Toxicology Reports 1 (2014) 385–390

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Short communication

Anti-inflammatory potential of native Australian herbs polyphenols

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ARTICLE INFO

Article history:
Received 11 April 2014
Received in revised form 6 June 2014
Accepted 25 June 2014
Available online 15 July 2014

Keywords:
Polyphenols
Herbs
Anise myrtle
Lemon myrtle
Tasmannia pepper leaf
COX-1
COX-2
iNOS

ABSTRACT

The anti-inflammatory potential of hydrophilic polyphenolic-rich extracts obtained from native Australian herbs: anise myrtle, lemon myrtle and Tasmannia pepper leaf, and a reference sample bay leaf, was evaluated using the lipopolysaccharide (LPS)-activated murine macrophage RAW 264.7 model. Pretreatment with all herbal extracts at non-cytotoxic concentrations reduced the LPS-induced protein levels of pro-inflammatory enzymes, cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). Concomitant decrease in accumulation of their products, prostaglandin E_2 (PGE_2) and nitric oxide (NO), respectively, was observed. A suppression of LPS-induced expression of COX-2 and iNOS and decrease of NO and PGE_2 levels suggests potential anti-inflammatory properties of the extracts.

Anise myrtle, lemon myrtle and bay leaf selectively inhibited COX-2 and iNOS enzymes, while Tasmannia pepper leaf extract exhibited a pronounced inhibitory activity toward COX-1 and was the least effective inhibitor of iNOS. Anise myrtle and lemon myrtle are potentially more efficient anti-inflammatory agents than Tasmannia pepper leaf.

1. Introduction

Examining the indigenous use of plant foods may provide a wealth of potential candidates for isolation of health-promoting substances and native Australian plants provide an intriguing source. The Australian flora have developed in distinct isolation from other continents as a result of the separation of the Australian land masses from the supercontinent Gondwana and the rest of the world over 65 million years ago [1]. The conditions of geographic isolation, followed by a warming of the continent and subsequent onset of aridity, as well as the nutrient poor soils led to a unique adaptation of plants resulting in a more complex flora with approximately 85% of the vascular plants in Australia being endemic. Over centuries the Australian aborigines utilized a large body of edible plants. Some of these plants were reported to possess unique nutritious and organoleptic properties [10]. This characteristic offers opportunities to utilize them in the development of novel tastes and flavors.

Three endemic Australian herbs: Tasmannia pepper leaf, anise myrtle and lemon myrtle, which resemble a bay leaf, are produced commercially and are been incorporated in Australian cuisine. Hydrophilic polyphenolic-rich extracts obtained from these herbs contain highly bioactive

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http://dx.doi.org/10.1016/j.toxrep.2014.06.011

flavonoids, phenolic acids and tannins [19]. The dominant compounds of anise myrtle and lemon myrtle hydrophilic extracts are ellagic acid and derivatives, ellagic acid glycosides and ellagitannins, whether chlorogenic acid, quercetin and derivatives are the major components of Tasmannia pepper leaf extract. These phytochemicals are reported to possess numerous health-enhancing properties. For example, ellagic acid is a potent antioxidant, exhibits estrogenic and/or anti-estrogenic activities, anti-inflammatory, antimicrobial and prebiotic effects [5,14,15,18]. Chlorogenic acid possesses anti-diabetic, antilipidemic [13] and anti-inflammatory capacity [2,21] and has radioprotective action [4]. Polyphenolic-rich extracts of anise myrtle, lemon myrtle and Tasmannia pepper leaf suppress the activities of isolated α-glucosidase, pancreatic lipase and angiotensin converting enzyme (ACE) [19]. The same extracts exhibit antioxidant, cytoprotective and pro-apoptotic activities [20].

Inflammation is the normal physiological and immune response to pathogen invasion and cell injury. During the inflammatory response, immunocytes, such as monocytes and macrophages, are activated and secrete inflammatory mediators, such as nitric oxide (NO) and prostaglandin E_2 (PGE_2), respectively, *via* the inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). iNOS and COX-2 are involved in tumor progression through various mechanisms, including inhibition of apoptosis, stimulation of angiogenesis and promotion of tumor cell proliferation [3]. Therefore inhibition of iNOS and COX-2 activities is a viable approach to inhibit inflammation and carcinogenesis and to prevent cancer.

To date no research data is available on anti-inflammatory activities of the indigenous Australian herbs. Therefore the objective of this study was to evaluate the anti-inflammatory properties of purified polyphenolic-rich extracts obtained from commercially produced anise myrtle, lemon myrtle and Tasmannia pepper leaf using the lipopolysaccharide (LPS)-activated murine macrophages RAW 264.7. Commercially available bay leaf was used as a reference sample.

2. Materials and methods

2.1. Plant material

Anise myrtle (*Syzygium anisatum* Vickery, Craven and Biffen; AM) and lemon myrtle (*Backhousia citriodora* F. Muell, Myrtaceae; LM) were supplied by the Australian Rainforest Products (Lismore, NSW, Australian). Tasmannia pepper leaf (*Tasmannia lanceolata* R. Br., Winteracea; TPL) was obtained from the Diemen Pepper (Birchs Bay, Hobart, Tasmania, Australia). The reference sample bay leaf (*Laurus nobilis L.*, Lauraceae) was obtained from Hoyts Industries Pty. Ltd. (Moorabbin, Victoria, Australia).

2.2. Preparation and analysis of polyphenolic-rich extracts from plant sources

The polyphenolic-rich extracts were prepared and their composition evaluated as described previously [19].

2.3. Cell culture

RAW264.7 (murine macrophage) cell line was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in Dulbecco's Modified Eagle's medium (DMEM; Invitrogen) containing 10% fetal bovine serum (FBS, Invitrogen Corporation, Carlsbad, CA, USA), 100 μg/ml streptomycin and 100 units/ml penicillin (Invitrogen Corporation, Carlsbad, CA, USA) at 37 °C, humidified 5% carbon dioxide (CO_2) atmosphere. The experimental cells were cultured no more than 40 passages.

2.4. Western blotting

RAW 264.7 cells (1×10^6 cells) were pre-cultured in 25 cm^2 flasks for 24 h. After 24 h fresh serum-free medium was added for 2.5 h to eliminate the effect of FBS. Subsequently the cells were treated with different concentrations of polyphenolic extracts in PBS for 1 h before exposure to 40 ng/ml LPS for 12 h. Next cell lysates were prepared by adding lysis buffer (1 mg. ml^{-1} BPB, 0.1% glycerol, 0.1 M DTT, 0.04% pH7.2 SDS, 62.5 mM pH6.8 Tris–HCl). The whole-cell lysates were run on a 4–12% Bis-Tris gel (NuPAGE, Invitrogen Corporation) and transferred to PVDF membrane (Invitrogen Corporation) using iBLOT Gel Transfer System (Invitrogen Corporation). After blocking nonspecific sites with 5% non-fat milk powder in TBS/T, the membrane was washed, incubated with primary antibody, washed again, further incubated with secondary antibody, washed and incubated with chemiluminescent alkaline phosphatase substrate (Thermo Scientific Super-Signal West Femto Substrate, Thermo Fisher Scientific). Primary rabbit polyclonal antibodies against COX-1, COX-2, iNOS and α-tubulin, as well as horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody, purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), were added at concentrations of 1 μl per 1 ml TBS/T. The immunoactive proteins were detected and quantified using chemiluminescent imaging system (ImageQuant LAS 4000, GE Healthcare). The relative densities of COX-1, COX-2 and iNOS proteins presented in graphs were normalized to α-tubulin.

2.5. Measurement of nitrite concentration

Nitrite concentration in culture media was determined by the Griess reaction [24]. Briefly, RAW 264.7 cells (3×10^5 per well) were incubated for 24 h at 37 °C in 48-well plates. The medium was removed and fresh serum-free medium was added for 2.5 h. Subsequently, the cells were treated for 1 h with different concentrations of polyphenolic extracts, which did not suppress the proliferation, before exposure to 40 ng/ml LPS for 12 h. The culture supernatants were mixed with equal volumes of modified Griess reagent (Sigma–Aldrich) for 15 min at room temperature in the absence of light. Nitrite concentration was measured by absorbance levels at 540 nm against a sodium nitrite standard curve.