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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.

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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.

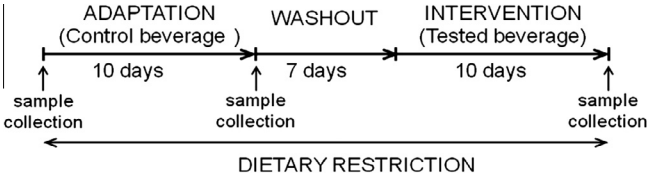

Fig. 1. Design of the intervention study.

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),

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