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The protective effect of the *Cornus mas* fruits (cornelian cherry) on hypertriglyceridemia and atherosclerosis through PPARα activation in hypercholesterolemic rabbits

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ABSTRACT

Cornelian cherry (*Cornus mas* L.) fruits have been used in traditional cuisine and in folk medicine in various countries. This study was conducted to evaluate the constituents and impact of cornelian cherry (*C. mas* L.) fruits lyophilisate on lipid levels, PPARα protein expression, atheromatous changes in the aorta, oxido-redox state, and proinflammatory cytokines in hypercholesterolemic rabbits. The HPLC–MS method was used for determining active constituents in cornelian cherry. In a subsequent *in vivo* study the protective effect of the cornelian cherry on diet-induced hyperlipidemia was studied using a rabbit model fed 1% cholesterol. Cornelian cherry (100 mg/kg b.w.) or simvastatin (5 mg/kg b.w.) were administered orally for 60 days. Two iridoids – loganic acid and cornuside – and five anthocyanins were identified as the main constituents of the cornelian cherry. The administering of the cornelian cherry led to a 44% significant decrease in serum triglyceride levels, as well as prevented development of atheromatous changes in the thoracic aorta. Cornelian cherry significantly increased PPARα protein expression in the liver, indicating that its hypolipidemic effect may stem from enhanced fatty acid catabolism. Simvastatin treatment did not affect PPAR-α expression. Moreover, the cornelian cherry had a significant protective effect on diet-induced oxidative stress in the liver, as well as restored upregulated proinflammatory cytokines serum levels. In conclusion, we have shown loganic acid to be the main iridoid constituent in the European cultivar of the cornelian cherry, and proven that the cornelian cherry could have protective effects on diet-induced hypertriglicerydemia and atherosclerosis through enhanced PPARα protein expression and *via* regulating oxidative stress and inflammation.

Abbreviations: LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; HMG-CoA, 3-hydroxy-3-methyl glutaryl coenzyme A; PPARα, peroxisome proliferator-activated receptor alpha; UPLC, ultra-performance liquid chromatography; Q-TOF, quadruple time of flight; TNFα, tumor necrosis factor α; IL-6, interleukin 6; MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase; GPx, glutathione peroxidase; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel; SREBP, sterol regulatory element-binding protein; VCAM-1, vascular cell adhesion molecule 1; ICAM-1, intercellular adhesion molecule 1; NF-κB, nuclear factor-kappa B; PPARγ, peroxisome proliferator-activated receptor gamma; MMP-9, matrix metalloproteinase 9; FRAP, ferric reducing/antioxidant power; AMPK, AMP-activated protein kinase.

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Introduction

Enhanced low-density lipoprotein (LDL-C) and decreased high-density lipoprotein cholesterols (HDL-C) in serum are established, independent risk factors in atherosclerosis and cardiovascular disorders. However, whether enhanced triglyceride (TG) levels are causal in atherosclerosis and cardiovascular disorders have been ambiguous for several decades. Available data, however, including recently published follow-up studies and meta-analyses implicate TG as an independent risk factor for unstable plaque formation, heart disease, and mortality in the general population (Grønholdt, 1999; Langsted et al., 2011; Miller et al., 2011). The medicinal products of choice in hypercholesterolemia are statins, and in hypertriglicerydemia, fibrates. The main hypolipidemic mechanism of action of statins has been evident since 1970s. Statins act through the inhibition of 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, the key enzyme in cholesterol synthesis in the liver. By contrast, fibrates have been used in medicine for over 80 years, although their mechanism of action has been unclear for many years. The studies done in the last decade have proven that fibrates increase expression of the peroxisome proliferator-activated receptor alpha (PPARα), nuclear receptors that play an important function in the regulation of the genes involved in lipid metabolism in the liver (Gervois and Mansouri, 2012). Generally, both statins and fibrates boast efficacy, although in some patients they cause insufficient lipid-lowering effects and their administering is long-term and often causes side effects that lead to discontinuation of treatment and the need to seek alternative treatments.

The family of Cornaceae includes approximately 40 species. The best-known species of *Cornus* is the Japanese cornelian cherry (*Cornus officinalis* Sieb. et Zucc.). There are many reports that either *C. officinalis* or iridoids isolated from it – loganin and morroniside – exert positive effects on lipid metabolism and possess antidiabetic effects (Park et al., 2009a,b; Yamabe et al., 2010; Park et al., 2010, 2011). The cornelian cherry (*Cornus mas* L.) is found in southern and central Europe and southwest Asia. In various countries, cornelian cherry was used for many years in traditional cuisine as well as in folk medicine. Unfortunately, nowadays cornelian cherry is not popular. Although it is available in many European countries, nevertheless it is consumed by only few people. In comparison to *C. officinalis*, little is known about the constituents and/or the effects of cornelian cherry. There are only a few recently published reports concerning iridoid constituents (Kucharska, 2012; West et al., 2012) and the possible impact of the cornelian cherry on lipid metabolism and the development of atherosclerosis (Rafieian-Kopaei et al., 2011; Asgary et al., 2013).

The aim of our study was to assess the constituents of lyophilisate from cornelian cherries and examine their effect on lipid metabolism, PPARα protein expression, lipid peroxidation, antioxidant and proinflammatory markers, and study the histopathological changes in the thoracic aortas and livers of hypercholesterolemic rabbits.

Materials and methods

Evaluation of cornelian cherry constituents

Plant materials and sample preparation

The cornelian cherries (*C. mas* L.) were obtained from the Bolestraszyce Arboretum and Institute of Physiography, Poland, in September 2010. The plant material was authenticated by Prof. Jakub Dolatowski, and the voucher specimen (BDPA 3 967) has been deposited at the Herbarium of Arboretum and Institute of Physiography in Bolestraszyce, Poland. The lyophilisate was prepared in the Department of Fruit, Vegetable and Cereals Technology at the Wroclaw University of Environmental and Life Science. The ripe cornelian cherries were washed, frozen, and stored frozen at −20 °C until lyophilisate processing. After freezing them and removing their pits the still-frozen samples were freeze-dried. During freeze-drying the pressure was reduced to 65 Pa. The temperature in the drying chamber was −60 °C, and the heating plate reached 30 °C. About 20 g of lyophilisate was obtained from 100 g of fruit. After freeze-drying, the samples were ground into powder using a laboratory mill.

Identification of compounds by UPLC–MS/MS

Compounds were identified through the method described by Sokół-Łętowska (2013), using the acquity ultra-performance liquid chromatography (UPLC) system coupled with a quadruple time of flight (Q-TOF) MS instrument (Waters Corp., Milford, MA, USA) with an electrospray ionization (ESI) source. The instrument was operated both in positive and negative ion mode, scanning *m*/*z* from 100 to 1500 Da at a scan rate of 2.0 s/cycle. Separation was achieved on the Acquity™ BEH C18 column (100 mm × 2.1 mm i.d., 1.7 μm; Waters). Detection wavelengths were set to 245 (iridoids) and 520 nm (anthocyanins). The mobile phase was a mixture of 4.5% formic acid (A) and acetonitrile (B). The gradient program was as follows: 0–1 min, 1% B; 1–12 min, 1–75% B; 12–12.5 min, 75–100% B; 12.5–13.5 min, 100% B; 13.5–14.5 min, 1% B. The flow rate was 0.45 ml/min and the injection volume was 5 μl. The column was operated at 30 °C. The major operating parameters for the Q-TOF MS were set as follows: capillary voltage 2.0 kV, cone voltage 45 V, cone gas flow of 11 l/h, collision energy 50 eV, source temperature 100 °C, desolvation temperature 250 °C, collision gas, argon; desolvation gas (nitrogen) flow rate, 600 l/h; data acquisition range, *m*/*z* 100–1000 Da; ionization mode, negative. The data were collected with Mass-Lynx™ V 4.1 software.

Determination of compounds by HPLC

Iridoids, anthocyanins, phenolic acid, and flavonols were determined by the method described by Kucharska (2012) using the Dionex HPLC (Sunnyvale, CA, USA) system equipped with a diode array detector model Ultimate 3000, a quaternary pump LPG-3400A, an autosampler EWPS-3000SI, a thermostated column compartment TCC-3000SD, and controlled by Chromeleon v.6.8 software. Separation was performed on a Cadenza CDC18 (75 mm × 4.6 mm, 5 μm) column (Imtakt, Japan) with a guard column. Oven temperature was set to 30 °C. The mobile phase was composed of solvent A (4.5% formic acid, v/v) and solvent B (acetonitrile). The applied elution conditions were: 0–1 min, 5% B; 1–20 min, 5–25% B; 20–21 min, 25–100% B, 21–26 min, 100% B, 26–27 min, 100–5% B; 27–30 min, 5% B. The flow rate was 1.0 ml/min, and the injection volume was 20 μl. Loganic acid and cornuside were detected at 245 nm and anthocyanins at 520 nm. Iridoids were quantified as loganic acid and anthocyanins as cyanidin 3-*O*-glucoside. The results were calculated as mg of compound in 1 g dry weight of cornelian cherry fruits extract (mg/g of dw). All determinations were performed in duplicate. The chemical structures of the HPLC-identified compounds are shown in Fig. 1.

The in vivo study

This experiment was performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and was approved by the Local Ethical Committee on Animal Research at the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences in Wrocław.