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Inhibition of microglial activation by elderberry extracts and its phenolic components

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ABSTRACT

Aims: Elderberry (*Sambucus* spp.) is one of the oldest medicinal plants noted for its cardiovascular, anti-inflammatory, and immune-stimulatory properties. In this study, we investigated the anti-inflammatory and anti-oxidant effects of the American elderberry (*Sambucus nigra* subsp. *canadensis*) pomace as well as some of the anthocyanins (cyanidin chloride and cyanidin 3-O-glucoside) and flavonols (quercetin and rutin) in bv-2 mouse microglial cells.
Main methods: The bv-2 cells were pretreated with elderberry pomace (extracted with ethanol or ethyl acetate) or its anthocyanins and flavonols and stimulated by either lipopolysaccharide (LPS) or interferon-γ (IFNγ). Reactive oxygen species (ROS) and nitric oxide (NO) production (indicating oxidative stress and inflammatory response) were measured using the ROS detection reagent DCF-DA and the Griess reaction, respectively.
Key findings: Analysis of total monomeric anthocyanin (as cyanidin 3-O-glucoside equivalents) indicated five-fold higher amount in the freeze-dried ethanol extract as compared to that of the oven-dried extract; anthocyanin was not detected in the ethyl acetate extracts. Elderberry ethanol extracts (freeze-dried or oven-dried) showed higher anti-oxidant activities and better ability to inhibit LPS or IFNγ-induced NO production as compared with the ethyl acetate extracts. The phenolic compounds strongly inhibited LPS or IFNγ-induced ROS production, but except for quercetin, they were relatively poor in inhibiting NO production.
Significance: These results demonstrated differences in anti-oxidative and anti-inflammatory effects of elderberry extracts depending on solvents used. Results further identified quercetin as the most active component in suppressing oxidative stress and inflammatory responses on microglial cells.

Introduction

There is increasing interest to investigate the health effects of dietary fruits and berries [40], botanicals that have been implicated for improved cardiovascular, anti-inflammatory and immune enhancing functions [2,33]. Elderberries (*Sambucus* spp.) are widely grown in Europe, Asia, North Africa and North America; the plant has been called "the medicine chest of country people". Although all parts of the plant have been used in folk medicine for centuries, the berries and the flowers are most commonly described in scientific literature [39]. The berries contain a wide variety of anthocyanins, flavonoids and other polyphenols [27,51]. The major flavonoids identified are quercetin and rutin and the primary anthocyanins are cyanidin-3-O-sambubioside and cyanidin 3-O-glucoside [4,39,50]. In addition, the presence of iridoid glycosides, sesquiterpenes and phytosterols has also been reported [45].

Among several berry species, elderberry contains the highest amount of total flavonols [29]. In addition, hydrophilic antioxidant capacity for elderberry is among the highest that was measured in fresh fruits [51]. Different extracts have been shown to possess anti-

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inflammatory, antiviral, anti-diabetic, anti-carcinogenic and immune-stimulatory activities [39,47]. Cyanidin3-O-glucoside has 3.5 times higher antioxidant activities as compared with vitamin E [48]. This compound has been shown to protect against oxidative damage [53], and suppress the production of nitric oxide (NO) induced by lipopolysaccharide (LPS) in macrophage cells [15,49]. The anthocyanins, such as cyanidin and protocatechuic acid are also effective in decreasing NO production [32]. In LPS stimulated RAW264.7 macrophages, quercetin and its glycoside, rutin, inhibited inducible nitric oxide synthase (iNOS) expression [5,24]. Although macrophages share many properties similar to microglial cells, studies to examine elderberry extracts on oxidative and inflammatory responses of microglial cells have not been extensive.

Inflammatory processes play a major role in the progression of a number of neurodegenerative diseases and activation of microglial cells, the resident macrophages in the central nervous system, is an important initial step of the inflammatory response [43]. Upon exposure to proinflammtory cytokines and/or lipopolysaccharides (LPS), specific signal transduction pathways which regulate the induction of iNOS and other oxidative and inflammatory mediators (see [12,44], for reviews) are activated. Therefore, suppression of microglial activation might lead to an earlier restoration of homeostasis and resolution of neuroinflammation. While LPS is known to directly activate TLR4 receptors and the NF-kB pathway, IFNγ, a cytokine derived from peripheral immune cells, is coupled to the JAK–STAT pathway. Some pro-inflammatory genes, such as that for iNOS, comprise of promoters that require activation of transcription factors involving NF-kB and the JAK–STAT pathways [34]. However, our earlier studies showed that LPS or IFNγ can each stimulate iNOS in bv-2 microglial cells, and this was attributed to the presence of cross-talk mechanisms through activation of ERK1/2 [7,41,42]. Despite that LPS and IFNγ can individually induce ROS and NO in microglial cells, it is not clear whether botanicals may exert differences in these pathways. In a recent study, there is evidence that the anti-inflammatory effects of certain botanical-derived compounds are stronger for IFNγ-stimulated inflammation than those for LPS [10].

In this study, freeze-dried or oven-dried elderberry pomace was extracted by ethanol or ethyl acetate and the effects of the extracts on oxidative and inflammatory responses upon stimulation of murine bv-2 microglial cells with LPS or IFNγ were determined. In addition, studies were extended to examine the effects of phenolic components, such as cyanidin chloride, cyanidin 3-O-glucoside, quercetin or rutin (Fig. 1).

Materials and methods

Materials

Quercetin, rutin (Sigma-Aldrich, St. Louis, MO), cyanidin 3-O-glucoside and cyanidin chloride (Indofine Chemical Comp., Hillsborough, NJ) were dissolved in DMSO as a stock solution. Dulbecco's modified Eagle's medium (DMEM), penicillin, streptomycin, 0.05% (w/v) trypsin/EDTA, and phosphate-buffered saline (PBS) were obtained from GIBCO (Gaithersburg, MD). Lipopolysaccharide (LPS) (rough strains) from Escherichia coli F583 (Rd mutant) was obtained from Sigma-Aldrich (St. Louis, MO). Murine interferon-γ (IFNγ) was purchased from R&D Systems (Minneapolis, MN). Fetal bovine serum was from Atlanta Biologicals (Lawrenceville, GA).

Elderberry materials and preparation of extracts

Elderberry (*Sambucus nigra* subsp. *canadensis*) extracts were derived from ripe berries harvested from 4-year-old plants grown in a commercial orchard near Hartsburg, Missouri. Berries were destemmed by a shaker, rinsed twice with lightly bleached water, and frozen in plastic food-grade buckets (9 kg) in a −20 °C freezer after the bleached water was completely drained. Thawed berries were heated to 82 °C for 5 min in a 230-liter steam-jacketed kettle and pressed immediately using a cider mill to separate the juice (60% w/w) and pomace (40% w/w). Elderberry pomace (e.g., seeds and skins) was then refrozen and stored at −20 °C. Samples of elderberry pomace were either freeze-dried or oven-dried (105 °C for 18–24 h) and subsequently extracted with 10 volumes of 95% ethanol or ethyl acetate (room temperature, overnight). Upon evaporation of the solvent under nitrogen, the dried residue was weighed, and stored at −20 °C. The extracts were re-dissolved in cell culture grade DMSO before adding to the cells.

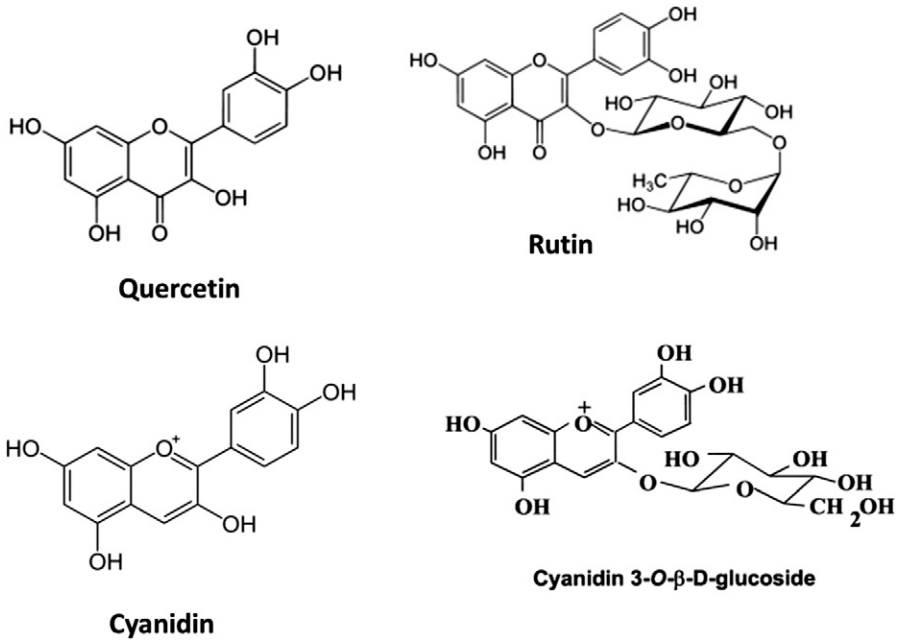

Fig. 1. Chemical structures of flavonols (quercetin, rutin) and anthocyanins (cyanidin, cyanidin 3-O-β-glucoside) present in elderberry.