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Chemical Analysis and Effect of Blueberry and Lingonberry Fruits and Leaves against Glutamate-Mediated Excitotoxicity

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ABSTRACT: Phenolic compounds are a large class of phytochemicals that are widespread in the plant kingdom and known to have antioxidant capacities. This study aimed to determine the antioxidant capacities as well as the content of total soluble phenolics, anthocyanins, tannins, and flavonoids in the fruits and leaves of blueberries and lingonberries growing in Newfoundland. This study also determined the potential neuroprotective effect of extracts from fruits and leaves against glutamate-mediated excitotoxicity, which is believed to contribute to disorders such as stroke and neurodegenerative diseases. Lingonberry and blueberry plants were found to be rich sources of phenolic compounds. Total antioxidant capacities in terms of radical scavenging activity and reducing power were much higher in leaves of both plants as compared to their fruits. These results were in correlation with phenolic contents including total flavonoids, anthocyanins, and tannins. Brain-derived cell cultures from rats were prepared and grown for about 2 weeks. Cell cultures were treated with glutamate (100 μM) for 24 h, and the effect of extracts was determined on cells subjected to this excitotoxicity. Glutamate treatment caused a ~23% cell loss when measured after 24 h of exposure. Whereas lingonberry fruit extract did not provide protection from glutamate toxicity, blueberry fruit extracts were extremely protective. Leaf extracts of both lingonberry and blueberry showed a significant neuroprotective effect. The greater protective effect of leaf extracts was in correlation with the levels of phenolics and antioxidant capacity. These findings suggest that berries or their components may contribute to protecting the brain from various pathologies.

KEYWORDS: *antioxidants, berries, oxidative stress, phenolic compounds*

INTRODUCTION

Overproduction of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, and peroxyl radicals, and reactive nitrogen species (RNS), such as nitric oxide and peroxynitrite radicals, could lead to oxidative stress and nitrosative stress, respectively. These reactive species can damage proteins, lipids, and DNA, leading to lipid peroxidation, altered signal transduction pathways, and the destruction of membranes and organelles, which could be responsible for the development of several disorders, including cancer, cardiovascular disease, diabetes, and neurodegeneration.[1−3] The brain is particularly susceptible to oxidative stress pertaining to its high oxygen demand, as well as the fact that it is enriched with polyunsaturated fatty acids. Moreover, a high iron concentration and low levels of antioxidants are also factors responsible for overproduction of ROS and RNS in brain cells.[2,4] It has been reported by several researchers that excessive production of oxidative and nitrosative compounds is associated with brain aging and neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases.[3−7] The balance between ROS and antioxidants in biological systems is referred to as redox homeostasis, which is essential for normal cell function.[8] To combat oxidative stress, there are several types of endogenous enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase, as well as nonenzymatic glutathione; and there are also several nonenzymatic antioxidants that are obtained primarily in the diet, including tocopherol, ascorbate, carotenoids, and various polyphenolic compounds.[1,2]

Polyphenols are a large class of natural compounds that have high antioxidant capacity and potential beneficial effects such as anti-inflammatory, anticancer, antifungal, antimicrobial, and antiulcer properties.[9] These classes of compounds also appear to have positive effects on the cardiovascular system, which may be due to their ability to act as free radical scavengers or by other mechanisms.[10] Polyphenols are abundant in plants, especially vegetables and fruits. Berry crops are very rich sources of polyphenolic antioxidants, particularly flavonoid compounds.[11] Because plant-derived supplements are considered as natural and hence potentially safer than synthetic drugs, there has been an increasing demand for "nutraceuticals".[12] The term nutraceutical was originally defined by Dr. Stephen DeFelice as daily nutritional supplements such as food or a part of food, which have beneficial effects in treating or preventing diseases.[13] Berries have been reported to have diverse health-promoting phytochemicals and are very rich sources of polyphenolics especially flavonoids, anthocyanins, and proanthocyanidins. An enormous body of research has been published suggesting that the dietary consumption of berries has positive effects on human health and diseases (see, e.g., refs 14 and 15). The health-promoting properties of berries are gaining continued interest in the berry market not only as antioxidants but also because of their bioactive properties in vivo.[14,16]

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In particular, the potential of berries and their constituents to protect the brain from aging and neurodegenerative disease has gained increased attention in recent years. For example, dietary supplementation with polyphenol-containing fruits can decrease age-related behavioral deficits in rats.[17] In a recent study conducted with a mouse model of Alzheimer's disease, treatment with berries rich in polyphenols decreased the extent of behavioral abnormalities associated with the disease.[18] Other experimental studies have shown that rats fed a diet enriched with blueberries can protect the brain against oxidative stress and associated learning deficits.[19] Surprisingly, a diet enriched with blueberries has been demonstrated to later protect animals from the damage induced by ischemic stroke.[20,21]

The present work aims to study the polyphenolic content, antioxidant capacity, and potential neuroprotective effects of fruit and leaf extracts from lowbush blueberries and lingonberries (also known as partridgeberries) that grow natively in Newfoundland, Canada. Following initial chemical analysis, cell cultures derived from rodent brains were subjected to high levels of glutamate, the most prominent endogenous excitatory neurotransmitter present in the mammalian central nervous system. Glutamate-induced "excitotoxicity" is a pathological process by which cells are damaged and killed by excessive stimulation from neurotransmitters such as glutamate and similar substances. This abnormal process produces oxidative and nitrosative stress and likely contributes to the pathology of traumatic brain injury, stroke, neurodegenerative disorders, and normal brain aging.[2,22,23] The plant species used in the current set of studies belong to the family Ericaceae and genus *Vaccinium* and are known to have numerous health benefits.[24−28] However, to our knowledge, the neuroprotective effects of blueberry leaf or lingonberries have not yet been studied, and hence this will be the first report in this field.

MATERIALS AND METHODS

Extract Preparation. Plant materials used in the present study are fruits and leaves of wild lowbush blueberry (*Vaccinium angustifolium* Aiton) and lingonberry (*Vaccinium vitis-idaea* L.). Blueberry samples were collected near Fort Amherst, St. John's, NL, Canada, in September 2011. Lingonberry samples were collected at Bauline, NL, Canada, in October 2011. Leaves and fruits were harvested and stored at −20 °C within an hour of collection. Sample extraction was carried out from the collected leaf and fruit samples in 80% (v/v) acetone with 0.2% formic acid in the ratio 1:2 and was subjected to 30 min of shaking on ice. This extraction procedure was used because acetone and formic acid were previously found to be the best extraction solvents among ethanol, methanol, and acetonitrile at various aqueous mixtures with different shaking periods.[29] The sample mixture was then centrifuged at 20000*g* for 20 min at 4 °C. Supernatant was collected, and the procedure was repeated with the residue. Both of the supernatants were mixed together, and the final concentrations for leaf and fruit samples were 25 and 250 mg/mL of fresh weight, respectively.

Biochemical Assays. Blueberry and lingonberry fruit and leaf extracts were further diluted 10 times for biochemical assays. Total soluble phenolic content in both leaves and fruits was determined using Folin−Ciocalteu reagent as described by Chandrasekara and Shahidi[30] with some modifications. Total soluble phenolic content of each sample was determined using a gallic acid standard curve and expressed as milligrams of gallic acid equivalents (GAE) per gram of berry or leaf fresh weight. Total anthocyanin content was measured by a pH differential method described by Foley and Debnath.[31] Absorption at 510 and 700 nm was measured in buffers at pH 1.0 and 4.5, and the difference between the two values was used to determine total anthocyanin content. Results are expressed as GAE. Total flavonoid content was measured by an aluminum chloride colorimetric assay,[32] and total flavonoid content was expressed as micromoles of catechin equivalent (CE) per gram of leaf or fruit. Proanthocyanidin contents (tannins) of sample extracts were determined according to the method developed by Chandrasekara and Shahidi,[30] and results were expressed as micromoles of CE per gram of leaf or fruit. Total antioxidant capacity of samples was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, which was conducted according to the method of Brand-Williams et al.[33] with some modifications. The gallic acid standard curve was used to express the results as GAE. The reducing power of extracts was determined according to the method described by Chandrasekara and Shahidi.[30] The results were expressed as ascorbic acid equivalents (AsA) using appropriate standard curves.

Cell Culture. The cell culture experiments utilized rat pups and were conducted in the School of Pharmacy at the Health Science Centre, Memorial University. All procedures using rat pups were approved by the Institutional Animal Care Committee of Memorial University of Newfoundland (protocol 12-20-JW). The brains of 1−3-day-old rat pups, which were supplied by Animal Care Services, were dissected, and cortical cultures were prepared as previously described.[34] Briefly, dissociated cortices were diluted in serum-containing media [Basal Medium Eagles (GIBCO, Grand Island, NY, USA) containing 10% horse serum (GIBCO), an antibiotic−antimycotic solution at a final concentration of 100 units/mL penicillin G, 100 μg/mL streptomycin sulfate, and 250 ng/mL amphotericin B (Sigma, St. Louis, MO, USA), 0.5% glucose (Sigma), 1 mM sodium pyruvate (GIBCO), and 1% N2 supplements (GIBCO)] to a concentration of 500,000 cells/mL; cells were then plated in 0.3 mL aliquots onto Costar 24-well polystyrene plates (Corning, NY, USA) coated overnight with poly-L-ornithine (500 μg/mL; Sigma). Poly-L-ornithine promotes the adhesion of cells to the culture wells. All cultures were maintained in a humidified incubator (5% CO_2, 37 °C). Half of the media in cultures was replaced 2 days after plating and then twice per week, with serum-free media containing 2% B27 supplements (GIBCO). In general, glia formed a confluent monolayer that adhered to the membrane substrate, whereas neurons adhered to the underlying glia. We have previously found that these cultures contain approximately 12% neurons as determined by NeuN, which is a neuronal marker expressed strongly in nuclei and perikarya,[34] with the remaining cells representing the glial population. Approximately 95% of NeuN-negative cells stained positively for glial fibrillary acidic protein (Invitrogen, Camarillo, CA, USA), suggesting that the majority of glial cells in these cultures are composed of astrocytes. These cultures were used for experiments at 9−16 days in vitro (DIV).

Glutamate Exposure and Extract Treatment. The cell culture experiments were carried out after the samples of extracts were filtered through a sterile filter of 40 μm pore size. We first tested whether the solvent used in the extraction of blueberry and lingonberry fruits and leaves had any effect on cell cultures before treating cells with the extract itself and determined the appropriate amount of solvent that did not affect the cultures. Glutamate was dissolved in sterile distilled water (dH_2O). Cell cultures were exposed to glutamate (100 μM) in a volume of 3 μL/0.3 mL of cell culture media, and control cultures received an equivalent volume of sterile dH_2O. Cell cultures were treated with 1 μL of lingonberry and blueberry fruit extracts (250 mg/mL) and leaf extract (25 mg/mL) at the time of glutamate exposure and were treated with the leaf and fruit extracts for 24 h. We found that cultures treated with 1 μL of solvent alone had no significant change in cell number after 24 h. For each treatment plate, at least two control treatments were performed using dH_2O as well as two glutamate treatments. Experiments were performed in at least three separate culture preparations, and each condition was represented by at least six samples.

Imaging, Histology, and Cell Counts. After 24 h of treatment, cell cultures were fixed for 20 min with 4% paraformaldehyde, according to the method of Engel et al.[35] Images were captured using a Zeiss Observer A1 microscope and a Pixelfly qe CCD camera (pco, Kelheim, Germany). Five images at a magnification of 200× were captured from different regions of each well. Images of 4′,6-diamidino-2-phenylindole (DAPI)-positive cells were captured with a DAPI

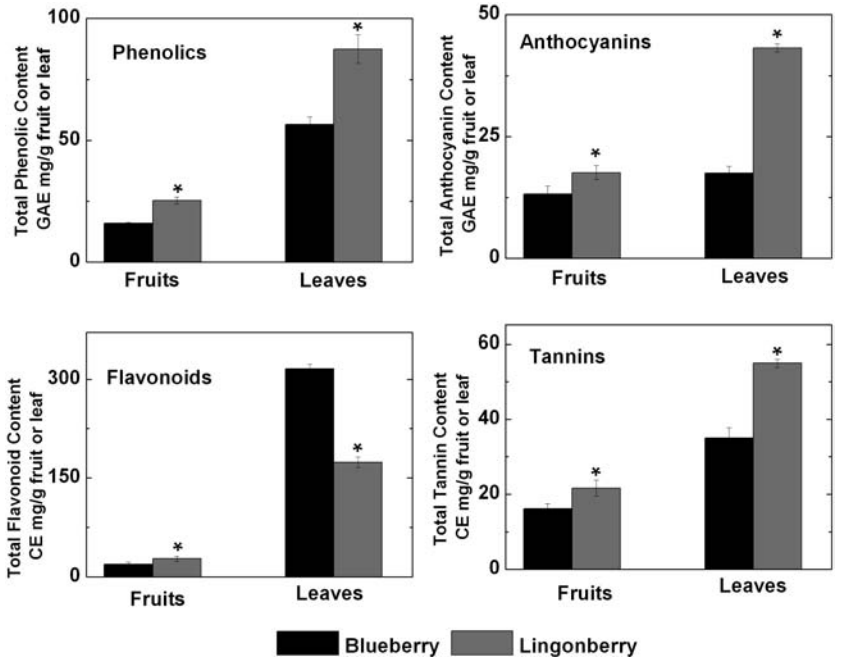

Figure 1. Total phenolic, anthocyanin, flavonoid, and tannin contents in blueberry and lingonberry extracts from fruits and leaves. Data are expressed as the mean ± SE, $n = 3$, for each experiment. ∗ indicates values differ significantly from blueberry data at $p < 0.05$ (nonparametric unpaired t test). The content of total soluble phenolics, flavonoids, anthocyanins, and tannins was significantly higher in the leaves of both species versus the respective fruits. GAE, gallic acid equivalents; CE, catechin equivalents.

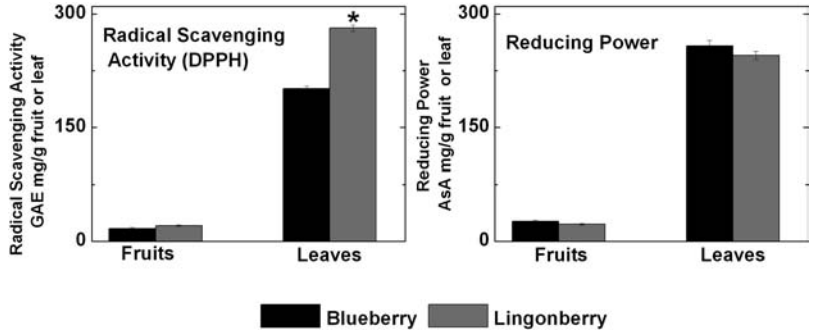

Figure 2. Total radical scavenging activity and reducing power in blueberry and lingonberry extracts from fruits and leaves. Data are expressed as the mean ± SE, $n = 3$, for each experiment. ∗ indicates values differ significantly from blueberry data at $p < 0.05$ (nonparametric unpaired t test). GAE, gallic acid equivalents; AsA, ascorbic acid equivalents.

optical filter set. At least two investigators who were blind to the treatment conditions were used to count the amount of cells in each image. The mean of two readings for each well was generated and calculated accordingly. Data on the number of DAPI-positive cells is expressed as a percent of control values for each given experimental day. Condensed nuclei data are expressed as the percent of the total amount of DAPI-positive cells that contained condensed nuclei within each condition. The percent of condensed nuclei was calculated using the program Image J.[36] Representative images of cellular morphology in control and glutamate-treated cultures were captured using differential interference contrast microscopy.

Statistical Analysis. All of the biochemical experiments were repeated at least three times. Data in the text and the figures are expressed as the mean ± SE of three replicates. Statistically significant differences were determined by the nonparametric unpaired t test using the statistical program SPSS (IBM Inc.). In all cases the confidence coefficient was set at 0.05. The data for the bioactivity (cell culture) experiments were analyzed with one-way ANOVA ($p < 0.05$) followed by Tukey's multiple-comparisons test using the statistical program GraphPad Prism (La Jolla, CA, USA). Data represented in figures are expressed as the means ± SE of at least six wells per condition. Significance was also set at $p < 0.05$ for these experiments.

RESULTS

Biochemical Assays. The content of total soluble phenolics, flavonoids, anthocyanins, and tannins was found to be significantly higher in lingonberry fruits as compared to blueberry fruits (Figure 1). This same trend was observed for leaves except for flavonoid content, in which the leaves of blueberry had a higher content as compared to those of lingonberry. The content of total soluble phenolics, flavonoids, anthocyanins, and tannins was significantly higher in the leaves of both species versus the fruits ($p < 0.05$). This latter finding correlated well with total radical scavenging capacity and reducing power, in which the leaves had much higher activity compared to fruits (Figure 2). The reducing power of the leaves of both plants was similar, but the radical scavenging capacity was significantly higher in lingonberry leaves versus blueberry leaves ($p < 0.05$). There was no significant difference

between the blueberry and lingonberry fruits in scavenging activity and reducing power.

Cell Culture Experiments. In preliminary studies we found that treatment of cultures with 10 μM glutamate produced a cell loss of 17.0 ± 5.7%, which was not statistically significant. However, 100 μM glutamate treatment for 24 h caused a significant loss of cells of 23.1 ± 5.1%; therefore, we used a concentration of 100 μM glutamate for the remaining experiments. Analysis of cultures using light microscopy indicated that the cells in control (untreated) cultures had intact cell bodies and that cells consistent with neuronal morphology also displayed intact, smooth neurites (Figure 3).

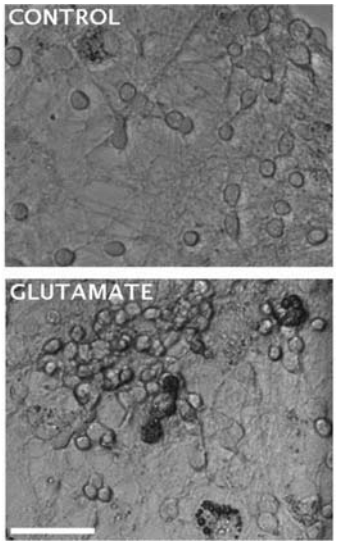

Figure 3. Light microscopic images of untreated cells (control; top) and cells treated with 100 μM glutamate for 24 h (bottom). Note that cells in control cultures had intact cell bodies and that cells consistent with neuronal morphology also displayed intact, smooth neurites. After treatment with glutamate for 24 h, many cells had disrupted cell bodies, and there was an increase in dark punctae. Scale bar = 50 μm and applies to both images. Cell cultures are 12 DIV.

After treatment with glutamate for 24 h, many cells had disrupted cell bodies, and there was an increase in dark punctae, which may be indicative of condensed nuclei in dead or dying cells.[37] We quantified the potential neuroprotective effects of the berry extracts by counting the amount of DAPI-stained cells that were present in cultures under various conditions (Figures 4 and 5). Treatment with glutamate appeared to increase the amount of brighter, condensed nuclei in cultures, which is often indicative of delayed cell death[34] (Figure 4). Glutamate-exposed cell cultures treated with blueberry and lingonberry fruit extracts showed 112.9 ± 15.1 and 79.1 ± 12.4% of control values, respectively, indicating high protection from blueberry fruit, but no protection from lingonberry fruit (Figure 5). Cultures treated with leaf extracts of blueberry and lingonberry showed 101.8 ± 5.1 and 106.9 ± 8.6% of control values, respectively, which was significantly different versus glutamate treatment alone, indicating a highly protective effect of the leaf extracts from both species (Figure 5). We also quantified the percentage of cells displaying condensed nuclei in the same cultures in which we determined the extent of cell loss. Glutamate caused an increase in the amount of condensed nuclei after 24 h of exposure, but this increase was not statistically significant. Interestingly, there also appeared to be an increase in condensed nuclei in cultures treated with blueberry or lingonberry leaf extract, but again these findings were not statistically significant.

DISCUSSION

In the current study, we were able to detect high levels of polyphenolic compounds, such as anthocyanins, flavonoids, and tannins, in blueberries and lingonberries (partridgeberries) growing natively in Newfoundland. We also observed that the level of phenolic compounds was higher in the leaves of both species compared to the respective fruits. The leaves also had much higher total antioxidant capacity than the fruits, as indicated by radical scavenging activity and reducing power, which is consistent with our previous findings in lingonberry cultivars.[29] These biochemical data are generally in line with the biological activities of the extracts on glutamate exposed rat brain cultures. For example, although the fruit extract of blueberries was highly protective against glutamate toxicity, lingonberry fruits were not protective. However, the extracts from leaves of both plant species were highly neuroprotective in our cell culture model.

Glutamate plays an important role in normal neurophysiology, such as a variety of cognitive functions,[38] and is involved as well with synaptic plasticity, which is believed to be the cellular mechanism of learning and memory.[39,40] However, increased glutamate levels result in glutamate-mediated excitotoxicity, which can lead to cell damage and death.[41] This phenomenon could be due to excessive glutamate release or inadequate uptake of glutamate by glial cells through transporters. This pathological process is believed to contribute to brain aging and neurodegeneration over many years, but can occur very rapidly during severe insults such as stroke and traumatic brain injury.[3,42] Excitotoxicity generally causes an excessive elevation of intracellular Ca^{2+} levels,[22,37] which causes changes in the normal functioning of neurons and can overactivate several Ca^{2+}-dependent enzymes leading to changes in normal cellular processes.[42−44] As a consequence of the activation of calcium-dependent enzymes such as xanthine oxidase,[45] phospholipase A_2,[42,46] and nitric oxide synthase, free radicals including ROS and RNS are produced in cells.[22] Excitotoxicity is also responsible for oxidative dysfunction in mitochondria,[47] which could lead to further ROS generation. Therefore, when cell cultures were exposed to glutamate, receptors were overactivated, which likely led to oxidative and nitrosative stress, and ultimately damaged and killed cells. However, elevated levels of Ca^{2+} can also activate other enzymes, such as endonucleases and proteases, which can degrade DNA and proteins, respectively.[42] Therefore, cell death may have occurred through mechanisms other than oxidative and nitrosative stress, and the protective effect of our berry extracts may not have been due to antioxidant and/or antinitrosative properties, but rather to other mechanisms. However, Ahn et al.[48] found that 100 μM glutamate causes hippocampal cell death by altered calcium signaling and nitrosative stress. Also, in our culture system, neurons generally constitute only ∼12% of the cells, with the remaining cells mostly made up by astrocytes. We observed ∼23% total cell death after glutamate exposure, suggesting that much of the cell death is represented by glial cell death. It is possible that glial cells died due to excessive glutamate uptake and cell swelling; however, Chen et al.[37] found that astrocytes exposed to glutamate primarily died due to oxidative stress. This finding suggests that berry extracts would likely protect glia through

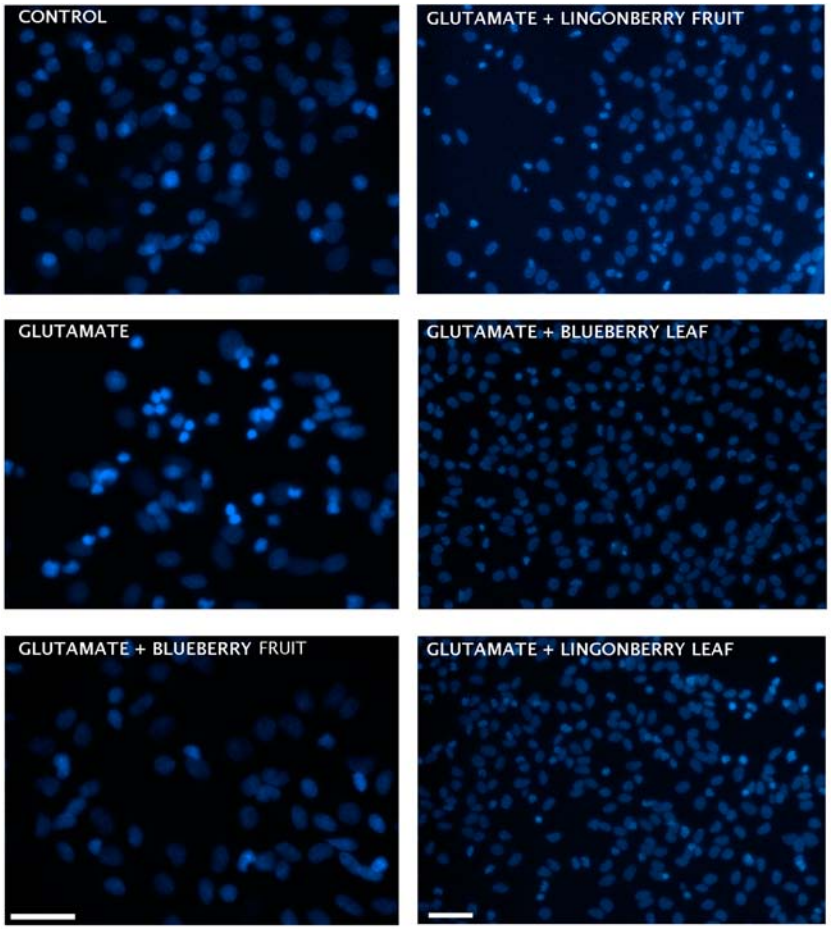

Figure 4. Representative images of cortical cells stained with DAPI (blue) in control conditions, after 24 h of treatment with 100 μM glutamate, and after 24 h treatment with 100 μM glutamate in the presence of blueberry and lingonberry fruit and leaf extracts. Note the presence of several condensed nuclei after treatment with glutamate, which may be indicative of delayed cell death. Images on the right are at a magnification of 200×, whereas images on the left have been enlarged to better represent nuclear morphology. Both scale bars = 50 μm. The scale bar in the glutamate + blueberry fruit images applies to all three images on the left, whereas the scale bar in the glutamate + lingonberry leaf image applies to the three images on the right. Cell cultures in images on the left are 16 DIV, whereas those on the right are 15 DIV.

antioxidant properties. Our qualitative finding of increased dark punctae and altered morphology in glutamate-treated cells is also consistent with astrocytes exposed to glutamate toxicity.[37]

Many polyphenolic compounds are potent free radical scavengers, and a plethora of literature is available on the antioxidant capacities of phenolic compounds[49−52] and their protective effects.[53−55] Other groups have found similar findings compared to ours using nervous system cell culture models to assess potential neuroprotective effects of various polyphenolic compounds. For example, Ahn et al. found that a proanthocyanidin extract from grapes could inhibit hippocampal cell death by decreasing nitrosative stress.[48] Similar results have been found with other antioxidant compounds, as carotenoids from *Pittosporum tobira* have been shown to protect rat cortical cells against exposure to 100 μM glutamate for 24 h,[56] the same treatment protocol used in the current study. An extract from the fruit of *Alpinia oxyphylla* was shown to protect cortical neurons against exposure to 30 μM glutamate and also had an effect on condensed nuclei, similar to our findings.[57] However, in a model of glutamate toxicity in hippocampal cultures, a seed extract of *Cassia obtusifolia* provided no protection to both neurons and glia.[58] It is interesting that only the extract from lingonberry fruit did not show any protection against toxicity in our model. This may be due to the exact chemical profile of the fruit versus the other extracts. Although the overall content of polyphenolics in lingonberry fruit appeared higher than that of blueberry fruit, it is possible that specific compounds in the extracts are responsible for the neuroprotection. For example, Bhuiyan et al. found that exposure to 50 μM glutamate killed 40% of cortical neurons, but the addition of the specific polyphenol cyanidin-3-glucoside offered no protection.[59]

Previous studies have shown that dietary polyphenols can cross the blood−brain barrier,[60] and anthocyanins specifically have been detected in brain tissue after oral administration to rodents.[61−63] Estimates of specific anthocyanins in brain tissue are generally in the subnanomolar range (~0.2−0.25 nmol/g tissue).[62,63] It is difficult to make direct comparisons to such studies with the current work, as we added whole extract and not specific polyphenols. The final concentration of the extracts we added was 0.833 μg/mL of fruit extract and 0.083 μg/mL of leaf extract. We have previously conducted chemical analysis of commercially available lingonberry extracts (unpublished data) and have found that these lingonberry extracts contain an estimated 63.7 mg of cyanidin-3-galactoside/100 mg of fresh extract weight. Assuming that our fresh lingonberry extracts contain a similar amount of this compound, this would translate to the cultured cells being exposed to approximately a 10 nM

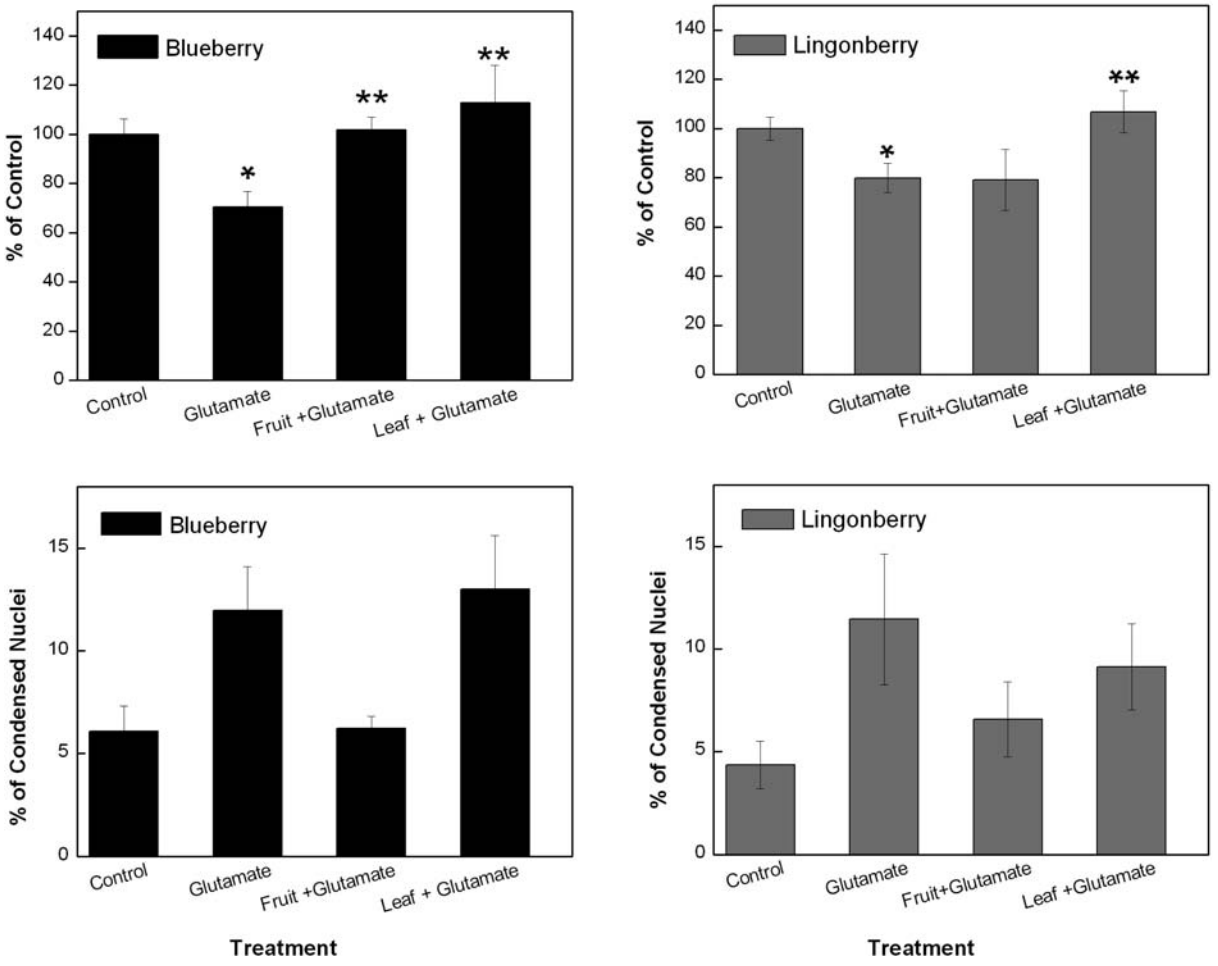

Figure 5. Summary of the effects of blueberry (left) and lingonberry (right) fruit and leaf extracts on glutamate-mediated cell death. Cells were treated with 100 μM glutamate alone or in the presence of extracts. (Top graphs) The amount of DAPI-positive nuclei was quantified, and data are expressed as percent of control values. $n = 6-16$. *, $p < 0.05$ versus control; **, $p < 0.05$ versus glutamate only (one-way ANOVA with Tukey's post hoc analysis). Bottom graphs show the percentage of nuclei that exhibited a condensed morphology in the same culture wells used to generate the data in the top graphs.

concentration of fruit extract and 1 nM in leaf extract. Talavera et al.[62] detected a level of another cyanidin compound (cyanidin-3-glucoside) of 0.25 nmol equivalent/g of tissue. Therefore, the amount of extract that we added to cultures is likely slightly higher than what might be achieved in the brain after oral administration. In addition, the polyphenolic compounds contained in our extracts may not be the predominate forms that would actually enter the brain, as a recent study found that although anthocyanins have a fairly high bioavailability, they also undergo significant metabolism, producing diverse metabolites.[64] Nonetheless, we feel that our system could be used to screen specific polyphenols at various concentrations for potential neuroprotective potential and also to study the mechanisms of action of protection.

Overall, the findings suggest that consumption of blueberries and lingonberries could have a positive effect on human health. For example, the high polyphenolic content and antioxidant capacity of these two species would be potentially beneficial for the prevention of disorders such as cardiovascular disease and diabetes.[1] Also, we provide further evidence that these species can be beneficial for the brain as well. It is possible that the consumption of berries, or a tea made from the leaves of the plants, as well as supplements produced from the extracts of the berries and/or leaves could slow brain aging or inhibit the development of neurodegenerative disorders. Therefore, ingestion of berries or supplements produced from them could possibly increase the antioxidant and antinitrosative capacity of the brain. Also, other studies have shown that rats fed a diet enriched with blueberries can protect the brain against oxidative stress[19] as well as stroke,[20,21] which suggests that the outcome from such typically debilitating disorders can be improved if the antioxidant levels in the brain are increased through diet. Therefore, future studies are aimed at analyzing the threshold of dietary consumption of berry products and potential neuroprotection.

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Author Contributions

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

Ca^{2+}, calcium; CE, catechin equivalent; DAPI, 4′,6-diamidino-2-phenylindole; DIV, days in vitro; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalents; RNS, reactive nitrogen species; ROS, reactive oxygen species

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