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The effects of bilberries, blackcurrants and their constituent anthocyanins on heart health in humans

KEYWORDS: Berries, anthocyanins, polyphenols, supplementation, cardiovascular disease, atherosclerosis.

Abstract Berries have a long history of use in humans, as they have been shown to have numerous positive health effects. In this review the focus is on the effects of bilberries and blackcurrants and their purified extracts containing higher levels of anthocyanins on human heart health. Anthocyanins have an effect on several different areas of cardiovascular health, including improvement of endothelial function, anti-oxidative effects, anti-inflammatory effects and the normalization of HDL and LDL lipoproteins in the blood. Purified anthocyanin supplements derived from bilberry and blackcurrant show positive effects against all these cardiovascular disease targets.

INTRODUCTION

Berries have been consumed for many centuries because of their beneficial effects for vitality, prevention of viral infections, eye health, bladder and kidney health, and gastro-intestinal health (1). The interest in dietary supplementation with berries has increased in recent times because of the recognition of their beneficial effects on cardiovascular disease, cancer prevention, neurodegenerative diseases, aging skin protection, inflammation-related diseases, type 2 diabetes and metabolic syndrome (2, 3).

Bilberries and blackcurrants are an important source of micro- and macronutrients. Their beneficial health effects have been associated with the high levels of bioactive substances they contain, such as phenolic acids, flavonoids and especially anthocyanins. Anthocyanins are the largest group of water-soluble pigments in the plant kingdom and are responsible for the red, blue and purple colors of many fruits and vegetables. Products with high anthocyanin content include berries, red grapes, red cabbage, eggplant and the black variants of soybean and rice. Anthocyanins are classed as flavonoids, part of the (poly)phenolic group of natural products, and are usually found in the form of flavylum cations. Anthocyanins are classified according to the number and location of the hydroxy and methoxy groups attached to the flavan nucleus, and by the nature of the attached mono-, di- or tri-saccharides, which can contain glucose, galactose, arabinose, rhamnose and xylose groups (4,5). The six main

anthocyanidins found in nature are pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin, with cyanidin as the most widely occurring (Figure 1).

Anthocyanin glycoside	R ₁	R ₂	R ₃
Cyanidin-3-O-arabinoside	OH	H	arabinoside
Cyanidin-3-O-galactoside (C3Gal)	OH	H	galactoside
Cyanidin-3-O-glucoside (C3G)	OH	H	glucoside
Cyanidin-3-O-rutinoside (C3R)	OH	H	rhamnosyl-glucoside
Delphinidin-3-O-galactoside (D3Gal)	OH	OH	galactoside
Delphinidin-3-O-glucoside (D3G)	OH	OH	glucoside
Delphinidin-3-O-rutinoside (D3R)	OH	OH	rhamnosyl-glucoside
Malvidin-3-O-glucoside	OMe	OMe	glucoside
Pelargonidin-3-O-glucoside	H	H	glucoside
Petunidin-3-O-glucoside (P3G)	OMe	OH	glucoside

Figure 1. Main anthocyanidin-glycosides occurring in nature.

The average anthocyanin content of bilberry is around 430 mg and that of blackcurrant is around 270 mg per 100 g of edible fruit (6). Bilberries contain a wide variety of at least 16 different anthocyanins; the main ones are delphinidin-3-glucoside (D3G), delphinidin-3-galactoside (D3Gal), cyanidin-3-glucoside (C3G), cyanidin-3-galactoside

(C3Gal) and petunidin-3-glucoside (P3G). Blackcurrant contains only 4 anthocyanins: C3G, cyanidin-3-rutinoside (C3R), D3G and delphinidin-3-rutinoside (D3R).

In recent years there has been increased scientific support for the positive effects of anthocyanins in the area of heart health. Epidemiological studies have shown positive correlations between regular flavonoid intake and a reduced risk of cardiovascular disease (CVD), CVD mortality and coronary heart disease (CHD) mortality (7,8). A recent epidemiological study showed that a high anthocyanin intake reduced the risk of myocardial infarction (9).

CVD covers a variety of heart and circulatory pathologies including coronary heart disease, congenital heart disease, stroke, angina and myocardial infarction. All have different pathology profiles, however a common factor is an impaired endothelial function. Oxidative stress can worsen the situation by oxidizing lipoproteins which can be more easily taken up by macrophages. Fat-laden macrophages can become foam cells leading to inflammation of the blood vessel wall. This then expands, leading to atherosclerosis and restriction of blood flow (10).

The objective of this review is to provide an overview of human studies performed with bilberry, blackcurrant and their constituent anthocyanins and their effects on heart health, especially in relation to endothelial function, anti-oxidative effects, inflammation and atherosclerosis.

ENDOTHELIAL FUNCTION

Endothelial cells line the blood vessels and play an important role in vascular health. Impaired endothelial function leads to a lower ability of the blood vessel to dilate because of the lower availability of nitric oxide (NO) (a vasodilator), and other vasoactive molecules such as endothelin-1 and prostacyclin. Nitric oxide synthase (NOS) is present in the endothelial cell, and converts arginine into NO. This diffuses into the vascular smooth muscle cells and activates guanylyl cyclase (GC), leading to the production of cGMP. This second messenger activates a chemical cascade leading to vasodilation (see Figure 2).

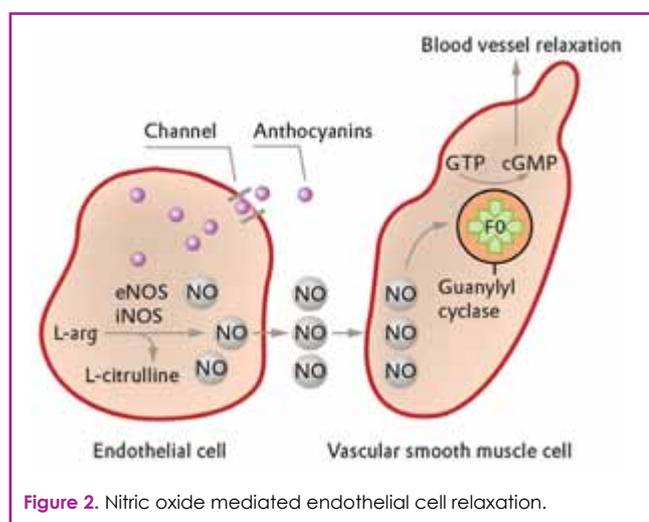


Figure 2. Nitric oxide mediated endothelial cell relaxation.

Endothelial dysfunction has been recognized as an early marker for atherosclerosis (11). Several risk factors including hypertension, smoking, chronic inflammation and oxidative stress (reactive oxygen species or ROS formation) worsen endothelial function. Biomarkers used to measure endothelial function in humans are Flow Mediated Dilation (FMD), plasma nitrite and nitrate, and cellular adhesion markers as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1) (12).

Several randomized placebo-controlled trials have been performed to determine the effect of blackcurrant juice and purified anthocyanins from bilberry and blackcurrant on endothelial function. They show that a short-term blackcurrant juice consumption in healthy individuals (single consumption of 250 mL 20% blackcurrant juice, providing 50.5 mg total cyanidin and delphinidin) does not have a significant effect on endothelial dependent or endothelial independent vascular reactivity, and on biomarkers of endothelial function (plasma nitrite, nitrate, ICAM-1 and VCAM-1) (13). A blackcurrant juice supplementation study in healthy volunteers with a habitual consumption of less than 2 portions of fruit and vegetables per day (4 x 250 mL blackcurrant juice providing 143 mg anthocyanins per day for 6 weeks) found a significant increase in FMD. These effects are attributed to the improved endothelial function, as the endothelium-independent glyceryl trinitrate-induced dilation was not affected. Oxidative stress, measured as F2-isoprostanes, was significantly lowered after supplementation (14). As the long-term blackcurrant supplementation study does show an improvement in endothelial function, the lack of effect in the earlier trial is most likely due to low amount of active constituents consumed.

It is not only blackcurrant juice which shows positive effects on endothelial function. Supplementation studies using purified anthocyanins from bilberry and blackcurrant in hypercholesterolemic subjects (320 mg anthocyanins per day for 12 weeks) showed significantly increased FMD and cGMP levels. The cGMP levels and FMD were found to be positively correlated. These effects can be attributed to an improved endothelial function, as the endothelium-independent vasodilation (not NO dependent) was not influenced by anthocyanin supplementation. Also on exposure to the NO-cGMP inhibitor L-N-monomethyl arginine (L-NMMA), the beneficial effects on FMD were abolished. This has been confirmed in a rat aortic ring model in the same study. The levels of VCAM-1 were also significantly decreased (15). A similar study using the same anthocyanins, the same dosage and type of subjects over 24 weeks also found that the endothelial adhesion factor VCAM-1 was significantly decreased in plasma. It was confirmed in a porcine arterial endothelial cell line that the anthocyanins C3G and D3G are most likely to be responsible for the significant reduction of VCAM-1 (12).

It is therefore likely that the positive effects of anthocyanins, or anthocyanin-rich products, on endothelial function in humans is mediated via the activation of NO-cGMP signaling and reducing the plasma levels of cellular adhesion molecules, thereby lowering the progression towards atherosclerosis.

ANTI-OXIDATIVE EFFECTS

Oxidative damage plays a significant role in the progression of CVD (16). As described above, the presence of NO is needed to maintain normal endothelial function. However, reactive oxygen species can interact with NO and reduce its bioavailability (17). Anthocyanins are powerful anti-oxidative compounds, which have the ability to scavenge radicals and chelate metal ions preventing the cells from oxidative damage (18).

Blackcurrant juice was assessed for its anti-oxidative potential. Blackcurrant juice supplementation in healthy subjects (250 mL juice per day for 1 week) showed significantly increased serum anti-oxidative status (serum SH groups and PON1 lactonase activity) (19). A recent human intervention study with bilberry pomace extract demonstrated the anti-oxidative effects at the molecular level. It was found that the metabolite phloroglucinol aldehyde (PGA), formed after digestion of the anthocyanin-rich extract activates the NRF2/ARE stress-response mechanism. The transcription factor nuclear factor E2 (Nrf) is activated by anthocyanins. It moves to the nucleus and activates the antioxidant response element (ARE) sequence. Upon activation several genes coding for anti-oxidative and anti-inflammatory factors are expressed (20).

INFLAMMATION

Inflammation in CVD plays an important role in the progression of atherosclerosis. During oxidative stress lipoproteins in the blood are oxidized, and can be taken up more rapidly by macrophages in the blood. Oxidized lipoproteins can harm vascular endothelial cells as the loaded macrophages can turn into foam cells (13). These foam cells attach to the endothelial wall and become fatty streaks. More inflammatory mediators will be attracted to this place, the fatty streak will increase, and will be strengthened with fibrin to form a plaque. The inflammatory response of the body involves macrophages, which play a central role in the regulation of NO, prostaglandins and cytokines. Transcription factor NF-KB plays a central role in the inflammatory response.



Activation of NF-KB leads to activation of target genes, and pro-inflammatory mediators are secreted (21).

Supplementation with bilberry juice in subjects with at least one risk factor for CVD (330 mL juice per day for 4 weeks) resulted in a significant decrease in the plasma concentrations of inflammatory mediators CRP, IL-6, IL-15 and MIG. All of these are target genes activated by NF-KB (21).

Several randomized placebo-controlled trials supplementing purified anthocyanins from bilberry and blackcurrant showed positive effects on inflammatory mediators. Supplementation in healthy subjects (300 mg anthocyanins per day for 3 weeks) resulted in a significant decrease in NF-KB controlled pro-inflammatory mediators. IL-4, IL-13, IL-8, 'regulated upon activation, normal T cell expressed and secreted' (RANTES), and IFN- α were significantly decreased after anthocyanin supplementation. No change was found in TNF- α , CRP and IL-1 β . IL-4, IL-8 and IL-13 are pro-inflammatory cytokines that induce NF-KB, and IFN- α also induces NF-KB. Within the same study monocytes were exposed to the same purified anthocyanins and a LPS-induced activation of NF-KB was significantly suppressed. This shows that the anthocyanins are most likely to inhibit the NF-KB activation, and decrease the amount of pro-inflammatory mediators (22). A similar study in subjects with hypercholesterolemia (320 mg anthocyanins per day for 24 weeks) also found anti-inflammatory effects; decreased levels of CRP and IL-1 β , cytokines which were not found to be affected in healthy individuals (15, 22). The exposure and target group could explain this difference. In a HepG2 cell line assay the single anthocyanins C3G and D3G inhibited the IL-6 and IL-1 β induced CRP production in a dose dependent manner. The anti-inflammatory effects found with simultaneous exposure of C3G and D3G were stronger compared with the effects of exposure to the single anthocyanins, indicating a synergistic effect (15).

Not all the studies done with purified anthocyanins gave positive effects on inflammatory markers. Another randomized, placebo-controlled, double-blind trial performed in pre-hypertensive subjects (640 mg purified anthocyanins from bilberry and blackcurrant per day for 4 weeks) showed no significant difference in anti-inflammatory markers (23). The lack of effect can be due to the small sample size, low power of the study, target group and/or time window.

It is most likely that the anti-inflammatory effects of anthocyanins, or anthocyanin-rich products are mediated either via the direct inhibition of NF-KB or via the inhibition of its activators, preventing the activation of pro-inflammatory reactions in the body.

ATHEROSCLEROSIS

Atherosclerosis is characterized by thickened artery walls, narrowed by plaques consisting of white blood cells, dead cells, triglycerides and cholesterol, which impedes the blood flow. The process starts with oxLDL uptake by macrophages. This can be caused by either an increased uptake of LDL cholesterol, or a reduced presence of HDL.

HDL facilitates the reverse cholesterol transport, meaning that it promotes the efflux of cholesterol from macrophages, foam cells and atherosclerotic plaques, so that it can be transported to the liver and excreted as bile (24). This is shown in Figure 3.

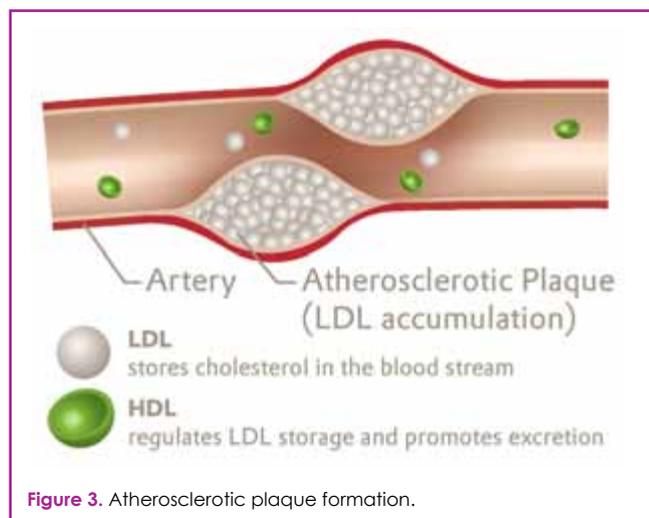


Figure 3. Atherosclerotic plaque formation.

Short-term supplementation with blackcurrant juice in healthy subjects (single dose of 250 mL 20% juice) does not show a significant effect on plasma triacylglycerides and non-esterified fatty acid concentration levels (13). A similar study with blackcurrant juice in healthy subjects (250 ml 100% juice for 1 week) showed a significant decrease in macrophage cholesterol content; however the cholesterol efflux rate from the cells was not affected. As the efflux rate has not changed, it is most likely that the uptake of cholesterol has been inhibited (19). The difference between the two results could be due to dose, exposure time and the subjects on which the study was performed.

Supplementation with purified anthocyanins from a mixture of bilberry and blackcurrant in dyslipidemic subjects (320 mg per day for 12 weeks) showed significant increases in HDL cholesterol, decreases in LDL cholesterol, and increased cellular cholesterol efflux to serum. The mass and the activity of plasma cholesteryl ester transfer protein (CETP) was significantly decreased and its change was correlated with the change in LDL cholesterol, while the change in HDL cholesterol was found to be correlated to the cellular cholesterol efflux to serum (24). A similar study with the same anthocyanins (320 mg per day for 12 weeks) in hypercholesterolemic subjects showed significant decrease in LDL cholesterol, and an increase in HDL cholesterol. The change in cGMP (marker of endothelial function) and HDL were positively related to FMD, therefore the improvement in serum lipid profile could have contributed to the improvement in endothelial function (15). A study using anthocyanin supplementation in hypercholesterolemic subjects for 24 weeks showed a significant decrease in LDL cholesterol, and an increase in HDL cholesterol. No significant changes in the levels of triacylglycerides and total cholesterol were observed. The decrease in cholesterol was positively correlated to the change in CRP. CRP has the ability to bind LDL or oxLDL and promote the uptake via macrophages. Thus, the change in serum lipid profiles could be mediated via the anti-inflammatory effects of anthocyanins (15). Another

randomized, placebo-controlled, double-blind trial in pre-hypertensive subjects (640 mg anthocyanins per day for 4 weeks) showed a significant increase in HDL cholesterol, but no change in LDL cholesterol. The lack of effect on LDL can be due to the small sample size, low power of the study, initial health status of the target group or time window (23).

The improvements in serum lipid profiles after anthocyanin consumption contribute to a lower risk of atherosclerotic plaque formation. The anthocyanins promote cholesterol efflux to the serum and HDL cholesterol, removing the excess of cholesterol from the blood vessels, while simultaneously CETP and LDL cholesterol are limited, reducing the availability of cholesterol for incorporation into the plaques.

CONCLUSION

Anthocyanins have been revealed to be powerful compounds in the support of heart health because of their action on multiple targets. In the pathology of cardiovascular disease they improve endothelial function, anti-oxidative markers, anti-inflammatory effects, and also improve the lipoprotein profile. These targets cannot be seen in isolation, as for instance an improvement found in the lipoprotein profile is also found to be correlated to endothelial function (12). By influencing these targets, anthocyanins contribute to the maintenance and improvement of heart health. Whether the effects observed are directly caused by the anthocyanins themselves, or their metabolites is a current focus of research. The bioavailability is found to be extremely low in humans; <0.1% of the intact anthocyanins are excreted in urine. This indicates that after ingestion anthocyanins undergo an extensive first-pass metabolism, and enter the systemic circulation as metabolites. The concentration of the phenolic acid metabolites is found to be significantly higher in the blood plasma than the concentration of the parent compound. It is therefore likely that not only the parent compounds, but also the metabolites are bioactive, and thus likely to be responsible for the beneficial health effects (25, 26).

Although bilberries and blackcurrants are important natural sources of anthocyanins and other phenolic compounds, they require a relatively long exposure time before a positive effect on heart health is observed. Short term studies gave weak or modest results at best (19), whereas studies performed with purified anthocyanins (320 mg to 640 mg/day, over several weeks) showed positive effects on several aspects of cardiovascular health. This is most likely due to the higher dosages of anthocyanins found in supplements. An edible 100 gram portion of bilberry contains ca. 430 mg and blackcurrant ca. 270 mg total anthocyanins (6). Consumption of an effective anthocyanin dose over the long term only through berries would require a large daily intake, which may stress the digestive system, especially with tannin-rich berries. The use of supplementation with purified anthocyanins, or extracts containing high concentrations of anthocyanins, should be explored in more detail to determine their effects on long term supplementation in large, well-designed placebo-controlled randomized design trials with surrogate markers or clear clinical end points of CVD, while simultaneously monitoring the absorption and metabolism (26-29). The current available studies are encouraging and

have shown that supplementation using purified anthocyanins can lead to effective dietary levels of anthocyanins, which have been found to have positive effects on biomarkers of human heart health.

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