Research Article

Berry anthocyanins as novel antioxidants in human health and disease prevention

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Edible berries, a potential source of natural anthocyanin antioxidants, have demonstrated a broad spectrum of biomedical functions. These include cardiovascular disorders, advancing age-induced oxidative stress, inflammatory responses, and diverse degenerative diseases. Berry anthocyanins also improve neuronal and cognitive brain functions, ocular health as well as protect genomic DNA integrity. This chapter demonstrates the beneficial effects of wild blueberry, bilberry, cranberry, elderberry, raspberry seeds, and strawberry in human health and disease prevention. Furthermore, this chapter will discuss the pharmacological benefits of a novel combination of selected berry extracts known as OptiBerry, a combination of wild blueberry, wild bilberry, cranberry, elderberry, raspberry seeds, and strawberry, and its potential benefit over individual berries. Recent studies in our laboratories have demonstrated that OptiBerry exhibits high antioxidant efficacy as shown by its high oxygen radical absorbance capacity (ORAC) values, novel antiangiogenic and antiatherosclerotic activities, and potential cytotoxicity towards *Helicobacter pylori*, a noxious pathogen responsible for various gastrointestinal disorders including duodenal ulcer and gastric cancer, as compared to individual berry extracts. OptiBerry also significantly inhibited basal MCP-1 and inducible NF-κβ transcriptions as well as the inflammatory biomarker IL-8, and significantly reduced the ability to form hemangioma and markedly decreased EOMA cell-induced tumor growth in an *in vivo* model. Overall, berry anthocyanins trigger genetic signaling in promoting human health and disease prevention.

Keywords: Antioxidant / Antiangiogenic / Antiatherosclerosis / Berry anthocyanins / Diabetes

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1 Introduction

Numerous independent investigations have demonstrated the correlation between consumption of fresh fruits and vegetables with the prevention, delay or onset of chronic degenerative diseases including cancer [1–3]. Fresh fruits and vegetables are rich sources of a large number of diverse nutrients, including vitamins/antioxidants, trace minerals/micronutrients, phytosterols, novel enzymes, dietary fiber, and potent chemoprotectants [1–3]. These structurally diverse phytonutrients may possess complementary and overlapping or identical mechanisms of potential disease-preventive action, including novel antioxidant, antibacterial, antiviral, and antiangiogenic properties, enhanced production of detoxification enzymes, enhancing immune health, reduction of platelet aggregation, promotion of a healthy lipid profile, reducing hypertension, and impacting hormone metabolism [2–8]. It has been well demonstrated that a combination of fruits may exhibit additive or synergistic effects on enhancing the antioxidant efficacy and status in human subjects [9, 10]. In addition, our studies have demonstrated that a novel combination of edible berry extracts including blueberry, bilberry, elderberry, cranberry, raspberry seeds and strawberry exhibit significantly superior antioxidant, antiangiogenic and antibacterial potential as compared to the individual berries, 20 different combinations of these six berries, and a grape seed proanthocyanidin extract (GSPE), which demonstrated superior antioxidant potential as compared to vitamins C, E, and β-carotene in earlier studies [8, 11].

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Abbreviations: EC, endothelial cells; **EOMA,** endothelioma; **GSPE,** grape seed proanthocyanidin extract; **T3,** triiodothyronine; **VEGF,** vascular endothelial growth factor

2 Anthocyanins: Sources, chemistry, and bioavailability

Edible berries and selected fruits are rich sources of both anthocyanins and flavonoid glycosides, which are responsible for the red, violet, purple, and blue color of the fruits [12, 13]. Anthocyanins present in various fruits and vegetables especially in edible berries provide the natural pigmentation and exhibit a wide range of antioxidant protection and therapeutic benefits including the integrity of genomic DNA, potent cardioprotective, neuroprotective, antiinflammatory, and anticarcinogenic properties [1, 2, 5, 9–26]. Anthocyanins are also the integral components and natural colorants in red wines [16]. The 3-glucoside anthocyanins: delphinidin, cyanidin, petunidin, and malvidin are present in red wines. However, malvidin 3-glucoside, malvidin 3-glucoside acetate, and malvidin 3-glucoside coumarate are the predominant compounds of all edible berries [22, 23]. In addition to imparting color to the food, anthocyanins may prevent auto-oxidation of lipids as well as lipid peroxidation in biological systems [26].

The positively charged oxygen atom in the anthocyanin molecule makes it a more potent and distinct hydrogen-donating antioxidant compared to oligomeric proanthocyanidins (OPCs) and other flavonoids [12]. Furthermore, the uniqueness of anthocyanin antioxidants critically depends on their ability for electron delocalization and to form resonating structures following changes in pH, which doesnot take place in other popular antioxidants [18–20]. In aqueous phase, anthocyanins exist as a mixture of four molecular species and their relative color depends upon pH. At pH 1–3, the flavylium cation is red colored, at pH 5, the colorless carbinol pseudobase is generated, and at pH 7–8 the blue purple quinoidal base is formed [27].

In the United States, the intake of anthocyanins in humans has been estimated to be 180–215 mg/day. Anthocyanins can be identified in human blood plasma after consumption of berries using HPLC, while anthocyanin-like compounds have also been identified in human serum [14]. Anthocyanins exhibit two absorbance maxima at 270–280 nm and 510–540 nm, respectively [15]. Pedersen *et al.* [25] assessed the effects of consumption of 500 mL of blueberry and cranberry juice by healthy female subjects. A significant increase in plasma antioxidant capacity was observed following consumption of cranberry juice, while consumption of blueberry juice had no such effects [25].

3 Antioxidant potential

Anthocyanins have been demonstrated to be novel antioxidants and potent inhibitors of lipid peroxidation as compared to other classic antioxidants [12–17, 19–30]. Endothelial dysfunction has been proposed to play an important role in the initiation and development of vascular diseases and anthocyanins improve endothelial function [29–31]. The enrichment of endothelial cells (EC) with elderberry anthocyanins conferred significant protective effects of EC against diverse oxidative stressors [31, 32]. Vascular EC can incorporate anthocyanins into the membrane and cytosol, conferring significant protective effects against oxidative insult, which may have important implications on preserving EC functions and preventing against vascular diseases [31].

Dietary consumption of blueberry polyphenolics has demonstrated significant protection against free radicals and oxidative stress within red blood cells *in vivo* [32]. Black raspberry has also been shown to provide significant antioxidant protection in the gut epithelium of weanling pigs due to its high anthocyanin content [33]. A comparative assessment was performed on total antioxidant status in serum following consumption of strawberries, spinach, red wine, or vitamin C in eight elderly women. It was concluded that the consumption of strawberries, spinach, or red wine, which are rich in antioxidant phenolic compounds, can increase the serum antioxidant capacity in humans [34].

In our laboratories, extensive studies were conducted on six edible berry extracts including wild blueberry, wild bilberry, cranberry, elderberry, raspberry seed, and strawberry, and accordingly a novel synergistic combination of these six berry extracts was developed, OptiBerry, which demonstrated optimal ORAC value in conjunction with high cellular uptake and low cytotoxicity, as shown by lactate dehydrogenase leakage [35]. The rationale behind this development was that individual berries contain specific anthocyanin derivative(s) such as petunidin, malvidin, pelargonidin, peonidin, delphinidin, cyanidin, and others, which demonstrate specific health benefits including cardiovascular health, ocular health, dermal health, protection against urinary tract infection, and others, however, a novel combination of these six edible berries will have a combination of these anthocyanin derivatives which synergistically should exhibit superior health benefits. This novel synergistic combination of six edible berry extracts exhibited significantly superior antioxidant, antiangiogenic, and antibacterial potential as compared to the individual berries, 20 different combinations of these berries, and a GSPE, which exhibited superior antioxidant potential as compared to vitamins C, E, and β-carotene [8, 11, 36]. Subsequently, we demonstrated OptiBerry's unique antioxidant potential in a whole body scenario using a hyperbaric oxygen-induced oxidative stress model in a state-of-the-art EPR [37].

4 Angiogenesis, vascular endothelial growth factor (VEGF), and hemangioma

"Angiogenesis" defines the growth of new blood vessels, an important natural process which takes place in the animal or human body contributing to both health and diseased conditions [38]. Angiogenesis occurs in the healthy body for healing wounds and for restoring blood flow to tissues after ischemic injury or insult, while unwanted growth of blood vessels may lead to varicose veins, tumor formation, and cancer metastases, and an antiangiogenic approach can serve as a therapeutic intervention [35, 38, 39]. The healthy body controls angiogenesis through angiogenesis-stimulating growth factors or angiogenesis inhibitors. Black raspberry extract was demonstrated to be antiangiogenic in a novel human tissue based *in vitro* angiogenesis assay. Active black raspberry fractions can be promising for cancer therapy. They are natural and contain active ingredients which have potent antiangiogenic properties and are safe to be used in multiple doses [40].

VEGF, a novel biomarker of angiogenesis, plays a crucial role for the vascularization of tumors [41]. The effects of multiple berry extracts on inducible VEGF expression by human HaCaT keratinocytes were evaluated in our laboratory. Six individual berry extracts (wild blueberry, bilberry, cranberry, elderberry, raspberry seed, and strawberry) and OptiBerry were investigated. All of the six berry extracts and OptiBerry demonstrated significant inhibition of both H_2O_2- as well as TNFα-induced VEGF expression by human keratinocytes, while OptiBerry exhibited the greatest effect [42]. In the same experimental setting, antioxidants such as GSPE or α-tocopherol did not influence inducible VEGF expression, while pure flavonoids such as ferrulic acid, catechin, and rutin suppressed oxidant-inducible VEGF expression. OptiBerry was also shown to impair angiogenesis in a matrigel assay model [42]. Thus, structural characteristics of berry anthocyanins is responsible for their inhibitory potential on inducible VEGF expression and release [12, 35].

The efficacy of OptiBerry was tested in a model of proliferating hemangioma, a unique model to assess *in vivo* angiogenesis. Macrophages are commonly involved in proliferating hemangiomas. The CC chemokine MCP-1 (monocyte chemotactic protein-1), a major accessory facilitating angiogenesis, has been shown to be responsible for recruiting macrophages to the infection or inflammation sites, and antagonists to MCP-1 are considered to be antiangiogenic [43]. MCP-1 transcription in angiogenesis is mediated by several transcription factors among which NF-$\kappa\beta$ is a key player. The endothelioma (EOMA) cells derived from spontaneously arising hemangioma were activated with TNFα (400 IU/mL) for 12 h and elevated levels of basal MCP-1 transcription in these EOMA cells were observed. Pretreatment of these EOMA cells with OptiBerry significantly inhibited basal MCP-1 transcription as well as NF-$\kappa\beta$ activity [41]. Subsequently, 8 wk old 129P3/J mice were injected (s.c.) 100 μL of EOMA cell suspension (5×10^6 cells) with or without OptiBerry pretreatment. OptiBerry pretreatment did not result in hemangioma formation in all mice and significantly reduced the average mass of tumor growth below 50% [42]. Histological analysis demonstrated that OptiBerry markedly decreased infiltration of macrophages in hemangiomas [42]. Thus, both antioxidant and antiangiogenic properties of edible berry anthocyanins may act synergistically to promote significant health benefits.

5 Anticancer properties

Blueberry, bilberry, cranberry, strawberry, lingonberry, tart cherry, black raspberry, and red raspberry as such, and their extracts, have exhibited potential cancer chemopreventive properties [23, 44–53]. Anthocyanins and ellagic acid are integral constituents in these berries, which exhibit novel anticarcinogenic activities. Freeze-dried extracts of strawberries (*Fragara ananassa*) or black raspberries (*Rubus ursinus*), along with ellagic acid, display potent chemopreventive activity which appear to involve cellular transformation and interference of uptake, activation, detoxification, and/or intervention of DNA binding and DNA repair [44]. Research on black raspberries has shown that they inhibit azoxymethane-induced colon cancer, esophageal tumorigenesis as well as show antiproliferative effect in liver cancer cells [46–48], while freeze-dried strawberries have been shown to be potent inhibitors of esophageal cancer [45]. An ethanolic extract of black raspberry has recently been demonstrated to suppress cell proliferation and nitric oxide synthase activity as well as induce both apoptosis and terminal differentiation in human oral squamous cell carcinoma cells [50]. Thus, black raspberry may serve as a promising chemopreventative agent [50].

5.1 *Helicobacter pylori* and inflammatory response

Approximately 50% of the world's population is infected with *H. pylori*, which has been demonstrated as a causative factor for diverse gastrointestinal diseases including duodenal ulcer and gastric cancer [36]. *H. pylori* is slowly developing resistance to clarithromycin, a proven antibiotic agent against *H. pylori* infection. In our laboratory, we evaluated the *in vitro* bactericidal activities of various berry extracts including blueberry, bilberry, elderberry, cranberry, strawberry and raspberry seeds, and OptiBerry, with or without clarithromycin on *H. pylori* (ATCC strain 49503) [36]. All these samples tested at all concentrations, inhibited the growth of *H. pylori*, compared with controls, with maximum inhibition with OptiBerry. At the lowest concentration of 0.25%, significant inhibition of *H. pylori* was observed with elderberry (30%), bilberry (50%), blueberry (50.5%), and OptiBerry (62%). A concentration-dependent increase in inhibition of *H. pylori* with the higher concentrations of 0.5 and 1% of all the berry extracts was observed. Modest increases in bactericidal effect were seen with the 0.5% concentration of strawberry, raspberry, and cranberry extracts, compared with the increases noted for elderberry, bilberry, blueberry, and OptiBerry. At the 1% concentration, all extracts showed >70% inhibition, with cranberry, elderberry, bilberry, and blueberry extracts showing >90% inhibition, and OptiBerry exhibiting 100% inhibition. The addition of clarithromycin to the 0.25% berry concentrations, led to a significant increase in the bactericidal effects of the elderberry, bilberry, blueberry, and OptiBerry extracts against *H. pylori* compared with other berry extracts alone [36]. When clarithromycin was added to the 0.5% berry concentrations, a significant increase in the inhibition of *H. pylori* was observed with all the extracts tested. Finally, when clarithromycin was added to the 1% berry concentrations, >90% inhibition was noted for all extracts, with elderberry, bilberry, blueberry, and OptiBerry exhibiting 100% inhibition [36].

5.2 Inhibitory effect of berry anthocyanins on *H. pylori*-induced IL-8 production in gastric MKN 45 cells

In a recent pilot study, we assessed the effect of OptiBerry on IL-8 (one of the major mediators of the inflammatory response and also a potent angiogenic factor) inhibition in *H. pylori*-treated cultured human gastric cancer cells MKN45 (JCRB0254, DSMZ, Germany), and this is being reported for the first time. *H. pylori* ATCC 49503 cells were obtained from ATCC (Bethesda, MD) and used in this study. OptiBerry dramatically inhibited *H. pylori*-induced IL-8 production in MKN45 gastric cells.

Cultured human gastric MKN45 cells (1×10^6 cells) were grown in RPMI media and treated with or without 0.5% OptiBerry, 10 ng of TNFα (R&D systems, Minneapolis, MN) and/or *H. pylori* (1×10^8 cells). Supernatants were collected at 6, 12, and 24 h of treatment, centrifuged and IL-8 levels measured using assay kits (R&D Systems, Minneapolis, MN).

A significant increase in IL-8 production was observed in cultured MKN45 following treatment with *H. pylori* cells. Approximately, 20, 22, and 25% increases in IL-8 production were observed at 6, 12, and 24 h of treatment, respectively. Addition of OptiBerry completely inhibited IL-8 production in the *H. pylori*-treated cultured MKN45 cells at all time points. Figure 1 demonstrates the effect of *H. pylori* on IL-8 production in cultured MKN45 cells and the time-dependent inhibition by OptiBerry.

6 Cardioprotection

Berry anthocyanins act as a novel cardioprotectant by maintaining vascular permeability, reducing inflammatory responses and platelet aggregation, and offer superior vascular protection as compared to other cardioprotective drugs [29–32]. Hypertension, atherosclerosis, and arteriosclerosis can reduce the flexibility of arterial walls, which contributes to poor blood flow and plaque formation [29–32]. Rat aortas exposed to anthocyanin-enriched blueberry extract *in vitro* exhibited relaxation caused by endothelium-generated nitric oxide [54]. In another study, treatment of rats with bilberry anthocyanosides (*Vaccinium myrtillus*) for 12 days before the induction of hypertension kept the blood–brain barrier permeability normal and limited the increase in vascular permeability in the skin and the aorta wall [55].

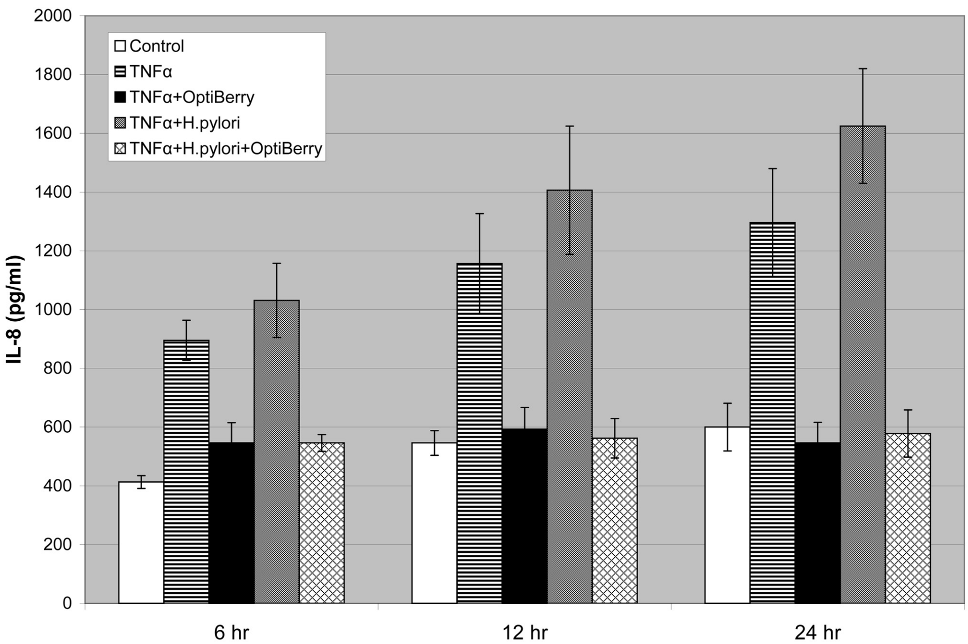

Figure 1. Effect of *H. pylori* on IL-8 production in cultured MKN45 cells and the time-dependent inhibition by OptiBerry.

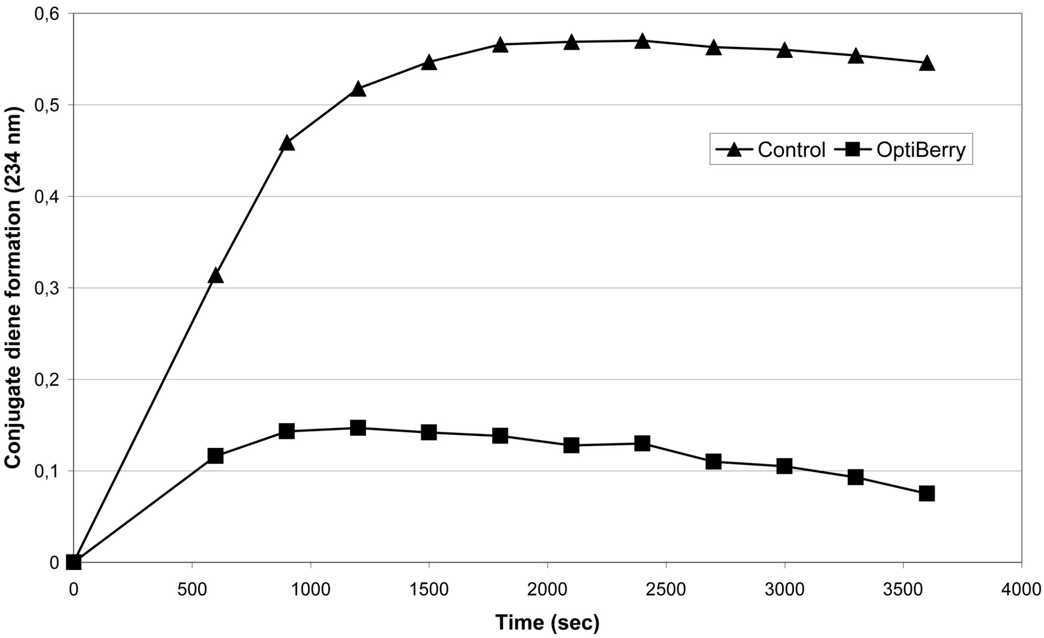

Figure 2. LDL + VLDL oxidation time curves. The values are control (0.570) *vs.* OptiBerry (0.121), demonstrating that OptiBerry significantly reduces oxidation of the lipoproteins (p <0.05).

In another study, hamsters given oral doses (10 mg *per* 10 g body weight) of a commercial product containing 36% bilberry anthocyanosides for 2 or 4 wk exhibited better capillary perfusion and fewer sticking leukocytes in the capillaries as compared to the untreated controls [56].

6.1 Atherosclerosis

Hypercholesterolemia, an integral constituent of the atherosclerotic index and a significant cardiovascular risk factor, is prevalent in the U.S. population [57]. Atherosclerosis is a disease of the arteries in which fatty plaques develop on the inner arterial wall, which eventually obstructs blood flow [57]. Risk factors for atherosclerosis include genetics, diet, lifestyle, smoking, circulating lipid and cholesterol levels, and molecular and circulating signals of chronic vascular inflammation. The protective ability of anthocyanins against atherosclerosis is based partly on their antioxidant properties.

6.2 Antiatherosclerotic activity of anthocyanins in hamster

Hamsters have a similar lipid profile to hypercholesterolemic humans when fed a hypercholesterolemic diet of 0.2% cholesterol and 10% coconut oil [57]. The efficacy of OptiBerry supplementation in hamsters against the incidence of atherosclerosis was assessed in male hamsters. The results of this study are being reported for the first time. The animals were divided into two groups of nine animals with equal weights (about 80 g each) and given powder chow containing 10% coconut oil and 0.2% cholesterol mixed with water and made into a brownie. Treated group animals were fed with chow containing 1% OptiBerry. The animals were weighed after 12 wk of feeding the atherogenic diet. Plasma was obtained by cardiac puncture and analyzed for cholesterol and triglycerides using Sigma enzyme assay kits (St. Louis, MO, USA) and colorimetry. Aortas were dissected and cleaned, and en face samples were stained with oil red O which visualizes lipid-laden foam cells, the initial stage of atherosclerosis. SigmaScan software was used to determine the% of aortal surface covered with foam cells. LDL + VLDL was isolated from pooled plasma samples of each group and oxidized with cupric ion at pH 7.6, protein concentration of 70 μg/mL at 37°C. The formation of conjugated dienes was monitored at 234 nm over time using the Genesys 5 spectrophotometer (HTC, Hickory, NC).

All of the animals in the experimental group gained significantly less weight than the control group (Approximately 8% decrease in weight gain) indicating a weight reduction from appetite loss or increased metabolism. Table 1 demonstrates the effect of OptiBerry on body weight, lipid profile, and on the percentage of aorta covered with foam cells, a biomarker of atherosclerosis *in vivo.* No significant changes were observed in triglycerides and cholesterol levels. The atherosclerotic index (% of aorta covered with foam cells) was significantly reduced by 36.6% following supplementation of OptiBerry as compared to the untreated controls.

The LDL + VLDL oxidation curves in Fig. 2 show that the control oxidizes at the greatest rate (slope) and OptiBerry has a significantly lower rate of oxidation of the athe-

Table 1. Body weight, lipid profile, and% atherosclerosis data (mean + SD)

Group	Final weight (g)	Plasma triglycerides (mg/dL)	Plasma cholesterol (mg/dL)	% Atherosclerosis
Control	140 ± 4	163 ± 62	298 ± 83	20.5 ± 1.7
OptiBerry	129 ± 3*	177 ± 111	287 ± 97	13.0 ± 3.1*

Each value represents the mean + SD of 4–6 animals. Statistically significant compared to control. *$p < 0.05$.

rogenic lipoproteins ($p < 0.05$). Thus OptiBerry which is high in anthocyanin content and has higher ORAC value demonstrated least oxidization of the lipoproteins.

Overall, OptiBerry supplementation resulted in less body weight gain as compared to the controls, which might have happened due to its antiangiogenic potential. Furthermore, Optiberry may provide significant health benefits by dramatically ameliorating the incidence of atherosclerosis as demonstrated by reducing the formation of foam cells as well as its reduced ability to oxidize functional lipoproteins.

7 Neuroprotection

Brain functions such as balance, coordination, short-term memory, and information retrieval can be impaired with advancing age, but research has shown that eating blueberries can reverse age-related and oxidative stress-induced decline in brain functions [3, 4, 22, 31, 32, 58–62]. Berries have been shown to enhance dopamine release in the brain, which improves the ability of brain cells to enhance intracellular communication [3, 4, 22, 31, 32, 58–62]. Strawberry supplementation was shown to enhance striatal muscarinic receptor sensitivity, and this appeared to be reflected in the reversal of cognitive behavioral deficits [58, 59]. Strawberries or blueberries have also been shown to reverse age-induced declines in β-adrenergic receptor function in cerebellar Purkinje neurons, while blueberries were shown to prevent and/or reverse age-related declines in cerebellar noradrenergic receptor function [3].

In another study, Fisher F344 rats (6–8 months old) were fed diets containing vitamin E, aqueous blueberry extract and dried strawberry extract, or dried spinach extract for 8 wk, and then subjected to 48 h of 100% oxygen-induced damage similar to that found in aged rats. All antioxidant diets prevented decreases in nerve growth factor in the basal forebrain and other adverse effects were reduced. In a separate investigation, rats given intraperitoneal injections of bilberry anthocyanins (200 mg/kg/day) for 5 days had significantly more triiodothyronine (T3) in their brains than rats given only the solvent (26% alcohol). T3 enters the brain by a specific transport in the capillaries; therefore, anthocyanins may mediate T3 transport at the capillary level. Bilberry-treated animals exhibited superior memory, better vision, and better control of sensory input [60, 63].

8 Diabetes and vision

The leaves and fruits of *V. myrtillus* (containing 25% anthocyanidins)(VMA) have been used for centuries in Europe to ameliorate the symptoms of diabetes [63]. Consumption of VMA with breakfast cereals and other whole grain foods could add another level of protection against the onset of diabetes [63]. An aqueous alcohol extract of *V. myrtillus* leaves produced a 26% reduction in plasma glucose levels in rats made diabetic by the drug streptozotocin. Plasma triglycerides decreased in proportion with the amount of bilberry leaf extract given to rats (1.2 or 3.0 g/kg body weight) fed a hyperlipidemic diet [63]. Antihyperglycemic potential of anthocyanins has also been demonstrated by Matsui *et al.* [64].

The berry anthocyanins appear to benefit vision in several ways, including improving night vision by enhanced generation of retinal pigments, increasing circulation within the capillaries of the retina, decreasing macular degeneration and diabetic retinopathy, and improving or preventing glaucoma, retinitis pigmentosa, and cataracts [65]. Bilberry has been demonstrated to improve eyesight particularly night vision. It is worthwhile to mention that bilberry jam was extensively used by British Air Force Pilots during World War I and II before their bombing mission. Since carotenoids with vitamin A activity are found in *Vaccinium* species, some of the benefits pertaining to vision are attributable to these compounds. A double-blind, placebo-controlled study showed that oral doses of anthocyanins are important for generation of visual purple, which helps to convert light into electrical signals for the brain. Adapto-electroretinograms (AERG) of two sets of six subjects were made before treatment at 1 and 3 h postadministration. Subjects given the bilberry adapted to the light within 6.5 min, compared with 9 min for the control group. In another study, 50 patients with senile cataracts were given a combination of bilberry extract standardized to contain 25% anthocyanosides (180 mg twice daily) and vitamin E in the form of dl-tocopheryl acetate (100 mg twice daily) administered for 4 months. The progression of cataracts was stopped in 96% of the subjects treated ($n = 25$) compared to 76% in the control group ($n = 25$) [66].

In a double-blind study, 14 diabetic or/and hypertensive outpatients with vascular retinopathy underwent therapy with VMA (160 mg b.i.d.) or placebo ($n = 20$) for 1 month. At the end of the month, patients placebo-treated received the active drug for one additional month. Ophthalmoscopic and fluoroangiographic findings recovered before and after treatments showed an improvement ranging from 77 to 90% of anthocyanosides-treated patients. In another randomized, double-blind, placebo-controlled trial, 50 patients, 21 men and 29 women (mean age 67 year, range 48–81) suffering from mild senile cortical cataract underwent therapy with Vitamin E plus VMA (2 tabs b.i.d.) for 4 months. VMA was able to stop lens opacity progress in 97% of the cataracts. No adverse-drug reactions were recorded [21].

Long-term administration of berry-derived supplements including anthocyanins is safe and can inhibit the development of the early stages of diabetic retinopathy, and this finding warrants further investigation to unveil the mechanism. Furthermore, anthocyanins can offer an achievable, safe, and inexpensive adjunct therapy to inhibit the development of retinopathy and can normalize hyperglycemia [21, 67, 68].

In a recent study, Tsuda *et al.* [69] explored the gene expression profiles in human adipocytes treated with anthocyanins and demonstrated that anthocyanins can regulate adipocytokine gene expression to positively modulate adipocyte function to control obesity and diabetes.

9 Conclusions

Anthocyanins, the natural colorants/pigments of fruits and vegetables, are novel, safe, and proven antioxidants and chemopreventive agents. A broad spectrum of *in vitro, in vivo*, and human studies have demonstrated that anthocyanins promote antioxidant status, healthy vision, urinary tract health, and dermal health as well as exhibit potential health benefits including cardiovascular, neuroprotective, anticarcinogenic potential, and antidiabetic properties. Recent studies have examined and demonstrated the potential cancer chemopreventive, antiangiogenic, and other benefits of berry extracts especially freeze-dried berries including blueberry, bilberry, strawberry, and black raspberries. OptiBerry, a novel synergistic blend of wild blueberry, bilberry, cranberry, elderberry, raspberry seed, and strawberry, was shown to be safer and more potent as compared to the individual berries and other combinations of berry extracts tested [11, 35–37, 42]. It demonstrated superior bioavailability, antioxidative, antiangiogenic, antibacterial, antiinflammatory, and antiatherosclerotic properties. Although more human studies are warranted to establish the broad spectrum of health benefits of anthocyanins, the currently available data and recent findings are very encouraging.

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