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Effects of polyphenol-rich chokeberry juice on cellular antioxidant enzymes and membrane lipid status in healthy women

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ABSTRACT

The effect of long-term polyphenol-rich chokeberry juice consumption on activities of antioxidant enzymes and membrane lipid status in erythrocytes of 25 healthy women was examined. Percentages of membrane fatty acids, superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were assessed at a baseline, in the middle and at the end of a 3-month-long consumption period. A significant increase in C22:6n-3, n-3 polyunsaturated fatty acids (PUFAs), total PUFAs and unsaturation index and a significant decrease in monounsaturated fatty acids (MUFAs) and n-6:n-3 ratio were found. Significantly higher SOD and GP_x activities were also recorded at the end of the study. Serum lipids and glucose were stabile during the consumption period, while the levels of thiobarbituric acid–reactive substances (TBARS), as serum indicator of lipid peroxidation, were reduced significantly. These results indicate a positive impact of regular chokeberry juice consumption on cellular oxidative damage and suggest its putative role in the protection against oxidative stress.

1. Introduction

Epidemiological studies have shown that regular consumption of fruits, vegetables and other foods rich in antioxidants is associated with a lower level of oxidative stress, as well as incidences of cardiovascular and other chronic diseases (Arts & Hollman, 2005; Hooper et al., 2008). Numerous indices and methods have been used to assess the oxidative stress, defined as an imbalance between reactive oxygen species (ROS) production and their removal by antioxidants. Among various indices, products of lipid peroxidation are the most common group used to evaluate the individual oxidative (antioxidant/pro-oxidant) status (Dotan, Lichtenberg, & Pinchuk, 2004).

Lipid peroxidation is a result of complex reactions which lead to the formation of peroxyl radicals and hydroperoxides, in the first place. These initial products further decompose either into isoprostanes or various aldehydes, determined

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Abbreviations: GPx, glutathione peroxidase; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances.

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as thiobarbituric acid–reactive substances, (TBARS) expressed as malondialdehyde (MDA) equivalents, as the most widely used index of lipid peroxidation (Moore & Roberts, 1998). Biological membranes, which give outer boundaries to the cells, are the first line of contact with other substances, including promoters of oxidative damage (Cyboran, Oszmiański, & Kleszczyńska, 2012). The structure and functions of membranes are impaired by the oxidation of lipids in their phospholipid bilayer (Rice-Evans, Miller, & Pganga, 1997). Among fatty acids of cell membranes, polyunsaturated fatty acids (PUFAs) are the most prone to oxidative damage due to their high content of unsaturated bonds, which are the main site of interaction with free radicals. Considering this, a relative concentration of PUFAs has been suggested as an indirect marker of membrane lipid peroxidation (Solans et al., 2000; Suarez, Ramirez-Tortosa, Gil, & Faus, 1999).

It has been shown that plant extracts have potential to protect an organism against the harmful effects of oxidative damage promoters (Robards, Prenzler, Tucke, Swatsitang, & Glover, 1999). Polyphenols, the most abundant plant secondary metabolites, exhibit the strongest protective effect regarding cellular oxidative damage. Polyphenols are also considered to be the most important dietary antioxidants, due to their high antioxidant capacity and presence in plant foods (Scalbert, Manach, Morand, Remesy, & Jimenez, 2005).

Recent studies have revealed polyphenols' antioxidant potential in protecting biological membranes by two distinct mechanisms: indirect, by scavenging free radicals in medium and direct, by their presence in the membrane (Cyboran et al., 2012; Raudoniūtė et al., 2011). It has been shown that polyphenols interact with the lipid phase of membrane with a tendency to incorporate into the outer hydrophilic part of phospholipid bilayer (Bonarska-Kujawa, Pruchnik, Oszmiański, Sarapuk, & Kleszczyńska, 2011; Cyboran et al., 2012).

Berry fruits are well known as a rich source of polyphenols, especially anthocyanins, which contribute to their strong antioxidant activity (Szajdek & Borowska, 2008). The contribution to the total antioxidant capacity was found to be dependent on phenolic compounds' structure and their content in different berries. Also, it has been shown that chokeberry (*Aronia melanocarpa* L.) has a significantly higher content of polyphenols and consequently higher antioxidant activity than other berries (Oszmiański & Wojdylo, 2005). The ability of chokeberry to attenuate oxidative damage and protect from its deleterious consequences contributes to numerous health promoting effects, with protection from cardiovascular diseases being one of the most important (Chrubasik, Li, & Chrubasik, 2010). Studies have shown that long-term consumption of chokeberry products has beneficial effects on risk predictors of cardiovascular diseases, such as blood pressure level, lipid profile and concentration of fasting plasma glucose (Broncel et al., 2010; Naruszewicz, Laniewska, Millo, & Dłuzniewski, 2007; Skoczynska et al., 2007). Furthermore, *in vitro* studies revealed that chokeberry extract, as a rich source of polyphenols, has an ability to incorporate in phospholipid bilayer and this way protect the cell against other substances, including free radicals (Bonarska-Kujawa et al., 2011). A direct antioxidant effect of dietary polyphenols *in vivo*, due to extensive metabolism and low bioavailability (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005), has been doubtful, but the indirect effects on an antioxidant system are more convincing (Duchnowicz, Nowicka, Koter-Michalak, & Broncel, 2012). Despite the fact that data on the *in vivo* effects are limited, stimulation of erythrocytes' antioxidant enzymes was found in humans after the supplementation with chokeberry extract (Broncel et al., 2010). Additionally, Bernabé et al. (2013) recently reported that 6 months consumption of citrus-based juice with 5% of aronia extract resulted in the decrease of lipid peroxidation, measured as a level of urinary isoprostanes. This effect was observed both in metabolic syndrome subjects and in the control group.

Taking into account all these facts, the aim of our study was to investigate membrane fatty acid profile and status of antioxidant enzymes in erythrocytes obtained from healthy female volunteers, before and after 3 month of regular polyphenol-rich organic chokeberry juice consumption.

2. Material and methods

2.1. *Subjects and study design*

The study was approved by the Ethical Committee of Faculty of Pharmacy, University of Belgrade and has been undertaken according to the Helsinki Declaration (24 February 2012, register number 304/1). All subjects gave written informed consent prior to the enrolment. Twenty-five apparently healthy women volunteers with mean age 35.2 ± 7.7 were involved in the study. They were instructed to consume 100 mL of polyphenol-rich organic chokeberry juice daily for 3 months, as part of their regular diets. Exclusion criteria (Table 1) were the presence of chronic diseases treated with drugs, a BMI under

Table 1 – Selection criteria of study participants.

Inclusion criteria	Exclusion criteria
Apparently healthy women	Pregnant and lactating women
Age 20–47 years old	Peri-menopause and post-menopause
Body mass index between 18.5 and 29.9 kg/m^2	Chronic diseases (i.e. cardiovascular diseases, diabetes, inflammatory diseases, cancer or allergy), thyroid abnormalities
Stable body weight (±3 kg) during the last 3 months	Use of oral contraceptives, corticosteroids and hormones
Non-smokers	Use of antacids or laxatives at least once a week
Alcohol consumption ≤30 g/day	Irregular or unbalanced dietary pattern
	Food intolerance or allergy to the juice components
	Parallel participation in other dietary intervention study