6·25	7·20	8·19	6·37	7·23	8·02	5·83	6·20	7·21	5·92	6·54	7·04	6·20	6·43	7·04
Thrombocytes (10^{9}/litre)	187	225	267	181	223	254	201	220	245	194	207	235	188	192*	217	184	190	242
Packed cell volume (litres)	0·43	0·45	0·46	0·42	0·44	0·46	0·43	0·44	0·46	0·40	0·44	0·46	0·40	0·44	0·45	0·40	0·44	0·46
Na (mmol/l)	139	140	140	137	138	141	139	140	142	136	137	139	140	142*	142	137	140*	143
K (mmol/l)	4·13	4·26	4·38	4·07	4·28	4·51	4·11	4·36	4·67	3·98	4·06	4·22	4·17	4·30*	4·58	4·11	4·46*	4·65
Cl (mmol/l)	102	103	104	102	104	107	102	106	106	100	104	104	103	106*	108	101	103	106
Urea (mmol/l)	4·7	6·3	6·8	4·9	5·7	7·7	5·4	6·0	7·0	4·0	5·0	66	4·2	6·7*	70	4·0	5·0	70
Creatinine (μmol/l)	69	82	89	71	79	92	73	79	95	6	76	83	6·4	73	80	5·6	71	83
Bilirubin (μmol/l)	5	6	9	6	8*	10	6	8	9	6	9	10	7	10*	13	10	13*	14
ALT (μkat/l)	0·40	0·47	0·59	0·35	0·45	0·55	0·39	0·44	0·59	0·29	0·33	0·40	0·24	0·37	0·48	0·33	0·34*	0·71
AST (μkat/l)	0·42	0·45	0·51	0·39	0·44	0·48	0·39	0·47	0·53	0·38	0·43	0·45	0·38	0·40*	0·43	0·40	0·49*	0·53
ALP (μkat/l)	1·30	1·56	1·84	1·26	1·46	1·81	1·27	1·57	1·74	1·35	1·59	1·76	1·41	1·54	2·12	1·55	1·75*	2·14
GMT (μkat/l)	0·37	0·46	0·54	0·35	0·49	0·66	0·38	0·44	0·61	0·20	0·27	0·52	0·24	0·40	0·55	0·24	0·33*	0·61
LD (μkat/l)	2·52	2·73	3·05	2·74	3·04*	3·31	2·67	3·04*	3·42	2·48	2·74	3·05	2·70	2·78	2·89	2·47	2·57	2·80
CRP (mg/l)	1	2	3	1	2	3	1	2	3	1	1	1	1	1*	2	1	1*	3
Cholesterol (mmol/l)	4·23	4·90	5·61	4·51	4·78	5·46	4·64	5·00	5·68	4·92	5·49	6·23	4·02	5·09*	5·97	4·61	5·45	6·28
TAG (mmol/l)	1·30	1·65	2·28	1·23	1·87	2·21	1·40	1·72	2·65	1·21	1·34	2·34	0·98	1·31	2·59	1·41	1·61	2·11
HDL (mmol/l)	1·24	1·35	1·48	1·21	1·29	1·49	1·19	1·25	1·46	1·09	1·13	1·54	1·05	1·20	1·34	1·10	1·19	1·29
LDL (mmol/l)	2·15	2·71	3·47	2·28	2·85	3·33	2·48	2·91	3·18	2·99	3·83	4·02	2·23	3·12*	3·66	2·78	3·72	4·51
ApoA1 (g/l)	1·43	1·48	1·58	1·41	1·50	1·60	1·39	1·52	1·66	1·30	1·48	1·88	1·36	1·50	1·94	1·38	1·46	1·92
ApoB (g/l)	0·73	0·86	1·09	0·73	0·93	1·05	0·79	0·93	1·06	0·83	1·04	1·15	0·69	0·93	1·21	0·78	0·94	1·23
PSA_{tot} (μg/l)	4·20	6·18	11·40	3·76	5·59*	7·10	3·53	4·53	7·54	2·99	3·99	11·25	2·70	4·24	10·20	3·43	5·37	9·80
PSA_{free} (μg/l)	0·87	1·03	1·18	0·77	0·91*	1·09	0·73	0·90*	1·36	0·66	0·73	0·82	0·50	0·69	0·93	0·69	0·73	0·76
TST (nmol/l)	10·7	14·2	16·2	11·4	14·4	16·5	12·6	15·9	17·5	12·8	17·6	22·4	16·9	20·4	28·0	10·9	18·7	24·1
Se (μmol/l)	0·89	1·13	1·37	0·91	1·02	1·34	0·82	1·04	1·29	0·49	0·84	1·11	0·71	0·78	0·85	0·68	0·91	1·20

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GMT, γ-glutamyl transpeptidase; LD, lactate dehydrogenase; CRP, C-reactive protein; PSA_{tot}, total prostate-specific antigen; PSA_{free}, free prostate-specific antigen; TST, testosterone.
* Median was significantly different from that at day 0 ($P<0·05$).

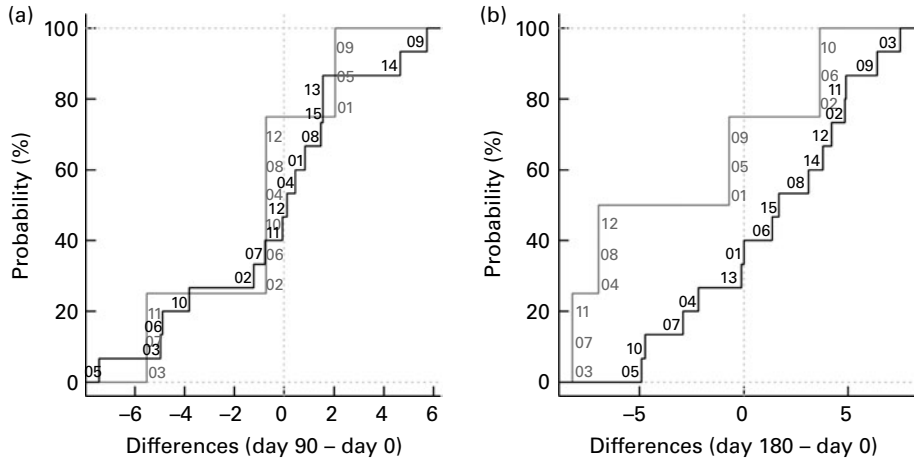

Fig. 2. Effect of cranberry (*Vaccinium macrocarpon*) on free prostate-specific antigen:total prostate-specific antigen ratio after 90 and 180 d of consumption. The values are expressed as difference values based on day 90 and day 0 (a) and day 180 and day 0 (b) of study. (—), Cranberry group; –, control group. * $P<0{\cdot}05$ *v.* control. The numbers near to the lines correspond with the number of each participant.

Anti-adherence activity of urine

Four biofilm-positive micro-organisms were used: *Escherichia coli* FB42, *Enterococcus faecalis* FB16 and *Candida parapsilosis* BC 12 (clinical strain; Collection of the Department of Microbiology, Faculty of Medicine, Masaryk University, Brno, Czech Republic) and *Staphylococcus epidermidis* CCM 7221 from the Czech Collection of Micro-organisms (CCM; Faculty of Sciences, Masaryk University, Brno, Czech Republic). The bacteria were stored in cryotubes at −76°C. The biofilm formation was detected using a modification of the adherence assay[21]. The experiment was repeated three times.

Statistical analysis

The data were analysed using the non-parametric Wilcoxon two-tailed tests (paired-samples and independent-samples) to test the statistical significance of differences in all parameters on days 0, 90 and 180. The level of significance was 5 %. Values are presented as 1st quartile, median and 3rd quartile or mean value and standard deviation. Box and empirical cumulative distribution plots were used as graphic illustration of significant differences in progression over 3 and 6 months between the groups.

Results

At baseline both groups had similar clinical and demographic characteristics except for significant differences in BMI and Q_{max} values (Table 1). The daily dose of CFP contained 223 mg organic acids, 1·65 mg anthocyanins, 29·5 mg condensed tannins and 52 mg total phenols. Patients who received cranberry for 6 months had a statistically significant lower IPSS and QoL score than controls. A lower IPSS score reflected improvement in the irritative and obstructive symptoms (Table 2). All parameters of urination (Q_{max}, average urinary flow rate (Q_{ave}), prostate bladder voiding and post-void residual urine volumes) were significantly improved in at least 70 % of participants of the cranberry group (Fig. 1); in the control group, the tested parameters did not change with the exception of post-void residual urine volume where a statistically significant deterioration was found (Table 3). Haematology values were unchanged with the exception of a significant increase in erythrocytes in the cranberry group, which, however, was within physiological limits (Table 4). PSA_{tot} decreased in approximately 80 % of patients in the cranberry group while, in contrast, the PSA_{free}:PSA_{tot} ratio mostly increased (Table 4; Fig. 2). Although changes in the values of several 'safety' markers were statistically significantly different, after 6 months for both groups, the fluctuation was within normal physiological limits. From this point of view, the cranberry group compared with the control group stabilised and this might be true for oxidative stress markers as well (Table 5). Differences in urine adherence *ex vivo* in both groups were not significantly different. No adverse events were recorded.

Discussion

Plant extracts for urinary tract disorders have long been used in traditional medicine. Today, botanical diuretics, antimicrobials and anti-adherence agents, renal protectives and herbs for patients with LUTS or BHP are requested by patients, even though accepted only with reservation by urologists[22,23]. Cranberry fruit and juice are noted for their ability to inhibit the binding of pathogenic *E. coli* strains and other microbes to the bladder epithelium. This effect has been attributed to proanthocyanidins (condensed tannins), even if a more simple explanation might be the direct antibacterial action of hippuric acid[14]. Cranberry prophylaxis is also recommended to women with recurrent urinary tract infection. In a recent publication, for example, the antibiotic Trimethoprim was shown to have minimal advantage over cranberry extract in the prevention of recurrent urinary tract infections in women and had side effects[24]. LUTS refer to a complex of irritative and obstructive voiding symptoms that are common in both ageing women and men. Prostate enlargement and BPH affect primarily older men. The incidence of LUTS associated with BPH increases dramatically with advancing age[25]. Unfortunately, no trials have yet been published assessing the effect of cranberry components on men with indicated

Table 5. Markers of oxidative stress in control and cranberry groups
(First quartiles, medians and third quartiles)

	Cranberry group									Control group								
	Day 0			Day 90			Day 180			Day 0			Day 90			Day 180		
Parameter	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile	1st quartile	Median	3rd quartile
PMDA (nmol/g protein)	49·11	60·46	76·40	50·54	74·31	77·40	50·40	57·80	76·60	51·23	60·55	70·45	47·10	51·20*	63·87	47·67	61·79	69·77
SHG_{tot} (μmol/g protein)	3·06	3·65	4·06	2·40	4·10	4·93	2·83	3·72	4·21	3·10	3·52	3·56	3·01	3·13	3·43	3·00	3·40	3·74
AOPP (μmol/l)	189·9	199·0	231·2	160·6	202·4	257·5	161·2	203·2	240·5	159·0	222·3	302·7	145·3	180·4*	274·3	139·9	155·3*	286·5
TAC (nA)	4·92	5·72	6·64	5·08	6·01	6·50	5·29	5·82	6·29	4·41	5·39	6·19	4·35	5·72	6·50	4·51	4·72	6·36
MDA (nmol/g Hb)	0·31	0·37	0·44	0·32	0·34	0·41	0·33	0·39	0·44	0·29	0·35	0·42	0·25	0·36	0·41	0·25	0·41*	0·48
GSH (μmol/g Hb)	10·29	10·82	11·91	10·24	11·45	12·15	10·86	11·13	12·14	8·69	10·10	11·44	9·43	10·25	11·41	9·72	11·25	11·77
SOD (U/g Hb)	1·78	2·17	2·31	1·97	2·08	2·34	2·07	2·14	2·25	1·57	1·88	2·05	1·18	1·66	2·01*	1·50	1·95	2·06
GPX (μmol/min per g Hb)	22·58	27·09	29·12	22·26	26·76	29·14	23·28	25·08	29·87	21·23	22·77	26·52	18·89	19·89	27·36	23·77	24·41*	29·05
CAT (μmol/min per g Hb)	39·11	96·62	121·56	39·80	108·49	134·13	40·53	102·30	129·31	95·43	101·04	123·74	98·36	115·93*	117·98	111·21	117·60*	124·08
GST (μmol/min per g Hb)	36·01	44·42	53·46	39·23	46·08	59·58	40·48	46·46	56·54	16·43	51·17	60·47	16·95	51·05	61·31	18·05	49·10	62·94
GSR (μmol/min per g Hb)	4·35	5·62	6·25	5·09	6·08	7·69	5·09	6·53	7·13	2·26	4·58	5·74	2·84	4·81	5·33	1·86	4·87*	6·38

PMDA, plasma malondialdehyde; SHG_{tot}, total thiol groups; AOPP, advanced oxidation protein products; TAC, total antioxidant capacity; MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase, GPX, glutathione peroxidase; CAT, catalase; GST, glutathione transferase; GSR, glutathione reductase.
* Median was significantly different from that at day 0 ($P<0·05$).

LUTS and/or increased PSA levels. Recently published results have demonstrated that CP might be linked to a higher prostate cancer risk[26]. The present study was focused on men with diagnosed LUTS, increased PSA level, and histologically confirmed non-bacterial prostatitis. We selected cranberry whole fruit powder in preference to cranberry extract for the present study. Our previous work had shown equivalent efficacy between CFP and two different cranberry extracts[27]. The daily dose of 1500 mg dried cranberries was based on our double-blind study in young women[18]. This dose elicited urine anti-adherence activity but had no adverse effects. In participants taking cranberry for 6 months there was, in addition to a marked improvement in all urodynamic parameters (Fig. 1), a statistically significant decrease in the IPSS score, and an increase in the quality of life evaluated by the QoL questionnaire (Table 2). Taking cranberry affected the value of PSA (Table 4). In the cranberry group, both a decrease in the PSA value and an increase in the PSA_{free}: PSA_{tot} ratio (Fig. 2) without affecting CRP or testosterone levels were recorded. The use of selective 5-α-reductase inhibitors has often been linked to hormone changes associated with unpleasant sexual side effects, in particular, erectile dysfunction and decreased libido[28,29]. The treatment approach in patients with elevated PSA and histologically confirmed prostatitis is rather complicated and may involve long-term antibiotics with the expectation of lowering the PSA level. The decrease in PSA in the cranberry group demonstrates that prophylaxis by cranberry may be as effective as antibiotic treatment but without the risk of antimicrobial resistance and a minimum of adverse effects. The diuretic effects of cranberries may also have contributed to the reduction in LUTS in the cranberry group[30]. Cranberries contain several structurally different groups of compounds that modulate various cellular pathways in man including the urinary tract and the prostate. However, phenolics, as phenolic acids, anthocyanins and proanthocyanidins, that are metabolised mainly to hippuric acid, are assumed to be the active components. The synergistic effects of cranberry constituents may improve their bioactivity. Use of the whole berries may be more beneficial than single components and with minimal adverse effects.

Conclusions

Our trial is the first to evaluate cranberry in the treatment of LUTS specifically in men with BHP, elevated PSA levels and non-bacterial prostatitis. The present results show that dried cranberries improve prostate health very effectively both in men with elevated PSA in histologically proven prostatitis and for improving voiding dysfunction. In the cranberry group, no associations were found between dried powdered berry consumption and CRP levels. Unlike currently used medication for prostatitis and LUTS, cranberry has no adverse effects. Our findings may assist men suffering from LUTS, and also their clinicians, to decide on a treatment that is both inexpensive and natural, like cranberry. The limitations of the present study include the relatively small number of men. Given the probability that some responses on the IPSS and QoL questionnaires in the cranberry group may have been secondary to a placebo effect, there is a need to control for this in future clinical trials.

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The original authors and their contributions were as follows: A. V. was involved in the development of the protocol; V. Student and D. S. participated in the clinical observation of the subjects; J. Vrbkova carried out the statistical analysis; F. R. performed the microbial anti-adherent assay; R. R. performed the cranberry analysis; V. Simanek. and J. U. analysed the clinical chemistry data; J. Vostalova was responsible for the management of the study, and was the principal investigator and guarantor.

There is no conflict of interest.

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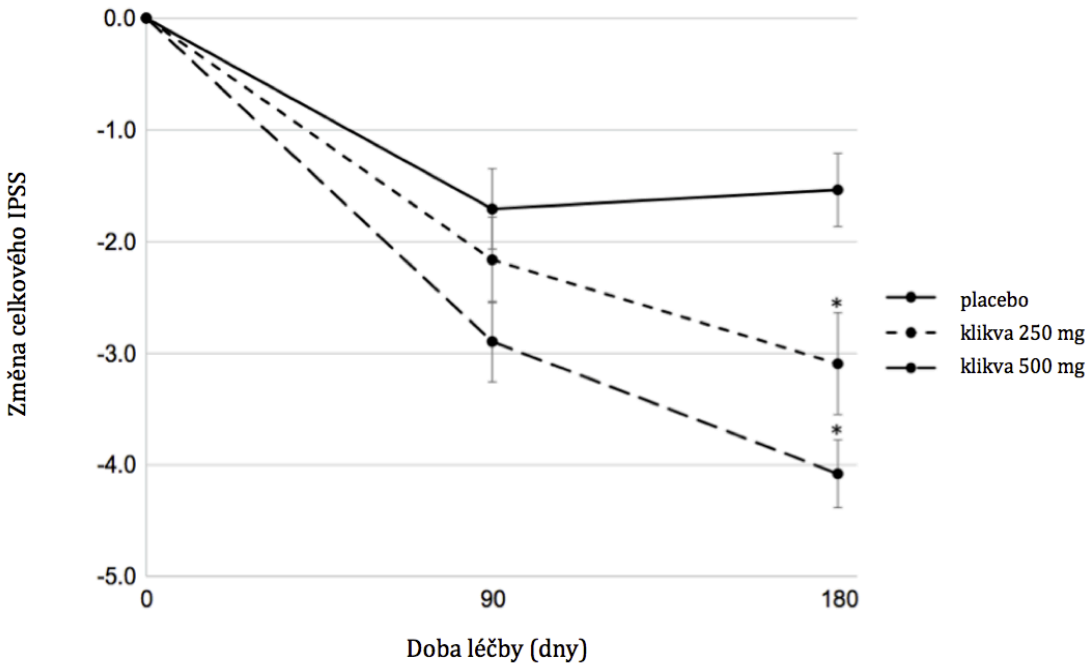
5.6 Vliv *V. macrocarpon* na urologické parametry mužů u mužů se symptomy dolních cest močových (příloha 6)

Vliv klikvy velkoplodé byl ověřen u dosud urologicky neléčených mužů s počínajícími LUTS (IPSS≤8, PSA<2.5 ng/ml). Celkem 124 mužů bylo randomizováno do tří skupin: placebo, 250 mg a 500 mg lyofilizovaného plodu klikvy velkoplodé/den po dobu šesti měsíců.

U vyšší dávky klikvy 500 mg byl prokázan vliv lyofilizovaný plodu klikvy velkoplodé na LUTS (pokles IPSS skóre o 3 body) (obr. 7).



Obr. 7. Vliv klikvy velkoplodé na hodnoty IPSS skóre ve skupině placebo, klikva 250 mg a 500 mg/den.
Hodnoty jsou vyjádřeny jako rozdíl mezi dnem 180 (T3) a dnem 0 (T1) studie.
$p < 0,05$ skupina klikva 250 mg i 500 mg *vs*. placebo

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ORIGINAL ARTICLE

Cranberry fruit powder (Flowens™) improves lower urinary tract symptoms in men: a double-blind, randomized, placebo-controlled study

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Abstract
Background Lower urinary tract symptoms (LUTS) and benign prostatic hyperplasia increase with age. To date, several medications are available to treat LUTS, including herbal remedies which offer less side effects but lack robust efficacy studies.
Methods This 6-month, randomized, double-blind, placebo-controlled study aimed at evaluating the dose effect of 250 or 500 mg cranberry powder (Flowens™) on LUTS and uroflowmetry in men over the age of 45. A total of 124 volunteers with PSA levels <2.5 ng/mL and an international prostate symptoms score (IPSS) score ≥8 were recruited and randomized. The primary outcome measure was the IPSS, evaluated at 3 and 6 months. Secondary outcome measures included quality of life, bladder volume (Vol), maximum urinary flow rate (Q_{max}), average urinary flow rate (Q_{ave}), ultrasound-estimated post-void residual urine volume (PVR), serum prostate-specific antigen, selenium, interleukin 6, and C-reactive protein at 6 months.
Results After 6 months, subjects in both Flowens™ groups had a lower IPSS (−3.1 and −4.1 in the 250- and 500-mg groups, $p = 0.05$ and $p < 0.001$, respectively) versus the placebo group (−1.5), and a dose–response effect was observed. There were significant differences in Q_{max}, Q_{ave}, PVR, and Vol in the Flowens™ 500-mg group versus baseline ($p < 0.05$). A dose-dependent effect on Vol was observed, as well as on PVR, for participants with a nonzero PVR. There was no effect on clinical chemistry or hematology markers.
Conclusions Flowens™ showed a clinically relevant, dose-dependent, and significant reduction in LUTS in men over 45.

Keywords *Vaccinium macrocarpon* · Cranberry · Lower urinary tract symptoms · Benign prostatic hyperplasia · IPSS

Background

Lower urinary tract symptoms (LUTS) become increasingly bothersome as men age, with a prevalence of moderate-to-severe symptoms rising to nearly 50 % of men in their eighties [1]. LUTS may be related to benign prostatic hyperplasia (BPH) that occurs in 50 % of men in their 50 and 90 % of men in their eighties [1] or can arise from age-related bladder detrusor dysfunction and other sympathetic conditions [2]. LUTS are measured using the international prostate symptoms score (IPSS), a validated tool, widely used among the medical community [3].

Although LUTS are not a life-threatening condition, its impact on quality of life (QoL) can be significant and treatment is necessary in most cases to avoid complications [4] and in certain cases, surgery may be recommended. Upon diagnosis, watchful waiting is recommended in approximately 34 % of cases in the USA [1].

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Registered pharmacological treatments for LUTS may be responsible for a variety of side effects. Complementary medicine is increasingly being used by men who wish to reduce LUTS [5]. Current herbal remedies include stinging nettle (*Urtica doica*), saw palmetto (*Serenoa repens*), African plum (*Pygeum africanum*), rye pollen (*Secale cereale*), South African star grass (*Hypoxis rooperi*), pumpkin seeds (*Cucurbita pepo* L.), pine (*pinus*), spruce (*picea*), flaxseed, and beta-sitosterol, which may exert inhibition of 5α-reductase, as well as anti-estrogenic, anti-proliferative, and anti-inflammatory effects [5].

Cranberry fruit (*Vaccinium macrocarpon* Ait., Ericaceae) was used by Native Americans to treat kidney and urinary ailments [6]. Cranberry fruit is recognized as a rich source of organic and phenolic acids, flavonols, flavan-3-ols, anthocyanins, proanthocyanidins (PACs), and pentacyclic triterpenoids, including ursolic and oleanic acids [7]. Preventive use of cranberry ingredients for urinary tract infections has encouraged this research on LUTS in men.

A recent study reported that a 6-month daily intake of 1500 mg cranberry powder significantly reduced the IPSS by 4.48, increased the urinary flow rate, and reduced total prostate-specific antigens (PSA) and post-void residual volume (PVR) in men with LUTS [8]. The aim of this study was to evaluate the effect of a 6-month daily intake of 250 or 500 mg of cranberry powder (Flowens™) on lower urinary tract (LUT) parameters in men with moderate-to-severe LUTS with IPSS score ≥8 and a PSA <2.5 ng/mL.

Methods

Flowens™ and placebo capsules

Flowens™ (dry cranberry powder, Batch No. 120906) supplied by NATUREX-DBS LLC., USA, was used. Capsules consisted of either 500 mg of Flowens™ or a combination of 250 mg of Flowens™ and 250 mg of placebo or 500 mg of placebo (low-density STAR-DRI® 1015A maltodextrin, canola oil, Red 40 Lake, sodium aluminum silicate, and Blue 1 Lake). The capsules were indistinguishable in appearance. All capsules were provided in identical plastic boxes with safe seal.

Study design and participants

The study was a 6-month, single-center, randomized, double-blind, placebo-controlled trial, consisting of three parallel treatment arms. The study was conducted at the Department of Urology at the university hospital in Olomouc in the Czech Republic, according to the guidelines laid down in the Declaration of Helsinki. All procedures involving human subjects were approved by the Ethics Committee of the University Hospital and Faculty of Medicine and Dentistry, Palacky University in Olomouc, Czech Republic (reference 55/12). Enrollment began in October 2012 with follow-up completed in July 2013. Inclusion criteria comprised IPSS score ≥8 and PSA values <2.5 ng/mL. Exclusion criteria included food allergies, prostatitis, chronic liver or kidney diseases, neurological, gastrointestinal or metabolic disorder, or any other chronic health condition. Subjects were also ineligible if they had prior invasive treatment for BPH or recent treatment with α-blockers (within 1 month) or 5α-reductase inhibitors (within 6 months) or phytotherapy (within 3 months). The primary endpoint of this study was the evaluation of LUTS using IPSS, evaluated at baseline, 3, and 6 months. Secondary endpoints included quality of life (QoL) at baseline, 3, and 6 months, as well as bladder voided volume (Vol), maximum urinary flow rate (Q_{max}), average urinary flow rate (Q_{ave}), ultrasound-estimated PVR, serum PSA, selenium, interleukin-6 (IL-6), and C-reactive protein (CRP), at baseline and 6 months.

Intervention and randomization

Written informed consent was obtained from the eligible participants. They were instructed not to make dietary or lifestyle changes during the study. Participants were randomly assigned to consume daily 500, 250 mg of Flowens™ or placebo for 6 months. The randomization plan was generated with online software QuickCalcs (GraphPad Software Inc., USA, last accessed on July 2, 2014) and carried out by clinical staff not directly involved in the study.

Participants were observed at baseline and 6 months for: (1) detailed medical history, (2) assessment of all concurrent medical drugs and therapies, (3) dietary habits, (4) completion of the IPSS questionnaire, including a question on QoL, (5) urinalysis, (6) uroflowmetry, (7) kidney and bladder ultrasound, and (8) blood laboratory analysis including PSA. At 3 months, only the physical examination and IPSS score were performed. The Flowens™ bottles were collected at 3 months and at the end of study. Compliance was assessed by performing remaining capsule counts.

Uroflowmetry

Q_{max} and Q_{ave} were measured using FlowMic (Medkonsult, Czech Republic). The Q_{max} and Q_{ave} were calculated by measuring the Vol per unit of time. PVR was assessed within 10 min of voiding using an ultrasound device BK Medical Viking 2400 with abdominal probe 3–7 MHz. Vol, and PVR were calculated using the formula for a prolate ellipsoid (width × length × height × 0.523).

Table 1 Summary of baseline characteristics and LUT measures

	Placebo ($n = 41$)	Flowens™ 250 mg ($n = 43$)	Flowens™ 500 mg ($n = 38$)
Age (years)	54.0 ± 5.1	53.3 ± 5.2	52.5 ± 5.4
Weight (kg)	89.3 ± 11.9	91.2 ± 11.9	90.1 ± 8.0
Height (cm)	178.5 ± 6.6	180.6 ± 6.6	180.7 ± 6.2
Body Mass Index	28.1 ± 3.8	27.9 ± 2.9	27.7 ± 3.0
Systolic blood pressure (mmHg)	131.1 ± 12.1	130.6 ± 10.1	132.1 ± 11.5
Diastolic blood pressure (mmHg)	80.1 ± 7.3	80.5 ± 7.2	80.9 ± 7.4
Heart beat (bpm)	68.9 ± 3.5	67.8 ± 4.6	68.1 ± 4.4
IPSS (score)	9.1 ± 2.0	9.7 ± 3.1	9.4 ± 2.0
PVR (mL)	15.0 ± 19.2	15.9 ± 23.2	17.8 ± 21.0
Q_{max} (mL/s)	22.0 ± 7.8	20.5 ± 7.1	19.5 ± 7.5
Q_{ave} (mL/s)	14.3 ± 5.2	12.5 ± 4.6	12.5 ± 5.5
Bladder volume (mL)	408.5 ± 117.9	339.9 ± 114.4	339.0 ± 118.9

Results are presented as mean ± standard deviation (SD)

Clinical chemistry and hematology

Basic biochemical and hematological parameters were determined in all samples using a HITACHI Modular Evo P analyzer (Hitachi, Japan). Serum PSA was determined using an Architect-type LEIA analyzer (Abbott Laboratories, Abbott Park, IL, USA). CRP was determined by a Quikread 101 and IL-6 by the system Modular® Analytics <E176>. Selenium in plasma was estimated by atomic absorption spectrometry using the AA6300 instrument (Shimadzu, Japan). Hemoglobin (Hb), hematocrit (Htc), erythrocytes (RBC), thrombocytes (PLT), and leukocytes (WBC) were measured in Na_2EDTA blood.

Statistical methods

The primary and secondary analyses were based on the per-protocol population that included all eligible participants who were treated during the entire length of the study. A Mann–Whitney *U* test was used to compare both treatment dose and placebo data. Differences versus baseline measures were performed using the Wilcoxon matched pairs test. *P* values <0.05 were considered to be significant.

An analysis of covariance was used to test whether there was an effect of the dose on the outcome measure at the end of treatment. The volume of urine among participants with PVR was modeled using a truncated Poisson distribution. A two-stage model was fit using the *hurdle* function in the 'pscl' package (Developed by Achim Zeileis and Simon Jackman, Stanford University) running on R version 3.0.0. Dose/250 mg, baseline PVR, and baseline IPSS were entered into this model.

Results

A total of 148 men were pre-screened for the study. A total of 124 men were randomized, 41 to the placebo group, 43 to the Flowens™ 250-mg group, and 40 to the Flowens™ 500-mg group. In the Flowens™ 500-mg group, two participants were lost to follow-up and were not included in the per-protocol analysis. Table 1 presents a summary of baseline characteristics and LUT function measures across the three groups of the analysis. Adherence with scheduled visits was 98.4 %. Compliance to the treatment was 100 %.

IPSS data with voiding and storage symptom subscore and QoL data during the 6-month treatment period are presented in Table 2. Uroflowmetry data are presented in Table 2.

At 6 months, mean difference and corresponding 95 % confidence interval (CI) were −1.5 (−2.2, −0.89) for the placebo group, −3.1 (−4.0, −2.2) for the Flowens™ 250-mg group, and −4.1 (−4.7, −3.5) for the Flowens™ 500-mg group (Fig. 1).

Analysis of covariance for IPSS at 6 months with baseline IPSS entered as a covariate showed a significant dose effect ($t_{119} = -4.8$, $p < 0.0001$) and a significant effect of baseline score ($t_{119} = 8.3$, $p < 0.0001$). In the Flowens™ 500-mg group, a significant reduction in voiding symptoms was observed at both visits ($p = 0.03$ and $p < 0.001$, respectively); as well as storage symptoms at 6 months ($p = 0.018$) (Table 2). At 6 months, analysis of covariance of Q_{max} and Q_{ave} found a statistically indeterminate effect of dose. 59, 49, and 50 % of the participants in the placebo, 250, and 500 mg Flowens™ groups reported a nonzero PVR, respectively, which represented a significant dose-dependent reduction in PVR of 0.09 (95 % CI 0.03–0.14)

Table 2 Participants IPSS score, voiding and storage symptom score, quality of life score, and uroflowmetry at baseline, 3, and 6 months after placebo, Flowens™ at 250- or 500-mg intake

	Group	Baseline, Mean ± SD (*p* value)		3 months, Mean ± SD (*p* value)		6 months, Mean ± SD (*p* value)		Relative change at 6 months (% change vs placebo)
Total IPSS score	Placebo	9.1 ± 2.0		7.4 ± 2.0		7.6 ± 2.6		−1.5 ± 2.1
	Flowens™ 250 mg	9.7 ± 3.1	(NS)	7.6 ± 3.7	(NS)	6.6 ± 3.4	0.05	−3.1 ± 3.0
	Flowens™ 500 mg	9.4 ± 2.0	(NS)	6.5 ± 2.6	(NS)	5.3 ± 2.5	<0.001*	−4.1 ± 1.9
Voiding/obstructive symptoms score	Placebo	4.9 ± 1.8		3.7 ± 1.6		3.9 ± 2.3		−1.0 ± 1.9
	Flowens™ 250 mg	5.1 ± 2.4	(NS)	3.6 ± 2.7	(NS)	3.4 ± 2.8	(NS)	−1.8 ± 2.1
	Flowens™ 500 mg	4.6 ± 1.8	(NS)	2.9 ± 1.7	0.03*	2.3 ± 1.4	<0.001*	−2.3 ± 1.8
Storage/irritative symptoms score	Placebo	4.2 ± 1.3		3.7 ± 1.4		3.7 ± 1.4		−0.5 ± 1.2
	Flowens™ 250 mg	4.6 ± 1.5	(NS)	3.8 ± 1.8	(NS)	3.3 ± 1.5	(NS)	−1.3 ± 1.6
	Flowens™ 500 mg	4.8 ± 1.6	(NS)	3.6 ± 2.0	(NS)	3.0 ± 1.9	0.018*	−1.8 ± 1.4
Quality of life	Placebo	2.4 ± 0.9		2.1 ± 0.8		2.0 ± 0.7		−0.4 ± 0.81
	Flowens™ 250 mg	2.3 ± 0.8	(NS)	2.2 ± 0.9	(NS)	2.0 ± 0.7	(NS)	−0.3 ± 0.7
	Flowens™ 500 mg	2.1 ± 0.6	(NS)	2.0 ± 0.7	(NS)	1.9 ± 0.5	(NS)	−0.2 ± 0.6
Q_{max} (mL/s)	Placebo	22.0 ± 7.8	–	–	–	21.9 ± 8.6	(NS)	−0.1 ± 5.2
	Flowens™ 250 mg	20.5 ± 7.1	–	–	–	21.4 ± 6.7	(NS)	+0.9 ± 5.0
	Flowens™ 500 mg	19.5 ± 7.5	–	–	–	21.7 ± 8.9	0.018§	+2.2 ± 5.8
Q_{ave} (mL/s)	Placebo	14.3 ± 5.2	–	–	–	14.2 ± 5.1	(NS)	−0.1 ± 2.8
	Flowens™ 250 mg	12.5 ± 4.6	–	–	–	13.2 ± 4.0	(NS)	+0.7 ± 3.4
	Flowens™ 500 mg	12.5 ± 5.4	–	–	–	13.8 ± 5.7	0.040§	+1.3 ± 3.9
PVR (mL)	Placebo	15.0 ± 19.2	–	–	–	14.4 ± 18.3	(NS)	−0.6 ± 24.5
	Flowens™ 250 mg	15.9 ± 23.2	–	–	–	13.6 ± 18.1	(NS)	−2.3 ± 26.3
	Flowens™ 500 mg	17.8 ± 21.0	–	–	–	9.9 ± 13.6	0.027§	−7.9 ± 21.4
Vol (mL)	Placebo	408.5 ± 117.9	–	–	–	364.3 ± 112.5	(NS)	−44.2 ± 92.0
	Flowens™ 250 mg	339.9 ± 114.4	–	–	–	368.6 ± 104.6	(NS)	+28.7 ± 112.6
	Flowens™ 500 mg	339.0 ± 118.9	–	–	–	393.0 ± 134.0	0.014§	+54.0 ± 122.5

Results are presented as mean ± SD

NS not significant

* Denotes a significant difference versus placebo using the Mann–Whitney *U* test

§ Denotes a significant difference versus baseline using the Wilcoxon matched pairs test

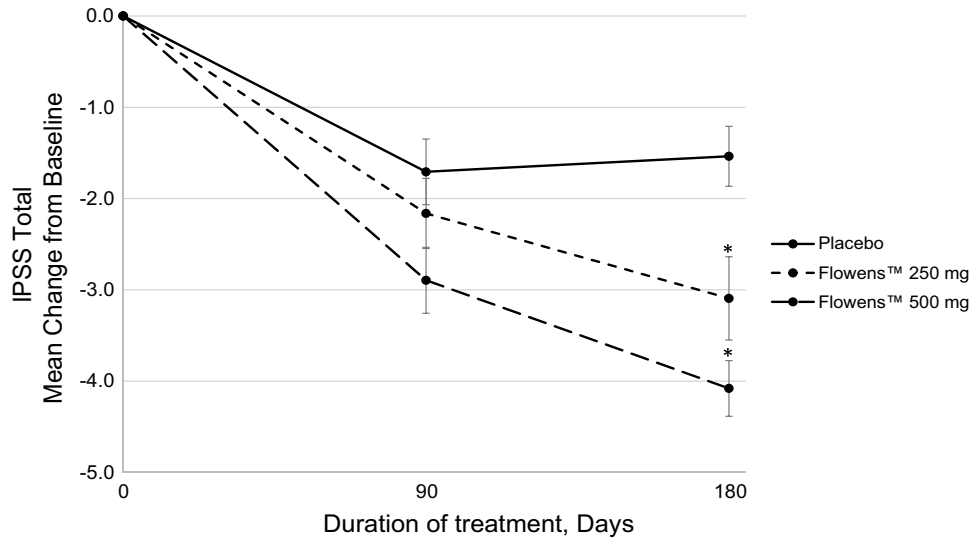

Fig. 1 Mean change in total IPSS score from baseline. Data represent the mean change ± the standard error of the mean. *$p < 0.05$ versus placebo based on analysis of covariance at the end of treatment

per 250-mg dose, for a given baseline PVR and IPSS ($z = -3.0$, $p = 0.003$). Vol measurements, with baseline Vol entered as a covariate, found a significant linear effect of dose ($t_{119} = 2.8$, $p = 0.005$).

All clinical hematology parameters were within normal range at the beginning and at the end of the study, thereby demonstrating the safety of the intervention product (data not shown).

Discussion

This double-blind, randomized, placebo-controlled study demonstrated the efficacy and the safety of the daily intake of Flowens™ at 250 or 500 mg in men with LUTS for 6 months. At 6 months, the decrease in IPSS score was significant and dose dependent (−3.1 and −4.1 in the 250 and 500-mg groups, $p = 0.05$ and $p < 0.001$, respectively) versus the placebo group (−1.5), while no side effects were observed. This decrease in IPSS score was >3 versus baseline for both doses, which is considered clinically meaningful by the American Urological Association [3]. In the 500-mg group, voiding symptoms were significantly reduced at 3 and 6 months versus placebo, and storage symptoms were significantly reduced versus placebo at 6 months. In addition, all parameters (Q_{max}, Q_{ave}, Vol, and PVR) were significantly improved versus baseline at the end of treatment ($p < 0.05$). However, urinary flow rate measures tend to suffer from some inaccuracy due to the natural variation in urinary flow with the same individual from test to test and to training effect. Urinary flow rate also decreases as men age, and a clinical cutoff value of 15 mL/s has been defined to identify those with higher risk of having bladder outlet obstruction [9], defining the current cohort as being moderately symptomatic. A PVR superior to 100 mL suggests abnormality and could require further tests [10]. Although a strong relationship has been found between PVR and prostate volume [11], PVR lacks precise clinical or urodynamic meaning [12], limiting direct conclusions linking PVR with the beneficial effect of Flowens™ on LUTS. Bladder volume was also significantly increased with Flowens™ dose, which may be related to an improvement in bladder detrusor activity [2, 13].

The results obtained with Flowens™ were superior to those observed with most other botanicals. For instance, *Serenoa repens* (Saw palmetto) was not superior to placebo at any dose after a 72-week trial [14]. In addition, only few *Serenoa repens* studies used validated symptom scores, and most were-short duration studies [15]. Purified beta-sitosterol, on the other hand, significantly improved IPSS score to an extent similar to that of Flowens™; a −4.9 weighted mean difference was observed in two different 6-month studies [16]; however, these studies were conducted in the early 1990s, and no additional trial has confirmed these results, except for a few open studies on stinging nettle [17, 18]. A Concord grape juice was overall not effective at reducing LUTS in men over 45 years old, albeit an improvement in Q_{max}. These results might be due the length of the study, as well as a low concentration in antioxidant compounds [19].

Potential mechanism of action may involve effects on the bladder detrusor contraction and relaxation (through muscarinic receptor agonist or α-blockers) or on dynamic and static prostatic components of voiding (through α-blockers, 5α-reductase inhibition, or phosphodiesterase-5 inhibition), modulation of micturition reflex, or reduction in inflammation [2, 20–24].

Conclusions

In this double-blind, randomized, placebo-controlled intervention study, 250 or 500 mg of Flowens™ taken once daily showed significant, clinically meaningful and dose-dependent reduction in LUTS, as demonstrated by a reduction in IPSS score of >3 after a 6-month period. Larger, multi-centric clinical studies with a longer follow-up period and side effects reporting may be warranted to confirm these data in order to recommend Flowens™ as a possible alternative in reducing LUTS for moderately symptomatic men.

Acknowledgments Financial support from NATUREX-DBS is gratefully acknowledged. The cranberry powder (Flowens™) was supplied by NATUREX-DBS, SAGAMORE, MA 02561.

Conflict of interest MR and EF are employed by NATUREX and NATUREX-DBS, respectively.

Ethical standard Ethics Committee of the University Hospital and Faculty of Medicine and Dentistry, Palacky University in Olomouc, Czech Republic (reference 55/12).

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5.7 Vliv *V. macrocarpon* na biochemické markery u mužů s rakovinou prostaty

(studie 7)

V randomizované, dvojitě slepé, placebem kontrolované studii byl ověřen účinek klikvy velkoplodé na pacienty s prokázaným KP (**studie 7**). Práškovitý plod *V. macrocarpon* byl užíván v dávce 1500 mg/den pacienty 30 dní před radikální prostatektomií. Dle výsledků bylo PSA v den operace o 22,5 % nižší ve skupině klikvy, naopak o 0,9 % vyšší v placebové skupině. Snížení sérového PSA může být spojeno s down-regulací beta-microseminoproteinu (MSMB). Signifikantní vzestup hladiny malondialdehydu v plazmě u obou skupin ukazuje, že užívání brusinky nemá u pacientů s karcinomem prostaty vliv na snížení oxidačního stresu. Výsledky prokazují, že intaktní plod obsahuje látky, které regulují expresi androgen-sensitivních genů což by mohlo oddálit biochemický návrat onemocnění. Naše výsledky o vlivu plodu *V. macrocarpon* jsou prvním *in vivo* potvrzením působení obsahových látek plodu klikvy na buňky nádoru prostaty u mužů. Ve studiích účinků plodu klikvy na karcinom prostaty byly experimenty prováděny pouze na šesti liniích nádorových buněk nebo laboratorním potkanu (1 studie) s vyvolaným nádorem prostaty. Aplikovány byly extrakty polyfenolů získané ze šťávy plodu (Weh et al., 2016).

Cranberry intervention in patients with prostate cancer prior to radical prostatectomy. Clinical, pathological and laboratory findings

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Background and Objectives. Recently, we described an inverse association between cranberry supplementation and serum prostate specific antigen (PSA) in patients with negative biopsy for prostate cancer (PCa) and chronic nonbacterial prostatitis. This double blind placebo controlled study evaluates the effects of cranberry consumption on PSA values and other markers in men with PCa before radical prostatectomy.
Methods. Prior to surgery, 64 patients with prostate cancer were randomized to a cranberry or placebo group. The cranberry group (n=32) received a mean 30 days of 1500 mg cranberry fruit powder. The control group (n=32) took a similar amount of placebo. Selected blood/urine markers as well as free and total phenolics in urine were measured at baseline and on the day of surgery in both groups. Prostate tissue markers were evaluated after surgery.
Results. The serum PSA significantly decreased by 22.5% in the cranberry arm (n=31, $P<0.05$). A trend to down-regulation of urinary beta-microseminoprotein (MSMB) and serum gamma-glutamyltranspeptidase, as well as upregulation of IGF-1 was found after cranberry supplementation. There were no changes in prostate tissue markers or, composition and concentration of phenolics in urine.
Conclusions. Daily consumption of a powdered cranberry fruit lowered serum PSA in patients with prostate cancer. The whole fruit contains constituents that may regulate the expression of androgen-responsive genes.

Key words: *Vaccinium macrocarpon*, randomized controlled trial, adenocarcinoma, PSA, cancer markers, urinary metabolites.

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INTRODUCTION

Prostate cancer (PCa) is the second leading cause of cancer death in men in Europe[1]. Advanced age, race, and a family history of PCa are identifiable risk factors associated with PCa occurrence. Physical activity and diet, modifiable risk factors, have been suggested to play a role in the pathogenesis of PCa (ref.[2]). Consuming a more plant-based diet for cancer prevention is recommended[3]. Several components derived from edible plants may contribute in reducing the risk of PCa (ref.[4]). Thus, food components (nutraceuticals) or complex plant extracts act as anti-oxidants, anti-inflammatory remedies, weak 5α-reductase inhibitors or target androgen receptor (AR) synthesis and signalling[5]. Molecular mechanisms of the anti-cancer action of natural products were mostly studied on human androgen-dependent and/or independent prostate cell lines. Only a few foods/nutraceuticals have been tested in humans as chemoprotective agents against prostate cancer. Two very recent reviews of intervention clinical trials have been published on: silymarin/silibinin, broccoli sprouts/sulforaphane, pomegranate extract, tomatoes/lycopene, grapes, green tea extract, polyphenols, soybean/genistein, flaxseed/lignans and α-linolenic acid, and fish oil/omega-3 fatty acids in men following surgery or radiation for PCa (ref.[6,7]).

Cranberry fruit contains several types of components which play a role in its health benefits. These include vitamins C, E, K, organic and phenolic acids, saccharides, flavan-3-ols, flavonols, anthocyanins, anthocyanidins, proanthocyanidins and triterpenoids, shown to possess anti-bacterial, anti-viral, anti-oxidant, anti-inflammatory, anti-angiogenic and anti-cancer activities[8,9]. Most of this evidence, with the exception of preventing urinary tract infections, was derived from studies on prostate cancer cell lines[10-13]. Our recent clinical study reported that daily intake of 1500 mg cranberry powder for 6 months significantly reduced total prostate-specific antigen (PSA) elevated from chronic non-bacterial prostatitis, and/or benign prostate hyperplasia[14]. The primary objective of this study was to investigate the effect of the cranberry fruit powder (CFP) supplementation on PSA response in men with PCa. The post-treatment differences between the cranberry and placebo group on basic physiological biomarkers, selected blood inflammatory markers, urine and prostate tissue markers were secondary outcome measures.

MATERIALS AND METHODS

Cranberry material

Cranberry fruit powder (CFP; PACRAN® EU-SP_06104, Batch No: A333/132/A12) was supplied by NATUREX-DBS, USA). For structure and content of selected compounds in CFP see Appendix. Capsules of two kinds contained: 500 mg of CFP or 500 mg of placebo of the following composition: low density STAR-DRI® 1015A maltodextrin, canola oil, Red 40 Lake, sodium aluminium silicate and Blue 1 Lake. CFP capsules were indistinguishable in appearance from the placebo capsules. All capsules were provided in plastic boxes with safe seal labelled PACRAN®.

Ethics statement, study design, patients, randomization and treatment dose

The study was conducted at the Department of Urology according to the guidelines laid down in the Declaration of Helsinki (2008 revision). All procedures involving human subjects were approved by the Ethics Committee of the University Hospital and Faculty of Medicine and Dentistry, Palacky University in Olomouc, Czech Republic (reference number 55/12). All participants signed a letter of informed consent.

The study design was a single-center, randomized, placebo-controlled intervention trial consisting of two parallel treatment arms. The enrollment began in May 2012 with follow-up complete in May 2013. To be eligible, the patient had to have a pathological diagnosis of adenocarcinoma of the prostate from prostate biopsy. Other inclusion criteria were BMI<37, normal liver function test, normal range of blood pressure and heart rate. The exclusion criteria were current antibiotic use (antibiotics reduce the intestinal microflora) or history of hormonal and radiation therapies or chemotherapy. Before study entry, patients medical history was taken and a physical examination, complete blood count and clinical chemistry profile.

The subjects (n=64) were randomly assigned to either cranberry group, a daily dose 1500 mg dry cranberry fruit powder (n=32) or placebo group (n=32) at least 21 days before surgery. The randomization plan for treatment assignment to patients was generated using on line software QuickCales (GraphPad Software Inc., USA). Suggested clinical dosing of cranberry fruit powder was based on our study in men at risk of prostate disease[14]. Patients were instructed not to consume food rich in color pigment (anthocyanin-containing fruit) or soy products or to make other dietary or lifestyle changes during the study. Compliance was assessed by performing a remaining capsule counts at the end of the intervention when the patients were admitted to the hospital for surgery.

Blood and urine samples for clinical chemistry, hematology and urine analysis were collected on the first day at registration and after intervention immediately before surgery. The prostatectomy specimens were embedded in paraffin, step-sectioned, and microscopically examined.

Clinical chemistry and hematology

Basic biochemical and hematological parameters were determined. Total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), triacylglycerols (TAG), C-reactive protein (CRP), alanine aminotransferase (ALT), gamma-glutamyltranspeptidase (GGT), glycaemia, creatinine, blood urea nitrogen (BUN) and interleukin-6 (IL-6) were quantified in serum using a Cobas (Hitachi, Japan). Testosterone (TST) and prostate specific antigen (PSA) in serum was determined using an Architect i2000SR (Abbott, USA). Insuline-like growth factor-1 (IGF-1), insulin-like growth factor binding protein (IGFP) and free testosterone (fTST) were quantified in serum using a RIA kits by Cobra 5005 analyzer (Packard BioInstruments, USA).

Selected hematological parameters were measured.

Selected parameters for evaluation of oxidative stress total sulphydryl groups (TSHG), lipid peroxidation products such as malondialdehyde in plasma (PMDA) and erythrocytes (MDA), glutathione (GSH), glutathione peroxidase (GSHPer), glutathione reductase (GSHRed), superoxide dismutase (SOD) and catalase in erythrocytes were carried out as described by Vidlar et al.[14]. Paraoxonase 1 (PON1) activity was measured according to Sumegova et al.[15]. Plasma 8-hydroxydeoxyguanosine (8-OHdG) was measured by OxiSelect™ Oxidative DNA Damage ELISA, 8-OHdG Quantification kit. Plasma selenium and zinc were determined by inductively coupled plasma mass spectrometry (Agilent 7700x, Agilent Japan) using octapole reaction cell in He mode to eliminate spectral interference. The determination was performed by external calibration. Calibration solutions were prepared by diluting certified reference materials - water calibration solutions of Se and Zn with concentration 1.000 +/- 0.002 mg/L (Analytika Ltd, Czech Republic). Prior to ICP-MS determination blood plasma samples were digested in microwave mineralizator (Meg 1200 mls, Milestone, Italy) with mixture of HNO_3 (Analpure, Analytika Ltd, Czech Republic) and H_2O_2 (analytical grade, Analytika Ltd., Czech Republic).

RNA urine markers

Urine samples were collected and analyzed as described previously[16]. Briefly, a portion of the first voided urine after an "attentive" prostate massage was used. Total RNA was isolated, quantified and reverse transcribed. The quantitative real-time PCR (qPCR) reactions were performed with specific primers and probes for AMACR, PCA3, EZH2, MSMB, PSA and TRPM8 on LightCycler® 480, Roche. After relative quantification ($\Delta C_t = C_{t\ target} - C_{tPSA}$) inverse values of ΔC_t ($-\Delta C_t$) were used for subsequent statistical analysis and visualization[17]. As the first step, we evaluated the urine samples after treatment with placebo or cranberry. With respect to mild changes between placebo and cranberry arms, we also analyzed the first urine samples (before treatment) for 39 patients [other patient samples were not included for the following reasons: urine samples were not collected (n=3), urine RNA had low concentration (n=3) or results of qRT-PCR

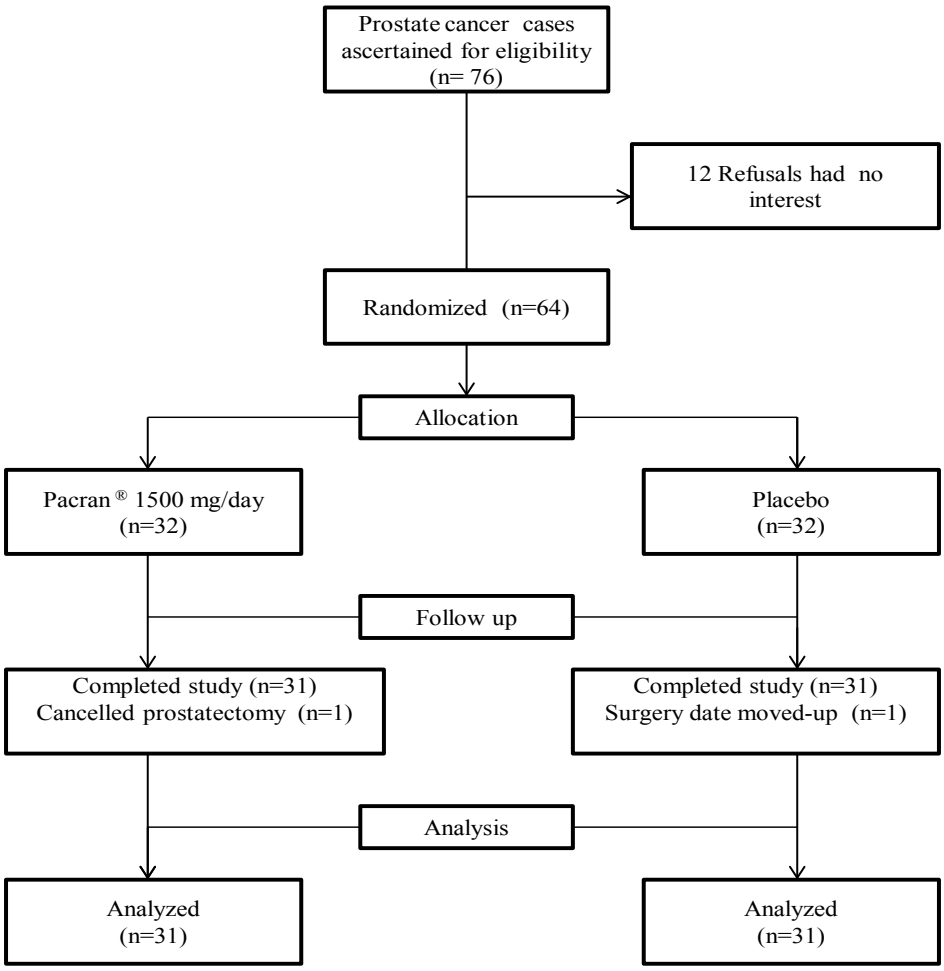

Fig. 1. CONSORT trial flow diagram.

after treatment were non-evaluable because of negative or low expression of PSA mRNA (n=17).

Specimen collection and immunohistochemistry

The prostatectomy specimens were formalin-fixed, paraffin-embedded, and sectioned for routine examination by indirect immunohistochemistry. Five-micron thick sections with representative tumor and surrounding areas were stained with the following antibodies and dilutions: Ki-67 at 1:200 (clone MIB-1, Dako); Chromogranin A at 1:100 (clone 5H7, Novocastra); PSMA at 1:100 (clone YPSMA, Abcam); AR at 1:100 (clone AR441, Dako); NF-κB p65 at 1:400 (clone F-6, Santa Cruz) and COX-2 at 1:50 (clone M-19, Santa Cruz). Visualization was performed by the DakoREAL™ EnVision™+ Dual Link System-HRP and Dako Liquid DAB plus Substrate Chromogen System.

Statistical methods

Data were analyzed with statistical software R (http://www.r-project.org/). Instead of absolute values, the differences in time were used for computations with continuous parameters. Student's two-sample t-test (between groups comparison of differences in time) and Student's one-sample t-test (tests of location – zero differences in time) were used for normally distributed data (tested with the Shapiro-Wilk normality test), otherwise nonparametric Wilcoxon two-sample or one-sample tests were used. Categorical data were analyzed with Fisher's exact rank tests. Graphs of empirical cumulative distribution functions were used as a graphical data representation.

RESULTS

Patient recruitment is depicted in Fig. 1. We invited 76 patients aged 45 to 75 years who were scheduled to undergo radical prostatectomy as their primary treatment and at least 21 days before surgery. Twelve patients had no interest to take part in a trial. There were no significant differences between the cranberry and placebo groups with regard to age, body mass index (BMI), plasma concentrations of zinc and selenium (Table 1). Median range of Gleason score was 7 (6.7) for cranberry and 7 (7.7) for placebo group. With regard to other factors, patients in the cranberry group had a statistically significant higher initial PSA values than those of the placebo group. Table 2 shows preoperative (clinical) and postoperative (pathological) T stage for patients in both groups. There is no significant difference between cranberry and placebo can-

Table 1. Patient baseline demographics and clinical characteristics.

Parameter	Unit	Cranberry group (n=31)	Placebo group (n=31)
Age	Years	62.5/66.0/68.0	61.0/64.0/68.5
BMI	kg/m^2	24.8/27.8/28.7	25.8/26.9/29.1
Initial PSA	ng/mL	6.2/8.8/13.6	3.7/5.4/9.3$^{\#}$
Zinc	mg/L	0.981/1.174/1.438	1.034/1.225/1.577
Selenium	ng/mL	97.1/120.1/131.0	102.7/120.1/142.7
Prostate volume	mL	49.3/57.5/65.0	40.5/49.0/61.0
Gleason score		n (%)	n (%)
5		2 (6.45)	2 (6.45)
6		7 (22.58)	4 (12.90)
7		19 (61.29)	25 (80.65)
8		1 (3.23)	0
9		2 (6.45)	0

The values are expressed as first quartile, median and third quartile.
$^{\#}$Significant difference between Cranberry and Placebo groups by two-sample Wilcoxon test $P<0.1$

Table 2. Histopathological preoperative and postoperative T stage.

Preoperative T stage, n				Postoperative T stage, n			
Cranberry group		Placebo group		Cranberry group		Placebo group	
T1a-T1c	21	T1a-T1c	22	pT2a	3	pT2a	2
T2a	2	T2a	4	pT2b	3	pT2b	2
T2b	5	T2b	2	pT2c	17	pT2c	20
T2c	3	T2c	3	pT3a	5	pT3a	4
				pT3b	3	pT3b	3

Table 3. Markers of hematology.

		Cranberry group		Placebo group	
Parameter	Unit	Start Day	End Day	Start Day	End Day
Htc		0.43/0.44/0.46	0.42/0.44/0.46$^{**;\#\#}$	0.43/0.44/0.46	0.43/0.45/0.46$^{\#\#}$
Hb	g/L	145.5/151.0/155.0	142.5/149.0/156.0	143.0/150.0/156.0	145.0/149.0/157.5
PLT	x10(9)/ L	187.5/232.0/257.0	184.0/207.0/249.0	189.5/223.0/266.5	190.0/233.0/258.5
RBC	x10(12)/L	4.71/4.91/5.20	4.64/4.88/5.17	4.74/4.92/5.11	4.70/4.91/5.21
WBC	x10(9)/L	5.54/6.40/7.25	5.32/6.20/7.03	5.90/6.49/7.62	5.80/7.42/7.85

The values are expressed as first quartile, median and third quartile.
**Significant difference between value of Start day by one-sample Wilcoxon test $P<0.05$
$^{\#\#}$Significant difference between value of Cranberry and Placebo group by two-sample Wilcoxon test $P<0.05$
**One-sample Wilcoxon test, $P<0.05$; $^{\#\#}$Two-sample Wilcoxon test, $P<0.05$
Abbreviations used: Hct (hematocrit), Hb (hemoglobin), PLT (platelets), RBC (red blood cells), WBC (white blood cells).

cer staging scores before (P=0.617) or after prostatectomy (P=0.943).

Hematology values were unchanged with the exception of hematocrit value but the fluctuations were within normal physiological limits (Table 3).

Serum PSA concentration decreased by 22.5% in the cranberry group, whereas the concentration increased by 0.9% in the placebo group over the study period (Table 4). There was also a trend towards increased IGF-1 and decreased GGT in the cranberry group. There were no significant differences in other clinical chemistry parameters.

The oxidative stress markers are shown in Table 5. Highly statistically significant differences for the cranberry group were found for erythrocyte malondialdehyde (MDA), plasma malondialdehyde (PMDA), total thiol groups (TSHG), catalase and for placebo group in MDA, PMDA, paraoxonase 1 (PON1) and glutathione peroxidase (GSHPer).

Table 6 shows selected markers for detection of prostate cancer in urine. A trend to down-regulation of beta-microseminoprotein (MSMB) after cranberry supplementation was observed along with up-regulation of PCA3.

The results of immunohistochemical evaluation are shown in Table 7. There were no significant differences between cranberry and placebo groups.

Phenolics in the urine were analysed directly without enzymatic cleavage and then after further incubation with a mixture of deconjugation enzymes, β-glucuronidase and sulfatase (for details see Appendix, Table II and III). There was no significant difference in free or total pheno-

Table 4. Markers of clinical chemistry.

		Cranberry group		Placebo group	
Parameter	Unit	Start Day	End Day	Start Day	End Day
ALT	μkat/L	0.37/0.43/0.49	0.39/0.44/0.55	0.35/0.45/0.49	0.37/0.45/0.53
GGT	μkat/L	0.41/0.47/0.77	0.37/0.45/0.76*	0.37/0.58/0.72	0.39/0.49/0.84
Creatinine	μmol/L	78.5/83.0/89.5	76.5/86.0/90.0	74.5/81.0/87.0	77.5/82.0/88.0
BUN	mmol/L	4.8/5.1/5.8	4.8/5.4/5.8	4.6/5.2/5.7	4/4.9/5.6
Glucose	mmol/L	5.2/5.7/6.4	5.1/5.5/6.2	5.1/5.6/6.4	5.3/5.7/6.7
TAG	mmol/L	1.05/1.38/1.66	1.028/1.29/1.69	1.315/1.67/2.51	1.235/1.65/2.19
Cholesterol	mmol/L	4.32/4.95/5.90	4.23/4.92/5.94	4.67/5.22/6.01	4.64/5.26/6.29
LDL	mmol/L	2.25/2.69/3.82	2.24/2.84/3.78	2.55/3.01/3.66	2.46/3.07/3.81
HDL	mmol/L	1.21/1.39/1.79	1.26/1.41/1.63	1.08/1.28/1.34	1.17/1.23/1.47
CRP	mg/L	0.75/1.20/2.20	0.70/1.00/1.60	0.60/1.10/2.75	0.38/1.05/2.55
Il-6	ng/L	1.5/2.9/3.95	2/2.9/3.75	1.525/2.6/3.8	1.5/2.7/3.65
IGF-1	μg/L	105/137/161	113/149/174*	113/138/170	121/142/164
IGFP	μg/L	2089/2590/2855	2148/2568/2853	2342/2581/2879	2337/2541/3090
TST	nmol/L	11.92/15.39/21.57	11.26/15.17/17.68	11.34/14.58/18.79	11.86/14.81/17.03
fTST	nmol/L	24.63/30.55/39.75	19.75/27.75/36.10	25.00/31.30/35.70	24.25/28.50/36.20*
PSA	ng/mL	6.23/8.83/13.59	4.54/6.84/13.03**	3.68/5.38/9.33	3.62/5.43/9.05**

The values are expressed as first quartile, median and third quartile.
*Significant difference between value of Start day by one-sample Wilcoxon test $P<0.1$
**Significant difference between value of Start day by one-sample Wilcoxon test $P<0.05$
*One-sample Wilcoxon test, *P<0.1*; **One-sample Wilcoxon test, $P<0.05$

Table 5. Markers of oxidative stress in blood.

		Cranberry group		Placebo group	
Parameter	Unit	Start Day	End Day	Start Day	End Day
MDA	nmol/g[a]	18.51/20.67/23.62	23.66/26.72/29.71**	18.65/20.98/25.28	23.18/27.41/32.45**
GSH	μmol/g[a]	6.97/7.85/8.92	6.93/8.01/9.03	6.97/8.04/8.58	7.01/7.84/9.07
PMDA	nmol/g[b]	82.8/103.5/119.8	81.2/115.1/151.8**	70.9/105.0/132.7	84.3/113.6/135.9**
TSHG	μmol/g[b]	6.50/7.24/8.12	6.99/7.69/8.56**	6.47/6.79/8.49	6.88/7.48/8.42*
PON1	μkat/L	0.83/1.50/2.00	0.79/1.31/2.14	0.75/1.23/2.29	0.70/1.43/2.43*
8-OHdG	μg/L	7.57/9.03/11.20	8.55/9.87/11.95	6.90/8.74/11.02	6.66/9.19/11.18
Catalase	μkat/g[a]	1.12/1.19/1.43	1.17/1.30/1.46**	1.07/1.32/1.49	1.11/1.39/1.47
GSX	μkat/g[a]	1.54/2.17/2.92	1.40/1.87/2.37*	1.86/2.25/2.84	1.18/1.69/2.19**
GSR	μkat/g[a]	0.21/0.25/0.29	0.22/0.25/0.28	0.20/0.23/0.28	0.21/0.24/0.31*
SOD	U/g[a]	3.09/3.28/3.51	3.00/3.41/3.65	3.10/3.34/3.62	3.17/3.39/3.83

The values are expressed as first quartile, median and third quartile.
[a] g of hemoglobin; [b] g of protein
*Significant difference between value of Start day by one-sample Wilcoxon test $P<0.1$
**Significant difference between value of Start day by one-sample Wilcoxon test $P<0.05$
*One-sample Wilcoxon test, *P<0.1*; **One-sample Wilcoxon test, $P<0.05$

lics between the groups. No anthocyanins or proanthocyanidins were detected in urine.

DISCUSSION

Cranberry products, fresh or dry fruit, juice, dietary supplements containing juice powder or powdered fruit, are used mainly in protection against recurrent urinary tract infection in women[21] and improvement of lower urinary parameters in men with moderate to severe LUTS (ref.[22]). Studies on the possible effects of cranberry and its components on prostate cancer were realized only in DU 145 human prostate cancer cells which are androgen independent. Treatment of cranberry proanthocyanidin-enriched fraction inhibited matrix metalloproteinase-2 and -9 (MMP) activity through the induction and/or inhibition of specific temporal MMP regulators[10]. MMP activity is associated with tumor cell invasion and metastasis. Cranberry fruit contains a high amount of ursolic acid and its esters. The ability of these components to inhibit MMP-2 and MMP-9 was determined in DU 145 cells[11]. Treatment of DU145 cells with the whole cranberry extract, flavonol-enriched or proanthocyanidin-enriched fractions induced apoptosis in cells through caspase-8 activation[12].Treatment of whole cranberry extract significantly decreased the cellular viability of DU145 cells. It also decreased the proportion of cells in the G2-M phase and increased the proportion in the G1 phase of the cell cycle. These alterations in cell cycle

Table 6. RNA urine markers.

		Cranberry group		Placebo group	
Parameter	Units	Start Day	End Day	Start Day	End Day
RNA	ng/mL	8.23/17.46/37.23	5.16/10.55/15.22*	7.37/17.19/42.52	7.48/13.70/33.25
Ct PSA	dCt	32.30/34.21/35.61	30.79/34.17/35.85	31.41/34.02/35.83	32.70/34.66/36.97
AMACR	-dCt	-4.21/-1.90/-0.79	-4.25/-2.61/-0.87	-3.47/-2.19/-0.60	-3.45/-2.35/-1.12
PCA3	-dCt	-5.41/-4.44/-3.34	-4.26/-3.30/-2.71**	-5.27/-3.08/-2.68	-3.87/-3.26/-2.58
TRPM8	-dCt	-4.58/-3.98/-3.59	-4.31/-4.03/-3.03	-4.92/-4.41/-3.69	-5.09/-4.56/-4.33
MSMB	-dCt	-0.55/0.29/0.70	-0.72/-0.18/0.76*,#	-0.56/-0.27/0.51	-0.74/-0.13/0.53#
EZH2	-dCt	-2.16/0.81/1.93	-1.73/-0.20/1.13	-1.40/0.04/2.19	-2.52/0.46/1.92

The values are expressed as first quartile, median and third quartile.
*Significant difference value of Start day by one-sample Wilcoxon test $P<0.1$;**Significant different value of Start day by one-sample Wilcoxon test $P<0.05$
#Significant difference value of Cranberry and Placebo group by two-sample Wilcoxon test $P<0.1$
*One-sample Wilcoxon test, $P<0.1$
**One-sample Wilcoxon test, $P<0.05$
#Two-sample Wilcoxon test, $P<0.1$

Table 7. Specific markers in *ex vivo* prostate tissue.

Parameter	Unit	Cranberry group	Placebo group
Ki67	% of positivity	3.0/5.0/10.0	3.0/7.5/10.0
Chromogranin A[a]	% of positivity	0.0/0.0/0.5	0.0/0.0/0.0
PSMA[b]	See legend[b]	1.00/2.00/3.00	1.00/2.00/2.75
AR[c]	Histoscore	90/150/150	90/100/150
p65 NF-κB[c]	Histoscore	100/150/200	150/175/200
COX-2[c]	Histoscore	150/200/200	150/200/200

The values are expressed as first quartiles, median and third quartiles.
[a]Percentage of positivity above 5% was present only in two patients from Cranberry group.
[b]PSMA was evaluated as follows: 0, absent; 1, weak positivity in some glands; 2, medium positivity in less than half of glands; 3, strong positivity in more than half of glands or medium positivity in majority of glands.
[c] Histoscore; % of positivity multiplied by staining intensity (0, absent; 1, weak; 2, moderate; and 3, strong), resulting in histoscore from 0 to 300.

were associated with changes in cell cycle regulatory proteins[13].

Findings from the above *in vitro* studies suggest that whole cranberry extract or at least 3 key types/categories of components, flavonols, proanthocyanidins or triterpenoids, are associated with biological alteration of cell targets and may be protective for PCa. The effects of cranberry on PCa in humans, to the best our knowledge, have not yet been reported. In this study, we evaluated, in a randomized, double-blind, placebo control trial (RCT) the effects of CFP on blood, urine and prostate tissue markers in PCa patients. The daily dose 1500 mg CFP was given to men with PCa for at least 21 days (mean, SD were 31 ± 9 days in the cranberry group and 35 ± 8 days in placebo group) before radical prostatectomy. The intervention was well-tolerated. On the day of surgery, there was a marked decrease of 22.5% in PSA level in the cranberry arm and a small increase of 0.9% in the placebo arm. This is in good concordance with our previous study on cranberry effects in men with non-bacterial prostatitis[14]. The down-regulation of serum PSA may be related a trend to down-regulation of beta-microseminoprotein (MSMB) which has been reported to be androgen regulated[18-20]. Unexpectedly, there was enhanced expression of PCA3 in the cranberry arm. The trend to downregulation of MSMB after cranberry supplementation is more reliable than upregulation of PCA3 due to larger number of successful test results for both time points (19 for MSMB and only 8 PCA3 in the cranberry arm). Interestingly, 6/9 patients with downregulation of MSMB had also downregulation of serum PSA. Furthermore, Martinez-Pinero et al. showed that urinary PCA3 is not a reliable marker of cancer stage or response to androgen-deprivation therapy in advanced prostate cancer[23]. The significant increase of malondialdehyde levels in plasma and erythrocytes between start and end day in both groups may be evidence that cranberry consumption was not effective in inhibiting oxidative stress in PCa patients.

CONCLUSION

These data suggest that further studies to evaluate cranberry consumption as a prophylactic against the biochemical recurrence of prostate cancer in patients after surgery is warranted.

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Author contributions: VS: manuscript preparation; AV: management of clinical trial; JB: prostate cancer markers; ZK: morphological examinations; JVr: statistical evaluation; MK: patient recruitment and data collection; PK: analytical methods; JVo: laboratory assays.

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6 Závěr

V klinických studiích jsme se zaměřili na ověření vlivu silymarinu (komplexní směsi flavonolignanů ze semen *Silybum marianum*) v kombinaci se selenem/L-argininem a plodu klikvy velkoplodé (*Vaccinium macrocarpon*) na vybraná urologická onemocnění dobře hodnotitelná parametry laboratorní medicíny. Oba zkoumané rostlinné materiály jsou charakterizované, jak co do složení a znalosti chemických struktur sekundárních metabolitů, tak i farmakologických účinků získaných z klinických studií.

Námi zvolená urologická onemocnění LUTS, prostatitida a karcinom prostaty patří k nejčastějším u mužů a IMC u žen. Tato onemocnění významně snižují kvalitu života pacienta a u karcinomu prostaty i ohrožují život samotný. Moderní medicína má účinné a ověřené možnosti léčby u LUTS (farmakoterapie i chirurgická léčba), u IMC a prostatitidy (antibiotika), u lokalizovaného karcinomu prostaty radikální léčbu (prostatektomie nebo radioterapie) a u pokročilého metastazujícího nádoru prostaty (hormonální léčbu, chemoterapii nebo biologickou léčbu).

Je zcela zřejmé, že tyto léčebné postupy jsou jasně indikované a nelze je nahradit alternativní léčbou. Nicméně je zde stále prostor k aplikaci přírodních látek a to hlavně v prevenci nebo zvýšení účinnosti „klasické“ léčby. Týká se to zejména pacientů s mírnými potížemi LUTS, prevence recidivy IMC a u karcinomu prostaty v rámci chemoterapie či oddálení biochemického relapsu karcinomu prostaty.

Nezastupitelnou roli však hraje samotný přístup pacienta k léčbě. Prokázalo se, že samotný životní styl a složení denní diety má vliv na vznik nebo i průběh onemocnění. Komplexní interakce mezi sloučeninami obsaženými v dietě s mikro-biomem trávicího ústrojí, genetickými, epigenetickými a metabolickými procesy mají klíčový vliv na imunitu a celkové zdraví lidského organismu. Při výběru přírodních látek, u kterých jsme ověřili jejich vliv na vybraná urologická onemocnění, jsme vycházeli z předpokladu, že jednotlivé komponenty směsi či extraktu většinou nemají srovnatelný účinek s účinkem komplexních směsí těchto látek.

Na druhou stranu je třeba si uvědomit, že studie účinnosti komplexních směsí přírodních látek jsou ztížené jejich obtížnou standardizací a údaje týkající se jejich účinku a bezpečnosti byly získány z časově limitovaných studií na relativně malém počtu pacientů.

Jak ukázaly předložené klinické studie nelze užívání fytopřípravku silymarin v kombinaci s L-argininem/selenem a celého plodu *V. macrocapon* zamítnout ani favorizovat. Je však třeba najít to správné uplatnění, např. v prevenci či doplnění léčby.

Výsledky našich studií, i přes omezený počet, potvrdily prospěšnost užívání fytopřípravků u zvolených urologických onemocnění. Snížení počtu recidiv IMC u žen při užívání celého plodu *V.macrocarpon* bylo statisticky průkazné, bez nežádoucích účinků a pacientky projevily zájem o další užívání. Podobná situace byla u mužů s LUTS, kteří byli spokojeni se zlepšením urodynamických parametrů močení po dobu užívání *V.macrocarpon* či kombinace silymarinu se selenem/L-argininem. Jako „vedlejší“ efekt studií bylo zachycení a následná pravidelná péče u mužů, kteří by jinak s návštěvou urologa váhali.

U ověření vlivu přírodních látek na karcinom prostaty je situace obtížnější. Pro průkaz snížení rizika karcinomu jsou nutné studie dlouhodobé a s velkým počtem pacientů, což bylo nad naše možnosti. Naše studie však prokázala, že plod *V. macrocarpon* obsahuje sloučeniny, které zasahují do signálních drah v nádorových buňkách prostaty, což poukazuje na další možný cíl výzkumu v této oblasti.

Závěrem bych chtěl zdůraznit, že přírodní léčba v žádném případě **nenahrazuje již léčbu doporučovanou a zavedenou, ale také ji nelze odmítnout.** Je třeba **najít to správné pole pro její použití**, ať už v rámci prevence či podpůrné léčby. U mnoha pacientů dialog na téma o použití přírodních látek na diagnostikované onemocnění vede ke zlepšení komunikace a k zvýšení důvěry k lékaři, většímu zájmu o samotné zdraví a změně či zlepšení životního stylu, což má významný vliv na zdárný průběh léčby onemocnění.

7 Seznam používaných zkratek

5ARi	inhibitor enzymu 5α-reduktázy
AST	aspartátaminotransferáza
BMI	body mass index
BHP	benigní hyperplazie prostaty
CFP	lyofilizovaný plod kikvy (cranberry fruit powder)
CRP	C-reaktivní protein
ChP	chronická prostatida
ED	erektilní dysfunkce
EFSA	European Food Safety Agency
HPFS	Health Professionals Follow-Up Study
IGF 1	insulin-like growth factor-1
IIEF-5	International Index of Erectile Function
IMC	infekce močových cest
IPSS	International Prostate Symptom Score
LNCaP	buňky adenokarcinomu prostaty
KP	karcinom prostaty
KZ	klinická zkouška
LUTS	symptomy dolního močového traktu
MDA	malondialdehyd
mRNA	mediátorová RNA
NCCAM	National Center for Complementary and Alternative Medicine
NF-κB	jaderný faktor κB
NPC	Nutritional Prevention of Cancer
PCPT	Prostate Cancer Prevention Trial
PLT	trombocyty
PMDA	malondialdehyd v plazmě
PSA	prostatický specifický antigen
Q_{pr}	průměrný průtok
Q_{max}	maximální průtok
QoL	kvalita života
REDUCE	REduction by DUtasteride of prostate Cancer Events trial
SHG_{tot}	celkové SH skupiny v plazmě
SELECT	Selenium and Vitamin E Cancer Prevention Trial
SM-Se	silymarin-selen
TNFα	tumor nekrotizující faktor α
TRUS	transrektální ultrasonografie
TST	testosteron
V	objem močového měchýře
V_{rez}	reziduální objem moči po mikci