PharmaNutrition 12 (2020) 100187

Contents lists available at ScienceDirect

PharmaNutrition

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

A polyphenol rich sugarcane extract as a modulator for inflammation and neurological disorders

Jin Ji[a], Matthew Flavel[b,d,*], Xin Yang[b], Oliver C.Y. Chen[a], Luke Downey[c], Con Stough[c], Barry Kitchen[b,d]

[a] PulchriBio Intl, Cambridge, MA, USA
[b] TPM Bioactives Division, The Product Makers Pty Ltd, Melbourne, Australia
[c] Swinburne Centre of Psychopharmacology, Swinburne University, Melbourne, Australia
[d] La Trobe University, School of Life Sciences, Melbourne, Australia

ARTICLE INFO

Keywords:
Polyphenol rich sugarcane extract
Neuroprotective
TNF-α inhibition
Nrf2 activation
Oxidative stress
Monoamine oxidase

ABSTRACT

Evidence suggests that enhanced oxidative stress and mild, chronic inflammation is associated with the onset and progression of some neurological disorders. Hypothesis: A polyphenol rich sugarcane extract may be active in protecting against this inflammation and neurological degeneration. Study design: A series of cell culture strains and *in vitro* gene expression studies were used to understand the neuroprotective activity. Methods: Extract was prepared according to a patented extraction process and dose responses for TNF-α inhibition, Nrf-2 activation, MAO inhibition, DNA damage marker and AChE inhibition were prepared. This was in addition to a gene array assay for 84 genes related to neurogenesis. Results: PRSE displayed anti-inflammatory effect, possibly through inhibiting pro-inflammatory cytokines and activating Nrf2-ARE transcription pathway, and may exert neurological benefits through suppressing neuro-inflammation. In particular, subsequent studies with neuronal cells indicate that PRSE may exert additional neurological benefits through promoting genes that encode neurogenesis-related growth factors and regulate neuronal differentiation, preserving neuronal DNA from oxidative stress damage, and inhibiting key neuronal therapeutic targets such as MAO and AChE as a pathway to attenuate oxidative stress Conclusion: We report promising preclinical studies for the therapeutic intervention of a new polyphenol rich sugarcane extract on inflammation and neurological disorder modulation.

1. Introduction

Increasing body of evidence shows that oxidative stress and inflammation play a major role in the pathogenesis of neuronal system. Oxidative stress is induced by excessive reactive oxygen and nitrogen species (ROS/RNS) production in a cellular system, which overwhelms the capacity of antioxidant defense system. Also called free radicals, these reactive species if not controlled cause damages in lipids, cell membranes, proteins, and DNA/RNA. The brain is particularly susceptible to oxidative damage due to its high level of polyunsaturated fatty acids that are easily peroxidizable, and low level of intrinsic antioxidants to protect the brain from oxidation. Oxidative stress due to the accumulation of ROS is closely associated with the pathogenesis of neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease, as well as neuropsychiatric disorders such as anxiety and depression [1].

One major consequence of oxidative stress is the enhanced signal transduction leading to the activation of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), a key cytokine that triggers downstream cellular feedback loops governing inflammation. The resulting inflammation is the key defense response of the immune system to oxidative stress. While necessary, the inflammation mediated by oxidative stress lacks specificity and often causes significant uncontrolled damage. Thus, controlling these non-specific pro-

Abbreviations: PRSE, polyphenol rich sugarcane extract; ROS, reactive oxygen or nitrogen species; RNS, reactive nitrogen species; TNF-α, tumor necrosis factor-α; Nrf2, nuclear factor erythroid 2-related factor 2; ARE, antioxidant response element; MAO, monoamine oxidase; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; EDTA, ethylene diamine teraacetic acid; DMEM, Dulbecco's modified eagle medium; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); HBSS, Hank's balanced salt solution; ANOVA, analysis of variance; LPS, lipopolysaccharide; AChE, acetylcholinesterase

* Corresponding author at: The Product Makers, 50-60 Popes Road, Keysborough, Victoria, 3173, Australia.
E-mail address: mflavel@tpm.com.au (M. Flavel).

https://doi.org/10.1016/j.phanu.2020.100187
Received 10 February 2020; Received in revised form 5 March 2020; Accepted 6 March 2020
Available online 12 March 2020
2213-4344/

inflammatory cytokines has become a therapeutic target for inflammation related diseases or disorders. As another defensive mechanism, key proteins such as nuclear factor erythroid 2-related factor 2 (Nrf2), a redox-sensitive transcription factor are deployed by a cellular system to counter oxidative stress. Nrf2 is a member of the cap'n'collar family of leucine zipper transcription factors. In the nucleus, Nrf2 heterodimerizes with small proteins such as JUN, MAF and binds to DNA sites containing anti-oxidant response elements (ARE) and other sites to regulate transcription of cytoprotective genes that protect against oxidative stress [2]. Insufficient Nrf2 activation has been associated with impairments in cognition, Alzheimer's disease, Parkinson's disease, and other neurodegenerative diseases [3]. Numerous studies have reported that inflammation is intimately associated with oxidative stress and is suspected to be a key pathway of oxidative stress mediated neuronal damage [4]. In particular, inhibition of pro-inflammatory cytokine tumor necrosis factor (TNF-α) and activation of cellular defense pathway Nrf2-ARE are two upstream, fundamental therapeutic targets to intercept the onset of oxidative-mediated inflammation. Dimethyl fumarate (DMF, trade name Tecfidera®), a compound used as a treatment for patients with relapsing forms of multiple sclerosis, has been observed to activate the Nrf2 pathway, and inhibit the expression of adhesion molecules and cytokines, further suggesting the close relation between oxidative stress and neurological health, with cytokine inhibition and Nrf2 activation being potential functional pathways for investigation [5].

Recognizing the weakness in the endogenous antioxidant defense system in the brain, synthetic compounds and antioxidants obtained from natural plants and food source have been exploited for use in preventing inflammation and relieving oxidative stress for improved neurological health [6]. Among various categories of antioxidants, polyphenols (polyphenolic compounds) are known to be a major group of naturally occurring phytochemicals that can function as antioxidants *via* absorbing reactive oxygen and nitrogen species (ROS/RNS) facilitated by the conjugated phenol ring structure they contain in common. Studies have been reported on the use of polyphenol dietary intervention to delay the onset of Alzheimer's disease and reduce the occurrence of Parkinson's disease [7]. In addition, daily consumption of increased level of flavonoids, a major subgroup of polyphenols, in the diet and in beverages was found to associate with reduced incidences of dementia and aging by up to 50 % [8]. In mouse study of Alzheimer's disease, a polyphenolic extract from blueberries and grapes improved object recognition, and prevented cognitive impairment associated with aging and Alzheimer's disease [9]. Several putative mechanisms of action of dietary polyphenols in neuronal health include: (1) promoting hippocampal neurogenesis; (2) protecting neurons from oxidative damage and inflammation; and (3) preventing neurodegeneration through regulating key mitochondrial proteins such as monoamine oxidase (MAO) and acetylcholinesterase (AChE).

Sugarcane (*Saccharum officinarum*), a tall, perennial grass indigenous to tropical South Asia, Southeast Asia, and New Guinea, has been predominantly a food source up until the recent discovery of polyphenols and other bioactives in the plant. Pharmacological studies identified several polyphenolic compounds such as phenolic acids, flavonoids, and different glycosides in sugarcane juices and its unrefined products (*e.g.*, molasses), as well as fatty acids in its stems and leaves [10]. Since then, sugarcane has attracted increased research attention in its potential therapeutic usage. In a rat study relevant to neurological therapy, Duarte-Almeida et al. demonstrated the protective effect of sugarcane extract against MeHgCl-induced neuro-intoxication in rats, and its potent inhibitive effect of *ex vivo* lipoperoxidation of rat brain homogenates. The authors further attributed such *in vivo/ex vivo* neurological effects to the anti-oxidation functions of the phenolic compounds and other antioxidants present in sugarcane [11]. In another relevant study, Colonnello et al., demonstrated that sugarcane aqueous extract inhibited cell dysfunction in the cerebrum of rats with neurotoxicity and decreased physiological stress in Caenorhabditis elegans exposed to oxidative stress and excitotoxicity [12].

We recently reported that a polyphenol rich sugarcane extract (PRSE) obtained from sugarcane molasses using a patented hydrophobic extraction process contains a high level of phenolic compounds including tricin, apigenin, and luteolin, and displays a full spectrum of *in vitro* antioxidant property that effectively absorbs five different species of reactive oxygen/nitrogen radicals [13]. Using several *in vitro* cellular models for diabetes investigation, we demonstrated that PRSE deters the uptake of glucose and fructose in intestinal cells and restores insulin production in dysfunctional β-cells - key diabetic management functions presumably stemmed from the antioxidant properties of PRSE. In this paper, considering the intricate connections among anti-oxidation, anti-inflammation, and neurological health, we employed human cell study models to explore the anti-inflammatory property of this sugarcane extract (PRSE) and its potential in the prevention/protection of neurological diseases. We first studied the anti-inflammatory effect of PRSE *via* its impact on pro-inflammatory cytokine TNF-α and defense pathway Nrf2 activation in cells. We then examined the effect of PRSE on neuronal health from three areas of biological studies: (1) Promotion effect of PRSE on neurogenesis; (2) Neuroprotective effect of PRSE from oxidative stress *via* DNA damage control; and (3) Inhibition effect of PRSE on key neuronal therapeutic targets such as MAO and AChE as potential pathways to attenuate oxidative stress.

2. Materials and methods

2.1. Reagents

Unless otherwise specified, all chemicals were obtained from Sigma-Aldrich, St. Louis, MO, USA. The cell culture, cell media, and other biological reagents were obtained from sources indicated in the method description below.

2.2. Study material

A proprietary, polyphenol rich sugarcane plant extract (PRSE) obtained from sugarcane molasses was used in this study. The material was developed and prepared by The Product Makers Pty Ltd (Melbourne, Australia) based on a patented hydrophobic resin procedure described previously [13]. In short, deionized water is added to sugarcane mill molasses with constant stirring until the final Brix is 20. 500 g of wet weight pre-treated ion exchange resin and polymeric adsorbents (Dow Chemical, Australia) were then added to 1 L of above 20 Brix feed stock with gentle stirring. The mixture is then filtered under vacuum, and the resin particles collected and washed with 1 L of deionized water twice. The washed resins are then suspended in 1 L of 70 % ethanol, stirred for 10 min, and the filtrate collected by vacuum filtration. This suspension/filtration process was repeated twice more with each filtrate collected. Finally, the three batches of 70 % ethanol filtrates were combined, and the ethanol removed under vacuum. The aqueous extract was then lyophilized into a free-flowing brown powder (polyphenol-rich sugarcane extract PRSE) with a final moisture level of 2–4 % w/w. The chemical composition of this extract has been described previously [13,14]. These studies reported that the extract produced by the methods described in the present paper to have a total polyphenol content of 22.1 % of the extract or 221 mg/g GAE (as gallic acid equivalency) with major phenolic contributors to this including apigenin (1.89 μg /g), luteolin (5.30 μg/g), tricin (27.40 μg/g), diosmin (227.00 μg/g), syringic acid (107.57 μg/g) and chlorogenic acid (74.29 μg/g) amongst others.

For all the cell-based studies, PRSE solids were suspended in 50 % EtOH to yield a 50 mg/ml mixture. After 30 s of thorough vortex, the mixture was ultrasonicated in an ice bath for 15 min and vortexed occasionally during this time. Following the ultrasonication, the mixture was vortexed again for 30 s and centrifuged at 1780 *g* (or 3900 rpm @105 mm radius) for 15 min at 4 °C, and the resulted supernatant was

collected to serve as the stock extract (50 mg/ml) and stored under 4 ˚C until use. For each *in vitro* study, a series of dilutions of the stock extract were used according to specific study requirements.

2.3. Cellular TNF-α inhibition

THP-1, human monocyte-like cells, were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in RPMI1640 medium supplemented with 10 % heat-inactivated fetal calf serum (FCS), 100 U/mL penicillin, and 100 μg/mL streptomycin, and maintained under 37 ˚C, 5 % CO_2 with 95 % humidity before use.

To transform monocytes to macrophages for cellular TNF-α inhibition studies, THP-1 cells were pre-treated with 10 ng/ml phorbol 12-myristate 13-acetate (PMA) for 48 h. The viability of the induced macrophages were assessed using a standard CellTiter-Glo® Luminescent Cell Viability Assay (Promega) according to the manufacturer's protocol. Subsequently, the macrophages were stimulated with 1 μg/mL lipopolysaccharide (LPS) and then incubated with different concentrations of PRSE, or curcumin which is used as the study positive control, or phosphate-buffered solution at pH 7.4 (PBS 7.4, used as the negative control) for 24 h at 37 ˚C in the presence of 5 % CO_2. The cell culture supernatants were then collected for enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instruction. The ELISA data were then recorded and normalized against negative control, and inhibition curves and IC50 s were generated using Prism, version 8 (GraphPad Software Inc, CA, USA).

2.4. Cellular Nrf2 activation

Human hepatocellular carcinoma cells (HepG2) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM, HyClone, Logan, Utah, USA) supplemented with 10 % fetal bovine serum (FBS, HyClone, Logan, Utah, USA) and antibiotics (50 U/mL of penicillin and 50 μg/ml streptomycin), and maintained under 37 ˚C, 5% CO_2 with 95 % humidity before use.

One day prior to the cellular Nrf2 activation study, HepG2 cells were seeded at a density of $3-5 \times 10^5$ per well on white clear-bottom 96-well plates. The cells were incubated overnight with 100 μL of growth medium (MEM/EBSS (Hyclone), 10 % FBS, 1% non-essential amino acids, 1 mM Na-pyruvate, 1% Pen/Strep) at 37 ˚C in 5% CO_2. On the day of assay, Nrf2 reporters were transfected into the cells. The cells were then incubated at 37˚ in a CO_2 incubator (5% CO_2) overnight. The next day after the transfection, the media was replaced by Opti-MEM media (Gibco) containing 0.5 % FBS and 1 % NEAA and cells were treated by various concentrations of PRSE or quercetin (assay positive control), or PBS 7.4 (negative control). After 18 h of treatment, the cells were thoroughly washed with PBS 7.4 and lysed by 20 μL of cell culture lysis buffer (Promega, Madison, WI, USA), followed by addition of 100 μL of luciferase assay substrate (Promega, Madison, WI, USA). Luminescence of the subsequent lysate was measured by a multimode plate reader (BioTek Instruments, Inc., Winooski, VT, USA). The activation of Nrf2 is expressed as the fold change of the negative control.

2.5. PCR array study of neurogenesis-related genes

All *in vitro* neurological studies were carried out using Human SH-SY5Y neuroblastoma cells obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Upon delivery, the neuroblastoma cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM)/F-12 without L-glutamine (Invitrogen) supplemented with 0.5 % fetal calf serum (FCS), 100 U/ml penicillin and 0.1 mg/ml streptomycin for 8 days at 37 ˚C in 5% CO2. The cells were then grown in a medium supplemented with 1 μM all trans-retinoic acid in plates coated with 0.1 mg/ml poly-L-lysine and 1 mg/ml growth factor reduced Matrigel Matrix without phenol red (BD Biosciences) until use.

RNA isolation and quality control: After treatment with 0.25 mg/ml of PRSE and vehicle control, human SH-SY5Y neuroblastoma cells were harvested for RNA isolation. The cells were lysed by adding Trizol Reagent (Invitrogen), followed by incubation on ice for 10 min. RNA was isolated using RNeasy Micro columns (Qiagen) according to the manufacturer's instructions. RNA quantity and purity were determined by NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies). The total RNA was used to synthesize the template cDNA following "The RT2 First Strand Kit" from SABiosciences (Qiagen, Valencia, CA).

Human neurogenesis RT2 Profiler™ polymerase chain reaction (PCR) Array: The template cDNA from each sample was mixed with 2X RT2 qPCR master mix (SABiosciences, Qiagen, Valencia, CA) and then transferred to each well on the 96-well PCR array plate comprising predispensed gene-specific primer sets (SABiosciences, Qiagen, Valencia, CA). Each array consisted of primer sets of 84 genes related to neurogenesis. This comprised of genes that are important for cell proliferation, cell cycle, cell migration, cell differentiation, synaptic functions, apoptosis, growth factors, and cytokines. Each array also comprised five housekeeping genes (HKGs, negative controls) and three RNA and three PCR quality controls (positive controls). Reactions were carried out in PCR array kits using Applied Biosystems Real-Time PCR Instrument. The PCR amplification followed a two-step cycling program: 10 min denaturation at 95 ˚C, 40 cycles of 95 ˚C for 15 s, and 60 ˚C for 1 min. PCR amplification results of the investigated genes were normalized against those of the housekeeping controls and recorded.

2.6. Cellular DNA damage control

The human neuroblastoma SH-SY5Y cells were seeded on a 24-well cell culture plate and cultured for 24 h in the cell culture medium described above. The cells were then incubated with various concentrations of PRSE or withanone (used as the positive control), or PBS 7.4 (negative control) for 24 h, and then challenged with 250 μM glutamate for 24 h. Upon the completion of the 24 -h dosing period, the cells were washed twice with culture media, then fixed with a 3.7 % formaldehyde solution for 30 min. The assay plates were then washed with DPBS 2x on a Biotek plate washer and treated with a permeabilization solution of 0.5 % (v/v) Triton X-100 for 10 min. The plates were washed 1x and the cells were blocked for 30 min prior to antibody staining. After blocking, the plates were washed 1x and a primary antibody cocktail consisting of mouse-anti-phospho-histone H2A.X and Hoechst 333,342 was administere. Plates were incubated at room temperature for 1 h and then washed 2x with a plate washer (Biotek). Secondary antibody, Cy3-donkey-anti-rabbit-IgG, was added into each well and followed by one-hour incubation at room temperature. The plates were washed 2x with the plate washer leaving 200 μl of DPBS in each well. The plates were sealed and read on an ArrayScan™ VTI HCS Reader (Thermo Fisher Scientific, Waltham, MA) using the Compartmental Analysis Bioapplication software (Thermo Fisher Scientific, Waltham, MA). The data were then recorded and normalized against the negative control, and inhibition curves and IC50 s were generated *via* Prism software, version 8 (GraphPad Software Inc, CA, USA).

2.7. Cellular MAO inhibition

For cellular MAO inhibition study, the human neuroblastoma SH-SY5Y cells were co-incubated with with various concentration of PRSE or comparison material (harmine), and 60 μM of kynuramine for 90 min at 37 ˚C, followed by the addition of NaOH (0.5 M) to medium. The fluorescence was measured at 330 nm excitation and 460 nm emission. The data were then recorded and processed, and inhibition curves and IC50 s were generated *via* Prism software, version 8 (GraphPad Software Inc, CA, USA).

2.8. Acetylcholinesterase inhibition assay

In the acetylcholinesterase (AChE) inhibition assay, AChE activity was measured using a modified 96-well microplate assay based on Ellman's method [15] and an acetylcholinesterase inhibitor screening kit (Bioassay Systems LLC, Hayward, CA, USA). Here, thiocholine produced by the action of acetylcholinesterase reacts with 5,5'-dithiobis (2-nitrobenzoic acid) to form a yellow color product. The intensity of the product color, measured at 412 nm, is proportionate to the enzyme activity of the test sample. A series of concentrations of PRSE and assay positive control were evaluated, and the percentage of inhibition was determined by normalizing the enzyme activity of the test sample against background control (PBS 7.4). Donepezil hydrochloride, a known AChE inhibitor drug, was used as the assay control.

2.9. Statistical analysis

A statistical analysis was performed for all the study results as described previously [13]. In short, a correlation analysis was first carried out to confirm that there is a relationship between the two variable (x, y) pairs of the study results. A statistical hypothesis testing was then performed using the Kruskal-Wall test, a non-parametric alternative to a one-way ANOVA to confirm statistical significance. The statistical analysis was performed using the SAS® software, version 9 (SAS Institute, Inc., Cary, NC).

3. Results

3.1. Inhibition of pro-inflammatory cytokine TNF-α in cells

Treatment of induced human macrophages with PRSE effectively inhibited the expression of cytokine TNF-α, a key cell signaling protein that governs the immune cells and inflammation. As shown in Fig. 1A,

PRSE inhibited the expression of cytokine TNF-α in human macrophages in a dose dependent manner, with the half maximal inhibitory concentration (IC50) being at 36.31 μg/mL (Fig. 1A). As a comparison, curcumin, known to have anti-inflammatory activity, at the dose of 10 μM (3.68 μg/mL) inhibited the expression of TNF-α by 48 %, a magnitude comparable to that of the IC50. The results are summarized in Table 1.

3.2. Cellular activation of Nrf2 by PRSE

We carried out Nrf2 activation studies in a Nrf2-ARE reporter-HepG2 cellular system, a cellular model designed for use as a luciferase reporter-based screening assay for activators of Nrf2. PRSE effectively activated Nrf2 in a dose-dependent manner (Fig. 1B). RPSE at the dose of 62.5 μg/mL nearly doubled the activity of Nrf2 and at the dose of 500 μg/mL, enhanced the activation by 51-fold. Quercetin, a flavonol known to activate Nrf2-ARE pathway, induced Nrf2 activation in a dose-dependent manner. Treating the cells with 50 μM (15.11 μg/mL) of quercetin, Nrf2 activation was increased by 50-fold, comparable to that by 500 μg/mL PRSE. The results are also summarized in Table 1.

3.3. PRSE regulates four neurogenesis-related genes in human neuroblastoma cells

To assess the overall effect of PRSE on neurogenesis, we studied the impact of PRSE on 84 neurogenesis-related key genes in human neuroblastoma cells, an *in vitro* model often used for neuronal function and differentiation investigation. A PCR array technique was used to determine the expression of 84 neurogenesis-related key genes as well as housekeeping genes and control genes. The list of 84 neurogenesis-related genes studied is presented in the Supplemental Material. For the analysis of the gene expression data, the ΔΔCt method was used, where the level of gene expression in human neuronal cells is presented as fold-regulation, a biologically meaningful way representing the change of gene expression. Gene expression level greater than 1 indicates a positive or up-regulation, and the gene expression level less than 1 indicates a negative or down-regulation. Gene expression level values 3 or greater, or -3 or less indicates a significant change observed.

Fig. 2 is a scatter plot showing the regulation effect of PRSE on 84 neurogenesis-related genes. Among the 84 genes investigated, treatment of PRSE induced significant up-regulation of four neuronal functions related genes: NEUROG1, NEUROG2, NTF3, and OLIG2. Table 2 listed these four genes and their key functions in the neuronal system. The PCR array data showing the impact of PRSE on the expression of 84 genes is exhibited in the Supplemental Material.

3.4. Mitigation of oxidative insult induced neuronal DNA damage

In this study, we monitored neuronal DNA damage triggered by oxidative insult induced by pathogenically high level of glutamate treatment of human neuroblastoma cells, and studied whether PRSE extract could protect these neuronal model cells from the oxidative stress. The neuronal DNA damage response γH2AX, a biomarker for DNA double-strand breaks, is used as the biomarker of neuronal DNA damage. Results in Fig. 3 show that pathogenically high level of glutamate significantly increased the level of γH2AX in human neuronal model cells, and treatment of the cells with PRSE preserved the

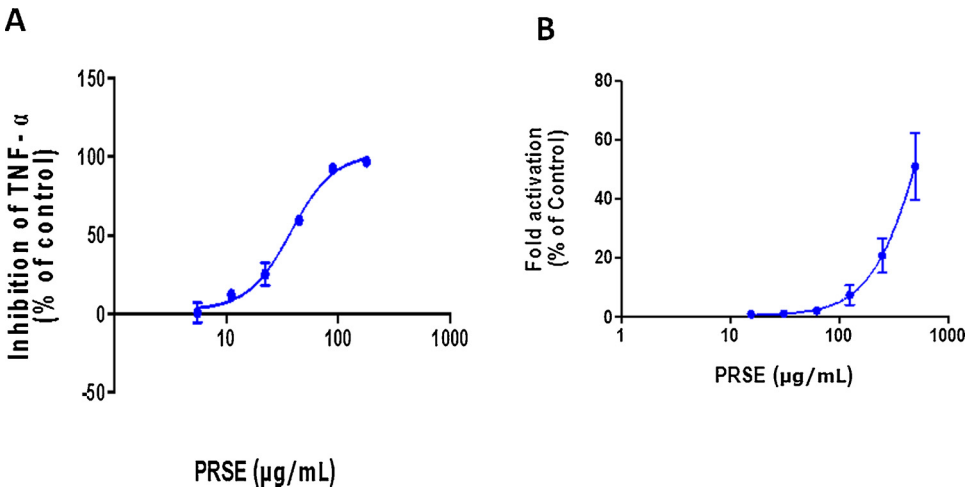

Fig. 1. Effect of PRSE pro-inflammatory cytokine TNF-α and transcription factor Nrf2 in human cells. 1A. Concentration-dependent inhibition effect of PRSE on pro-inflammatory cytokine TNF-α in a cellular TNF-α inhibition model. 1B. PRSE enhances Nrf2 activity in human HepG2 cells in a dose-dependent manner.

Table 1
Effect of PRSE *vs*. performance standards on the inflammatory and neurological biomarkers studied.

Function	Biomarker	Study Material	EC50 (μg/ml)	Maximum effect achieved	Effect
Anti-inflammatory	TNF-α	PRSE	36.3	97.00 % ± 3.11 %	Inhibition
		Curcumin	3.7	47.05% ± 3.45 %	Inhibition
	Nrf2 activation	PRSE	316.2	50.88 ± 11.35	Fold Activation
		Quercetin	11.8	67.29 ± 0.56	Fold Activation
Neuroprotection	DNA damage marker (γH2AX)	PRSE	501	48.23% ± 0.56 %	Inhibition
		Withanone	0.8	72.69% ± 0.23 %	Inhibition
	MAO	PRSE	524.8	46.60% ± 1.83 %	Inhibition
		Harmine	2.0*	92.77 % ± 0.95 %	Inhibition
	AChE	PRSE	120.8	100.00% ± 15.26 %	Inhibition
		Donepezil hydrochloride	15.8*	106.70 % ± 19.38 %	Inhibition

integrity of neuronal DNA as observed by the reduction of DNA damage biomarker γH2AX. The level of the reduced cellular DNA damage is dependent on the treatment concentration of PRSE. Treatment of neuronal model cells with 500 μg/mL of PRSE had reduced the neuronal DNA damage by 50 %. In a parallel study, withanone, a phytonutrient showing neuroprotective effect in previous studies also exhibited protective effect for neuronal DNA from oxidative stress in a concentration-dependent manner. Treating the neuronal model cells with 0.76 μg/mL of withanone reduced the neuronal DNA damage by 50 %. The results are also summarized in Table 1.

3.5. PRSE inhibits MAO and AChE, two therapeutic targets for neurological disorder treatment

Monoamine oxidases (MAOs) are a family of enzymes that catalyze the oxidation of monoamines and inactivate neurotransmitters. Previous studies associated MAOs with a number of psychiatric and neurological disorders. In this assay, we treated human neuroblastoma cells with various concentration of PRSE and observed a dose-dependent inhibition effect. As shown in Fig. 4A, treating cells with 500 μg/mL of PRSE induced 50 % of inhibition of MAO. Harmine, a harmala alkaloid belonging to the beta-carboline family of compounds and a known MAO inhibitor, exhibited a strong concentration-dependent inhibition effect, showing the half maximal inhibitory concentration (IC50) at 7.46 nM.

We also evaluated the inhibition effect of PRSE on acetylcholinesterase (AChE), another therapeutic target for the management of dementia and Alzheimer's disease. Data in Fig. 4B indicated that PRSE effectively inhibited AChE, with an IC50 of 120.8 μg/mL. Donepezil hydrochloride, an AChE inhibitor medication used to treat mild to moderate dementia in patients with Alzheimer's disease, showed strong inhibition effect with an IC50 of 37.86 nM (15.75 ng/mL). The above results are also summarized in Table 1.

4. Discussion

4.1. PRSE induces anti-inflammatory responses, possibly through TNF-α inhibition and Nrf2 activation pathways

Discovery of rich phytochemicals in sugarcane plants has triggered exploration of the use of sugarcane plants for modulation of inflammatory responses and neurological disorders symptomatology. Sugarcane stalks contain high level of water (~70 %), sugar (~16 %), and fiber (~13 %), with only ~1–3 % of the stalks is composed of non-sugar compounds. To collect the non-sugar phytochemicals effectively for investigation, we applied a patented hydrophobic extraction process to obtain a polyphenol rich sugarcane extract PRSE. Previous characterization work indicated that PRSE contains 221 mg GAE/g of polyphenol, a high level of polyphenols comparing with other natural materials such as ground turmeric spice and cacao powder, and displayed a full spectrum antioxidant properties against five primary reactive oxygen/nitrogen species [13]. Inflammation is a key defense response of immune system relevant to oxidative stress, and volumes of studies associated anti-inflammatory properties with antioxidants. In our studies, we explored anti-inflammatory properties of PRSE from two functional pathways: (1) Inhibition of pro-inflammatory cytokines (*e.g.*, TNF-α); (2) Activation of Nrf2-ARE pathway.

TNF-α is a key pro-inflammatory cytokine that triggers physiologic cascade of cytokines and subsequent production of interleukins. As an upstream responder, TNF-α has long been a target for anti-

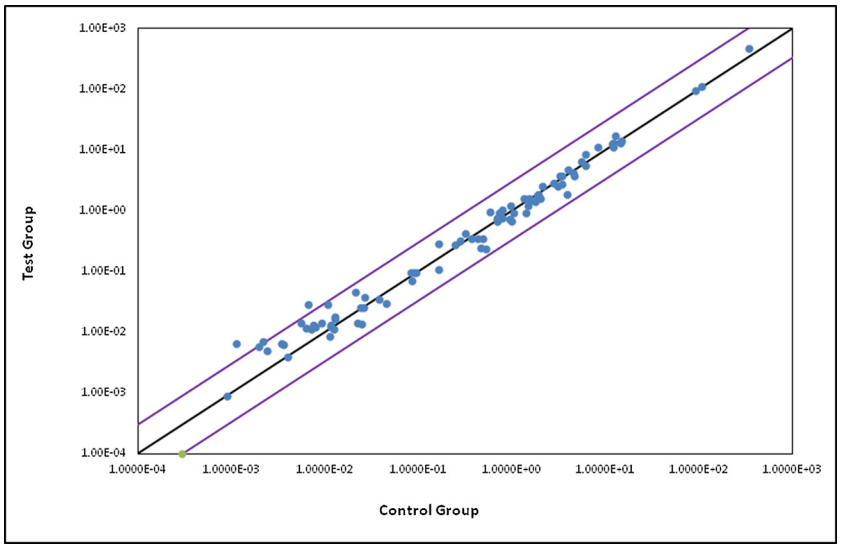

Fig. 2. Scatter plot of the regulation effect of PRSE on 84 neurogenesis-related genes. The black line indicates fold-changes of 1 (*i.e.*, no change). The purple lines indicate the threshold for significant fold-change in gene expression, which is defined as 3. Four genes were displayed outside of the threshold (purple lines), indicating a significant change of gene expression has been observed for these genes.

Table 2
Significant up-regulation effect of PRSE on four neurogenesis-related genes.

Gene symbol	Description of the Gene	Gene expression Level* (fold regulation comparing to control group)	Key Function
NEUROG1	Neurogenin 1	5.7	Neuronal Differentiation Transcription Factor
NEUROG2	Neurogenin 2	2.9	Neuronal Differentiation, Neuronal Migration Transcription Factor
NTF3	Neurotrophin 3	4.2	Neuronal Differentiation
OLIG2	Oligodendrocyte lineage transcription factor 2	3.1	Neuronal Differentiation, Neuronal Cell Fate Determination

* The gene expression level is presented as fold-regulation, a biologically meaningful way representing the change of gene expression comparing with a background control. For a fold change of > 1, the result is reported as a fold up-regulation. For a fold change < 1, the negative inverse of the result is shown as a fold down-regulation. Values of fold change up- or down-regulation > = 3 or < = 3 were defined as significant.

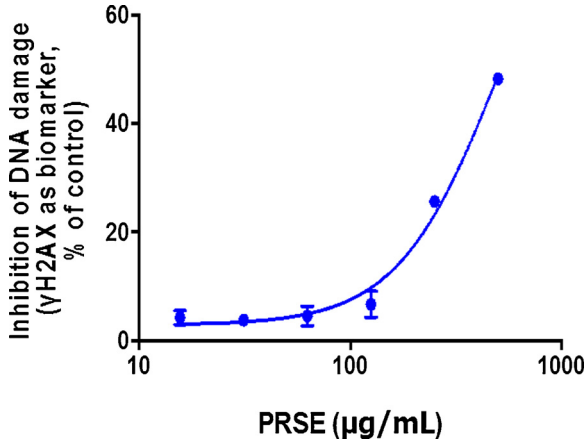

Fig. 3. PRSE mitigates neuronal DNA damage induced by oxidative insult: concentration-dependent mitigation effect of PRSE on neuronal DNA damage (γH2AX as biomarker) induced by oxidative insult.

inflammatory therapeutics with etanercept and infliximab being two well-known, FDA-approved TNF-α blockers for treatments of rheumatoid arthritis (RA) and other chronic inflammatory diseases. Our studies show that PRSE effectively inhibited the onset of TNF-α in LPS-stimulated human macrophages with an IC50 of 36.31 μg/mL. In comparison, curcumin, a polyphenol mostly found in turmeric and an anti-inflammatory medication well-practiced in Traditional Indian Medicine, induced ~40 % of inhibition at 10 μM (3.6 μg/mL). This finding should also be considered in light of recent human clinical trials which reported that curcumin exhibited pro-cognitive and mood measures, and that such effects were attributed to curcumin's anti-inflammatory properties [16]. One consideration in comparing the inhibition effect of above substances is that PRSE is a mixture material, unlike its comparison material curcumin that is of pure form (> 90 % purity). As a botanic plant extract, PRSE by nature contains various polyphenol components that function independently or synergistically or contradictorily. Besides these potential bioactives, PRSE contains other components such as metals and minerals that may not contribute to the specific inhibition effect being studied. Presumably, pure compounds such as curcumin are more concentrated and therefor more potent. This explains the ~10-fold difference in the inhibition potency between PRSE and curcumin observed in the above study. This consideration is also applicable when interpreting the rest of the study findings of this work.

Following TNF-α inhibition study, we further investigated whether Nrf2 activation, a key therapeutic target for modulating inflammation and many other physiological conditions, a possible function pathway for PRSE. Under basal conditions, Nrf2, a redox-sensitive transcription factor involved in cellular responses to oxidative stress, is a short-lived protein located mainly in the cytoplasm. In response to oxidative stress, Nrf2 activates, translocates into the nucleus and elicits the antioxidant response by initiating a series of gene products. The Nrf2 activation and the subsequent cascading events have been recognized an effective pathway to counter oxidative stress-mediated inflammation. Our studies show that PRSE initiated strong Nrf2 activation in a concentration-dependent manner. At 62.5 μg/mL, PRSE nearly doubled the activation of Nrf2. When treating the cells with 500 μg/mL of PRSE, we recorded a strong, 50-fold of Nrf2 activation. A number of studies reported the capability of natural products in activating Nrf2 pathway. In a similar Nrf2 reporter-HepG2 cellular assay, 56 compounds in the families of trans stilbenes were screened. Up to 69-fold activation of Nrf2 were recorded in this study, with families of nonfluoro trans stilbenes inducing the highest levels of Nrf2 activation [17]. In a parallel study that we conducted, 50-fold of Nrf2 activation was induced by treating the cells with 15.11 μg/mL of quercetin, a phytochemical known to be a Nrf2 activator. Comparing with these findings, PRSE demonstrated to be a potent Nrf2 activator that induced up to 50-fold of Nrf2 activation.

Beyond an anti-inflammatory target, Nrf2 activation has also been

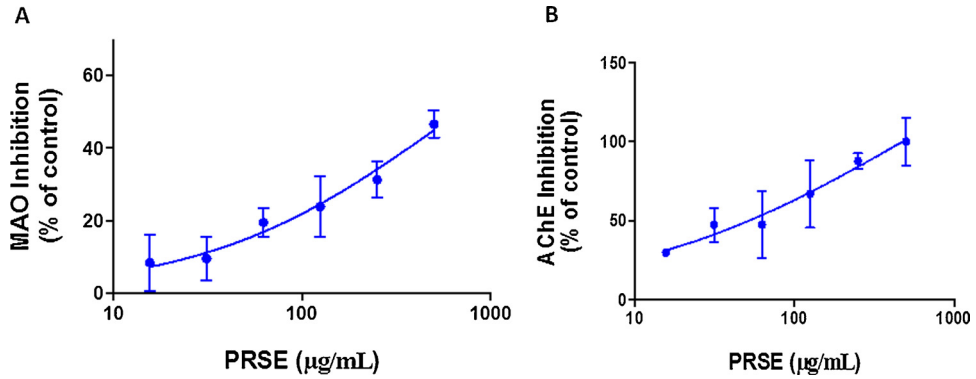

Fig. 4. PRSE inhibits MAO and AChE, two therapeutic targets for neurological disorder treatment. 4A. Concentration-dependent inhibition effect of PRSE on the expression of MAO in human neuronal cells. 4B: Concentration-dependent inhibition effect of PRSE on AChE activity.

reported an effective pathway to modulate neurological functions, suggesting it a direct therapeutic target for the treatment of neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease. The existing data strongly support the use of Nrf2 activators to treat multiple sclerosis (MS) [18] while ongoing studies in Parkinson's disease, Alzheimer's disease, and Huntington's disease are expected to shed more light on the significance of Nrf2 activation in these diseases as well. Our observation of PRSE as an effective Nrf2 activator suggests the potential use of PRSE in inflammation interception as well as neurological disorder modulation. This finding substantiated the subsequent studies on the effect of PRSE on neurological conditions.

4.2. PRSE induces upregulation effect on four neurogenesis-related genes

As a botanic plant extract, PRSE by nature is a mixture material containing various polyphenol components. The impact of PRSE in neurological health and its potential pathway are largely unknown. To gain an overall understanding in this function area, we evaluated the impact of PRSE on the expression of a broad range of neurogenesis-related genes in a human neuronal cell model *via* a PCR array technique. Among the 84 human neurogenesis-related genes investigated, treatment of PRSE induced significant up-regulation of four genes that are involved in a number of neuronal function areas: transcription factor (NEUROG2), neuronal differentiation and migration (NEUROG1, NEUROG2, NTF3), and neuronal cell fate determination (OLIG2). Table 2 lists the four genes along with their key functions, and detailed functional info on each of the regulated genes can be found in public databases. Among the four regulated genes, PRSE showed clear up-regulation of NTF3 (4.2 folds of regulation), which supports the survival and differentiation of existing neurons and to promote the growth and differentiation of new neurons and synapses. Studies have shown a close relation between neurotrophic factors regulation and neuronal stress management, and neurotrophic factor therapy (NFT therapy) has been a therapeutic approach for Parkinson's disease [19]. PRSE also showed clear up-regulation of NEUROG1 (2.9 folds) and NEUROG2 (5.7 folds), which are vertebrate neuronal determination genes that have been shown to specify distinct neuronal identities in different regions of the nervous system [20]. Another gene that PRSE up-regulates is Olig2, a basic helix-loop-helix transcription factor necessary for the oligodendroglial development in the nervous system particularly in the spinal cord. Although the functional role of Olig2 in oligodendrocyte migration and differentiation remains elusive, some studies suggest that up-regulation of Olig2 promotes the regeneration and remyelination of oligodendrocyte in lesions of drug-induced demyelination animal models [21] Other studies in mice further suggested Olig2 up-regulation as a therapeutic target for myelin repair [22].

Our data suggest that PRSE up-regulates multiple neurogenesis-related genes that govern the neuronal survival and stress management, neuronal differentiation, and other neuronal transcription and development functions. These findings agree with previously reports that discovered neurogenesis promotion effect by polyphenols [23]. As an initial exploratory investigation that covered a broad range of the neurologically significant genes, these results call for design of future studies aiming to test PRSE as a neurological function modulator.

4.3. PRSE protects neuronal cells from oxidative stress by mitigating DNA damage

Oxidative stress induces damage to DNA/RNA, and such damage is elevated in central and peripheral nervous systems of patients with neurodegenerative diseases. In this study, we monitored neuronal DNA damage triggered by oxidative stress in human neuronal cells, and studied whether PRSE extract could mitigate DNA damage and protect neuronal cells. A pathogenically high level of glutamate treatment of the cells was used to trigger neuronal oxidative stress. At an optimal level, glutamate is a major excitatory neurotransmitter in the central nervous system that articulates signaling network involved in brain functions including cognition, memory and learning. However, at low and high doses, glutamine leads to a depletion of intrinsic antioxidant glutathione and the accumulation of oxidants, triggering oxidative insult in brain and neurotoxic cascades. The neuronal DNA damage response γH2AX, a highly sensitive and specific biomarker for DNA damage initiation and repair, is monitored as the indicator of neuronal DNA damage. Our studies indicate that treating the cells with pathogenically high level of glutamate treatment triggered a significant level of neuronal DNA damage observed as elevated level of damage biomarker γH2AX, and the damage level reduced and cells recovered in the medium supplemented with PRSE. The cellular DNA damage reduction is concentration-dependent. Treatment of neuronal model cells with 500 μg/mL of PRSE had reduced the neuronal DNA damage by 50 %. Withanone, a phytonutrient that has shown positive neuroprotective effect in previous studies also exhibited protective effect for neuronal DNA from oxidative stress damage in a concentration-dependent manner. Treating the neuronal model cells with 0.76 μg/mL of withanone reduced the neuronal DNA damage by 50 %.

4.4. PRSE inhibits neurological disorder treatment target such as MAO, potentially exerts its therapeutic effect through oxidative stress attenuation

Monoamine oxidases (MAOs) are a family of enzymes that catalyze the oxidation of monoamines and inactivate neurotransmitters. MAO dysfunction has been found responsible for a number of psychiatric and neurological disorders, and monoamine oxidase inhibitors have been used in the treatment of Parkinson's disease, depression, and potentially Alzheimer disease. In our study, MAO target came into focus due to its close association with oxidative stress. Previous studies reported that one of the main function mechanisms of MAO's involvement in neurodegeneration is *via* oxidative stress, with finding suggested that MAO inhibitors exert neurological treatment effects by altering the redox state of neuronal and glial cells, mitigating oxidative stress and subsequent neuroinflammation [24].

We attempted this upstream target through cellular MAO inhibition studies, and discovered that PRSE was able to inhibit MAO activity in a concentration-dependent manner (Fig. 4A). Treating human neuroblastoma cells with 500 μg/mL of PRSE induced a 46.6 % of MAO inhibition, equivalent to the inhibition effect induced by ~0.0024 μg/mL of harmine, a harmala alkaloid belonging to the beta-carboline family of compounds also a known MAO inhibitor (Table 1). This finding resonated with a previous animal study where curcumin a polyphenolic compound, and its metabolite tetrahydrocurcumin were observed to inhibit MAO and reverse Parkinson's disease symptoms [25].

Synthetic MAO inhibitors are currently in clinical use for the treatment of depression and Parkinson's disease. Despite the strong clinical efficacy of MAO inhibitors, the prescription of MAO inhibitors has declined in the past decades largely due to the strict dietary restriction placed on patients, although such restriction has been questioned [26]. Results of many animal and clinical studies suggest the use of natural polyphenolic compounds in modulating mental health including cognition, mood, neuroplasticity, and antidepressant-like properties. The phytochemicals can be used alone, or as an adjunctive to prescription drugs to improve the mental state. Our findings agree with the approach that applies phytochemicals to modulate monoamine oxidase production.

In a separate enzymatic study, we also evaluated the inhibition effect of PRSE on AChE, another therapeutic target for the neurological diseases including dementia and Alzheimer's disease. AChE is a family of enzymes that catalyzes the hydrolysis of the neurotransmitter acetylcholine into choline and acetic acid, and terminates the impulse transmission. Inhibition of AChE leads to accumulation of the neurotransmitter acetylcholine and hyperstimulation of nicotinic and muscarinic receptors, which has been used as an approach to disrupt neurotransmission in a diseased stage [27]. AChE inhibitors such as

donepezil and rivastigmine are a major category of current medication for cognition enhancement and Alzheimer's disease treatment. PRSE effectively inhibited AChE in a dose-dependent manner, with an IC50 of 120.8 μg/mL (Fig. 4B). In a parallel study, donepezil hydrochloride, one of the approved AChE inhibitor drugs for cognition and Alzheimer's disease, exhibited an IC50 of 37.86 nM (15.75 ng/mL).

To understand what constituent(s) might have contributed to the observed anti-inflammatory and neurological disorder modulation effects in PRSE, we conducted preliminary targeted polyphenols analysis with Liquid Chromatography-Mass Spectrometry (LC–MS) technique and discovered three major groups of polyphenols in PRSE: tricin (27.40 μg/g), luteolin (5.30 μg/g), and apigenin (1.89 μg/g) [13]. These findings are encouraging as tricin and its glucose derivatives have been reported to protect against cerebral ischemia by reduction of inflammation biomarker such as NF-κB [28], while luteolin and apigenin also have been related to neuroprotective effects observed in *in vivo* studies [29]. Further phytochemical profiling and characterization studies are undergoing in our laboratory to provide more insight on the key bioactive(s) in PRSE.

5. Conclusion

Based on a patented hydrophobic extraction process, we obtained a polyphenol rich sugarcane extract that exhibits significant anti-inflammatory properties, potentially through inhibiting pro-inflammatory cytokines such as TNF-α, and activating Nrf2-ARE transcription pathway. Further studies indicated that PRSE significantly regulates four neurogenesis-related genes, protects neuronal cells from oxidative stress challenge potentially through preservation of neuronal DNA integrity, prevents neurodegeneration through regulating key proteins such as monoamine oxidase (MAO), and provides moderate preservation of endogenous antioxidants.

There has been abundant evidence linking transcription factors and cytokine production to overall inflammatory responses that occur in the central nervous system. The link appears to be underpinned by two common processes: first, transcription factors and cytokines are capable of interactions with critical proteins and lipid kinase signaling cascades in the brain. Positive influence of these transcription factors and cytokines can lead to inhibition of apoptosis triggered by neurotoxic species and to a promotion of neuronal survival and synaptic plasticity; second, the transcription factors and cytokines induce beneficial effects on the vascular system, leading to changes in cerebrovascular blood flow capable of causing enhanced vascularization and neurogenesis - two events important in the maintenance of cognitive performances. Together, these processes act to maintain neuronal homeostasis and play important roles in neuronal stress adaptation. Our findings in this work further demonstrated the close association of inflammation and neurological diseases, and presents for the first time, the potential effect of a polyphenol rich sugarcane extract in anti-inflammation and neurological disorder management.

Our findings also agree with previous reports on the use of polyphenols rich food and diet to mitigate inflammation and neurological disorders. Faria and colleagues reported that dietary polyphenols are able to cross blood-brain barrier and control neuronal disease pathogenesis at a molecular and symptomatic level [30]. Our studies supported these findings on the cellular level. One consideration in applying natural plant extracts is the possible synergistic or competing effects from various components in the extract. In a review article, Scheepens and colleagues discussed in depth designed synergies to improve oral bioavailability as well as efficacy of beneficial polyphenols [31]. In this current study, we reported the combined effect from PRSE, a mixture plant extract material. While we have completed preliminary phytochemical profiling work that suggest the possible bioactives, we are carrying out further composition profiling and characterization studies to provide more insight on the key bioactive(s) in PRSE.

We recognize that current study findings are based on human cells, which is still a long way towards the translation into clinical success. Regardless, our work encourages further *in vitro* and *in vivo* studies to verify these potential therapeutic effects, and explore signal pathway in more depth. In our laboratories, a number of follow up confirmation studies are underway, including further cellular studies and *in vivo* trials on the effect of PRSE on neurogenesis, depression, and cognition. The success of these studies provides an opportunity for discovering valuable bioactives extracted during sugar production. This could potentially result in new phyto-therapeutics for inflammation and neurological disorders based on a globally abundant crop. In a clinical setting it is unknown to what extent and how the metabolites studied would be altered by factors such as the microbiome. However, the results collected to date indicate that it is worthwhile attempting to translate these results into the increased complicity of the clinical setting. Human trials are then the next step to understand to what extent these extracts can promote healthy cognition and prevent neurological damage in humans.

Ethic statement

This work did not include any human subjects or animal experiments.

Declaration of Competing Interest

Yang X., Flavel M, and Kitchen B. are employees of The Product Maker Pty Ltd, Melbourne, Australia, the producer of PRSE. Ji J, Chen O. C.Y., Downey L., and Stough C. have no conflict of interest.

Acknowledgement

This work was supported by The Product Makers, Pty Ltd, Melbourne, Australia.

References

[1] S. Salim, Oxidative stress and the central nervous system, J. Pharmacol. Exp. Ther. 360 (1) (2017) 201–205.
[2] L.E. Tebay, H. Robertson, S.T. Durant, S.R. Vitale, T.M. Penning, A.T. Dinkova-Kostova, J.D. Hayes, Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy status and the pathways through which it attenuates degenerative disease, Free Radic. Biol. Med. (2015) 108–146 Mechanisms of activation of the transcription factor Nrf2 by redox stressors, nutrient cues, and energy stat88.
[3] M. Sandberg, J. Patil, B. D'Angelo, S.G. Weber, C. Mallard, NRF2-regulation in brain health and disease: implication of cerebral inflammation, Neuropharmacology 79 (2014) 298–306.
[4] J. Himmelfarb, Poor nutritional status and inflammation: Linking oxidative stress and inflammation in kidney disease: which is the chicken and which is the egg? Semin. Dial. 17 (6) (2004) 449–454.
[5] R. Bomprezzi, Dimethyl fumarate in the treatment of relapsing–remitting multiple sclerosis: an overview, Ther. Adv. Neurol. Disord. 8 (1) (2015) 20–30.
[6] F.C. Lau, B. Shukitt-Hale, J.A. Joseph, The beneficial effects of fruit polyphenols on brain aging, Neurobiol. Aging 26 (1) (2005) 128–132.
[7] H. Checkoway, K. Powers, T. Smith-Weller, G.M. Franklin, W.T. Longstreth Jr., P.D. Swanson, Parkinson's disease risks associated with cigarette smoking, alcohol consumption, and caffeine intake, Am. J. Epidemiol. 155 (8) (2002) 7.
[8] D. Commenges, V. Scotet, S. Renaud, H. Jacqmin-Gadda, P. Barberger-Gateau, J.F. Dartigues, Intake of flavonoids and risk of dementia, Eur. J. Epidemiol. 16 (4) (2000) 357–363.
[9] A. Dal-Pan, S. Dudonne, P. Bourassa, M. Bourdoulous, C. Tremblay, Y. Desjardins, F. Calon, Cognitive-enhancing effects of a polyphenols-rich extract from fruits without changes in neuropathology in an animal model of Alzheimer's disease, J. Alzheimer Dis. 55 (1) (2017) 115–135.
[10] A. Singh, U.R. Lal, H.M. Mukhtar, P.S. Singh, G. Shah, R.K. Dhawan, Phytochemical profile of sugarcane and its potential health aspects, Pharmacogn. Rev. 9 (17) (2015) 45.
[11] J.M. Duarte-Almeida, A.V. Novoa, A.F. Linares, F.M. Lajolo, M.I. Genovese, Antioxidant activity of phenolics compounds from sugar cane (Saccharum officinarum L.) juice, Plant Foods Hum. Nutr. 61 (4) (2006) 187.
[12] A. Colonnello, I. Kotlar, M.E. de Lima, A. Ortíz-Plata, R. García-Contreras, A.A. Soares, A. Santamaría, Comparing the effects of ferulic acid and sugarcane aqueous extract in in vitro and in vivo neurotoxic models, Neurotox. Res. 34 (3)

(2018) 640–648.
[13] J. Ji, X. Yang, M. Flavel, Z.P. Shields, B. Kitchen, Antioxidant and anti-diabetic functions of a polyphenol-rich sugarcane extract, J. Am. Coll. Nutr. (2019) 1–11.
[14] M.A. Deseo, A. Elkins, S. Rochfort, B. Kitchen, Antioxidant activity and polyphenol composition of sugarcane molasses extract, Food Chem. (January (11)) (2020) 126180.
[15] G.L. Ellman, K.D. Courtney, V. Andres Jr., R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol. 7 (2) (1961) 88–95.
[16] K.H. Cox, A. Pipingas, A.B. Scholey, Investigation of the effects of solid lipid curcumin on cognition and mood in a healthy older population, J. Psychopharmacol. 29 (5) (2015) 642–651.
[17] L.M. Deck, L.J. Whalen, L.A. Hunsaker, R.E. Royer, D.L. Vander Jagt, Activation of anti-oxidant Nrf2 signaling by substituted trans stilbenes, Bioorg. Med. Chem. 25 (4) (2017) 1423–1430.
[18] FDA, U. S, FDA Approves New Multiple Sclerosis Treatment: Tecfidera, Food and Drug Administration, US, 2013.
[19] S.B. Rangasamy, K. Soderstrom, R.A. Bakay, J.H. Kordower, Neurotrophic factor therapy for Parkinson's disease, Prog. Brain Res. 184 (2010) 237–264.
[20] T. Shaker, D. Dennis, D.M. Kurrasch, C. Schuurmans, Neurog1 and Neurog2 coordinately regulate development of the olfactory system, Neural Dev. 7 (1) (2012) 28.
[21] B. Tan, J. Yu, Y. Yin, G. Jia, W. Jiang, L. Yu, The Olig family affects central nervous system development and disease, Neural Regen. Res. 9 (3) (2014) 329.
[22] A. Wegener, C. Deboux, C. Bachelin, M. Frah, C. Kerninon, D. Seilhean, B. Nait-Oumesmar, Gain of Olig2 function in oligodendrocyte progenitors promotes remyelination, Brain 138 (1) (2014) 120–135.
[23] I. Figueira, G. Garcia, R.C. Pimpão, A.P. Terrasso, I. Costa, A.F. Almeida, A. Filipe, Polyphenols journey through blood-brain barrier towards neuronal protection, Sci. Rep. 7 (1) (2017) 11456.
[24] G. Alper, F.K. Girgin, M. Ozgönül, G. Menteş, B. Ersöz, MAO inhibitors and oxidant stress in aging brain tissue, Eur. Neuropsychopharmacol. 9 (3) (1999) 247–252.
[25] A. Rajeswari, M. Sabesan, Inhibition of monoamine oxidase-B by the polyphenolic compound, curcumin and its metabolite tetrahydrocurcumin, in a model of Parkinson's disease induced by MPTP neurodegeneration in mice, Inflammopharmacology 16 (2) (2008) 96–99.
[26] J.G. Fiedorowicz, K.L. Swartz, The role of monoamine oxidase inhibitors in current psychiatric practice, J. Psychiatr. Pract. 10 (4) (2004) 239.
[27] M.B. Colovic, D.Z. Krstic, T.D. Lazarevic-Pasti, A.M. Bondzic, V.M. Vasic, Acetylcholinesterase inhibitors: pharmacology and toxicology, Curr. Neuropharmacol. 11 (3) (2013) 315–335.
[28] W.L. Jiang, Y. Xu, S.P. Zhang, H.B. Zhu, J. Hou, Tricin 7-glucoside protects against experimental cerebral ischemia by reduction of NF-κB and HMGB1 expression, Eur. J. Pharm. Sci. 45 (1–2) (2012) 50–57.
[29] S.F. Nabavi, N. Braidy, O. Gortzi, E. Sobarzo-Sanchez, M. Daglia, K. Skalicka-Woźniak, S.M. Nabavi, Luteolin as an anti-inflammatory and neuroprotective agent: a brief review, Brain Res. Bull. 119 (2015) 1–11.
[30] A. Faria, D. Pestana, D. Teixeira, J. Azevedo, V. De Freitas, N. Mateus, C. Calhau, Flavonoid transport across RBE4 cells: a blood-brain barrier model, Cell. Mol. Biol. Lett. 15 (2) (2010) 234.
[31] A. Scheepens, K. Tan, J.W. Paxton, Improving the oral bioavailability of beneficial polyphenols through designed synergies, Genes Nutr. 5 (1) (2010) 75.