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Cytotoxic Effects of Bilberry Extract on MCF7-GFP-Tubulin Breast Cancer Cells

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ABSTRACT 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 (~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.

KEY WORDS: • ***anthocyanin*** • ***bilberry extract*** • ***cytotoxicity*** • ***mitosis***

INTRODUCTION

ANTHOCYANINS ARE THE BRIGHT color-containing compounds in bilberries, blueberries, and other fruits.[1] There are many reports regarding the anticancer effects of anthocyanin complexes derived from bilberries (*Vaccinium myrtillus*) and other related berries.[1–4] Purified anthocyanins have been shown to inhibit lipid peroxidation, cyclooxygenase, and human tumor cell proliferation.[5] Anthocyanin-rich extracts from berries have been shown to inhibit multiple biomarkers of colon cancer cells[6] and proliferation of human gastric adenocarcinoma cells.[7] Anthocyanins have also been shown to induce apoptosis in cancer cells, including human gastric adenocarcinoma,[7,8] hepatoma,[9] and human leukemia[10,11] cells, and in uterine and colon cell lines.[12] They also inhibit migration and invasion of human lung carcinoma cells[13] and potently inhibit the epidermal growth factor receptor.[14] The effects of anthocyanins[7] and specifically pro-delphinidin[15,16] have been shown to affect cell cycle progression at the G_0/G_1 phase in human gastric adenocarcinoma AGS cells and human non–small cell lung cancer A549 cells, respectively.

Because consumption of berries is the predominant means of anthocyanin ingestion, this means human exposure to these putative anticancer agents is via a mixture of several anthocyanins as well as other berry molecular components. Given the potential for synergistic action of mixtures of anthocyanins[17] and that these anthocyanins are typically consumed through diet in the form of berries, we wanted to analyze the cytotoxic effects of a full extract from bilberries.

One of the major mechanisms of cancer suppression involves induction of apoptosis.[18,19] Bilberry extract has been shown to induce apoptotic cell bodies and nucleosomal DNA fragmentation in human leukemia HL60 cells[8] and in human colon cancer HT-29 cells.[20] To study bilberry extract's ability to inhibit proliferation in association with induction of apoptosis, we used a sulforhodamine B cell proliferation assay together with terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) and

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