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FENO-MYRTILLUS®

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ANTI-CANCER EFFECTS

Bilberry extract (Antho50) selectively induces redox-sensitive caspase 3-related apoptosis in chronic lymphocytic leukemia cells by targeting the Bcl-2/Bad pathway.

Sci Rep. 2015 Mar 11; 5:8996. doi: 10.1038/srep08996.

Defect in apoptosis has been implicated as a major cause of resistance to chemotherapy observed in B cell chronic lymphocytic leukaemia (B CLL). This study evaluated the pro-apoptotic effect of an anthocyanin-rich dietary bilberry extract (Antho 50) on B CLL cells from 30 patients and on peripheral blood mononuclear cells (PBMCs) from healthy subjects, and determined the underlying mechanism. Antho 50 induced concentration- and time-dependent pro-apoptotic effects in B CLL cells but little or no effect in PBMCs. Among the main phenolic compounds of the bilberry extract, delphinidin-3-O-glucoside and delphinidin-3-O-rutinoside induced a pro-apoptotic effect. Antho 50-induced apoptosis is associated with activation of caspase 3, down-regulation of UHRF1, a rapid dephosphorylation of Akt and Bad, and down-regulation of Bcl-2. Antho 50 significantly induced PEG-catalase-sensitive formation of reactive oxygen species in B CLL cells. PEG-catalase prevented the Antho 50-induced induction of apoptosis and related signaling. The present findings indicate that Antho 50 exhibits strong pro-apoptotic activity through redox-sensitive caspase 3 activation-related mechanism in B CLL cells involving dysregulation of the Bad/Bcl-2 pathway. This activity of Antho 50 involves the glucoside and rutinoside derivatives of delphinidin. They further suggest that Antho 50 has chemotherapeutic potential by targeting selectively B CLL cells.

Tumor suppression effects of bilberry extracts and enzymatically modified isoquercitrin in early preneoplastic liver cell lesions induced by piperonyl butoxide promotion in a two-stage rat hepatocarcinogenesis model.

Exp Toxicol Pathol. 2014 Aug; 66(5-6):225-34. doi: 10.1016/j.etp.2014.02.002. Epub 2014 Mar 26.

To investigate the protective effect of bilberry extracts (BBE) and enzymatically modified isoquercitrin (EMIQ) on the hepatocarcinogenic process involving oxidative stress responses, we used a two-stage hepatocarcinogenesis model in N-diethylnitrosamine-initiated and piperonyl butoxide (PBO)-promoted rats. We examined the modifying effect of co-administration with BBE or EMIQ on the liver tissue environment including oxidative stress responses, cell proliferation and apoptosis, and phosphatase and tensin homolog (PTEN)/Akt and transforming growth factor (TGF)- β /Smad signalings on the induction mechanism of preneoplastic lesions during early stages of hepatocellular tumor promotion. PBO increased the numbers and area of glutathione S-transferase placental form (GST-P)(+) liver cell foci and the numbers of Ki-67(+) proliferating cells within GST-P(+) foci. Co-administration of BBE or EMIQ suppressed these effects with the reductions of GST-P(+) foci (area) to 48.9-49.4% and Ki-67(+) cells to 55.5-61.4% of the PBO-promoted cases. Neither BBE nor EMIQ decreased microsomal reactive oxygen species induced by PBO. However, only EMIQ suppressed the level of thiobarbituric acid-reactive substances to 78.4% of the PBO-promoted cases. PBO increased the incidences of phospho-PTEN(-) foci, phospho-Akt substrate(+) foci, phospho-Smad3(-) foci and Smad4(-) foci in GST-P(+) foci. Both BBE and EMIQ decreased the incidences of phospho-PTEN(-) foci in GST-P(+) foci to 59.8-72.2% and Smad4(-) foci to 62.4-71.5% of the PBO-promoted cases, and BBE also suppressed the incidence of phospho-Akt substrate(+) foci in GST-P(+) foci to 75.2-75.7% of the PBO-promoted cases. These results suggest that PBO-induced tumor promotion involves facilitation of PTEN/Akt and disruptive TGF- β /Smad signalings without relation to oxidative stress responses, but this promotion was suppressed by co-treatment with BBE or EMIQ through suppression of cell proliferation activity of preneoplastic liver cells.

Cytotoxic effects of bilberry extract on MCF7-GFP-tubulin breast cancer cells.

J Med Food. 2010 Apr; 13(2):278-85. doi: 10.1089/jmf.2009.0053.

Bilberry (European blueberry) has been reported to have many biological effects, including anticancer activity. In this study, we investigated the antiproliferative effects of bilberry extract in relation to its ability to induce apoptosis and affect microtubule assembly and organization in MCF7 human breast cancer cells. We observed that bilberry extract inhibited cell proliferation in a concentration-dependent fashion with a 50% inhibitory concentration of 0.3-0.4 mg/mL, in concert with induction of apoptotic cell death. At these concentrations there was no selective inhibition of mitosis or any other cell cycle stage, nor was there any apparent effect on the microtubule or actin cytoskeletons. However, somewhat higher extract concentrations (0.5-0.9 mg/mL) did cause an increase in the fraction of cells at the G(2)/M phase of the cell cycle, together with destruction of microtubules and formation of punctate tubulin aggregates in the cells. Bilberry extract at 0.3-0.4 mg/mL did not appreciably inhibit microtubule polymerization in vitro, but significant inhibition of polymerization (approximately 30%) did occur at higher extract concentrations (0.5-1 mg/mL). We conclude that bilberry extract as ingested by humans, not just the purified anthocyanins it contains, inhibits proliferation of and induces apoptosis in breast cancer cells at its lowest effective concentrations via a mechanism that does not involve action on microtubules or on mitosis. We further conclude that at somewhat higher concentrations the extract modifies microtubule organization in cells and causes accumulation of cells at mitosis by a direct action on microtubules.

Pilot study of oral anthocyanins for colorectal cancer chemoprevention.

Cancer Prev Res (Phila). 2009 Jul; 2(7):625-33. doi: 10.1158/1940-6207.CAPR-08-0201.

Naturally occurring anthocyanins possess colorectal cancer chemopreventive properties in rodent models. We investigated whether mirtocyan, an anthocyanin-rich standardized bilberry extract, causes pharmacodynamic changes consistent with chemopreventive efficacy and generates measurable levels of anthocyanins in blood, urine, and target tissue. Twenty-five colorectal cancer patients scheduled to undergo resection of primary tumor or liver metastases received mirtocyan 1.4, 2.8, or 5.6 grams (containing 0.5-2.0 grams anthocyanins) daily for 7 days before surgery. Bilberry anthocyanins were analyzed by high performance liquid chromatography (HPLC) with visible or mass spectrometric detection. Proliferation was determined by immunohistochemistry of Ki-67 in colorectal tumor. Concentrations of insulin-like growth factor (IGF)-I were measured in plasma. Mirtocyan anthocyanins and methyl and glucuronide metabolites were identified in plasma, colorectal tissue, and urine, but not in liver. Anthocyanin concentrations in plasma and urine were roughly dose-dependent, reaching approximately 179 ng/gram in tumor tissue at the highest dose. In tumor tissue from all patients on mirtocyan, proliferation was decreased by 7% compared with preintervention values. The low dose caused a small but nonsignificant reduction in circulating IGF-I concentrations. In conclusion, repeated administration of bilberry anthocyanins exerts pharmacodynamic effects and generates concentrations of anthocyanins in humans resembling those seen in Apc(Min) mice, a model of FAP adenomas sensitive to the chemopreventive properties of anthocyanins. Studies of doses containing <0.5 gram bilberry anthocyanins are necessary to adjudicate whether they may be appropriate for development as colorectal cancer chemopreventive agents.

Berries as chemopreventive dietary constituents--a mechanistic approach with the ApcMin/+ mouse.

Asia Pac J Clin Nutr. 2008; 17 Suppl 1:123-5.

Berries contain a number of compounds that are proposed to have anticarcinogenic properties. We wanted to see if pure ellagic acid, natural ellagitannins and three wild berries have any effect on the adenoma formation in Apc- mutated Min/+ mice. Min/+ mice were fed high-fat AIN93-G diets containing 10% (w/w) freeze-dried bilberry (Vaccinium myrtillus), lingonberry (Vaccinium vitis-idaea), cloudberry (Rubus chamaemorus), cloudberry seeds or cloudberry pulp or pure ellagic acid at 1564 mg/kg for 10



weeks. Beta-Catenin and cyclin D1 protein levels in the adenomas and in the normal-appearing mucosa were determined by Western blotting and immunohistochemistry. Early changes in gene expression in the normal-appearing mucosa were analyzed by Affymetrix microarrays. Three wild berries significantly reduced tumour number (15-30%, $p < 0.05$), and cloudberry and lingonberry also reduced tumour size by over 60% ($p < 0.01$). Cloudberry resulted in decreased levels of nuclear beta-catenin and cyclin D1 and lingonberry in the level of cyclin D1 in the large adenomas ($p < 0.05$). Affymetrix microarrays revealed changes in genes implicated in colon carcinogenesis, including the decreased expression of the adenosine deaminase, ecto-5f-nucleotidase and PGE2 receptor subtype EP4. Ellagic acid had no effect on the number or size of adenomas in the distal or total small intestine but it increased adenoma size in the duodenum when compared with the control diet ($p < 0.05$). Neither cloudberry seed nor pulp had any effect on the adenoma formation. Berries seem to have great potential as a source of chemopreventive components.

Bilberry inhibits angiogenesis in chick chorioallantoic membrane.

Biofactors. 2008; 33(3):161-4.

Angiogenesis is the process of generating new blood vessels from preexisting vessels and is considered essential in many pathological conditions. Bilberry has been traditionally used as a folk medicine for some countries. The purpose of the present study was to evaluate the effect of bilberry in chick chorioallantoic membrane angiogenesis model in vivo. In this well characterized model, bilberry inhibited angiogenesis in a concentration-dependent manner. Compared with the normal group, bilberry group has significant decreased vessels proliferation. These results provide evidence that bilberry inhibits angiogenesis and may be useful for treating angiogenesis-dependent human diseases.

Effect of cyanidin-3-glucoside and an anthocyanin mixture from bilberry on adenoma development in the ApcMin mouse model of intestinal carcinogenesis--relationship with tissue anthocyanin levels.

Int J Cancer. 2006 Nov 1; 119(9):2213-20.

Anthocyanins are dietary flavonoids, which can prevent carcinogen-induced colorectal cancer in rats. Here, the hypotheses were tested that Mirtoselect, an anthocyanin mixture from bilberry, or isolated cyanidin-3-glucoside (C3G), the most abundant anthocyanin in diet, interfere with intestinal adenoma formation in the Apc(Min) mouse, a genetic model of human familial adenomatous polyposis, and that consumption of C3G or Mirtoselect generates measurable levels of anthocyanins in the murine biophase. Apc(Min) mice ingested C3G or Mirtoselect at 0.03, 0.1 or 0.3% in the diet for 12 weeks, and intestinal adenomas were counted. Plasma, urine and intestinal mucosa were analyzed for presence of anthocyanins by high-pressure liquid chromatography with detection by UV spectrophotometry (520 nm) or tandem mass spectrometry (multiple reaction monitoring). Ingestion of either C3G or Mirtoselect reduced adenoma load dose-dependently. At the highest doses of C3G and Mirtoselect adenoma numbers were decreased by 45% ($p < 0.001$) or 30% ($p < 0.05$), respectively, compared to controls. Anthocyanins were found at the analytical detection limit in the plasma and at quantifiable levels in the intestinal mucosa and urine. Anthocyanin glucuronide and methyl metabolites were identified in intestine and urine. Total anthocyanin levels in mice on C3G or Mirtoselect were 43 ng and 8.1 microg/g tissue, respectively, in the intestinal mucosa, and 7.2 and 12.3 microg/ml in the urine. The efficacy of C3G and Mirtoselect in the Apc(Min) mouse renders the further development of anthocyanins as potential human colorectal cancer chemopreventive agents worthwhile.

Anthocyanin-rich extracts inhibit multiple biomarkers of colon cancer in rats.

Nutr Cancer. 2006; 54(1):84-93.

The aim of the present study was to investigate the chemoprotective activity of anthocyanin-rich extracts (AREs) from bilberry (*Vaccinium myrtillus* L.), chokeberry (*Aronia meloncarpa* E.), and grape (*Vitis vinifera*) by assessing multiple biomarkers of colon cancer in male rats treated with a colon carcinogen, azoxymethane. Fischer 344 male rats were fed the AIN-93 diet (control) or AIN-93 diet supplemented with AREs for 14 wk. Biomarkers that were evaluated included the number and multiplicity of colonic aberrant crypt foci (ACF), colonic cell proliferation, urinary levels of oxidative DNA damage, and expression of cyclooxygenase (COX) genes. To assess the bioavailability, levels of anthocyanins in serum, urine, and feces were evaluated. Total ACF were reduced ($P<0.05$) in bilberry, chokeberry, and grape diet groups compared with the control group. The number of large ACF was also reduced ($P<0.05$) in bilberry and chokeberry ARE-fed rats. Colonic cellular proliferation was decreased in rats fed bilberry ARE and chokeberry ARE diets. Rats fed bilberry and grape ARE diets had lower COX-2 mRNA expression of gene. High levels of fecal anthocyanins and increased fecal mass and fecal moisture occurred in ARE-fed rats. There was also a significant reduction ($P<0.05$) in fecal bile acids in ARE-fed rats. The levels of urinary 8-hydroxyguanosine were similar among rats fed different diets. These results support our previous *in vitro* studies suggesting a protective role of AREs in colon carcinogenesis and indicate multiple mechanisms of action.

Induction of apoptosis in cancer cells by Bilberry (*Vaccinium myrtillus*) and the anthocyanins.

J Agric Food Chem. 2003 Jan 1; 51(1):68-75.

Among ethanol extracts of 10 edible berries, bilberry extract was found to be the most effective at inhibiting the growth of HL60 human leukemia cells and HCT116 human colon carcinoma cells *in vitro*. Bilberry extract induced apoptotic cell bodies and nucleosomal DNA fragmentation in HL60 cells. The proportion of apoptotic cells induced by bilberry extract in HCT116 was much lower than that in HL60 cells, and DNA fragmentation was not induced in the former. Of the extracts tested, that from bilberry contained the largest amounts of phenolic compounds, including anthocyanins, and showed the greatest 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. Pure delphinidin and malvidin, like the glycosides isolated from the bilberry extract, induced apoptosis in HL60 cells. These results indicate that the bilberry extract and the anthocyanins, bearing delphinidin or malvidin as the aglycon, inhibit the growth of HL60 cells through the induction of apoptosis. Only pure delphinidin and the glycoside isolated from the bilberry extract, but not malvidin and the glycoside, inhibited the growth of HCT116 cells.

ANTI-MICROBIAL EFFECTS

Relationship between polyphenol content and anti-influenza viral effects of berries.

J Sci Food Agric. 2013 Jul; 93(9):2239-41. doi: 10.1002/jsfa.6031.

BACKGROUND: Berries are known to have many kinds of biological activities. We focused on their antiviral effect, which has not yet been well evaluated. RESULTS: We compared the anti-influenza viral effects of berries belonging to the genus Vaccinium - 35 species of blueberry (Vaccinium cyanococcus), the Natsuhaze (Vaccinium oldhamii), bilberry (Vaccinium myrtillus) and cranberry (Vaccinium oxycoccos)- with those belonging to the genus Ribes, i.e. blackcurrant (Ribes nigrum). Only Elliott and Legacy among Northern Highbush varieties but many Rabbiteye varieties such as Austin, Baldwin, Brightblue, Festival, T-100 and Tifblue showed anti-influenza viral activity. Natsuhaze, bilberry, cranberry and blackcurrant had high antiviral effects. A relationship was observed between the antiviral effect and total polyphenol content. CONCLUSIONS: Antiviral effects were found to differ markedly between berry species. Rabbiteye varieties tended to have higher antiviral effects than Northern, Southern and Half Highbush blueberry varieties. We also found that Natsuhaze, which has recently been harvested in Japan as a potential functional food, had an antiviral effect comparable to that of bilberry, cranberry and blackcurrant. There was a positive relationship between antiviral activity and polyphenol content, indicating the possibility that polyphenol is one of the key factors in the antiviral effects of berries.

Inhibition of adhesion of Neisseria meningitidis to human epithelial cells by berry juice polyphenolic fractions.

Phytother Res. 2011 Jun; 25(6):828-32. doi: 10.1002/ptr.3349. Epub 2010 Nov 17.

The adhesion of pathogens to host tissues is the requirement for the initiation of the majority of infectious diseases. It was shown recently that the binding of Neisseria meningitidis pili to immobilized human epithelial cells is inhibited by molecular size fractions (10-100 kDa) of berry juices. Additionally, the isolated meningococcal pili bound to polyphenolic fractions of berry juices. The present study investigated the antiadhesive effects of berry juice polyphenolics against living meningococcal bacteria in a human epithelial cell culture model. The ability of bilberry, cranberry, crowberry and lingonberry juice polyphenolic fractions to inhibit the attachment of N. meningitidis bacteria to HEC-1B human epithelial cells in a cell culture model was examined. The antibacterial effect of the fractions was tested using a microtiter broth microdilution assay. The most effective adhesion inhibition of 75% was achieved with cranberry juice polyphenolic fraction followed by crowberry (63%), bilberry (63%) and lingonberry (57%) juice polyphenolic fractions. Bacterial survival rates after incubation with the fractions varied between 75-100%. The present results suggest berry juice polyphenols as inhibitors of adherence of N. meningitidis. Thus the binding of meningococci to berry juice polyphenols might be protective for the host against the infection.

Inhibition activity of wild berry juice fractions against Streptococcus pneumoniae binding to human bronchial cells.

Phytother Res. 2011 Jan; 25(1):122-7. doi: 10.1002/ptr.3240.

Bacterial adhesion to the cell surface is a crucial step before infection can take place. Inhibition of bacterial binding offers a novel preventive approach against infections. Cranberry (Vaccinium macrocarpon Ait.) juice has been found to have antiadhesive activity against different bacteria. Streptococcus pneumoniae is an important pathogen and the most common cause for pneumonia, meningitis, and otitis media. In this study the inhibitory activity of cranberry (Vaccinium oxycoccos L.), bilberry (Vaccinium myrtillus L.) and crowberry (Empetrum nigrum and Empetrum hermaphroditum L.) juice fractions against pneumococcal binding was tested using human bronchial cells (Calu-3) as an adhesion model. In addition, the antimicrobial

activity of the berry juice fractions was tested. It was found that the studied berry juice fractions had antiadhesion activity and cranberry juice was the most active. The adhesion inhibition activity of cranberry juice was nearly 90% at a concentration of 8.7 mg/g of soluble solids. The antimicrobial activity of the studied berry juice fractions was found to be remarkable; pneumococcal growth was inhibited totally at a concentration of ~86 mg/g. Both antiadhesion and antimicrobial activities were reduced after solid-phase extraction of the berry juices, which may suggest molecular synergistic effects of the berry juice molecules against *S. pneumoniae*. The findings indicate that cranberry, bilberry and crowberry juices have potential against pneumococcal infections.

Comparative study of anthocyanin composition, antimicrobial and antioxidant activity in bilberry (*Vaccinium myrtillus* L.) and blueberry (*Vaccinium corymbosum* L.) fruits.

Acta Pol Pharm. 2009 Jul-Aug; 66(4):399-408.

*Simultaneous comparison of bilberry (*Vaccinium myrtillus* L.) and blueberry (*Vaccinium corymbosum* L) fruits for their anthocyanin composition, antimicrobial and antioxidant activity is reported. The aim of this study was to investigate and to compare anthocyanin composition, antimicrobial and antioxidant activity in bilberry and blueberry fruits and their skins. The investigations revealed that the highest amount of total anthocyanins was observed in fruits skins of blueberry cultivars. The results, obtained by chromatographic analysis, indicated that cyanidin is a dominant anthocyanidin in bilberry and malvidin in blueberry samples. Extracts of "Herbert", "Coville", "Toro" blueberry cultivars and bilberry fruits revealed antimicrobial properties. *Citrobacter freundii* (ATCC 8090) and *Enterococcus faecalis* (ATCC29212) were the most sensitive among eight tested Gram-negative and Gram-positive bacteria. Significant differences between berry and skin extracts were not established. Studies with fruits showed that the strongest antioxidant activity possesses blueberry cultivar "Berkeley" (82.13 +/- 0.51%). Meanwhile, the amount of quenched free radicals in bilberry samples was 63.72 +/- 1.11%, respectively. The lowest antioxidant activity was estimated in blueberry cultivar "Coville". Accordingly, the strongest antiradical properties were estimated in blueberry cultivar "Ama" fruit skins. Bilberry fruit skin samples possess strong antiradical activity as well (82.69 +/- 0.37%).*

Berry phenolics: antimicrobial properties and mechanisms of action against severe human pathogens.

Nutr Cancer. 2006; 54(1):18-32.

*Antimicrobial activity and mechanisms of phenolic extracts of 12 Nordic berries were studied against selected human pathogenic microbes. The most sensitive bacteria on berry phenolics were *Helicobacter pylori* and *Bacillus cereus*. *Campylobacter jejuni* and *Candida albicans* were inhibited only with phenolic extracts of cloudberry, raspberry, and strawberry, which all were rich in ellagitannins. Cloudberry extract gave strong microbicidal effects on the basis of plate count with all studied strains. However, fluorescence staining of liquid cultures of virulent *Salmonella* showed viable cells not detectable by plate count adhering to cloudberry extract, whereas *Staphylococcus aureus* cells adhered to berry extracts were dead on the basis of their fluorescence and plate count. Phenolic extracts of cloudberry and raspberry disintegrated the outer membrane of examined *Salmonella* strains as indicated by 1-N-phenyl-naphthylamine (NPN) uptake increase and analysis of liberation of [¹⁴C] galactose- lipopolysaccharide. Gallic acid effectively permeabilized the tested *Salmonella* strains, and significant increase in the NPN uptake was recorded. The stability of berry phenolics and their antimicrobial activity in berries stored frozen for a year were examined using *Escherichia coli* and nonvirulent *Salmonella enterica* sv. Typhimurium. The amount of phenolic compounds decreased in all berries, but their antimicrobial activity was not influenced accordingly. Cloudberry, in particular, showed constantly strong antimicrobial activity during the storage.*

ANTIOXIDATIVE AND ANTI-INFLAMMATORY EFFECTS

Fractionation of an anthocyanin-rich bilberry extract and in vitro antioxidative activity testing.

Food Chem. 2015 Jan 15; 167:418-24. doi: 10.1016/j.foodchem.2014.07.004.

*The incidence of chronic diseases increases with advancing age of the population. A commonly discussed cause of chronic diseases is oxidative stress, which occurs in the body when there is an imbalance between the formation and inactivation of so-called reactive oxygen species (ROS). Epidemiological data suggest that a 'healthy diet', with a high content of flavonoids indicates preventive properties and correlates with an inverse effect with respect to the risk of chronic diseases. Berries (especially bilberries, *Vaccinium myrtillus* L.) are an important source of these flavonoids. In this study, we investigated, in vitro, the antioxidative properties of fractions obtained from a commercially available anthocyanin-rich bilberry extract (BE). As markers for antioxidative activity, the intracellularly generated ROS levels, oxidative DNA damage, and total glutathione (tGSH) levels were determined in the human colon cell lines Caco-2 and HT-29. In Caco-2 cells, the ROS levels and, in both cell lines, the oxidative DNA damage, were significantly reduced in the presence of the original BE and phenolcarboxylic acid-rich fraction. Total GSH levels were slightly increased after pretreatment with BE, phenolcarboxylic acid and the polymeric fractions, but not with the anthocyanin fraction. In summary, the BE and the therefrom-isolated phenolcarboxylic acid-rich fraction, showed the most potent antioxidative activity whereas the polymeric and anthocyanin-rich fraction, in total, were less active.*

Anthocyanins from fruit juices improve the antioxidant status of healthy young female volunteers without affecting anti-inflammatory parameters: results from the randomised, double-blind, placebo-controlled, cross-over ANTHONIA (ANTHOcyanins in Nutrition Investigation Alliance) study.

Br J Nutr. 2014 Sep 28; 112(6):925-36. doi: 10.1017/S0007114514001482. Epub 2014 Aug 4.

Anthocyanins (ACN) can exert beneficial health effects not only through their antioxidative potential but also through modulation of inflammatory parameters that play a major role in CVD. A randomised cross-over study was carried out to investigate the effects of ACN-rich beverage ingestion on oxidation- and inflammation-related parameters in thirty healthy female volunteers. The participants consumed 330 ml of beverages (placebo, juice and smoothie with 8.9 (SD 0.3), 983.7 (SD 37) and 840.9 (SD 10) mg/l ACN, respectively) over 14 d. Before and after each intervention, blood and 24 h urine samples were collected. Plasma superoxide dismutase (SOD) and catalase activities increased significantly after ACN-rich beverage ingestion ($P < 0.001$), whereas after placebo juice ingestion no increase could be observed. Plasma glutathione peroxidase and erythrocyte SOD activities were not affected. An increase in Trolox equivalent antioxidant capacity could also be observed after juice ($P < 0.001$) and smoothie ($P < 0.01$) ingestion. The plasma and urinary concentrations of malondialdehyde decreased after ACN-rich beverage ingestion ($P < 0.001$), whereas those of 8-OH-2-deoxyguanosine as well as inflammation-related parameters (IL-2, -6, -8 and -10, C-reactive peptide, soluble cluster of differentiation 40 ligand, TNF- α , monocyte chemoattractant protein-1 and soluble cell adhesion molecules) were not affected. Thus, ingestion of ACN-rich beverages improves antioxidant enzyme activities and plasma antioxidant capacity, thus protecting the body against oxidative stress, a hallmark of ongoing atherosclerosis.

Effect of plant extracts on H₂O₂-induced inflammatory gene expression in macrophages.

J Inflamm Res. 2014 Jun 24; 7:103-12. doi: 10.2147/JIR.S61471. eCollection 2014.

BACKGROUND: Arctium lappa (AL), Camellia sinensis (CS), Echinacea angustifolia, Eleutherococcus senticosus, Panax ginseng (PG), and Vaccinium myrtillus (VM) are plants traditionally used in many herbal formulations for the treatment

of various conditions. Although they are well known and already studied for their anti-inflammatory properties, their effects on H₂O₂-stimulated macrophages are a novel area of study. **MATERIALS AND METHODS:** Cell viability was tested after treatment with increasing doses of H₂O₂ and/or plant extracts at different times of incubation to identify the optimal experimental conditions. The messenger (m)RNA expression of TNF α , COX2, IL1 β , NF κ B1, NF κ B2, NOS2, NFE2L2, and PPAR γ was analyzed in macrophages under H₂O₂ stimulation. The same genes were also quantified after plant extract treatment on cells pre-stimulated with H₂O₂. **RESULTS:** A noncytotoxic dose (200 μ M) of H₂O₂ induced active mRNA expression of COX2, IL1 β , NFE2L2, NF κ B1, NF κ B2, NOS2, and TNF α , while PPAR γ was depressed. The expression of all genes tested was significantly ($P < 0.001$) regulated by plant extracts after pre-stimulation with H₂O₂. COX2 was downregulated by AL, PG, and VM. All extracts depressed IL1 β expression, but upregulated NFE2L2. NF κ B1, NF κ B2, and TNF α were downregulated by AL, CS, PG, and VM. NOS2 was inhibited by CS, PG, and VM. PPAR γ was decreased only after treatment with *E. angustifolia* and *E. senticosus*. **CONCLUSION:** The results of the present study indicate that the stimulation of H₂O₂ on RAW267.4 cells induced the transcription of proinflammatory mediators, showing that this could be an applicable system by which to activate macrophages. Plant extracts from AL, CS, PG, and VM possess *in vitro* anti-inflammatory activity on H₂O₂-stimulated macrophages by modulating key inflammation mediators. Further *in vitro* and *in vivo* investigation into molecular mechanisms modulated by herbal extracts should be undertaken to shed light on the development of novel modulating therapeutic strategies.

Comparative antioxidant activity of cultivated and wild Vaccinium species investigated by EPR, human neutrophil burst and COMET assay.

Eur Rev Med Pharmacol Sci. 2013; 17(15):1987-99.

OBJECTIVES: The *Vaccinium* (*V.*) spp. berries are considered a source of antioxidants, mainly belonging to polyphenols, specifically flavonoids and anthocyanins. Wild genotypes generally contain more antioxidants than cultivated counterparts. So, seven different antioxidant assays on extracts from cultivated and wild *Vaccinium* berries were performed, to evaluate their difference in terms of bioactivity on oxidative protection and minimum dosage to have a significant action. **MATERIALS AND METHODS:** Four cell-free antioxidant assays (ABTS radical scavenging and electronic paramagnetic resonance using Fremy's salt, superoxide anion and hydroxyl radical), and three assays on human cells (two luminol amplified chemiluminescence, LACL, one on DNA damage, COMET) were used to measure the effects of cultivated blueberry (*V. corymbosum*) and wild bilberry (*V. myrtillus*) on the differently induced oxidative stress. Concentrations vs activity patterns were obtained by successive dilutions of extracts in order to identify both EC₅₀ and minimum significant activity (MSA). **RESULTS:** All the assays (except for the hydroxyl radical scavenging) showed a good relationship mainly with anthocyanin and polyphenol content and the significant greater activity of wild *Vaccinium* extracts. In fact, LACL data gave an EC₅₀ of 11.8 and an MSA of 5.2 g were calculated as fresh weight dosage in cultivated berries, compared with lower doses in wild berries, EC₅₀ of 5.7 g and MSA of 3.4 g. **CONCLUSIONS:** Wild *Vaccinium* extracts averaged 3.04 and 2.40 fold more activity than cultivated extracts by EC₅₀ and MSA, respectively.

Bilberry and blueberry anthocyanins act as powerful intracellular antioxidants in mammalian cells.

Food Chem. 2012 Oct 15; 134(4):1878-84. doi: 10.1016/j.foodchem.2012.03.092.

Berry anthocyanins have pronounced health effects, even though they have a low bioavailability. The common mechanism underlying health protection is believed to relate to antioxidant activity. Berry extracts, chemically characterised for their phenolic content, were prepared from bilberries (*Vaccinium myrtillus*L.) and blueberries (*Vaccinium corymbosum*L.); the bilberry extract was further purified to obtain the anthocyanin fraction. The antioxidant activity of each extract was examined at the cellular level. For this purpose a specific assay, known as cellular antioxidant activity assay (CAA), was implemented in different cell lines: human colon cancer (Caco-2), human hepatocarcinoma (HepG2), human endothelial (EA.hy926) and rat vascular smooth muscle (A7r5). Here we show for the first time that anthocyanins had intracellular antioxidant activity if applied at very

low concentrations (<1 µg/l; nM range), thereby providing a long-sought rationale for their health protecting effects in spite of their unfavorable pharmacokinetic properties.

Modulation of inflammatory gene expression by a bilberry (*Vaccinium myrtillus* L.) extract and single anthocyanins considering their limited stability under cell culture conditions.

J Agric Food Chem. 2012 Sep 12; 60(36):8902-10. doi: 10.1021/jf3028842.

*Studies with nonintestinal models indicate that anthocyanin-rich extracts can modulate inflammatory gene expression and may help prevent development of inflammatory bowel diseases (IBD). This work investigated the influence of a bilberry (*Vaccinium myrtillus* L.) extract (BE) and comprising anthocyanins on pro-inflammatory genes in IFN-γ/IL-1β/TNF-α stimulated human colon epithelial cells (T84) by qRT-PCR and cytokine arrays. Moreover, the stability of selected anthocyanins under cell culture conditions was examined to assess their anti-inflammatory properties. BE and single anthocyanins significantly inhibited the expression and secretion of IBD-associated pro-inflammatory mediators (TNF-α, IP-10, I-TAC, sICAM-1, GRO-α) in the stimulated cells. The anti-inflammatory activity thereby strongly depends on the aglycon structure (hydroxylation and methylation pattern) and the sugar moiety. In contrast to anthocyanidins, which were highly unstable in cell culture medium, suggesting that their degradation products might contribute to the inhibitory effects assigned to the parent compounds, anthocyanins have higher stability and may directly contribute to BE's effects.*

Antioxidative protection of dietary bilberry, chokeberry and *Lactobacillus plantarum* HEAL19 in mice subjected to intestinal oxidative stress by ischemia-reperfusion.

BMC Complement Altern Med. 2011 Jan 27; 11:8. doi: 10.1186/1472-6882-11-8.

BACKGROUND: Ischemia-reperfusion (I/R) in the intestines is an inflammatory condition which activates leukocytes and reactive oxygen species (ROS) and leads to lipid peroxidation and DNA damage. Bilberry and chokeberry fruits are rich sources of polyphenols which may act as antioxidants and prevent lipid peroxidation. Lactic acid bacteria (LAB) may improve microbial status in the intestines and increase the metabolic activity towards polyphenolic degradation. The aim of the study was to clarify antioxidative effects of bilberry and chokeberry fruits alone and with addition of a LAB-strain, *Lactobacillus plantarum* HEAL19, in an I/R-model in mice. **METHODS:** Male BALB/cJ mice were fed the experimental diets for 10 days. Diets consisted of standard chow supplemented with either bilberry (*Vaccinium myrtillus*) or chokeberry (*Aronia × prunifolia*) powder alone or in combination with the LAB-strain *Lactobacillus plantarum* HEAL19. I/R-injury was induced by holding superior mesenteric artery clamped for 30 minutes followed by reperfusion for 240 minutes. Thereafter, colonic and caecal tissues and contents were collected. Malondialdehyde (MDA) was used as indicator of lipid peroxidation and was measured by a calorimetric assay, lactobacilli were cultured on Rogosa agar plates and Enterobacteriaceae on VRBG agar plates, anthocyanins and phenolic acids were analysed by HPLC-DAD-ESI-MSn. **RESULTS:** MDA was significantly decreased in the colon of groups fed bilberry alone ($p = 0.030$) and in combination with *L. plantarum* HEAL19 ($p = 0.021$) compared to the IR-control but not in chokeberry-fed groups. Supplementation with bilberry or chokeberry alone reduced the total number of lactobacilli on the mucosa. Higher concentrations of anthocyanins were found in the colon than in the caecum content of mice. A more varied composition of different anthocyanins was also observed in the colon content compared to the caecum of bilberry-fed mice. Phenolic acids formed by microbial degradation of the dietary polyphenols in the gut could be detected. More phenolic metabolites were found in the intestines of bilberry-fed mice than in the chokeberry-fed ones. **CONCLUSIONS:** Bilberry alone and in combination with *L. plantarum* HEAL19 exerts a better protection against lipid peroxidation than chokeberry. These dietary supplements may be used to prevent or suppress oxidative stress.

Bilberry juice modulates plasma concentration of NF-kappaB related inflammatory markers in subjects at increased risk of CVD.

Eur J Nutr. 2010 Sep; 49(6):345-55. doi: 10.1007/s00394-010-0092-0. Epub 2010 Feb 2.

PURPOSE: Bilberries are abundant in polyphenols. Dietary polyphenols have been associated with strategies for prevention and treatment of chronic inflammatory diseases. We investigated the effect of bilberry juice on serum and plasma biomarkers of inflammation and antioxidant status in subjects with elevated levels of at least one risk factor for cardiovascular disease (CVD). METHODS: In a randomized controlled trial, participants consumed either bilberry juice (n = 31) or water (n = 31) for 4 weeks. RESULTS: Supplementation with bilberry juice resulted in significant decreases in plasma concentrations of C-reactive protein (CRP), interleukin (IL)-6, IL-15, and monokine induced by INF-gamma (MIG). Unexpectedly, an increase in the plasma concentration of tumor nuclear factor-alpha (TNF-alpha) was observed in the bilberry group. CRP, IL-6, IL15, MIG, and TNF-alpha are all target genes of nuclear factor- kappa B (NF-kappaB), -a transcription factor that is crucial in orchestrating inflammatory responses. Plasma quercetin and p-coumaric acid increased in the bilberry group, otherwise no differences were observed for clinical parameters, oxidative stress or antioxidant status. Furthermore, we studied the effect of polyphenols from bilberries on lipopolysaccharide (LPS)-induced NF-kappaB activation in a monocytic cell line. We observed that quercetin, epicatechin, and resveratrol inhibited NF-kappaB activation. CONCLUSIONS: These findings suggest that supplementation with bilberry polyphenols may modulate the inflammation processes. Further testing of bilberry supplementation as a potential strategy in prevention and treatment of chronic inflammatory diseases is warranted.

Age-associated changes in oxidative damage and the activity of antioxidant enzymes in rats with inherited overgeneration of free radicals.

J Cell Mol Med. 2006 Jan-Mar; 10(1):206-15.

Reactive oxygen species have been hypothesized to play an important role in the process of aging. To investigate the correlation between oxidative stress and accumulation of protein and DNA damage, we have compared the age-dependent levels of protein carbonyl groups and the activities of antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase in cytosol and mitochondrial extracts from liver cells of Wistar and OXYS rats. The latter strain is characterized by increased sensitivity to free radicals. Faster age-dependent increase in the level of protein carbonyl groups was found in OXYS as compared with Wistar rats. A complicated enzyme-specific pattern of age-dependent changes in the activities of antioxidant enzymes was observed. Long-term uptake of dietary supplements Mirtilene forte (extract from the fruits of Vaccinium myrtillus L.) or Adrusen zinco (vitamin E complex with zinc, copper, selenium and omega-3 polyunsaturated fatty acids) sharply decreased the level of protein oxidation in cytosol and mitochondrial extracts of hepatocytes of Wistar and of OXYS rats. Both dietary supplements increased the activity of catalase in the liver mitochondria of OXYS rats. Our results are in agreement with the shorter life-span of OXYS and with the mitochondrial theory of aging, which postulates that accumulation of DNA and protein lesions leads to mitochondrial dysfunction and accelerates the process of aging.

Antioxidant properties of prepared blueberry (Vaccinium myrtillus) extracts.

J Agric Food Chem. 2005 Aug 24; 53(17):6896-902.

A blueberry extract (A) and two anthocyanin-derived extracts (B and C) were prepared. The contents of polyphenols, flavonoids, anthocyanins, and anthocyanin-derived pigments of the extracts were determined for the first time. The pigment profile of blueberry extract A corresponded to 15 anthocyanins, whereas extract B was mainly composed of anthocyanin-pyruvic acid adducts of the blueberry original anthocyanins and extract C was mainly composed of the respective vinylpyranoanthocyanin-catechins (portisins). The extracts' abilities to inhibit lipid peroxidation, induced by 2,2'-azobis(2-methyl-propanimidamide) dihydrochloride in a liposomal membrane system were examined. The antioxidant capacities of the extracts were evaluated

through monitoring oxygen consumption and by measuring the formation of conjugated dienes. All of the extracts provided protection of membranes against peroxy radicals by increasing the induction time of oxidation. This effect increased with the polyphenol content and with the structural complexity of the anthocyanin-derived pigments of the extracts. The pigments present in extract C seemed to induce a higher protection of the liposome membranes toward oxidation. In addition, the antiradical properties and the reducing power of the extracts were determined by using DPPH and FRAP methods, respectively. The results from these assays were in agreement with those obtained with the liposome membranes.

Inhibition of protein and lipid oxidation in liposomes by berry phenolics.

J Agric Food Chem. 2004 Dec 1; 52(24):7419-24.

The antioxidant activity of berry phenolics (at concentrations of 1.4, 4.2, and 8.4 μg of purified extracts/mL of liposome sample) such as anthocyanins, ellagitannins, and proanthocyanidins from raspberry (*Rubus idaeus*), bilberry (*Vaccinium myrtillus*), lingonberry (*Vaccinium vitis-idaea*), and black currant (*Ribes nigrum*) was investigated in a lactalbumin-liposome system. The extent of protein oxidation was measured by determining the loss of tryptophan fluorescence and formation of protein carbonyl compounds and that of lipid oxidation by conjugated diene hydroperoxides and hexanal analyses. The antioxidant protection toward lipid oxidation was best provided by lingonberry and bilberry phenolics followed by black currant and raspberry phenolics. Bilberry and raspberry phenolics exhibited the best overall antioxidant activity toward protein oxidation. Proanthocyanidins, especially the dimeric and trimeric forms, in lingonberries were among the most active phenolic constituents toward both lipid and protein oxidation. In bilberries and black currants, anthocyanins contributed the most to the antioxidant effect by inhibiting the formation of both hexanal and protein carbonyls. In raspberries, ellagitannins were responsible for the antioxidant activity. While the antioxidant effect of berry proanthocyanidins and anthocyanins was dose-dependent, ellagitannins appeared to be equally active at all concentrations. In conclusion, berries are rich in monomeric and polymeric phenolic compounds providing protection toward both lipid and protein oxidation.

BIOAVAILABILITY

Practical application of flavonoid-poor menu meals to the study of the bioavailability of bilberry anthocyanins in human subjects.

Biosci Biotechnol Biochem. 2014; 78(10):1748-52. doi: 10.1080/09168451.2014.932667.

Practical application of flavonoid-poor menus was evaluated on the bioavailability of anthocyanins as model flavonoids. Detectable amounts of flavonoids were not found in plasma and urine collected from 13 participants, who took the menus. After ingesting bilberry anthocyanins (919 μmol), average plasma AUC_{0-6h}, C_{max}, T_{max} values and urinary recovery were 386.0 nmol h/mL, 139.1 nM, 1.31 h and 0.21%, respectively.

Is the antioxidative effectiveness of a bilberry extract influenced by encapsulation?

J Sci Food Agric. 2014 Aug; 94(11):2301-7. doi: 10.1002/jsfa.6558.

*BACKGROUND: Bilberries (*Vaccinium myrtillus* L.) have been suggested to have preventive properties against diseases associated with oxidative stress such as colon cancer or inflammatory bowel diseases. Therefore the gastrointestinal tract is regarded as a potential target for prevention. In this study the antioxidative properties of a commercially available anthocyanin-rich bilberry extract (BE) were investigated in comparison with four different BE-loaded microcapsule systems. As markers to describe the antioxidant status in this cellular system, intracellular reactive oxygen species (ROS) levels, oxidative DNA damage and total glutathione (tGSH) levels were monitored. RESULTS: Incubations with the BE-loaded capsule systems showed an increase in cellular glutathione levels and reduction of ROS levels at high BE concentrations (100-500 $\mu\text{g mL}^{-1}$) and a positive effect on the formation of DNA strand breaks (5-10 $\mu\text{g mL}^{-1}$ BE). The biological properties of BE-loaded pectin amide core-shell capsules, whey protein matrix capsules and coated apple pectin matrix capsules were comparable to those of the non-encapsulated BE. CONCLUSION: Overall, the BE and the encapsulated BE types tested have antioxidative activity under the studied assay conditions in terms of the prevention of oxidative DNA damage, the reduction of intracellular ROS and the enhancement of cellular tGSH.*

Effect of microformulation on the bioactivity of an anthocyanin-rich bilberry pomace extract (*Vaccinium myrtillus* L.) in vitro.

J Agric Food Chem. 2013 May 22; 61(20):4873-81. doi: 10.1021/jf305180j.

In cell culture were compared the different release rates of anthocyanins from a bilberry pomace extract encapsulated either in food grade whey protein-based matrix capsules (WPC) or in pectin amid-based hollow spherical capsules (PHS). The impact of the formulations on typical anthocyanin-associated biological end points such as inhibition of the epidermal growth factor receptor (EGFR) and suppression of cell growth in HT29 colon carcinoma cells was assessed. The purpose was to find whether the release rates are sufficient to maintain biological activity and whether encapsulation affected EGFR inhibitory and growth suppressive properties of the extract. Even though anthocyanin release from extract-loaded capsules was proven under cell culture conditions, the inhibitory potential toward the EGFR was diminished. However, non-encapsulated extract as well as both extract-loaded encapsulation systems diminished the growth of HT29 cells to a comparable extent. The loss of EGFR inhibitory properties by encapsulation despite anthocyanin release indicates substantial contribution of other further constituents not monitored so far. Taken together, both applied encapsulation strategies allowed anthocyanin release and maintained biological activity with respect to growth inhibitory properties. However, the loss of EGFR inhibitory effects emphasizes the need for biological profiling to estimate process-induced changes of plant constituent's beneficial potencies.

Preparation and comparative release characteristics of three anthocyanin encapsulation systems.

J Agric Food Chem. 2012 Jan 25; 60(3):844-51. doi: 10.1021/jf2047515.

Bilberries (Vaccinium myrtillus L.) and their major polyphenolic constituents, anthocyanins, have preventive activities inter alia against colon cancer and inflammatory bowel diseases. However, anthocyanins are sensitive to environmental conditions; thus their bioavailability in the gastrointestinal tract is an important determinant of their in vivo activity. In the study reported here, the potential benefits of encapsulating an anthocyanin rich bilberry extract (BE) on anthocyanin stability were investigated. Nonencapsulated BE and three different BE loaded microcapsule systems were incubated in simulated gastric fluid (SGF) and fed state simulated intestinal fluid (FeSSIF). After exposure to these media, released anthocyanins were identified and quantified by HPLC with UV/Vis detection. Although a rapid release of anthocyanins was observed within the first 20 min, encapsulation of anthocyanins doubled the amount of available anthocyanins after 150 min of incubation. These results illustrate the ability of encapsulation to inhibit early degradation of anthocyanins in the intestinal system.

Determination of anthocyanins in the urine of patients with colorectal liver metastases after administration of bilberry extract.

Biomed Chromatogr. 2011 Jun; 25(6):660-3. doi: 10.1002/bmc.1499.

Anthocyanins possess cancer chemopreventive properties in preclinical models. Their clinical pharmacology is only poorly understood. In this pilot study, anthocyanins and their metabolites were analysed in the urine of two patients with colorectal liver metastases. They received a single dose of 1.88 g standardized bilberry extract (mirtoselect) via either nasogastric or nasojejunal tube intra-operatively during liver resection. HPLC-MS/MS and HPLC-UV analysis showed there were more anthocyanins and metabolites in the urine of the patient who received mirtoselect via the stomach than via the jejunum. This result is consistent with information obtained in rodents which suggests the stomach is the predominant site for anthocyanin absorption.

Bioavailability of various polyphenols from a diet containing moderate amounts of berries.

J Agric Food Chem. 2010 Apr 14; 58(7):3927-32. doi: 10.1021/jf9024823.

Berries are a rich source of various polyphenols. The objective of this study was to investigate the bioavailability of polyphenols from berries. Middle-aged subjects (n = 72) consumed moderate amounts of berry or control products for 8 weeks in a randomized, placebo-controlled dietary intervention trial. Average intake of berries was 160 g/day (bilberries, lingonberries, black currants, and chokeberries). Plasma and urine polyphenols were analyzed by GC-MS and HPLC and berry polyphenols by HPLC. The total intake of polyphenols was 837 mg/day. Plasma quercetin, p-coumaric acid, 3-hydroxyphenylacetic acid, caffeic acid, protocatechuic acid, vanillic acid, homovanillic acid, and 3-(3-hydroxyphenyl)propionic acid increased significantly from the baseline in the berry group compared to the control group (p < 0.05). The urinary excretion of quercetin, p-coumaric acid, and 3-hydroxyphenylacetic acid increased significantly in the berry group compared to the control group (p < 0.05). In conclusion, a number of polyphenols are bioavailable from a diet containing moderate amounts of blue and red berries.

Distribution and excretion of bilberry anthocyanins [corrected] in mice.

J Agric Food Chem. 2009 Sep 9; 57(17):7681-6. doi: 10.1021/jf901341b.

The physiology and tissue distribution of bilberry anthocyanins were studied in mice. After oral administration of bilberry extract (100 mg/kg body weight), both unmodified and methylated anthocyanins appeared in the plasma. The plasma concentration of total anthocyanins reached a maximum of 1.18 +/- 0.3 microM after 15 min and then sharply decreased. Their urinary excretion

was highest between 0 and 6 h after administration and had ceased by 24 h. The total quantities of bilberry anthocyanins excreted into urine represented 1.88% (range, 0.62% to 2.45%) of consumed anthocyanins. Thirteen anthocyanins were identified in bilberry extracts. Of these, malvidin-3-glucoside and -3-galactoside were the principal anthocyanins in the plasma 60 min after administration. When mice were maintained for 2 weeks on a diet containing 0.5% of bilberry extracts, the plasma concentration of anthocyanins reached a maximum of 0.26 μ M. Anthocyanins were detected only in the liver, kidney, testes, and lung, with maximum tissue concentrations of 605, 207, 149, and 116 pmol/g, respectively. In these organs, malvidin-3-glucoside and -3-galactoside were the predominant anthocyanins. Anthocyanins were not detectable in the spleen, thymus, heart, muscle, brain, white fat, or eyes. We conclude that bilberry anthocyanins were absorbed into the body and distributed in specific organs, particularly the liver, kidney, and testis. The most common anthocyanins in tissues were malvidin glycosides.

Intact anthocyanins and metabolites in rat urine and plasma after 3 months of anthocyanin supplementation.

Nutr Cancer. 2006; 54(1):3-12.

Anthocyanins are polyphenols responsible for most red to purple colors in plants. Human consumption of these pigments is increasing because of their potential health benefits and use as natural colorants. With more than 600 different anthocyanins found in nature, the impact of chemical structure on their absorption and metabolism needs to be investigated. Urine and plasma samples were collected from 32 rats receiving control diet or chokeberry-, bilberry-, and grape-enriched (3.85 g cyanidin 3-galactoside equivalent/kg) diet for 14 wk. Below 2 micromol/l of anthocyanins and relatively higher levels of presumable metabolites were detected by high-performance liquid chromatography-photodiode array in the plasma. In the urine the total concentration of intact anthocyanins and methylated derivatives ranged from 17.4 (bilberry) to 52.6 (chokeberry) nmol/l. The type and number of anthocyanin glycosylations affected the absorption remarkably. Detection of an acylated anthocyanin in plasma and urine suggests bioavailability of these anthocyanin derivatives that are commonly found in commercially available colorants.

Bioavailability and tissue distribution of anthocyanins in bilberry (*Vaccinium myrtillus* L.) extract in rats.

J Agric Food Chem. 2006 Sep 6; 54(18):6578-87.

To clarify how structural diversity of anthocyanins relates to their *in vivo* function, bioavailability was precisely studied in rats using bilberry (*Vaccinium myrtillus* L.) extract (Bilberon 25) as an anthocyanin source that contains 15 different anthocyanins. The bilberry extract was orally or intravenously administered to rats, and the plasma levels of each anthocyanin were determined by high-performance liquid chromatography. As the result, all anthocyanins except peonidin 3-O- α -L-arabinoside were detectable in the blood plasma. The plasma concentration of anthocyanins as a whole reached the maximum level of 1.2 μ M at 15 min after oral administration of 400 mg/kg bilberry extract (153.2 mg/kg as anthocyanins) and then decreased with time. Uptake and decay profiles of each anthocyanin in the plasma were almost the same for all anthocyanins except a few with their maximum after 30 min. Among the anthocyanins carrying the same aglycone, the plasma level after 15 min of oral administration was as follows: galactoside > glucoside > arabinoside. Plasma clearance of anthocyanins after intravenous administration clearly showed that arabinoside disappeared more rapidly than glucoside and galactoside. On the other hand, when anthocyanins carrying the same sugar moiety were compared, the half disappearance time of plasma anthocyanins was in the following order: delphinidin > cyanidin > petunidin = peonidin > malvidin. The bioavailability of anthocyanins was in the range of 0.61-1.82% and was 0.93% as the anthocyanin mixture. The bioavailability of anthocyanins carrying the same aglycone was in the following order: Galactoside showed the highest followed by glucoside and arabinoside for cyanidin and delphinidin, but arabinoside and galactoside showed a higher bioavailability than glucoside for petunidin and malvidin. Anthocyanins recovered in urine and bile during the first 4 h after intravenous administration were only 30.8 and

13.4%, respectively. Anthocyanin profiles in tissues were quite different from those in blood plasma. The major anthocyanins distributed in liver and kidney were the O-methyl anthocyanins such as peonidin, malvidin, and other O-methyl anthocyanins derived from delphinidin, cyanidin, and petunidin-glycosides.

Anthocyanins are efficiently absorbed from the small intestine in rats.

J Nutr. 2004 Sep; 134(9):2275-9.

Anthocyanins are natural pigments that possess antioxidant activities and are implicated in various health effects. Recent studies showed that the stomach is a site of anthocyanin absorption. However, the fate of anthocyanins in the small intestine remains unknown. We therefore investigated anthocyanin absorption after in situ perfusion of the jejunum + ileum in rats. The intestine was perfused for 45 min with a physiological buffer supplemented with various anthocyanins. Purified anthocyanin glycosides (9.2 nmol/min) or blackberry (9.0 nmol/min) or bilberry (45.2 nmol/min) anthocyanins were perfused. A high proportion of anthocyanin glycosides was absorbed through the small intestine after perfusion. The rate of absorption was influenced by the chemical structure of the anthocyanin and varied from 10.7 (malvidin 3-glucoside) to 22.4% (cyanidin 3-glucoside). Regardless of the anthocyanins perfused, only glycosides were recovered in the intestinal lumen. After perfusion of a high amount of blackberry anthocyanins (600 nmol/min), native cyanidin 3-glucoside was recovered in urine and plasma from the aorta and mesenteric vein. Methylated and/or glucuronidated derivatives were also identified. Analysis of bile samples revealed that cyanidin 3-glucoside and its methylated derivatives (peonidin 3-glucoside + peonidin glucuronide) quickly appeared in bile. This study demonstrated that anthocyanin glycosides are rapidly and efficiently absorbed from the small intestine. Furthermore, anthocyanins are quickly metabolized and excreted into bile and urine as intact glycosides as well as methylated forms and glucuronidated derivatives.

BRAIN HEALTH

Anthocyanin suppresses the toxicity of A β deposits through diversion of molecular forms in in vitro and in vivo models of Alzheimer's disease.

Nutr Neurosci. 2016 Jan; 19(1):32-42. doi: 10.1179/1476830515Y.0000000042. Epub 2015 Aug 25.

The pathogenesis of Alzheimer's disease (AD) is strongly correlated with the aggregation and deposition of the amyloid beta (A β 1-42) peptide in fibrillar form, and many studies have shown that plant-derived polyphenols are capable of attenuating AD progression in various disease models. In this study, we set out to correlate the effects of anthocyanoside extracts (Vaccinium myrtillus anthocyanoside (VMA)) obtained from bilberry on the in vitro progression of A β fibril formation with the in vivo effects of this compound on AD pathogenesis. Methods Thioflavin T fluorescence assays and atomic force microscopy were used to monitor A β amyloid formation in in vitro assays. Effects of A β amyloids on cellular viability were assayed using cultured Neuro2a cells. Cognitive effects were probed using mice that simultaneously expressed mutant human A β precursor and mutant presenilin-2. Results Addition of VMA inhibited the in vitro formation of A β peptide fibrils and also reduced the toxicity of these aggregates toward Neuro2a cells. A diet containing 1% VMA prevented the cognitive degeneration in AD mice. Curiously, this diet-derived retention of cognitive ability was not accompanied by a reduction in aggregate deposition in brains; rather, an increase in insoluble deposits was observed compared with mice raised on a control diet. Discussion The paradoxical increase in insoluble deposits caused by VMA suggests that these polyphenols divert A β aggregation to an alternate, non-toxic form. This finding underscores the complex effects that polyphenol compounds may exert on amyloid deposition in vivo.

Anthocyanin-enriched bilberry and blackcurrant extracts modulate amyloid precursor protein processing and alleviate behavioral abnormalities in the APP/PS1 mouse model of Alzheimer's disease.

J Nutr Biochem. 2013 Jan; 24(1):360-70. doi: 10.1016/j.jnutbio.2012.07.006.

A growing body of epidemiological evidence suggests that fruit and vegetable juices containing various phenolic compounds can reduce the risk of Alzheimer's disease (AD). As the altered amyloid precursor protein (APP) processing leading to increased β -amyloid (A β) production is a key pathogenic feature of AD, we elucidated the effects of different polyphenols on neuroprotection and APP processing under different in vitro stress conditions. The effects of these compounds were also investigated in transgenic AD mice (APdE9). Free radical toxicity and apoptosis were induced in human SH-SY5Y neuroblastoma cells overexpressing APP751. Menadione-induced production of reactive oxygen species was significantly decreased upon treatment with myricetin, quercetin or anthocyanin-rich extracts in a dose-dependent manner. However, these extracts did not affect caspase-3 activation, APP processing or A β levels upon staurosporine-induced apoptosis. APdE9 mice fed with anthocyanin-rich bilberry or blackcurrant extracts showed decreased APP C-terminal fragment levels in the cerebral cortex as compared to APdE9 mice on the control diet. Soluble A β 40 and A β 42 levels were significantly decreased in bilberry-fed mice as compared to blackcurrant-fed mice. Conversely, the ratio of insoluble A β 42/40 was significantly decreased in blackcurrant-fed mice relative to bilberry-fed mice. Both berry diets alleviated the spatial working memory deficit of aged APdE9 mice as compared to mice on the control diet. There were no changes in the expression or phosphorylation status of tau in APdE9 mice with respect to diet. These data suggest that anthocyanin-rich bilberry and blackcurrant diets favorably modulate APP processing and alleviate behavioral abnormalities in a mouse model of AD.

Vaccinium myrtillus ameliorates unpredictable chronic mild stress induced depression: possible involvement of nitric oxide pathway.

Phytother Res. 2012 Apr; 26(4):488-97. doi: 10.1002/ptr.3584.

Chronic unpredictable stressors can produce a situation similar to clinical depression and such animal models can be used for the preclinical evaluation of antidepressants. Nitric oxide, a secondary messenger molecule, has been implicated in neurotransmission, synaptic plasticity, learning, aggression and depression. Vaccinium myrtillus (bilberry) extract is a potent inhibitor of reactive oxygen/nitrogen species and cytokine production. The present study investigated the role of nitric oxide in the antidepressant action of Vaccinium myrtillus in unpredictable chronic mild stress-induced depression in mice. Animals were subjected to different stress paradigms daily for a period of 21 days to induce depressive-like behavior. Pretreatment with L-arginine significantly reversed the protective effect of bilberry (500 mg/kg) on chronic stress-induced behavioral (immobility period, sucrose preference) and biochemical (lipid peroxidation and nitrite levels; endogenous antioxidant activities) in stressed mice. Furthermore, L-NAME (10 mg/kg) pretreatment with a sub-effective dose of bilberry (250 mg/kg) significantly potentiated the protective effect of bilberry extract. The study revealed that modulation of the nitric oxide pathway might be involved in antidepressant-like effects of Vaccinium myrtillus in stressed mice.

Effects of dietary supplementation with a combination of fish oil, bilberry extract, and lutein on subjective symptoms of asthenopia in humans.

Biomed Res. 2011 Dec; 32(6):387-93.

The aim of this study was to determine the effects of dietary supplementation with a combination of fish oil, bilberry extract, and lutein on subjective symptoms of asthenopia in humans by a double-blind, randomized, parallel-group, and placebo-controlled trial. In the Active group, eleven subjects ingested a supplement containing omega-3 fatty acid-rich fish oil (docosahexaenoic acid 783 mg/day, eicosapentaenoic acid 162 mg/day), bilberry extract (anthocyanidin 59 mg/day), and lutein (17.5 mg/day) in soft gel capsule form, every day for 4 weeks. In the Placebo group, nine subjects ingested placebo capsules. Before and after supplementation, subjects completed a questionnaire to determine their asthenopia symptoms and were also assessed for mental fatigue symptom by the visual analog scale (VAS) test. Asthenopia symptoms such as "stiff shoulder, low back pain", "frustration", "dry-eye", and "stuffy head" were improved in the Active group. Furthermore, a score of mental fatigue was improved after 4 weeks of supplementation, and no side effects were observed after the 4-week supplementation and a 2-week washout period in the Active group. These results suggest that dietary supplementation with the combination of omega-3 fatty acid-rich fish oil, bilberry extract, and lutein may safely improve subjective symptoms of asthenopia and mental fatigue in humans.

Effects of anthocyanins on psychological stress-induced oxidative stress and neurotransmitter status.

J Agric Food Chem. 2008 Aug 27; 56(16):7545-50. doi: 10.1021/jf800930s. Epub 2008 Jul 29.

There is strong evidence that oxidative stress participates in the etiology of neurodegenerative diseases such as Parkinson's, and Alzheimer's diseases. Moreover, emotional stress effects in the central nervous system play a vital role in homeostasis. The protective effect of anthocyanins on the cerebral oxidative stress was studied using the whiskers cut model. In mice, such treatment causes psychological or emotional distress leading to oxidative stress in tissues. To investigate the in vivo antioxidant activity of anthocyanins, an extract of Vaccinium myrtillus L., an anthocyanin mixture, was orally administered (100 mg/kg of body weight.) to mice for 7 days, and then psychological stress was assessed by cutting off their whiskers. Whisker removal increased both protein carbonyl formation and lipid peroxidation in the brain, heart, kidney, and liver. Further, the levels

of oxidative markers showed regional differences in the brain. Concomitantly, dopamine neurotransmitter levels were altered in both the midbrain and the brain cortex. Orally administered anthocyanins were also active in the brain, suppressing stress-induced cerebral oxidative stress and dopamine abnormalities in distressed mice. These effects of anthocyanin treatment suggest their possible usefulness for the treatment of cerebral disorders related to oxidative stress.

Protective activities of Vaccinium antioxidants with potential relevance to mitochondrial dysfunction and neurotoxicity.

Neurotoxicology. 2007 Jan; 28(1):93-100. Epub 2006 Jul 31.

Both the neurotransmitter dopamine (DA) and a neurotoxic metabolite, 6-hydroxy DA, can be oxidized to generate hydrogen peroxide and other reactive species (ROS). ROS promote oxidative stress and have been implicated in dopaminergic neurodegeneration, e.g., Parkinson's disease (PD). There is also evidence for a relation between catecholamine-mediated oxidative damage in dopaminergic neurons and the effects of these neurotransmitters on the redox state of cytochrome c (Cyt c). In neurons and other cells, oxidative stress may be enhanced by abnormal release of Cyt c and other mitochondrial proteins into the cytoplasm. Cyt c release can result in apoptosis; but sub-apoptogenic-threshold release can also occur, and may be highly damaging in the presence of DA metabolites. Loss of mitochondrial membrane integrity, a pathological situation of relevance to several aging-related neurodegenerative disorders including PD, contributes to release of Cyt c; and the level of such release is known to be indicative of the extent of mitochondrial dysfunction. In this context, we have used a Cyt c-enhanced 6-hydroxy DA oxidation reaction to gauge dietary antioxidant activities. Anthocyanin-rich preparations of *Vaccinium* species (*Vaccinium myrtillus*, *Vaccinium corymbosum*, and *Vaccinium oxycoccus*) as well as a purified glycosylated anthocyanidin were compared. The most potent inhibition of oxidation was observed with *V. myrtillus* preparation: 50% inhibition with 7 microM of total anthocyanins. This activity was 1.5-4 times higher than that for the other preparations or for the purified anthocyanin. Ascorbate (Vitamin C), at up to 4-fold higher concentrations, did not result in significant inhibition in this assay. Antioxidant activity in the assay correlated strongly ($r^2 > 0.91$, $P < 0.01$) with reported *Vaccinium* content of anthocyanins and total cyanidins, but not quercetin or myricetin. The results provide evidence for the high potency of anthocyanins towards a potentially neurotoxic reaction, and provide a basis for *in vivo* testing of these flavonoids and their physiological metabolites in the context of neuro- and mitochondrio-protective effects.

Opposite effects of antioxidants on anxiety in Wistar and OXYS rats.

Bull Exp Biol Med. 2006 Jun; 141(6):734-7.

Passive behavior in the open field test and high anxiety in an elevated plus-maze develop in OXYS rats by the age of 3 months and are regarded as manifestations of early aging. We studied the possibility of preventing these disturbances with vitamin E and whortleberry extract (20 mg/kg for 45 days from the age of 1.5 months). Whortleberry extract alone increased horizontal activity and reduced anxiety of OXYS rats, while anxiety in Wistar rats increased significantly after treatment with both preparations, and especially with vitamin E: the number of entries into open arms of the elevated plus-maze and the time spent there decreased. The results necessitate comprehensive evaluation of the aftereffects of long-term use of antioxidants, acknowledged geroprotectors intended for preventive use.

CARDIAC HEALTH

Polyphenol-rich juices reduce blood pressure measures in a randomised controlled trial in high normal and hypertensive volunteers.

Br J Nutr. 2015 Oct 14; 114(7):1054-63. doi: 10.1017/S0007114515000562.

Intake of fruits and berries may lower blood pressure (BP), most probably due to the high content of polyphenols. In the present study, we tested whether consumption of two polyphenol-rich juices could lower BP. In a randomised, double-blinded, placebo-controlled trial of 12 weeks, 134 healthy individuals, aged 50-70 years, with high normal range BP (130/85-139/89 mmHg, seventy-two subjects) or stage 1-2 hypertension (140/90-179/109 mmHg, sixty-two subjects), were included. They consumed 500 ml/d of one of either (1) a commercially available polyphenol-rich juice based on red grapes, cherries, chokeberries and bilberries; (2) a juice similar to (1) but enriched with polyphenol-rich extracts from blackcurrant press-residue or (3) a placebo juice (polyphenol contents 245.5, 305.2 and 76 mg/100 g, respectively). Resting BP was measured three times, with a 1 min interval, at baseline and after 6 and 12 weeks of intervention. Systolic BP significantly reduced over time (6 and 12 weeks, respectively) in the pooled juice group compared with the placebo group in the first of the three measurements, both for the whole study group (6.9 and 3.4 mmHg; $P=0.01$) and even more pronounced in the hypertensive subjects when analysed separately (7.3 and 6.8 mmHg; $P=0.04$). The variation in the BP measurements was significantly reduced in the pooled juice group compared with the placebo group (1.4 and 1.7 mmHg; $P=0.03$). In conclusion, the present findings suggest that polyphenol-rich berry juice may contribute to a BP- and BP variability lowering effect, being more pronounced in hypertensive than in normotensive subjects.

Polyphenol-rich bilberry ameliorates total cholesterol and LDL-cholesterol when implemented in the diet of Zucker diabetic fatty rats.

Rev Diabet Stud. 2013 Winter; 10(4):270-82. doi: 10.1900/RDS.2013.10.270.

BACKGROUND: Bilberries and blackcurrants are nutrient sources rich in bioactive components, including dietary fibers, polyphenols, and anthocyanins, which possess potent cardiovascular protective properties. Few studies investigating the cardio-protective effects of natural components have focused on whole bilberries or blackcurrants. OBJECTIVE: The aim of this trial was to investigate whether a diet enriched with bilberries or blackcurrants has beneficial effects on glucose metabolism, lipid profile, blood pressure, and expression of genes related to glucose and lipid metabolism. METHODS: Male Zucker Diabetic Fatty (ZDF) rats ($n=48$) were randomly assigned to either a control, bilberry-enriched, blackcurrant-enriched, or fiber-enriched diet for 8 weeks ad libitum. Real-time quantitative PCR analysis was performed on liver, adipose, and muscle tissue. Berry polyphenol content was determined by HPLC and LC-MS analysis. RESULTS: Bilberry enrichment reduced total (-21%, $p=0.0132$) and LDL-cholesterol (-60%, $p=0.0229$) levels, but increased HDL-cholesterol to a lesser extent than in controls. This may partly be due to the altered hepatic liver X receptor- α expression (-24%, $p<0.001$). Neither bilberries nor blackcurrants influenced glucose metabolism or blood pressure. Nevertheless, transcriptional analysis implied a better conservation of hepatic and adipocyte insulin sensitivity by bilberry enrichment. Anthocyanins constituted 91% and 87% of total polyphenol content in bilberries and blackcurrants, respectively. However, total anthocyanin content (3441 mg/100 g) was 4-fold higher in bilberries than in blackcurrants (871 mg/100 g). CONCLUSIONS: Bilberry consumption ameliorated total and LDL-cholesterol levels, but not HDL-cholesterol levels in ZDF rats. Neither bilberry nor blackcurrant enrichment delayed the development of diabetes or hypertension. Thus, in rats, bilberries may be valuable as a dietary preventive agent against hypercholesterolemia, probably by virtue of their high anthocyanin content.

Direct effects of *Vaccinium myrtillus* L. fruit extracts on rat heart mitochondrial functions.

Phytother Res. 2013 Apr; 27(4):499-506. doi: 10.1002/ptr.4750.

*In this study, the direct influence of bilberry (*Vaccinium myrtillus*) fruit extracts (aqueous and ethanolic) rich in anthocyanins on the oxidative phosphorylation of isolated rat heart mitochondria was investigated in vitro. Higher concentrations of bilberry extracts concentration-dependently inhibited mitochondrial state 3 respiration (by 23%-61%) with pyruvate plus malate, mildly (by 1.2- to 1.3-fold) uncoupled the oxidative phosphorylation, and increased (by 30%-87%) the state 4 respiration rate in the presence of exogenous cytochrome c. Succinate oxidation was less affected. Pure anthocyanins, the main components of used extracts, malvidin-3-glucoside, malvidin-3-galactoside, and cyanidin-3-galactoside, had no effect on oxidation of pyruvate plus malate. A statistically significant decrease in H₂O₂ production by mitochondria was found in the presence of bilberry fruit extracts. Our findings show that bilberry fruit anthocyanin-rich extracts possess direct effects on rat heart mitochondrial function in vitro. These findings give the first insights into the mechanism(s) of their action on cellular energy metabolism.*

Bilberry anthocyanin-rich extract alters expression of genes related to atherosclerosis development in aorta of apo E-deficient mice.

Nutr Metab Cardiovasc Dis. 2012 Jan; 22(1):72-80. doi: 10.1016/j.numecd.2010.04.011. Epub 2010 Aug 3.

Intake of anthocyanin-rich foods has been associated with a reduced risk of cardiovascular diseases. We recently reported that a nutritional supplementation with a bilberry anthocyanin-rich extract (BE) attenuates atherosclerotic lesion development in apolipoprotein E-deficient (apoE^{-/-}) mice. However, the mechanism(s) of their preventive action are not completely understood. Anthocyanins may alter mRNA levels of genes related to atherosclerosis in cultured macrophages and endothelial cells, but in vivo studies remain scarce. The aim of the present study was to explore the in vivo mechanisms of action of the same bilberry extract, administered by supplementation at a nutritional level, in the aorta of apo E^{-/-} mice using a global transcriptomic approach. This study focused on the early stage of atherosclerosis development for better assessment of BE action on initiation mechanisms of this pathology. After a two week period, plasma lipid and antioxidant capacity were evaluated and the global genomic analysis was carried out using pangenomic microarrays. BE supplementation significantly improved hypercholesterolemia whereas the plasmatic antioxidant status remained unchanged. Nutrigenomic analysis identified 1261 genes which expression was modulated by BE in the aorta. Bioinformatic analysis revealed that these genes are implicated in different cellular processes such as oxidative stress, inflammation, transendothelial migration and angiogenesis, processes associated with atherosclerosis development/protection. Some of the most significantly down-regulated genes included genes coding for AOX1, CYP2E1 or TXNIP implicated in the regulation of oxidative stress, JAM-A coding for adhesion molecules or VEGFR2 implicate in regulation of angiogenesis. Other genes were up-regulated, such as CRB3, CLDN14 or CDH4 potentially associated with increased cell-cell adhesion and decreased paracellular permeability. These results provide a global integrated view of the mechanisms involved in the preventive action of bilberry anthocyanin-rich extract against atherosclerosis.

***Vaccinium myrtillus* (Bilberry) Extracts Reduce Angiogenesis In Vitro and In Vivo.**

Evid Based Complement Alternat Med. 2010 Mar; 7(1):47-56. doi: 10.1093/ecam/nem151.

**Vaccinium myrtillus* (Bilberry) extracts (VME) were tested for effects on angiogenesis in vitro and in vivo. VME (0.3-30 µg ml⁻¹) and GM6001 (0.1-100 µM; a matrix metalloproteinase inhibitor) concentration-dependently inhibited both tube formation and migration of human umbilical vein endothelial cells (HUVECs) induced by vascular endothelial growth factor-A (VEGF-A). In addition, VME inhibited VEGF-A-induced proliferation of HUVECs. VME inhibited VEGF-A-induced phosphorylations of extracellular signal-regulated kinase 1/2 (ERK 1/2) and serine/threonine protein kinase family protein kinase B (Akt), but not that of phospholipase C_γ (PLC_γ). In an in vivo assay, intravitreal administration of VME inhibited the formation of neovascular tufts during oxygen-induced retinopathy in mice. Thus, VME inhibited angiogenesis both in vitro and in vivo, presumably by*

inhibiting the phosphorylations of ERK 1/2 and Akt. These findings indicate that VME may be effective against retinal diseases involving angiogenesis, providing it can reach the retina after its administration. Further investigations will be needed to clarify the major angiogenesis-modulating constituent(s) of VME.

Acute cardioprotective and cardiotoxic effects of bilberry anthocyanins in ischemia-reperfusion injury: beyond concentration-dependent antioxidant activity.

Cardiovasc Toxicol. 2010 Dec; 10(4):283-94. doi: 10.1007/s12012-010-9091-x.

Despite being reported to reduce the risk of cardiovascular diseases, little is known about acute direct effects of bilberry anthocyanins on whole mammalian heart under ischemia-reperfusion (I-R) conditions. Bilberry anthocyanins were prepared from the ripe bilberries and analyzed using HPLC-DAD. Their antioxidant activity was evaluated by measuring the intrinsic free radical-scavenging capacity and by cellular antioxidant assay (CAA) on endothelial cells, where we quantified the intracellular capacity to inhibit the formation of peroxy radicals. Experiments on the isolated rat hearts under I-R were carried out according to the Langendorff method. Perfusion with low concentrations of bilberry anthocyanins (0.01-1 mg/L) significantly attenuated the extent of I-R injury as evidenced by decreasing the release rate of LDH, increasing the postischemic coronary flow, and by decreasing the incidence and duration of reperfusion arrhythmias. High concentrations (5-50 mg/L) diminished cardioprotection and show cardiotoxic activity despite having their radical scavenging and intracellular antioxidant capabilities increased in a concentration-dependent manner. This study reveals the biphasic concentration-dependent bioactivity of bilberry anthocyanins under I-R, which results in strong cardioprotective activity in low concentrations and cardiotoxic activity in high concentrations.

Inhibitory actions of bilberry anthocyanidins on angiogenesis.

Phytother Res. 2010 Jan; 24 Suppl 1:S42-7. doi: 10.1002/ptr.2895.

The aim of this study was to examine the antiangiogenic properties and antioxidant activities (a) of the main anthocyanidins (delphinidin, cyanidin and malvidin) found as constituents in Vaccinium myrtillus (bilberry) anthocyanosides (VMA) and (b) of N-acetyl-L-cysteine (NAC). Each of these anthocyanidins concentration-dependently inhibited vascular endothelial growth factor (VEGF)-induced tube formation in a co-culture of human umbilical vein endothelial cells (HUVECs) and fibroblasts, the effect of each anthocyanidin being significant at 3 and/or 10 microM, while NAC significantly inhibited such tube formation at 1 microM (the only concentration tested). Moreover, each anthocyanidin (0.3-10 microM) and NAC (1-1000 microM) concentration-dependently scavenged the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The inhibitory effects against angiogenesis were similar among the anthocyanidins, as were those against the DPPH radical. Moreover, their radical-scavenging effects were induced by concentrations that were at or below those that induced their antiangiogenic effects. These findings indicate that the inhibitory effect of VMA on angiogenesis may depend on those of its main constituent anthocyanidins (delphinidin, cyanidin and malvidin), presumably via antioxidant effects.

Effect of Vaccinium myrtillus and its polyphenols on angiotensin-converting enzyme activity in human endothelial cells.

J Agric Food Chem. 2009 Jun 10; 57(11):4626-9. doi: 10.1021/jf900128s.

This study investigates if the connection between Vaccinium myrtillus and angiotensin-converting enzyme (ACE) might be an explanation of the pharmacological effects on circulation. Cultured endothelial cells from human umbilical veins were incubated with bilberry 25E extract. The main anthocyanidins combined in myrtillin chloride and separately in cyanidin, delphinidin, and malvidin, respectively, were examined concerning their effects on ACE. After 10 min of incubation with bilberry 25E, a significant,



dose-dependent inhibition of ACE activity was seen, and after incubation with myrtilin chloride a significant inhibition was seen. No effect was seen with the anthocyanidins. The effect seems to be dependent on this specific mixture of anthocyanins in the bilberry. *V. myrtillus* may thus have the potential to prevent and protect against cardiovascular diseases.

Atheroprotective effects of bilberry extracts in apo E-deficient mice.

J Agric Food Chem. 2009 Dec 9; 57(23):11106-11. doi: 10.1021/jf9035468.

Previous studies have demonstrated that the intake of berry foods was associated with a reduced risk of cardiovascular diseases. The aim of the present study was to evaluate the effects of two bilberry extracts, one rich in anthocyanins extracted from untreated bilberries (BE) and a second one extracted from yeast-fermented bilberries (FBE), on the development of atherosclerosis in apolipoprotein E-deficient mice (apo E(-/-)). Apo E(-/-) mice received for 16 weeks a diet supplemented with 0.02% of either BE or FBE. Atherosclerotic plaque area was measured in the aortic sinus. Supplementation of the diet with both bilberry extracts led to a significant inhibition of plaque development, whereas no effect on oxidative stress parameters or lipid profiles could be observed, suggesting the implication of other mechanisms of action. In addition, a better protection was observed with FBE, suggesting that the fermentation generates new bioactive compounds more effective in attenuating progression of the atherosclerotic lesions.

DIGESTIVE TRACT HEALTH

Bilberry-derived anthocyanins prevent IFN- γ -induced pro-inflammatory signalling and cytokine secretion in human THP-1 monocytic cells.

Digestion. 2014; 90(3):179-89. doi: 10.1159/000366055. Epub 2014 Nov 12.

BACKGROUND/AIMS: Anthocyanins are plant-derived dietary components that are highly abundant, for example, in bilberries. We have previously demonstrated that anthocyanins exert anti-inflammatory properties in mouse colitis models and ameliorate disease activity in ulcerative colitis patients. Here, we studied the molecular mechanisms through which anthocyanin-containing bilberry extract (BE) exerts anti-inflammatory effects in human monocytic THP-1 cells. METHODS: THP-1 cells were pre-incubated with BE 20 min prior to TNF- α or IFN- γ (100 ng/ml each) stimulation. Signalling protein activation was studied by Western blotting, mRNA expression by quantitative PCR and cytokine secretion by ELISA. RESULTS: IFN- γ -induced phosphorylation of STAT1 and STAT3 was significantly reduced by BE co-treatment. Consequently, levels of mRNA expression and/or cytokine secretion of MCP-1, IL-6, TNF- α , ICAM-1, and T-bet were lower with BE co-treatment. In contrast, BE enhanced TNF- α -mediated p65-NF- κ B phosphorylation but reduced ERK1/2 phosphorylation. BE co-treatment further increased TNF- α -induced mRNA expression and secretion of NF- κ B target genes, such as IL-6, IL-8, and MCP-1, while mRNA levels of ICAM-1 were reduced. CONCLUSIONS: BE co-treatment reduced IFN- γ -induced signal protein activation, pro-inflammatory gene expression, and cytokine secretion, whereas it enhanced TNF- α -induced responses. These findings suggest a distinct role for anthocyanins in modulating inflammatory responses that need to be further studied to fully understand anthocyanin-mediated effects.

Bilberry ingestion improves disease activity in mild to moderate ulcerative colitis - an open pilot study.

J Crohns Colitis. 2013 May; 7(4):271-9. doi: 10.1016/j.crohns.2012.07.010.

BACKGROUND AND AIMS: A significant fraction of patients with ulcerative colitis (UC) is not sufficiently controlled with conventional therapy or suffers from therapy related side effects. Anthocyanins, highly abundant in bilberries (Vaccinium myrtillus), were shown to have antioxidative and anti-inflammatory effects. We aimed to explore the therapeutic potential of bilberries in active UC. METHODS: In an open pilot trial with a total follow-up of 9 weeks the effect of a daily standardized anthocyanin-rich bilberry preparation was tested in 13 patients with mild to moderate UC. Clinical, biochemical, endoscopic and histologic parameters were assessed. RESULTS: At the end of the 6 week treatment interval 63.4% of patients achieved remission, the primary endpoint, while 90.9% of patients showed a response. In all patients a decrease in total Mayo score was detected (mean: 6.5 and 3.6 at screening and week 7, respectively; $p < 0.001$). Fecal calprotectin levels significantly decreased during the treatment phase (baseline: mean 778 μ g/g, range 192-1790 μ g/g; end of treatment: mean 305 μ g/g, range <30-1586 μ g/g; $p = 0.049$), including 4 patients achieving undetectable levels at end of treatment. A decrease in endoscopic Mayo score and histologic Riley index confirmed the beneficial effect. However, an increase of calprotectin levels and disease activity was observed after cessation of bilberry intake. No serious adverse events were observed. CONCLUSIONS: This is the first report on the promising therapeutic potential of a standardized anthocyanin-rich bilberry preparation in UC in humans. These results clearly indicate a therapeutic potential of bilberries in UC. Further studies on mechanisms and randomized clinical trials are warranted.

Effects of bilberry (*Vaccinium myrtillus*) in combination with lactic acid bacteria on intestinal oxidative stress induced by ischemia-reperfusion in mouse.

J Agric Food Chem. 2013 Apr 10; 61(14):3468-78. doi: 10.1021/jf400203h.

Intestinal ischemia-reperfusion (I/R) results in oxidative stress, inflammation, and tissue injuries. The present study investigates the antioxidative and anti-inflammatory effects of a dietary supplement of bilberry, either alone or in combination with Lactobacillus plantarum RES056, L. plantarum HEAL19, or Pediococcus acidilactici JAM046, in an I/R-induced model for oxidative stress in mice. A bilberry diet without addition of bacteria significantly decreased both lipid peroxidation ($p = 0.001$) and mucosal injury in the ileum. Of 14 anthocyanins identified in bilberry, anthocyanin arabinosides were the most resistant to absorption and microbial degradation in the intestines. Cyanidin-3-glucoside and delphinidin-3-glucoside seemed to be mostly absorbed in the stomach and upper part of the small intestine, while malvidin-3-galactoside, peonidin-3-glucoside, peonidin-3-galactoside, and petunidin-3-galactoside seemed to be digested by the microbiota in the cecum. Bilberry strongly influenced the composition of the cecal microbiota. In conclusion, a food supplement of bilberry protected small intestine against oxidative stress and inflammation induced by ischemia-reperfusion.

Bilberries and their anthocyanins ameliorate experimental colitis.

Mol Nutr Food Res. 2011 Nov; 55(11):1724-9. doi: 10.1002/mnfr.201100380.

Bilberries have positive effects in acute and chronic diarrhea. Patients with inflammatory bowel disease (IBD) report on improved symptoms upon ingestion. Bilberries contain approximately 10% of anthocyanins (ACs), which have anti-oxidative, anti-carcinogenic, and anti-inflammatory properties. We investigated whether experimental colitis can be ameliorated by dried bilberries or ACs. Acute and chronic dextrane sodium sulphate (DSS) colitis were induced in Balb/c mice by 2.5% DSS in the drinking water. Mice were fed with dried bilberries or ACs, respectively. Cytokines were determined in supernatants from mesenteric lymph nodes (MLNs) by ELISA and apoptosis was investigated by terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling assays. Oral administration of bilberries during acute DSS-induced colitis ameliorated disease severity and reduced secretion of IFN- γ and tumor necrosis factor from mesenteric lymph node cells. Dried bilberries also improved chronic DSS-colitis. Ingestion of ACs reduced intestinal inflammation in acute and chronic DSS-colitis with decreased histological scores and cytokine secretion. Both bilberries and ACs prevented inflammation-induced apoptosis in colonic epithelial cells. Taken together, ingestion of dried bilberries had positive effects on various parameters especially in acute DSS-colitis. Oral administration of ACs resulted in an amelioration of acute colitis as well as chronic colitis. These promising results justify a clinical study on their therapeutic effect in inflammatory bowel disease patients.

The protective effect and action mechanism of *Vaccinium myrtillus* L. on gastric ulcer in mice.

Phytother Res. 2011 Aug; 25(8):1160-5. doi: 10.1002/ptr.3413. Epub 2011 Feb 3.

Vaccinium myrtillus L. anthocyanoside (VMA) is used as a folk medicine to treat diseases related to gastric ulcers in northern Europe. However, the effects of VMA and its detailed mechanism on gastric ulcer have not been investigated sufficiently. Therefore, the aim of the present study was to investigate the protective effects of VMA on gastric mucosal damage in a murine gastric ulcer model. First the effects of VMA on ethanol-induced gastric ulcers in mice were investigated. Then, the levels of lipid peroxide in murine stomach homogenates were measured to investigate the antioxidative effects of VMA. In addition, the free radical scavenging activity of VMA and its main anthocyanidins were evaluated by electron spin resonance measurement. Oral administration of VMA (10, 30 and 100 mg/kg) significantly protected gastric mucosa against HCl/ethanol-induced gastric ulcers. Furthermore, VMA inhibited lipid peroxide levels in a concentration-dependent manner and showed high scavenging activity against the superoxide anion radical ($\cdot O(2) (-)$) and the hydroxyl radical ($\cdot OH$). Anthocyanidins also showed scavenging activity against the $\cdot O(2) (-)$, while only delphinidin showed high scavenging activity against the $\cdot OH$. These findings indicate that the protective effects of VMA on HCl/ethanol-induced gastric mucosal injury may be partially due to the antiperoxidative effects of anthocyanidins.

Effects of two natural medicine formulations on irritable bowel syndrome symptoms: a pilot study.

J Altern Complement Med. 2010 Oct; 16(10):1065-71. doi: 10.1089/acm.2009.0090.

OBJECTIVE: The study objective was to assess the effects and tolerability of two novel natural medicine formulations in improving bowel habit and abdominal symptoms in patients with irritable bowel syndrome (IBS). The DA-IBS formula was designed to treat diarrhea-predominant and alternating bowel habit IBS, and the C-IBS formula was designed to treat constipation-predominant IBS. **DESIGN:** This was a two arm, open-label, uncontrolled pilot study. **SETTINGS/LOCATION:** Subjects were recruited from the greater Lismore area (NSW, Australia) in 2001. **SUBJECTS:** The study included 31 patients who fulfilled the Rome II criteria for IBS. Twenty-one (21) patients were classified as suffering from diarrhea-predominant or alternating bowel habit IBS and 10 patients were classified with constipation-predominant IBS. **INTERVENTIONS:** The DA-IBS formula consisted of a mixture of dried, powdered bilberry fruit, slippery elm bark, agrimony aerial parts, and cinnamon quills. The C-IBS formula consisted of a mixture of dried powdered slippery elm bark, lactulose, oat bran, and licorice root. The aim of each formula was to normalize stool frequency and stool consistency. **RESULTS:** Ingestion of the DA-IBS formula was associated with a small, but significant increase in bowel movement frequency ($p = 0.027$). Subjects in the DA-IBS group also experienced reductions in straining ($p = 0.004$), abdominal pain ($p = 0.006$), bloating ($p < 0.0001$), flatulence ($p = 0.0001$), and global IBS symptoms ($p = 0.002$) during the treatment phase of the trial. Subjects in the C-IBS group experienced a 20% increase in bowel movement frequency ($p = 0.016$) and significant reductions in straining ($p < 0.0001$), abdominal pain ($p = 0.032$), bloating ($p = 0.034$), and global IBS symptom severity ($p = 0.0005$), as well as improvements in stool consistency ($p < 0.0001$). Both formulas were well-tolerated. **CONCLUSIONS:** The DA-IBS formula was not effective in improving bowel habit in individuals with diarrhea-predominant or alternating bowel habit IBS, although it did significantly improve a number of IBS symptoms. The C-IBS formula significantly improved both bowel habit and IBS symptoms in patients with constipation-predominant IBS. Further research is warranted on C-IBS, as a potentially useful therapeutic formula.

EYE HEALTH

Vaccinium myrtillus extract prevents or delays the onset of diabetes induced blood-retinal barrier breakdown.

Int J Food Sci Nutr. 2015 Mar; 66(2):236-42. doi: 10.3109/09637486.2014.979319. Epub 2015 Jan 13.

*Many dietary supplements have been sold through advertising their large number of beneficial effects. The aim of this study was to determine whether bilberries (*Vaccinium myrtillus*) help to prevent diabetes-induced retinal vascular dysfunction in vivo. *V. myrtillus* extract (VME; 100 mg/kg) was orally administered to streptozotocin-induced diabetic rats for 6 weeks. All diabetic rats exhibited hyperglycemia, and VME did not affect the blood glucose levels and body weight during the experiments. In the fluorescein-dextran angiography, the fluorescein leakage was significantly reduced in diabetic rats treated with VME. VME treatment also decreased markers of diabetic retinopathy, such as retinal vascular endothelial growth factor (VEGF) expression and degradation of zonula occludens-1, occludin and claudin-5 in diabetic rats. In conclusion, VME may prevent or delay the onset of early diabetic retinopathy. These findings have important implications for prevention of diabetic retinopathy using a dietary bilberry supplement.*

Protective effects of bilberry and lingonberry extracts against blue light-emitting diode light-induced retinal photoreceptor cell damage in vitro.

BMC Complement Altern Med. 2014 Apr 2; 14:120. doi: 10.1186/1472-6882-14-120.

*BACKGROUND: Blue light is a high-energy or short-wavelength visible light, which induces retinal diseases such as age-related macular degeneration and retinitis pigmentosa. Bilberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea*) contain high amounts of polyphenols (anthocyanins, resveratrol, and proanthocyanidins) and thus confer health benefits. This study aimed to determine the protective effects and mechanism of action of bilberry extract (B-ext) and lingonberry extract (L-ext) and their active components against blue light-emitting diode (LED) light-induced retinal photoreceptor cell damage. METHODS: Cultured murine photoreceptor (661 W) cells were exposed to blue LED light following treatment with B-ext, L-ext, or their constituents (cyanidin, delphinidin, malvidin, trans-resveratrol, and procyanidin B2). 661 W cell viability was assessed using a tetrazolium salt (WST-8) assay and Hoechst 33342 nuclear staining, and intracellular reactive oxygen species (ROS) production was determined using CM-H2DCFDA after blue LED light exposure. Activation of p38 mitogen-activated protein kinase (p38 MAPK), nuclear factor-kappa B (NF-κB), and LC3, an ubiquitin-like protein that is necessary for the formation of autophagosomes, were analyzed using Western blotting. Caspase-3/7 activation caused by blue LED light exposure in 661 W cells was determined using a caspase-3/7 assay kit. RESULTS: B-ext, L-ext, NAC, and their active components improved the viability of 661 W cells and inhibited the generation of intracellular ROS induced by blue LED light irradiation. Furthermore, B-ext and L-ext inhibited the activation of p38 MAPK and NF-κB induced by blue LED light exposure. Finally, B-ext, L-ext, and NAC inhibited caspase-3/7 activation and autophagy. CONCLUSIONS: These findings suggest that B-ext and L-ext containing high amounts of polyphenols exert protective effects against blue LED light-induced retinal photoreceptor cell damage mainly through inhibition of ROS production and activation of pro-apoptotic proteins.*

The protective effects of bilberry and lingonberry extracts against UV light-induced retinal photoreceptor cell damage in vitro.

J Agric Food Chem. 2013 Oct 30; 61(43):10345-53. doi: 10.1021/jf402772h.

Bilberry extract (B-ext) and lingonberry extract (L-ext) are currently used as health supplements. We investigated the protective mechanisms of the B-ext and L-ext against ultraviolet A (UVA)-induced retinal photoreceptor cell damage. Cultured murine photoreceptor (661W) cells were exposed to UVA following treatment with B-ext and L-ext and their main constituents (cyanidin, delphinidin, malvidin, trans-resveratrol, and procyanidin). B-ext, L-ext, and constituents improved cell viability and suppressed ROS generation. Phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK), c-Jun N-terminal kinase (JNK), and protein kinase B (Akt) were analyzed by Western blotting. B-ext and cyanidin inhibited phosphorylation of p38 MAPK, and B-ext also inhibited phosphorylation of JNK by UVA. L-ext, trans-resveratrol, and procyanidin alleviated the reduction of phosphorylated Akt levels by UVA. Finally, a cotreatment with B-ext and L-ext showed an additive effect on cell viability. Our findings suggest that both B-ext and L-ext endow protective effects against UVA-induced retinal damage.

Effect of fermented bilberry extracts on visual outcomes in eyes with myopia: a prospective, randomized, placebo-controlled study.

J Ocul Pharmacol Ther. 2013 Apr; 29(3):356-9. doi: 10.1089/jop.2012.0098.

PURPOSE: To investigate clinically the effects of yeast-fermented bilberry extract on visual outcomes in myopic eyes. METHODS: In a prospective, randomized, placebo-controlled, cross-over study, we examined 30 eyes of 30 middle-aged healthy volunteers (mean age±standard deviation, 39.5±7.2 years) with myopia [manifest spherical equivalent, -2.40±1.88 diopters (D)], who were randomly assigned to 1 of 2 oral regimens: fermented bilberry extract (400 mg/day) or placebo. We quantitatively assessed visual acuity, refraction, pupil constriction rate, accommodation, and mesopic contrast sensitivity (CS), before and 1 month after treatment. Only the right eyes were tested. The amplitude of accommodation and CS were measured with an accommodometer (D'ACOMO; WOC) and a CS unit (VCTS-6500; Vistech), respectively. From the CS, the area under the log contrast sensitivity function (AULCSF) was calculated. RESULTS: The mean amplitude of accommodation increased significantly, from 4.62±1.88 D before treatment, to 5.33±2.03 D after treatment in the study group (Wilcoxon signed-rank test, P=0.002). Moreover, the mesopic AULCSF was significantly increased, from 1.04±0.16 before, to 1.13±0.17 after, treatment (P=0.009). However, we found no significant changes in accommodation or AULCSF in the control group (P>0.05), or any significant changes in any other parameters in either group (P>0.05). CONCLUSIONS: The present data show that fermented bilberry extract is effective in causing increases in subjective accommodation and in mesopic CS in myopic eyes.

Bilberries potentially alleviate stress-related retinal gene expression induced by a high-fat diet in mice.

Mol Vis. 2012; 18:2338-51.

PURPOSE: Obesity- and diabetes-associated visual impairment and vascular dysfunctions are increasing as causes of vision loss. The detailed mechanisms of how obesity and diabetes affect eye health are still largely unknown, but animal models have been useful in exploring the effects of potential protective compounds, i.e., compounds characterized by antioxidant and anti-inflammatory properties. These properties occur in anthocyanins, and bilberries (European wild blueberries, Vaccinium myrtillus) are a major source of dietary anthocyanins in Nordic diets. The main aim of the present work was to study the protective effects of dietary bilberries (BB) on the level of gene expression in retinas in mice that develop obesity when fed a high-fat diet (HFD). METHODS: Mice (n=6 per group, four groups) were fed ad libitum a normal control diet (NCD), a HFD, or a diet with 5% bilberries (NCD+BB, HFD+BB) for 12 weeks. Food consumption, weight gain, and blood pressure were measured

during the feeding period and whole blood serum markers of obesity at sacrifice. Retinas were collected, and RNA extracted from all 24 mice and pooled samples from four mice per group were hybridized to Mouse-Ref8 V2 Expression BeadChips (Illumina platform) with 25,697 probes for genes and transcript variants. The expression profiles in the retinas were analyzed using R, PathVisio, and DAVID to screen for high fat-induced changes as well as for bilberry-induced changes in the HFD up- or downregulated transcripts. **RESULTS:** The HFD and HFD+BB groups gained weight from week 5 and final weight, blood glucose, serum free fatty acids, and systolic blood pressure as compared to mice fed the control diets (Mann-Whitney's U-test, $p < 0.05$). Bilberries had no significant effect on these parameters other than a trend to reduce systolic blood pressure in the HFD-fed mice (101 ± 4 versus 113 ± 9 mmHg, $p = 0.10$). Gene ontology enrichment analysis of 810 differentially expressed genes (F-test, $p < 0.05$) in the retina displayed differential regulation of genes in ontology groups, mainly pathways for apoptosis, inflammation, and oxidative stress, especially systemic lupus erythematosus, mitogen-activated protein kinase, and glutathione metabolism. Mice fed a HFD had increased retinal gene expression of several crystallins, while the HFD+BB mice showed potential downregulation of these crystallins when compared to the HFD mice. Bilberries also reduced the expression of genes in the mitogen-activated protein kinase (MAPK) pathway and increased those in the glutathione metabolism pathway. **CONCLUSIONS:** HFD feeding induces differential expression of several stress-related genes in the mouse retina. Despite minor effects in the phenotype, a diet rich in bilberries mitigates the upregulation of crystallins otherwise induced by HFD. Thus, the early stages of obesity-associated and stress-related gene expression changes in the retina may be prevented with bilberries in the diet.

Ginkgo biloba extract and bilberry anthocyanins improve visual function in patients with normal tension glaucoma.

J Med Food. 2012 Sep; 15(9):818-23. doi: 10.1089/jmf.2012.2241. Epub 2012 Aug 7.

Ginkgo biloba extract (GBE) and anthocyanins are considered beneficial for various vascular diseases. This study was performed to evaluate the effect of GBE and anthocyanins on visual function in patients with normal tension glaucoma (NTG) based on the vascular theory of mechanisms of glaucomatous optic nerve damage. Retrospective analysis was carried out by a chart review of 332 subjects (209 men and 123 women) who were treated with anthocyanins ($n = 132$), GBE ($n = 103$), or no medication (control, $n = 97$). Humphrey Visual Field (HVF) test, logarithm of the minimal angle of resolution best-corrected visual acuity (logMAR BCVA), intraocular pressure, blood pressure, and fasting blood glucose were determined before and after treatment. Complete ocular and systemic examinations were performed. The mean follow-up duration was 23.82 ± 9.84 (range, 12-59) months; the mean anthocyanin treatment duration was 24.32 ± 10.43 (range, 6-53) months, and the mean GBE treatment duration was 23.81 ± 10.36 (range, 6-59) months. After anthocyanin treatment, the mean BCVA for all eyes improved from $0.16 (\pm 0.34)$ to $0.11 (\pm 0.18)$ logMAR units ($P = .008$), and HVF mean deviation improved from $-6.44 (\pm 7.05)$ to $-5.34 (\pm 6.42)$ ($P = .001$). After GBE treatment, HVF mean deviation improved from $-5.25 (\pm 6.13)$ to $-4.31 (\pm 5.60)$ ($P = .002$). A generalized linear model demonstrated that the final BCVA was not affected by demographic differences among the groups. These results suggest that anthocyanins and GBE may be helpful in improving visual function in some individuals with NTG.

Vision preservation during retinal inflammation by anthocyanin-rich bilberry extract: cellular and molecular mechanism.

Lab Invest. 2012 Jan; 92(1):102-9. doi: 10.1038/labinvest.2011.132.

Anthocyanin-rich bilberry extract, a plant-derived antioxidant, has been utilized as a popular supplement for ocular health worldwide. However, it is unclear whether this extract has any biological effect on visual function, and the mechanism for such an effect is completely unknown. In this study, we generated a mouse model of endotoxin-induced uveitis (EIU) that shows retinal inflammation, as well as uveitis, by injecting lipopolysaccharide. We pretreated the mice with anthocyanin-rich bilberry extract and analyzed the effect on the retina. Anthocyanin-rich bilberry extract prevented the impairment of photoreceptor cell function, as measured by electroretinogram. At the cellular level, we found that the EIU-associated rhodopsin decreased and

the shortening of outer segments in photoreceptor cells were suppressed in the bilberry-extract-treated animals. Moreover, the extract prevented both STAT3 activation, which induces inflammation-related rhodopsin decrease, and the increase in interleukin-6 expression, which activates STAT3. In addition to its anti-inflammatory effect, the anthocyanin-rich bilberry extract ameliorated the intracellular elevation of reactive oxygen species and activated NF- κ B, a redox-sensitive transcription factor, in the inflamed retina. Our findings indicate that anthocyanin-rich bilberry extract has a protective effect on visual function during retinal inflammation.

Protective effects of bilberry (*Vaccinium myrtillus* L.) extract against endotoxin-induced uveitis in mice.

J Agric Food Chem. 2010 Apr 28; 58(8):4731-6. doi: 10.1021/jf904572a.

Endotoxin-induced uveitis (EIU), a useful animal model of ocular inflammation, is induced by injection of lipopolysaccharide (LPS). These experiments showed that the nitric oxide (NO) level significantly increased in the whole eye homogenate of BALB/C mice 24 h after footpad injection of LPS at a dosage of 100 mg/mouse. However, the elevated NO level was significantly reduced by oral administration of bilberry extract (containing 42.04% anthocyanins) at dosages of 50, 100, and 200 mg/kg/day for 5 days before the LPS injection. In addition, bilberry extract decreased malondialdehyde (MDA) level and increased oxygen radical absorbance capacity (ORAC) level, glutathione (GSH) level, vitamin C level, and total superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities. Moreover, bilberry extract increased expression of copper/zinc superoxide dismutase (CuZnSOD), manganese superoxide dismutase (MnSOD), and GPx mRNA. Taken together, bilberry extract showed protective effects against EIU, whereas the effects of bilberry extract (100 and 200 mg/kg/day, 5 days) were dose-dependent. In conclusion, these results provide new evidence to elucidate the beneficial effects of bilberry extract on eye health.

Protective effect of bilberry (*Vaccinium myrtillus* L.) extracts on cultured human corneal limbal epithelial cells (HCLEC).

Phytother Res. 2010 Apr; 24(4):520-4. doi: 10.1002/ptr.2974.

*The use of bilberry (*Vaccinium myrtillus* L.) as a food and medicine for improving human vision has a long history all over the world. However, there is lack of convincing evidence from rigorous clinical trials or scientific research. This study investigated the effects of different concentrations of bilberry extracts on the cell viability, cell cycle and the expression of hyaluronic acid and glycosaminoglycans of cultured human corneal limbal epithelial cells. The data showed that bilberry extracts had no cytotoxicity to the corneal limbal epithelial cells at a wide range of concentrations (10⁽⁻⁹⁾-10⁽⁻⁴⁾ M, equalized to the content of cyanidin-3-O-glucoside). Bilberry extract (10⁽⁻⁶⁾, 10⁽⁻⁵⁾ and 10⁽⁻⁴⁾ M) increased cell viability after 48 h incubation. The number of cells decreased in G(0)/G(1) phase and increased prominently in S and G(2)/M phases after treatment with bilberry extracts at a high concentration (10⁽⁻⁴⁾ M). The expression of glycosaminoglycans increased prominently after incubation with bilberry extracts (10⁽⁻⁷⁾ and 10⁽⁻⁴⁾ M) for 48 h while no significant changes were observed for the expression of hyaluronic acid. The results indicated that bilberry extract may be beneficial for the physiological renewal and homeostasis of corneal epithelial cells.*

Bilberry and its main constituents have neuroprotective effects against retinal neuronal damage in vitro and in vivo.

Mol Nutr Food Res. 2009 Jul; 53(7):869-77. doi: 10.1002/mnfr.200800394.

*Our aim was to determine whether a *Vaccinium myrtillus* (bilberry) anthocyanoside (VMA) and/or its main anthocyanidin constituents (cyanidin, delphinidin, and malvidin) can protect retinal ganglion cells (RGCs) against retinal damage in vitro and*

in vivo. In RGC cultures (RGC-5, a rat ganglion cell-line transformed using E1A virus) *in vitro*, cell damage and radical activation were induced by 3-(4-morpholinyl) sydnonimine hydrochloride (SIN-1, a peroxydinitrite donor). Cell viability was measured using a water-soluble tetrazolium salt assay. Intracellular radical activation within RGC-5 cells was evaluated using 5-(and-6)-chloromethyl-2,7-dichlorodihydrofluorescein diacetate acetyl ester (CM-H(2)DCFDA). Lipid peroxidation was assessed using the supernatant fraction of mouse forebrain homogenates. In mice *in vivo*, we evaluated the effects of VMA on N-methyl-D-aspartic acid (NMDA)-induced retinal damage using hematoxylin-eosin and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) stainings. VMA and all three anthocyanidins (i) significantly inhibited SIN-1-induced neurotoxicity and radical activation in RGC-5, (ii) concentration-dependently inhibited lipid peroxidation in mouse forebrain homogenates. Intravitreously injected VMA significantly inhibited the NMDA-induced morphological retinal damage and increase in TUNEL-positive cells in the ganglion cell layer. Thus, VMA and its anthocyanidins have neuroprotective effects (exerted at least in part via an anti-oxidation mechanism) in these *in vitro* and *in vivo* models of retinal diseases.

Bilberry (*Vaccinium myrtillus*) anthocyanins modulate heme oxygenase-1 and glutathione S-transferase-pi expression in ARPE-19 cells.

Invest Ophthalmol Vis Sci. 2007 May; 48(5):2343-9.

PURPOSE: To determine whether anthocyanin-enriched bilberry extracts modulate pre- or posttranslational levels of oxidative stress defense enzymes heme-oxygenase (HO)-1 and glutathione S-transferase-pi (GST-pi) in cultured human retinal pigment epithelial (RPE) cells. *METHODS:* Confluent ARPE-19 cells were preincubated with anthocyanin and nonanthocyanin phenolic fractions of a 25% enriched extract of bilberry (10(-6)-1.0 mg/mL) and, after phenolic removal, cells were oxidatively challenged with H(2)O(2). The concentration of intracellular glutathione was measured by HPLC and free radical production determined by the dichlorofluorescein diacetate assay. HO-1 and GST-pi protein and mRNA levels were determined by Western blot and RT-PCR, respectively. *RESULTS:* Preincubation with bilberry extract ameliorated the intracellular increase of H(2)O(2)-induced free radicals in RPE, though H(2)O(2) cytotoxicity was not affected. By 4 hours, the extract had upregulated HO-1 and GST-pi protein by 2.8- and 2.5-fold, respectively, and mRNA by 5.5- and 7.1-fold, respectively, in a dose-dependent manner. Anthocyanin and nonanthocyanin phenolic fractions contributed similarly to mRNA upregulation. *CONCLUSIONS:* Anthocyanins and other phenolics from bilberry upregulate the oxidative stress defense enzymes HO-1 and GST-pi in RPE, suggesting that they stimulate signal transduction pathways influencing genes controlled by the antioxidant response element.

Anthocyanosides of *Vaccinium myrtillus* (bilberry) for night vision--a systematic review of placebo-controlled trials.

Surv Ophthalmol. 2004 Nov-Dec; 49(6):618; author reply 618.

We have systematically reviewed placebo-controlled trials of V. myrtillus-extracted anthocyanosides for evidence of positive effects on night vision. Searches of computerized databases and citations in retrieved articles identified 30 trials with outcome measures relevant to vision in reduced light. Of these, 12 were placebo-controlled. The 4 most recent trials were all randomized controlled trials (RCTs) and were negative in outcome. A fifth RCT and 7 non-randomized controlled trials reported positive effects on outcome measures relevant to night vision. Negative outcome was associated with more rigorous methodology but also with lower dose level and extracts from geographically distinct sources that may differ in anthocyanoside composition. Healthy subjects with normal or above average eyesight were tested in 11 of the 12 trials. The hypothesis that V. myrtillus anthocyanosides improves normal night vision is not supported by evidence from rigorous clinical studies. There is a complete absence of rigorous research into the effects of the extract on subjects suffering impaired night vision due to pathological eye conditions. Evidence from methodologically weaker trials and auxiliary evidence from animal studies, trials of synthetic anthocyanosides, and a recent randomized controlled trial of Ribes nigrum (black currant) anthocyanosides may warrant further trials of V. myrtillus anthocyanosides in subjects with impaired night vision.

LIVER HEALTH

Mirtoselect, an anthocyanin-rich bilberry extract, attenuates non-alcoholic steatohepatitis and associated fibrosis in ApoE(*)3Leiden mice.

J Hepatol. 2015 May; 62(5):1180-6. doi: 10.1016/j.jhep.2014.12.011. Epub 2014 Dec 13.

BACKGROUND & AIMS: Anthocyanins may have beneficial effects on lipid metabolism and inflammation and are demonstrated to have hepatoprotective properties in models of restraint-stress- and chemically-induced liver damage. However, their potential to protect against non-alcoholic steatohepatitis (NASH) under conditions relevant for human pathogenesis remains unclear. Therefore, we studied the effects of the standardised anthocyanin-rich extract Mirtoselect on diet-induced NASH in a translational model of disease. METHODS: ApoE()3Leiden mice were fed a Western-type cholesterol-containing diet without (HC) or with 0.1% (w/w) Mirtoselect (HCM) for 20 weeks to study the effects on diet-induced NASH. RESULTS: Mirtoselect attenuated HC-induced hepatic steatosis, as observed by decreased macro- and microvesicular hepatocellular lipid accumulation and reduced hepatic cholesteryl ester content. This anti-steatotic effect was accompanied by local anti-inflammatory effects in liver, as demonstrated by reduced inflammatory cell clusters and reduced neutrophil infiltration in HCM. On a molecular level, HC diet significantly induced hepatic expression of pro-inflammatory genes Tnf, Emr1, Ccl2, Mpo, Cxcl1, and Cxcl2 while this induction was less pronounced or significantly decreased in HCM. A similar quenching effect was observed for HC-induced pro-fibrotic genes, Acta2 and Col1a1 and this anti-fibrotic effect of Mirtoselect was confirmed histologically. Many of the pro-inflammatory and pro-fibrotic parameters positively correlated with intrahepatic free cholesterol levels. Mirtoselect significantly reduced accumulation and crystallisation of intrahepatic free cholesterol, providing a possible mechanism for the observed hepatoprotective effects. CONCLUSIONS: Mirtoselect attenuates development of NASH, reducing hepatic lipid accumulation, inflammation and fibrosis, possibly mediated by local anti-inflammatory effects associated with reduced accumulation and crystallisation of intrahepatic free cholesterol.*

Purified anthocyanins from bilberry and black currant attenuate hepatic mitochondrial dysfunction and steatohepatitis in mice with methionine and choline deficiency.

J Agric Food Chem. 2015 Jan 21; 63(2):552-61. doi: 10.1021/jf504926n. Epub 2015 Jan 12.

The berries of bilberry and black currant are a rich source of anthocyanins, which are thought to have favorable effects on nonalcoholic steatohepatitis (NASH). This study was designed to examine whether purified anthocyanins from bilberry and black currant are able to limit the disorders related to NASH induced by a methionine-choline-deficient (MCD) diet in mice. The results showed that treatment with anthocyanins not only alleviated inflammation, oxidative stress, steatosis, and even fibrosis but also improved depletion of mitochondrial content and damage of mitochondrial biogenesis and electron transfer chain developed concomitantly in the liver of mice fed the MCD diet. Furthermore, anthocyanins treatment promoted activation of AMP-activated protein kinase (AMPK) and expression of peroxisome proliferator-activated receptor-gamma coactivator-1 α (PGC-1 α). These data provide evidence that anthocyanins possess significant protective effects against NASH and mitochondrial defects in response to a MCD diet, with a mechanism maybe through affecting the AMPK/PGC-1 α signaling pathways.

Anti-inflammatory effects of anthocyanins-rich extract from bilberry (*Vaccinium myrtillus* L.) on croton oil-induced ear edema and *Propionibacterium acnes* plus LPS-induced liver damage in mice.

Int J Food Sci Nutr. 2014 Aug; 65(5):594-601. doi: 10.3109/09637486.2014.886184. Epub 2014 Feb 19.

*Bilberry (*Vaccinium myrtillus* L.) has been known to play a protective role in human health due to its high anthocyanin content. This study investigated the anti-inflammatory effects of bilberry extract (BE, containing 42.04% anthocyanin) on *Propionibacterium acnes* (*P. acnes*) plus lipopolysaccharide (LPS) induced liver injury and croton oil-induced ear edema in mice. Results showed that BE could effectively inhibit croton oil-induced ear edema and liver inflammation provoked by *P. acnes* plus LPS, as reflected by the reduced plasma alanine aminotransferase and aspartate aminotransferase activities. These findings were confirmed by hepatic pathological examination. Moreover, BE administration markedly suppressed the increase of liver mRNA levels of iNOS, TNF- α , IL-1 β and IL-6, and the protein levels of iNOS, TNF- α and NF- κ B. In addition, liver malondialdehyde and NO contents were significantly reduced by BE treatment. These results indicated that BE has potent protective effects on acute and immunological inflammation, which might contribute to the study of the anti-inflammatory effects of natural products and healthy food.*

Bilberry extract protect restraint stress-induced liver damage through attenuating mitochondrial dysfunction.

Fitoterapia. 2010 Dec; 81(8):1094-101. doi: 10.1016/j.fitote.2010.07.004. Epub 2010 Jul 8.

The aim of the present study is to investigate the protective effect of bilberry extract on liver damage in restraint stressed mice. A remarkable increase in alanine aminotransferase (ALT) and reactive oxygen species (ROS) levels was observed in stressed mice. Treatment with bilberry extract restored ALT and ROS to normal levels, and enhanced mitochondrial complex II activity that was lowered in restraint stressed mice. The mitochondrial electron transfer chain (ETC)-related gene expression was measured by RT-PCR, and a significant up-regulation of complex II mRNA was observed for SDHA, B, C and D mRNA in bilberry extract-treated group compared with that in stressed group. Bilberry extract administration also profoundly elevated the Na(+)-K(+)-ATPase activity and mitochondria membrane potential ($\Delta\Psi(m)$), which was reduced in the stressed group. Bilberry extract exhibited protective effect by scavenging free radicals and attenuating mitochondrial dysfunction in the liver of restraint stressed mice. It may be used as a promising therapeutic agent in preventing and delaying the life-related disease.

Protective effects of bilberry (*Vaccinium myrtillus* L.) extract on KBrO₃-induced kidney damage in mice.

J Agric Food Chem. 2008 Jan 23; 56(2):420-5.

*Potassium bromate (KBrO₃) is an oxidizing agent used as a food additive which causes kidney damage as a potent nephrotoxic agent, and the mechanism may be explained by the generation of oxygen free radicals. Our experiments showed that single intraperitoneal administration of 200 mg/kg KBrO₃ could induce serious kidney damage, with an increase in serum blood urea nitrogen (BUN) and creatinine levels. Five-day oral administration of bilberry (*Vaccinium myrtillus* L.) extract at 50, 100, and 200 mg/kg resulted in a reversal in serum BUN and creatinine to normal levels and decreased kidney malondialdehyde (MDA), nitric oxide (NO), and xanthine oxidase (XOD) levels. Also, bilberry extract improved oxygen radical absorbance capacity (ORAC) levels in kidney tissue, which showed that bilberry extract reduced the degree of oxidative stress and kidney damage induced by KBrO₃. These findings demonstrate that the protective effect of bilberry extract is attributed to its free radical scavenging activity and lipid peroxidation inhibitory effect.*



Protective effects of bilberry (*Vaccinium myrtillus* L.) extract on restraint stress-induced liver damage in mice.

J Agric Food Chem. 2008 Sep 10; 56(17):7803-7. doi: 10.1021/jf800728m. Epub 2008 Aug 9.

Our experiments showed that 18 h restraint stress could induce serious liver damage, with an increase in plasma alanine aminotransferase (ALT) level (107.68 +/- 3.19 U/L vs 18.08 +/- 1.46 U/L). Meanwhile, we observed increased malondialdehyde (MDA) levels and lowered oxygen radical absorbance capacity (ORAC) values in plasma and liver of restraint mice compared with starved mice. Bilberry extract (containing 42.04% anthocyanins) was oral administrated to mice at 50, 100, and 200 mg/(kg x day) for five days, which remarkably decreased plasma ALT level to 17.23 +/- 2.49 U/L at the dose of 200 mg/(kg x day) and thus alleviated stress-induced liver damage. In addition, bilberry extracts increased glutathione (GSH) and vitamin C levels and significantly decreased MDA and nitric oxide (NO) levels in the liver tissues. These results suggest that bilberry extract plays an important role in protecting against restraint stress-induced liver damage by both scavenging free radicals activity and lipid peroxidation inhibitory effect. This study showed the beneficial health effects of bilberry extract through its antioxidative action.

ORAL HEALTH

Consumption of bilberries controls gingival inflammation.

Int J Mol Sci. 2015 May 11; 16(5):10665-73. doi: 10.3390/ijms160510665.

Bioactive molecules in berries may be helpful in reducing the risk of oral diseases. The aim of this study was to determine the effect of bilberry consumption on the outcome of a routine dental clinical parameter of inflammation, bleeding on probing (BOP), as well as the impact on selected biomarkers of inflammation, such as cytokines, in gingival crevicular fluid (GCF) in individuals with gingivitis. Study individuals who did not receive standard of care treatment were allocated to either a placebo group or to groups that consumed either 250 or 500 g bilberries daily over seven days. The placebo group consumed an inactive product (starch). A study group, receiving standard of care (debridement only) was also included to provide a reference to standard of care treatment outcome. Cytokine levels were assayed using the Luminex MagPix system. The mean reduction in BOP before and after consumption of test product over 1 week was 41% and 59% in the groups that consumed either 250 or 500 g of bilberries/day respectively, and was 31% in the placebo group, and 58% in the standard of care reference group. The analysis only showed a significant reduction in cytokine levels in the group that consumed 500 g of bilberries/day. A statistically significant reduction was observed for IL-1b ($p=0.025$), IL-6 ($p=0.012$) and VEGF ($p=0.017$) in GCF samples in the group that consumed 500 g of bilberries daily. It appears that berry intake has an ameliorating effect on some markers of gingival inflammation reducing gingivitis to a similar extent compared to standard of care.

Protective effect of bilberry extract as a pretreatment on induced oral mucositis in hamsters.

Oral Surg Oral Med Oral Pathol Oral Radiol. 2013 Dec; 116(6):702-8. doi: 10.1016/j.oooo.2013.07.004.

OBJECTIVE: This study evaluated the effect of standardized bilberry extract containing anthocyanosides on chemotherapy-induced oral mucositis in hamsters. STUDY DESIGN: Twenty-four hamsters were randomly chosen and assigned to groups. Groups A and B were pretreated with deionized water, whereas group C was pretreated with bilberry extract daily for 7 days. Groups B and C underwent chemotherapy by intraperitoneal injections of 5-fluorouracil (days 4, 9, and 14); group A received normal saline. Potentiation of oral mucositis was achieved by scratching both cheek pouches of all animals with needles (days 5, 6, and 14). The pouches were histopathologically examined on day 17 after visual examination and blood sampling by cardiac puncture. RESULTS: The bilberry extract group showed significantly lower oral mucositis clinical and histopathologic scores ($P < .05$) and less percentile of mean daily weight reductions compared with animals receiving vehicle. CONCLUSIONS: Frequent administration of bilberry extract had a protective effect on oral mucosal damage induced by 5-fluorouracil in an animal model.

METABOLIC HEALTH

Anti-hyperglycemic and anti-hyperlipidemic effects of *Vaccinium myrtillus* fruit in experimentally induced diabetes (antidiabetic effect of *Vaccinium myrtillus* fruit).

J Sci Food Agric. 2016 Feb; 96(3):764-8. doi: 10.1002/jsfa.7144. Epub 2015 Mar 19.

BACKGROUND: Diabetes mellitus (DM) is a metabolic disorder that is associated with an increased risk of cardiovascular disease. *Vaccinium myrtillus* (bilberry) is a useful plant with antidiabetic properties in traditional medicine. The aim of this study was to investigate the effects of bilberry against DM. Diabetes was induced using intraperitoneal injection of alloxan (120 mg kg⁻¹ body weight (BW)). Bilberry powder (2 g d⁻¹) and glibenclamide (positive control; 0.6 mg kg⁻¹ BW) were administered for 4 weeks following alloxan injection. Serum glucose, insulin, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very-low-density lipoprotein cholesterol (VLDL-C), triglycerides (TG) and C-reactive protein (CRP) were determined at baseline and at 2nd and 4th week of the study. **RESULTS:** Bilberry supplementation resulted in a significant reduction of glucose compared with the diabetic control as well as glibenclamide treatment. Bilberry elevated insulin, reduced TC, LDL-C, VLDL-C and TG levels, and prevented HDL-C decline. Serum insulin, TC and LDL-C levels were not affected by glibenclamide, and CRP did not significantly change with either bilberry or glibenclamide. Histological examinations revealed a significant elevation of islet size in the bilberry and glibenclamide-treated groups. **CONCLUSION:** Dietary supplementation with bilberry fruits may protect against impaired glucose and lipid metabolism in DM.

Nontargeted metabolite profiling discriminates diet-specific biomarkers for consumption of whole grains, fatty fish, and bilberries in a randomized controlled trial.

J Nutr. 2015 Jan; 145(1):7-17. doi: 10.3945/jn.114.196840. Epub 2014 Nov 12.

BACKGROUND: Nontargeted metabolite profiling allows for concomitant examination of a wide range of metabolite species, elucidating the metabolic alterations caused by dietary interventions. **OBJECTIVE:** The aim of the current study was to investigate the effects of dietary modifications on the basis of increasing consumption of whole grains, fatty fish, and bilberries on plasma metabolite profiles to identify applicable biomarkers for dietary intake and endogenous metabolism. **METHODS:** Metabolite profiling analysis was performed on fasting plasma samples collected in a 12-wk parallel-group intervention with 106 participants with features of metabolic syndrome who were randomly assigned to 3 dietary interventions: 1) whole-grain products, fatty fish, and bilberries [healthy diet (HD)]; 2) a whole-grain-enriched diet with the same grain products as in the HD intervention but with no change in fish or berry consumption; and 3) refined-wheat breads and restrictions on fish and berries (control diet). In addition, correlation analyses were conducted with the food intake data to define the food items correlating with the biomarker candidates. **RESULTS:** Nontargeted metabolite profiling showed marked differences in fasting plasma after the intervention diets compared with the control diet. In both intervention groups, a significant increase was observed in 2 signals identified as glucuronidated alk(en)-ylresorcinols [corrected *P* value (*P*_{corr}) < 0.05], which correlated strongly with the intake of whole-grain products (*r* = 0.63, *P* < 0.001). In addition, the HD intervention increased the signals for furan fatty acids [3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF)], hippuric acid, and various lipid species incorporating polyunsaturated fatty acids (*P*_{corr} < 0.05). In particular, plasma CMPF correlated strongly with the intake of fish (*r* = 0.47, *P* < 0.001) but not with intakes of any other foods. **CONCLUSIONS:** Novel biomarkers of the intake of health-beneficial food items included in the Nordic diet were identified by the metabolite profiling of fasting plasma and confirmed by the correlation analyses with dietary records. The one with the most potential was CMPF, which was shown to be a highly specific biomarker for fatty fish intake. This trial was registered at clinicaltrials.gov as NCT00573781.

Wild blueberries (*Vaccinium myrtillus*) alleviate inflammation and hypertension associated with developing obesity in mice fed with a high-fat diet.

PLoS One. 2014 Dec 12; 9(12):e114790. doi: 10.1371/journal.pone.0114790. eCollection 2014.

BACKGROUND: Low-grade metabolic inflammation and hypertension are primary mechanisms involved in obesity-associated adverse health effects. Berries, especially Nordic wild blueberries (hereafter referred to as bilberries), represent an important source of dietary anthocyanins, a group of polyphenols with potential beneficial effects to combat obesity-associated metabolic disturbances. METHODS: The effects of 5% or 10% (w/w) of whole bilberries (BB) were studied on the development of obesity and its metabolic disturbances in C57BL mice fed with a high-fat diet (HFD) for three months. Cytokines, inflammatory cells, systolic blood pressure, glucose tolerance, insulin sensitivity, weight gain, body fat, food consumption and energy metabolism were assessed. RESULTS: Bilberries ameliorated type 1 pro-inflammatory responsiveness induced by HFD. This was indicated by the altered cytokine profile and the reduced prevalence of interferon gamma-producing T-cells, in particular T helper type 1 cells. Bilberries also prevented the progression of obesity associated long term increase in systolic blood pressure in mice. CONCLUSIONS: Bilberries reduce the development of systemic inflammation and prevent the progression of chronic hypertension, thus supporting their potential role in alleviating the adverse health effects associated with developing obesity.

Effects of soluble and insoluble fractions from bilberries, black currants, and raspberries on short-chain fatty acid formation, anthocyanin excretion, and cholesterol in rats.

J Agric Food Chem. 2014 May 14; 62(19):4359-68. doi: 10.1021/jf5007566. Epub 2014 May 5.

Dietary fiber and flavonoids, important components in berries, are suggested to improve metabolic health. This study investigates whether soluble and insoluble fractions isolated from bilberry, black currant, and raspberry affect the formation of short-chain fatty acids (SCFAs), uptake and excretion of flavonoids, and levels of cholesterol differently. Cecal SCFA pools were higher in rats fed the soluble than the insoluble fractions (525 vs 166 μmol , $P < 0.001$), whereas higher concentrations of butyric acid were found in the distal colon and serum of rats fed the insoluble fractions (5 vs 3 $\mu\text{mol/g}$ and 58 vs 29 $\mu\text{mol/L}$, respectively, $P < 0.001$). The soluble bilberry fraction gave lower amounts of liver cholesterol (56 mg) than the other berry fractions (87 \pm 5 mg), formed the highest amount of SCFAs (746 vs 266 \pm 21 μmol), and contributed the highest intake of anthocyanins. Cyanidin-3-glucoside monoglucuronide was detected in the urine of all groups, whereas anthocyanins were found only in groups fed soluble black currant and raspberry.

A single supplement of a standardised bilberry (*Vaccinium myrtillus* L.) extract (36 % wet weight anthocyanins) modifies glycaemic response in individuals with type 2 diabetes controlled by diet and lifestyle.

J Nutr Sci. 2013 Jul 24; 2:e22. doi: 10.1017/jns.2013.16.

*Dietary strategies for alleviating health complications associated with type 2 diabetes (T2D) are being pursued as alternatives to pharmaceutical interventions. Berries such as bilberries (*Vaccinium myrtillus* L.) that are rich in polyphenols may influence carbohydrate digestion and absorption and thus postprandial glycaemia. In addition, berries have been reported to alter incretins as well as to have antioxidant and anti-inflammatory properties that may also affect postprandial glycaemia. The present study investigated the acute effect of a standardised bilberry extract on glucose metabolism in T2D. Male volunteers with T2D (n 8; BMI 30 (sd 4) kg/m²) controlling their diabetes by diet and lifestyle alone were given a single oral capsule of either 0.47 g standardised bilberry extract (36 % (w/w) anthocyanins) which equates to about 50 g of fresh bilberries or placebo followed by a polysaccharide drink (equivalent to 75 g glucose) in a double-blinded cross-over intervention with a 2-week washout period. The ingestion of the bilberry extract resulted in a significant decrease in the incremental AUC for both glucose*

($P = 0.003$) and insulin ($P = 0.03$) compared with the placebo. There was no change in the gut (glucagon-like peptide-1, gastric inhibitory polypeptide), pancreatic (glucagon, amylin) or anti-inflammatory (monocyte chemoattractant protein-1) peptides. In addition there was no change in the antioxidant (Trolox equivalent antioxidant capacity, ferric-reducing ability of plasma) responses measured between the volunteers receiving the bilberry extract and the placebo. In conclusion the present study demonstrates for the first time that the ingestion of a concentrated bilberry extract reduces postprandial glycaemia and insulin in volunteers with T2D. The most likely mechanism for the lower glycaemic response involves reduced rates of carbohydrate digestion and/or absorption.

Effects of sea buckthorn and bilberry on serum metabolites differ according to baseline metabolic profiles in overweight women: a randomized crossover trial.

Am J Clin Nutr. 2013 Oct;98(4):941-51. doi: 10.3945/ajcn.113.060590. Epub 2013 Aug 14.

BACKGROUND: Berries are associated with health benefits. Little is known about the effect of baseline metabolome on the overall metabolic responses to berry intake. **OBJECTIVE:** We studied the effects of berries on serum metabolome. **DESIGN:** Eighty overweight women completed this randomized crossover study. During the interventions of 30 d, subjects consumed dried sea buckthorn berries (SBs), sea buckthorn oil (SBo), sea buckthorn phenolics ethanol extract mixed with maltodextrin (SBe+MD) (1:1), or frozen bilberries. Metabolic profiles were quantified from serum samples by using $(1)H$ nuclear magnetic resonance spectroscopy. **RESULTS:** All interventions induced a significant ($P < 0.001-0.003$) effect on the overall metabolic profiles. The effect was observed both in participants who had a metabolic profile that reflected higher cardiometabolic risk at baseline (group B: $P = 0.001-0.008$) and in participants who had a lower-risk profile (group A: $P < 0.001-0.009$). Although most of the changes in individual metabolites were not statistically significant after correction for multiplicity, clear trends were observed. SB-induced effects were mainly on serum triglycerides and very-low-density lipoprotein (VLDL) and its subclasses, which decreased in metabolic group B. SBo induced a decreasing trend in serum total, intermediate-density lipoprotein (IDL), and low-density lipoprotein (LDL) cholesterol and subfractions of IDL and LDL in group B. During the SBe+MD treatment, VLDL fractions and serum triglycerides increased. Bilberries caused beneficial changes in serum lipids and lipoproteins in group B, whereas the opposite was true in group A. **CONCLUSION:** Berry intake has overall metabolic effects, which depend on the cardiometabolic risk profile at baseline. This trial was registered at clinicaltrials.gov as NCT01860547.

Berries reduce postprandial insulin responses to wheat and rye breads in healthy women.

J Nutr. 2013 Apr; 143(4):430-6. doi: 10.3945/jn.112.169771.

Starch in white wheat bread (WB) induces high postprandial glucose and insulin responses. For rye bread (RB), the glucose response is similar, whereas the insulin response is lower. In vitro studies suggest that polyphenol-rich berries may reduce digestion and absorption of starch and thereby suppress postprandial glycemia, but the evidence in humans is limited. We investigated the effects of berries consumed with WB or RB on postprandial glucose and insulin responses. Healthy females ($n = 13-20$) participated in 3 randomized, controlled, crossover, 2-h meal studies. They consumed WB or RB, both equal to 50 g available starch, with 150 g whole-berry purée or the same amount of bread without berries as reference. In study 1, WB was served with strawberries, bilberries, or lingonberries and in study 2 with raspberries, cloudberries, or chokeberries. In study 3, WB or RB was served with a mixture of berries consisting of equal amounts of strawberries, bilberries, cranberries, and blackcurrants. Strawberries, bilberries, lingonberries, and chokeberries consumed with WB and the berry mixture consumed with WB or RB significantly reduced the postprandial insulin response. Only strawberries (36%) and the berry mixture (with WB, 38%; with RB, 19%) significantly improved the glycemic profile of the breads. These results suggest that when WB is consumed with berries, less insulin is needed for maintenance of normal or slightly improved postprandial glucose metabolism. The lower insulin response to RB compared with WB can also be further reduced by berries.

Bilberries reduce low-grade inflammation in individuals with features of metabolic syndrome.

Mol Nutr Food Res. 2012 Oct; 56(10):1501-10. doi: 10.1002/mnfr.201200195.

*SCOPE: Low-grade inflammation is a hallmark of cardiometabolic risk. Bilberries (*Vaccinium myrtillus*) are rich in polyphenols with potential anti-inflammatory properties. We studied the impact of bilberries on inflammation and gene expression profile in peripheral blood mononuclear cells in subjects with metabolic syndrome. METHODS AND RESULTS: In randomized, controlled dietary intervention, the participants consumed either a diet rich in bilberries (n = 15) or a control diet (n = 12). The bilberry group consumed daily an equivalent dose of 400 g fresh bilberries, while the control group maintained their habitual diet. No differences were found between the groups in body weight, glucose, or lipid metabolism, but bilberry supplementation tended to decrease serum high-sensitivity C-reactive protein, IL-6, IL-12, and LPS concentrations. An inflammation score was significantly different between the groups (p = 0.024). In transcriptomics analyses (three participants with improved oral glucose tolerance test in the bilberry group), Toll-like receptor signaling, cytoplasmic ribosomal proteins, and B-cell receptor signaling pathways were differently regulated. QPCR analyses (n = 13 and 11 in the bilberry and control groups, respectively) showed decreased expression of MMD and CCR2 transcripts associated with monocyte and macrophage function associated genes. CONCLUSION: Regular bilberry consumption may reduce low-grade inflammation indicating decreased cardiometabolic risk in the long term.*

A diet high in fatty fish, bilberries and wholegrain products improves markers of endothelial function and inflammation in individuals with impaired glucose metabolism in a randomised controlled trial: the Sysdimet study.

Diabetologia. 2011 Nov; 54(11):2755-67. doi: 10.1007/s00125-011-2285-3. Epub 2011 Aug 26.

AIMS/HYPOTHESIS: Low-grade inflammation and endothelial dysfunction may play a role in the pathogenesis of type 2 diabetes and cardiovascular disease. We evaluated whether a diet high in fatty fish, bilberries and wholegrain products (Healthy Diet) improves biomarkers reflecting inflammation and endothelial dysfunction in individuals with impaired glucose metabolism. METHODS: We recruited individuals with impaired glucose metabolism and features of the metabolic syndrome into a 12 week, parallel design, dietary intervention trial conducted at the Department of Clinical Nutrition, University of Eastern Finland (Kuopio, Finland). Randomisation was performed by matching according to sex and medians of age, BMI and fasting plasma glucose of the study population at screening. The primary endpoint in the present study was the change in plasma inflammatory markers and the measurements were performed blinded to group assignment. High-sensitivity (hs) C-reactive protein (CRP) and E-selectin responses were also analysed separately in participants not using statins (n = 76). RESULTS: Altogether, 131 individuals were assigned to either the Healthy Diet (n = 44), a whole-grain-enriched diet (WGED) (n = 42) or a control (n = 45) diet, and 104 participants (mean ± SD: age 59 ± 7 years; BMI 31.1 ± 3.5 kg/m²) who had completed the study, were analysed (Healthy Diet n = 36, WGED n = 34 and control diet n = 34). Plasma E-selectin decreased only in the Healthy Diet group. This occurred in all group participants (p < 0.05) and also after excluding participants using statins (p < 0.05). Plasma hsCRP levels decreased in the Healthy Diet (median -17%, p < 0.05) and WGED (median -27%, p < 0.01) groups in participants not using statins. Controlling for confounding factors, including BMI or insulin sensitivity, did not alter the results. A greater increase in plasma concentration of very-long-chain n-3 fatty acids and in the intake of fibre during the study was associated with a greater decrease in plasma E-selectin (p < 0.05). The intake of test breads consumed during the Healthy Diet and WGED interventions was inversely associated with the change in hsCRP levels (p < 0.001). CONCLUSIONS/INTERPRETATION: Our results suggest that the combined effect of fatty fish, bilberries and wholegrain products may improve endothelial dysfunction and inflammation in overweight and obese individuals at high risk of developing diabetes.

Whole grain products, fish and bilberries alter glucose and lipid metabolism in a randomized, controlled trial: the Sysdimet study.

PLoS One. 2011; 6(8):e22646. doi: 10.1371/journal.pone.0022646.

BACKGROUND: Due to the growing prevalence of type 2 diabetes, new dietary solutions are needed to help improve glucose and lipid metabolism in persons at high risk of developing the disease. Herein we investigated the effects of low-insulin-response grain products, fatty fish, and berries on glucose metabolism and plasma lipidomic profiles in persons with impaired glucose metabolism. **METHODOLOGY/PRINCIPAL FINDINGS:** Altogether 106 men and women with impaired glucose metabolism and with at least two other features of the metabolic syndrome were included in a 12-week parallel dietary intervention. The participants were randomized into three diet intervention groups: (1) whole grain and low postprandial insulin response grain products, fatty fish three times a week, and bilberries three portions per day (HealthyDiet group), (2) Whole grain enriched diet (WGED) group, which includes principally the same grain products as group (1), but with no change in fish or berry consumption, and (3) refined wheat breads (Control). Oral glucose tolerance, plasma fatty acids and lipidomic profiles were measured before and after the intervention. Self-reported compliance with the diets was good and the body weight remained constant. Within the HealthyDiet group two hour glucose concentration and area-under-the-curve for glucose decreased and plasma proportion of (n-3) long-chain PUFAs increased (False Discovery Rate p-values <0.05). Increases in eicosapentaenoic acid and docosahexaenoic acid associated curvilinearly with the improved insulin secretion and glucose disposal. Among the 364 characterized lipids, 25 changed significantly in the HealthyDiet group, including multiple triglycerides incorporating the long chain (n-3) PUFA. **CONCLUSIONS:** The results suggest that the diet rich in whole grain and low insulin response grain products, bilberries, and fatty fish improve glucose metabolism and alter the lipidomic profile. Therefore, such a diet may have a beneficial effect in the efforts to prevent type 2 diabetes in high risk persons.

Purified anthocyanin supplementation improves endothelial function via NO-cGMP activation in hypercholesterolemic individuals.

Clin Chem. 2011 Nov; 57(11):1524-33. doi: 10.1373/clinchem.2011.167361. Epub 2011 Sep 16.

BACKGROUND: Anthocyanins have been shown to improve endothelial function in animal models. However, whether these compounds have similar beneficial effects in humans is largely unknown. **METHODS:** In a short-term crossover study, 12 hypercholesterolemic individuals were given oral anthocyanins (320 mg) isolated from berries or placebo. Brachial artery flow-mediated dilation (FMD) was assessed before and after the intervention. In a long-term intervention trial (12 weeks), 150 hypercholesterolemic individuals were given anthocyanins (320 mg/day, n = 75) or placebo (n = 75), after which we measured FMD, plasma cGMP, and other serum biomarkers. Another short-term intervention was conducted in the presence of NO-cGMP inhibitors in 6 people and in a rat aortic ring model (n = 8). **RESULTS:** Significant increases of FMD from 8.3% (0.6%) at baseline to 11.0% (0.8%) at 1 h and 10.1% (0.9%) at 2 h were observed after short-term anthocyanin consumption, concomitantly with increases of plasma anthocyanin concentrations (P < 0.05). In the study participants who received long-term anthocyanin intervention, compared with the control group, we observed significant increases in the FMD (28.4% vs 2.2%), cGMP (12.6% vs -1.2%), and HDL-cholesterol concentrations, but decreases in the serum soluble vascular adhesion molecule-1 and LDL cholesterol concentrations (P < 0.05). The changes in the cGMP and HDL cholesterol concentrations positively correlated with FMD in the anthocyanin group (P < 0.05). In the presence of NO-cGMP inhibitors, the effects of anthocyanin on endothelial function were abolished in human participants and in a rat aortic ring model. **CONCLUSIONS:** Anthocyanin supplementation improves endothelium-dependent vasodilation in hypercholesterolemic individuals. This effect involves activation of the NO-cGMP signaling pathway, improvements in the serum lipid profile, and decreased inflammation.

A bilberry drink with fermented oatmeal decreases postprandial insulin demand in young healthy adults.

Nutr J. 2011 May 21; 10:57. doi: 10.1186/1475-2891-10-57.

BACKGROUND: In traditional medicine, blueberries have been used to facilitate blood glucose regulation in type 2 diabetes. Recent studies in diabetic mice have indicated facilitated glycaemic regulation following dietary supplementation with extracts from European blueberries, also called bilberries, (*Vaccinium myrtillus*). The purpose of the present study was to investigate the impact of fermented oat meal drinks containing bilberries or rosehip (*Rosa canina*) on glycaemic and insulinaemic responses. *METHODS:* glycaemic and insulinaemic responses in young healthy adults were measured in two series. In series 1, two drinks based on oat meal (5%), fermented using *Lactobacillus plantarum* 299v, and added with fruit (10%); bilberries (BFOMD) or rose hip (RFOMD) respectively, were studied. In series 2, BFOMD was repeated, additionally, a drink enriched with bilberries (47%) was tested (BBFOMD). As control a fermented oat meal drink (FOMD) was served. *RESULTS:* in series 1 the bilberry- and rosehip drinks, gave high glucose responses similar to that after the reference bread. However, the insulin index (II) after the BFOMD was significantly lower (II = 65) ($P < 0.05$). In series 2 a favourably low insulin demand to BFOMD was confirmed. FOMD gave high glucose response (GI = 95) but, significantly lower insulin response (II = 76). BBFOMD gave remarkably low insulin response II = 49, and tended to lower glycaemia (GI = 79) ($P = 0.0684$). *CONCLUSION:* A fermented oat meal drink added with bilberries induced a lower insulin response than expected from the glycaemic response. The mechanism for the lowered acute insulin demand is still unclear, but may be related to some bio-active component present in the bilberries, or to the fermented oat meal base.

Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice.

J Nutr. 2010 Mar; 140(3):527-33. doi: 10.3945/jn.109.118216. Epub 2010 Jan 20.

Blueberries or bilberries contain large amounts of anthocyanins, making them one of the richest sources of dietary anthocyanin. These berries are widely consumed as fresh and dried fruits, jams, or juices. Considerable attention has been focused on the health benefits of bilberry fruits beyond their antioxidant content or their ability to improve vision. In this study, we tested the effect of dietary bilberry extract (BBE) on hyperglycemia and insulin sensitivity in type 2 diabetic mice. We found that dietary BBE ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase (AMPK). Dietary BBE significantly reduced the blood glucose concentration and enhanced insulin sensitivity. AMPK was activated in white adipose tissue (WAT), skeletal muscle, and the liver of diabetic mice fed BBE. This activation was accompanied by upregulation of glucose transporter 4 in WAT and skeletal muscle and suppression of glucose production and lipid content in the liver. At the same time, acetyl-CoA carboxylase was inactivated and PPARalpha, acyl-CoA oxidase, and carnitine palmitoyltransferase-1A were upregulated in the liver. These changes resulted in improved hyperglycemia and insulin sensitivity in type 2 diabetes. These findings provide a biochemical basis for the use of bilberry fruits and have important implications for the prevention and treatment of type 2 diabetes via activation of AMPK.



SKIN HEALTH / COSMETICS

Protective effect of *Vaccinium myrtillus* extract against UVA- and UVB-induced damage in a human keratinocyte cell line (HaCaT cells).

J Photochem Photobiol B. 2014 Mar 5; 132:27-35. doi: 10.1016/j.jphotobiol.2014.01.013. Epub 2014 Feb 8.

*Recently, the field of skin protection have shown a considerable interest in the use of botanicals. *Vaccinium myrtillus* contains several polyphenols and anthocyanins with multiple pharmacological properties. The purpose of our study was to examine whether a water-soluble *V. myrtillus* extract (dry matter 12.4%; total polyphenols 339.3mg/100 g fw; total anthocyanins 297.4 mg/100 g fw) was able to reduce UVA- and UVB-induced damage using a human keratinocyte cell line (HaCaT). HaCaT cells were pretreated for 1h with extract in a serum-free medium and then irradiated with UVA (8-40 J/cm²) and UVB (0.008-0.72 J/cm²) rays. All experiments were performed 24h after the end of irradiation, except for oxidative stress tests. The extract was able to reduce the UVB-induced cytotoxicity and genotoxicity (studied by comet and micronucleous assays) at lower doses. *V. myrtillus* extract reduced lipid peroxidation UVB-induced, but had no effect against the ROS UVB-produced. With UVA-induced damage *V. myrtillus* reduced genotoxicity as well as the unbalance of redox intracellular status. Moreover our extract reduced the UVA-induced apoptosis, but had no effect against the UVB one. *V. myrtillus* extract showed its free radical scavenging properties reducing oxidative stress and apoptotic markers, especially in UVA-irradiated cells.*

Anthocyanins, but not anthocyanidins, from bilberry (*Vaccinium myrtillus* L.) alleviate pruritus via inhibition of mast cell degranulation.

J Food Sci. 2012 Dec;77(12):H262-7. doi: 10.1111/j.1750-3841.2012.02974.x. Epub 2012 Nov 19.

We have previously reported that bilberry anthocyanins exhibit an anti-pruritic effect in a mouse model of allergic contact dermatitis. It has been reported that anthocyanins are particularly sensitive to thermal treatment and are easily hydrolyzed to anthocyanidins when exposed to high temperatures. The objective of this study was to compare the anti-pruritic effect of anthocyanin-rich quality-controlled bilberry extract and anthocyanidin-rich degraded extract using a mouse model of allergic contact dermatitis. BALB/c mice with allergic contact dermatitis induced by 4 weeks of repeated application of 2,4,6-trinitro-1-chlorobenzene (TNCB) were administered Bilberon-25 orally for 4 weeks after sensitization with TNCB. The effect of Bilberon-25 on pruritus was evaluated by measurement of scratching behavior. RBL-2H3 mast cells were used to investigate the effect of Bilberon-25 on degranulation in 48/80-stimulated mast cells. Compared with nonheated Bilberon-25, the proportion of anthocyanins in heated Bilberon-25 decreased, and the proportion of anthocyanidins was increased in heated-time dependent manner. Treatment with non-heated Bilberon-25 significantly attenuated the TNCB-induced increase in scratching behavior, whereas treatment with 2 h-heated Bilberon-25 did not. Moreover, 300 µg/mL nonheated Bilberon-25 showed significant inhibition of degranulation in RBL-2H3 mast cells, whereas 2 h-heated Bilberon-25 had no effect at any concentration studied. It is assumed that the inhibitory effect of bilberry anthocyanins on pruritus might be mediated, at least in part, by its inhibitory effect on mast cell degranulation. In conclusion, the anthocyanin-rich but not anthocyanidin-rich bilberry extract may be a useful dietary supplement for skin diseases involving pruritic symptoms, such as chronic allergic contact dermatitis, atopic dermatitis, and rhinitis.

Anthocyanins from bilberry (*Vaccinium myrtillus* L.) alleviate pruritus in a mouse model of chronic allergic contact dermatitis.

Pharmacognosy Res. 2011 Jul; 3(3):173-7. doi: 10.4103/0974-8490.85002.

BACKGROUND: Bilberry (*Vaccinium myrtillus* L.) is one of the richest sources of anthocyanins which are known to have anticancer, wound healing and anti-allergic effects. Here, we examined whether bilberry extract (Bilberon-25) alleviates pruritus in a mouse model of chronic allergic contact dermatitis. *MATERIALS AND METHODS:* BALB/c mice with chronic allergic contact dermatitis induced by 3 weeks of repeated application of 2,4,6-trinitro-1-chlorobenzene (TNCB) were administered Bilberon-25 orally for 3 weeks after sensitization with TNCB. The effects of Bilberon-25 on pruritus and inflammation were evaluated by measurement of scratching behaviour and ear swelling, respectively. *RESULTS:* Treatment with Bilberon-25 significantly attenuated the TNCB-induced increase in scratching behaviour, but dexamethasone did not. In contrast, ear swelling was ameliorated by dexamethasone treatment, and significantly decreased by Bilberon-25. Repeated application of TNCB induced a shift in the cutaneous cytokine milieu from a T helper cell type (Th)1 to a Th2 profile; Bilberon-25 and dexamethasone alleviated this Th2 predominance of the lesional skin.

CONCLUSION: Anthocyanins from bilberry might be beneficial for the treatment of chronic pruritus which can occur in patients with inflammatory skin diseases such as atopic dermatitis.

Lonicera caerulea and Vaccinium myrtillus fruit polyphenols protect HaCaT keratinocytes against UVB-induced phototoxic stress and DNA damage.

J Dermatol Sci. 2009 Dec; 56(3):196-204. doi: 10.1016/j.jdermsci.2009.08.004. Epub 2009 Sep 10.

BACKGROUND: Sunlight is a very potent environmental factor in skin pathogenesis and can induce skin cancer. UVB irradiation is known to cause oxidative stress, inflammation and especially DNA damage. Topical application of agents with UV absorbing, antioxidant and anti-inflammatory activities is a successful strategy in the protection of the skin against UV-caused damage. *OBJECTIVE:* To examine the ability of the phenolic fraction of *Lonicera caerulea* and *Vaccinium myrtillus* fruits to moderate UVB-induced damage. *METHODS:* HaCaT keratinocytes, a well-established in vitro system for investigations on UV radiation induced cell damage, were used to assess the effects of pre- and post-treatment with *L. caerulea* (LCE) and *V. myrtillus* (VME) phenolic fractions (5-50 mg/l) on keratinocyte damage induced by a solar simulator (295-315 nm). *RESULTS:* In this study, a model of UVB-induced damage to HaCaT was established. LCE and VME efficiently reduced the extent of DNA breakage (especially at concentrations of 25 and 10 mg/l) together with caspase-3 and -9 activity and DNA laddering induced by UVB (100 or 200 mJ/cm²). LCE and VME significantly decreased RONS generation and partially diminished IL-6 expression. LCE pre-treatment also prevented keratinocytes proliferation. *CONCLUSION:* The results suggest that the phenolic fraction of *L. caerulea* and *V. myrtillus* fruits suppress UVB-caused injury to keratinocytes. These results now need to be demonstrated in vivo.

Bilberry extract reduces UVA-induced oxidative stress in HaCaT keratinocytes: a pilot study.

Biofactors. 2008; 33(4):249-66.

Exposure to UVA radiation is known to cause many adverse biological effects by inducing the stricken cells to produce reactive oxygen species (ROS). In recent years the use of botanicals has received considerable interest in the skin protection. Bilberry (*Vaccinium myrtillus* L.) fruit contains several polyphenols with strong antioxidant and anti-inflammatory properties. In this study we evaluated potential UVA preventive effect of *V. myrtillus* fruit extract (VME; anthocyanins, 25% w/w) in HaCaT keratinocytes. Pre-treatment (1 h) or post-treatment (4 h) of HaCaT with VME resulted in attenuation of UVA-caused damage. Application of the extract significantly reduced UVA-stimulated ROS formation in keratinocytes. VME also prevented/reduced UVA-caused peroxidation of membrane lipids and depletion of intracellular GSH. The observed cytoprotective effect may be linked to the antioxidant activity of the plant constituents, namely anthocyanins.

REVIEWS

Health effects of *Vaccinium myrtillus* L.: evaluation of efficacy and technological strategies for preservation of active ingredients.

Mini Rev Med Chem. 2014; 14(7):567-84.

Bilberries are a rich dietary source of various phytonutrients, including anthocyanins which contribute greatly to their antioxidant capacity and have demonstrated a broad spectrum of biomedical functions. These include protection against cardiovascular disorders, age-induced oxidative stress, inflammatory responses and several degenerative diseases. Berry anthocyanins also improve neuronal and cognitive brain functions, ocular health as well as protecting genomic DNA integrity. In recent years, sales of many dietary supplements/pharmaceutical products containing anthocyanins in various dosages and formulations have been made by advertising their wide range of beneficial effects. However, there is a heightened risk of distributing deteriorated formulations to consumers due to lax regulations, in particular those applicable to phytochemical characterization and extract standardization, and in terms of quality regarding the stability of anthocyanins. Anthocyanin pigments readily degrade during industrial processing and this can have a dramatic impact on color quality and may also affect nutritional/pharmaceutical properties. This review aims to summarize the main health effects of bilberry extract used in several food supplements/pharmaceutical formulations focusing on some important aspects of anthocyanin degradation during processing and storage. It will also describe the main technological strategies which can give active ingredients greater stability, solubility and dispersibility in order to enhance formulation quality which is of great interest to the consumer and industry due to its direct and indirect impact on consumer health.

Bilberry (*Vaccinium myrtillus* L.).

Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition. Boca Raton (FL): CRC Press/Taylor & Francis; 2011. Chapter 4.

*Bilberry (*Vaccinium myrtillus* L.) is one of the richest natural sources of anthocyanins. These polyphenolic components give bilberry its blue/black color and high antioxidant content, and they are believed to be the key bioactives responsible for the many reported health benefits of bilberry and other berry fruits. Although bilberry is promoted most commonly for improving vision, it has been reported to lower blood glucose, to have anti-inflammatory and lipid-lowering effects, and to promote antioxidant defense and lower oxidative stress. Therefore, bilberry is of potential value in the treatment or prevention of conditions associated with inflammation, dyslipidemia, hyperglycemia or increased oxidative stress, cardiovascular disease (CVD), cancer, diabetes, and dementia and other age-related diseases. There are also reports that bilberry has antimicrobial activity. In this chapter, bilberry and its components and characteristics are described, and evidence for the health benefits of bilberry is presented and discussed.*