BLACK RASPBERRY COMPONENTS INHIBIT PROLIFERATION, INDUCE APOPTOSIS, AND MODULATE GENE EXPRESSION IN RAT ESOPHAGEAL EPITHELIAL CELLS.

We have shown that a diet containing freeze-dried black raspberries (BRB) inhibits the development of chemically induced cancer in the rat esophagus. To provide insights into possible mechanisms by which BRB inhibit esophageal carcinogenesis, we evaluated an ethanol (EtOH) extract of BRB, and two component anthocyanins (cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside) in BRB, for their effects on growth, apoptosis, and gene expression in rat esophageal epithelial cell lines. The EtOH extract and both anthocyanins selectively caused significant growth inhibition and induction of apoptosis in a highly tumorigenic cell line (RE-149 DHD) but not in a weakly tumorigenic line (RE-149). The uptake of anthocyanins from the EtOH extract into RE-149 DHD cells far exceeded their uptake into RE-149 cells, which may have accounted for the selective effects of the extract on growth and apoptosis of RE-149 DHD cells. The growth inhibitory and proapoptotic effects were enhanced by the daily addition of the EtOH extract and the anthocyanins to the medium. Interestingly, the EtOH extract did not alter cyclooxygenase-2 (COX-2) and nitric oxide synthase (i-NOS) expression in RE-149 DHD cells, whereas both anthocyanins downregulated the expressions of these genes. This differential effect may have been related to the relative amounts of anthocyanins in the extract vs. when they were added individually to the medium. We conclude that the selective effects of the EtOH extract on growth and apoptosis of highly tumorigenic rat esophageal epithelial cells in vitro may be due to preferential uptake and retention of its component anthocyanins, and this may also be responsible for the greater inhibitory effects of freeze-dried whole berries on tumor cells in vivo.

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INDUCTION OF APOPTOSIS IN HUMAN COLON CANCER HCT-116 CELLS BY ANTHOCYANINS THROUGH SUPPRESSION OF AKT AND ACTIVATION OF P38-MAPK.

Anthocyanins belong to a class of flavonoids that exhibit important antioxidant and anti-inflammatory actions as well as chemotherapeutic effects. However, little is known concerning the molecular mechanisms by which these activities are exerted. In this study, we investigated the anthocyanins isolated from Vitis coignetiae Pulliat for their potential anti-proliferative and apoptotic effects on human colon cancer HCT-116 cells. These anthocyanins inhibited cell viability and induce apoptotic cell death of HCT-116 cells in a dose-dependent manner. The apoptotic cell death was caspase-dependent and the anthocyanins regulated anti-apoptotic proteins (IAPs). In addition, apoptosis was associated with activation of p38-MAPK and suppression of Akt. In conclusion, this study suggests that the anthocyanins isolated from Vitis coignetiae Pulliat induce apoptosis might at least in part through activating p38-MAPK and suppressing Akt in human colon cancer HCT-116 cells.


INDUCTION OF APOPTOSIS AND INHIBITION OF INVASION IN HUMAN HEPATOMA CELLS BY ANTHOCYANINS FROM MEORU.

Anthocyanins belong to a class of flavonoids exhibiting antioxidant and anti-inflammatory actions as well as a variety of chemotherapeutic effects. However, little is known about the cellular and molecular mechanism of anticancer activity. In this study, we investigated the anthocyanins (delphinidin-3,5-diglucoside: cyanidin-3,5-diglucoside: petunidin-3,5-diglucoside: delphinidin-3-glucoside: malvidin-3,5-diglucoside: peonidin-3,5-diglucoside: cyanidin-3-glucoside: petunidin-3-glucoside: peonidin-3-glucoside: malvidin-3-glucoside = 27:63:8.27:1:2.21:2.21:6.7:1.25:5.72:1.25) [corrected] isolated from meoru (Vitis coignetiae Pulliat) exerted antiproliferative and anti-invasive and apoptotic effects on human hepatoma Hep3B cells. It was found that the anthocyanins could inhibit cell growth by 75% at the concentration of 400 microg/mL for 48 h. Flow cytometric analysis showed that the anthocyanins increased the amount of DNA fragments (sub-G1 fraction) in a dose-dependent manner, which is closely related to mitochondrial dysfunction and reduction in antiapoptotic proteins (Bcl-2, XIAP, cIAP-1, and cIAP-2). The anthocyanins also significantly inhibited the migration and invasion of Hep3B cells through a matrigel-coated chamber. Taken together this study indicates that the anthocyanins from meoru have antiproliferative and anti-invasive effects and may induce apoptosis through the activation of the mitochondrial pathway and inhibition of antiapoptotic proteins. This study provides evidence that the anthocyanins isolated from meoru might be useful in the treatment of human hepatitis B-associated hepatoma.

Consumption of flavonoid-rich foods and beverages is thought to reduce the risk of cardiovascular diseases. Whereas the biological activities of flavonoids have been characterized in vitro, there are no clear experimental data demonstrating that chronic dietary intake and intestinal absorption of flavonoids actually protects the heart against ischemia-reperfusion injury. We tested whether long-term consumption of specific flavonoids (anthocyanins) included in normal food could render the heart of rats more resistant to myocardial infarction. Maize kernels that differed specifically in their accumulation of anthocyanins were used to prepare rodent food in which anthocyanins were either present or absent. Male Wistar rats were fed the anthocyanin-rich (ACN-rich) or the anthocyanin-free (ACN-free) diet for a period of 8 wk. Anthocyanins were significantly absorbed and detected in the blood and urine of only rats fed the ACN-rich diet. In Langendorff preparations, the hearts of rats fed the ACN-rich diet were more resistant to regional ischemia and reperfusion insult. Moreover, on an in vivo model of coronary occlusion and reperfusion, infarct size was reduced in rats that ate the ACN-rich diet than in those that consumed the ACN-free diet (P<0.01). Cardioprotection was associated with increased myocardial glutathione levels, suggesting that dietary anthocyanins might modulate cardiac antioxidant defenses. Our findings suggest important potential health benefits of foods rich in anthocyanins and emphasize the need to develop anthocyanin-rich functional foods with protective activities for promoting human health.


**BERRY MEALS AND RISK FACTORS ASSOCIATED WITH METABOLIC SYNDROME.**

Background/Objectives: Nonalcoholic fatty liver disease is commonly associated with obesity, insulin resistance, dyslipidemia and type 2 diabetes, and can thus be regarded as the hepatic manifestation of metabolic syndrome. In this study we compared the effects of lifestyle intervention with and without industrial berry products, on risk factors associated with metabolic syndrome on slightly overweight women. Subjects/Methods: Sixty-one female volunteers (average age 42.9 years) were recruited and randomized for a 20-week dietary intervention trial with two parallel treatment groups, one lifestyle intervention group with berry products equaling with an average daily dose of 163 g of northern berries (berry group, diet 1, N=31, of which 28 completed the study) and the other group with lifestyle intervention only (control group, diet 2, N=30, of which 22 completed the study). Results:Increased berry consumption as part of the normal daily diet was the only lifestyle difference between the two intervention groups. The major effects achieved by diet 1 were changes in the levels of alanine aminotransferase (ALAT) and adiponectin (at P-values <0.001 and 0.002, respectively). A statistically significant difference between the two intervention groups was the higher decrease in the ALAT value in the berry group (P=0.003).Conclusions: The 23% decrease in the ALAT value, from 20.29 to 15.66 U/l in the berry group may be regarded as nutritionally significant by enhancing the liver function. This may contribute positively to the low-grade systemic inflammation in body and decrease the risk of cardiovascular diseases.

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**BERRIES MODIFY THE POSTPRANDIAL PLASMA GLUCOSE RESPONSE TO SUCROSE IN HEALTHY SUBJECtS.**

Sucrose increases postprandial blood glucose concentrations, and diets with a high glycaemic response may be associated with increased risk of obesity, type 2 diabetes and CVD. Previous studies have suggested that polyphenols may influence carbohydrate digestion and absorption and thereby postprandial glycaemia. Berries are rich sources of various polyphenols and berry products are typically consumed with sucrose. We investigated the glycaemic effect of a berry purée made of bilberries, blackcurrants, cranberries and strawberries, and sweetened with sucrose, in comparison to sucrose with adjustment of available carbohydrates. A total of twelve healthy subjects (eleven women and one man, aged 25-69 years) with normal fasting plasma glucose ingested 150 g of the berry purée with 35 g sucrose or a control sucrose load in a randomised, controlled cross-over design. After consumption of the berry meal, the plasma glucose concentrations were significantly lower at 15 and 30 min (P < 0.05, P < 0.01, respectively) and significantly higher at 150 min (P < 0.05) compared with the control meal. The peak glucose concentration was reached at 45 min after the berry meal and at 30 min after the control meal. The peak increase from the baseline was 1.0 mmol/l smaller (P = 0.002) after ingestion of the berry meal. There was no statistically significant difference in the 3 h area under the glucose response curve. These results show that berries rich in polyphenols decrease the postprandial glucose response of sucrose in healthy subjects. The delayed and attenuated glycaemic response indicates reduced digestion and/or absorption of sucrose from the berry meal.


**INGESTION OF BLACK CHOEKERRY FRUIT EXTRACT LEADS TO INTESTINAL AND SYSTEMIC CHANGES IN A RAT MODEL OF PREDIABETES AND HYPERLIPIDEMIA.**

This report presents a complex analysis of changes proceeding in the gut, blood and internal organs of rats with induced oxidative stress, glucose intolerance and hyperlipidemia after dietary supplementation with an extract from black chokeberry
ANTIOXIDANT CAPACITY AND OTHER BIOACTIVITIES OF THE FREEZE-DRIED AMAZONIAN PALM BERRY, EUTERPE OLERACEAE MART. (ACAI).

The fruit of Euterpe oleracea, commonly known as acai, has been demonstrated to exhibit significantly high antioxidant capacity in vitro, especially for superoxide and peroxyl scavenging, and, therefore, may have possible health benefits. In this study, the antioxidant capacities of freeze-dried acai fruit pulp/skin powder (OptiAcai) were evaluated by different assays with various free radical sources. It was found to have exceptional activity against superoxide in the superoxide scavenging (SOD) assay, the highest of any food reported to date against the peroxyl radical as measured by the oxygen radical absorbance capacity assay with fluorescein as the fluorescent probe (ORACFL), and mild activity against both the peroxynitrite and hydroxyl radical by the peroxynitrite averting capacity (NORAC) and hydroxyl radical averting capacity (HORAC) assays, respectively. The SOD of acai was 1,614 units/g, an extremely high scavenging capacity for O2^-. by far the highest of any fruit or vegetable tested to date. Total phenolics were also tested as comparison. In the total antioxidant (TAO) assay, antioxidants in acai were differentiated into “slow-acting” and “fast-acting” components. An assay measuring inhibition of reactive oxygen species (ROS) formation in freshly purified human neutrophils showed that antioxidants in acai are able to enter human cells in a fully functional form and to perform an oxygen quenching function at very low doses. Furthermore, other bioactivities related to anti-inflammatory and immune functions were also investigated. Acai was found to be a potential cyclooxygenase (COX)-1 and COX-2 inhibitor. It also showed a weak effect on lipopolysaccharide (LPS)-induced nitric oxide but no effect on either lymphocyte proliferation and phagocytic capacity.

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TOTAL OXIDANT SCAVENGING CAPACITIES OF EUTERPE OLERACEA MART. (ACAI) FRUITS.

The antioxidant capacities of 11 commercial and non-commercial samples of Euterpe oleracea Mart. (acai) fruit pulp were studied with the total oxidant scavenging capacity assay in a modified and automated version against three reactive oxygen species. The antioxidant capacities of all purple acai samples were found to be excellent against peroxyl radicals, good against peroxynitrite and poor against hydroxyl radicals compared with common European fruit and vegetable juices recently analysed. In all cases the correlation between sample concentration and antioxidant capacities was non-linear. The antioxidant capacities against all three reactive oxygen species of the fruit pulp from one white acai variety were very low. The phenolic compounds in purple acai fruit pulp were identified by high-performance liquid chromatography-mass spectrometry, and the two major anthocyanins, cyanidin-3-glucoside and cyanidin-3-rutinoside, were quantified by high-performance liquid chromatography-visible spectrometry. The contributions of the anthocyanins to the overall antioxidant capacities of the fruit were estimated to be only approximately 10%. Obviously, compounds not yet identified are responsible for the major part of the antioxidant capacities of the acai pulp.


PHYTOCHEMICAL AND NUTRIENT COMPOSITION OF THE FREEZE-DRIED AMAZONIAN PALM BERRY, EUTERPE OLERACEAE MART. (ACAI).

Euterpe oleracea is a large palm tree indigenous to the Amazon River and its tributaries and estuaries in South America. Its fruit, known as acai, is of great economic value to native people. In this study, a standardized freeze-dried acai fruit pulp/skin powder was used for all analyses and tests. Among many findings, anthocyanins (ACNs), proanthocyanidins (PACs), and other flavonoids were found to be the major phytochemicals. Two ACNs, cyanidin 3-glucoside and cyanidin 3-rutinoside were found to be predominant ACNs; three others were also found as minor ACNs. The total content of ACNs was measured as 3.1919 mg/g dry weight (DW). Polymers were found to be the major PACs. The concentration of total PACs was calculated as 12.89 mg/g DW. Other flavonoids, namely, homoorientin, orientin, isovitexin, scoparin, and taxifolin deoxyhexose, along with several unknown flavonoids, were also detected. Resveratrol was found but at a very low concentration. In addition, components including fatty acids, amino acids, sterols, minerals, and other nutrients were analyzed and quantified. Total polyunsaturated fatty acid,
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stimulate signal transduction pathways influencing genes controlled by the antioxidant response element.

phenolics from bilberry upregulate the oxidative stress defense enzymes HO$_1$ and GST$_	ext{pi}$ in RPE, suggesting that they stimulate signal transduction pathways influencing genes controlled by the antioxidant response element.

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ANTHOCYANINS, PHENOLICS, AND ANTIOXIDANT CAPACITY IN DIVERSE SMALL FRUITS: VACCINIUM, RUBUS, AND RIBES.

Fruits from 107 genotypes of Vaccinium L., Rubus L., and Ribes L., were analyzed for total anthocyanins (ACY), total phenolics (TPH), and antioxidant capacities as determined by oxygen radical absorbing capacity (ORAC) and ferric reducing antioxidant power (FRAP). Fruit size was highly correlated (r = 0.84) with ACY within Vaccinium corymbosum L., but was not correlated to ACY across eight other Vaccinium species, or within 27 blackberry hybrids. Certain Vaccinium and Ribes fruits with pigmented flesh were lower in ACY, TPH, ORAC, and FRAP compared to those values in berries with nonpigmented flesh. ORAC values ranged from 19 to 131 micromol Trolox equivalents/g in Vaccinium, from 13 to 146 in Rubus, and from 17 to 116 in Ribes. Though ACY may indicate TPH, the range observed in ACY/TPH ratios precludes prediction of ACY from TPH and vice versa for a single genotype. In general, TPH was more highly correlated to antioxidant capacity than ACY was. This study demonstrates the wide diversity of phytochemical levels and antioxidant capacities within and across three genera of small fruit.


PHARMACOKINETICS OF ANTHOCYANINS AND ANTIOXIDANT EFFECTS AFTER THE CONSUMPTION OF ANTHOCYANIN-RICH ACAI JUICE AND PULP (EUTERPE OLERACEA MART.) IN HUMAN HEALTHY VOLUNTEERS.

The acai berry is the fruit of the acai palm and is traditionally consumed in Brazil but has gained popularity abroad as a food and functional ingredient, yet little information exists on its health effect in humans. This study was performed as an acute four-way crossover clinical trial with acai pulp and clarified acai juice compared to applesauce and a non-antioxidant beverage as controls. Healthy volunteers (12) were dosed at 7 mL/kg of body weight after a washout phase and overnight fast, and plasma was repeatedly sampled over 12 h and urine over 24 h after consumption. Noncompartmental pharmacokinetic analysis of total anthocyanins quantified as cyanidin-3-O-glucoside showed Cmax values of 2,321 and 1,138 ng/L at t max times of 2.2 and 2.0 h, and AUC last values of 8,568 and 3,314 ng h L$^{-1}$ for pulp and juice, respectively. Nonlinear mixed effect modeling identified dose volume as a significant predictor of relative oral bioavailability in a negative nonlinear relationship for acai pulp and juice. Plasma antioxidant capacity was significantly increased by the acai pulp and applesauce. Individual increases in plasma antioxidant capacity of up to 2.3- and 3-fold for acai juice and pulp, respectively were observed. The antioxidant capacity in urine, generation of reactive oxygen species, and uric acid concentrations in plasma were not significantly altered by the treatments. Results demonstrate the absorption and antioxidant effects of anthocyanins in acai in plasma in an acute human consumption trial.

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BILBERRY (VACCINIUM MYRTILLUS) ANTHOCYANINS MODULATE HEME OXYGENASE-1 AND GLUTATHIONE S-TRANSFERASE-Pi EXPRESSION IN ARPE-19 CELLS.

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 dichlorofluorescin 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.

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ACTIVITY OF ANTHOCYANINS FROM FRUIT EXTRACT OF RIBES NIGRUM L. AGAINST INFLUENZA A AND B VIRUSES.
Earlier, we have detected antiviral activity in an extract from Ribes nigrum L. fruits (“Kurokarin”, name of the one species of black currant in Japanese) against influenza A and B viruses, and herpes simplex virus 1 (Knox et al., Food Processing 33, 21-23, 1998). In the present study, the antiviral activity of constituents of a Kurokarin extract and the mechanism of its antiviral action were examined. Kurokarin extracts were separated to fractions A to D by column chromatography. The major constituents of the fraction D were estimated as anthocyanins. The fraction D was further fractionated by thin-layer chromatography (TLC) to fractions A’ to G’. The fraction E’ consisted of 3-O-alpha-L-rhamnopyranosyl-beta-D-glucopyranosyl-cyanidin and 3-O-beta-D-glucopyra-nosyl-cyanidin, and the fraction F’ consisted of 3-O-alpha-L-rhamno-
pyranosyl-beta-D-glucopyranosyl-delphinidin and 3-O-beta-D-glucopyranosyl-delphinidin, identified by high performance liquid chromatography (HPLC) with standards and by high resolution mass spectrometry. The fractions D’ to G’ showed potent antiviral activity against influenza viruses A and B. The additive antiviral effect of a combination of the fractions E’ and F’ was assessed. Anthocyanins in the fraction F’ did not directly inactivate influenza viruses A and B, but they inhibited virus adsorption to cells and also virus release from infected cells.


CYTOTOXIC EFFECTS OF BILBERRY EXTRACT ON MCF7-GFP-TUBULIN BREAST CANCER CELLS.

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.

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