Journal of Functional Foods 23 (2016) 359–369

Available online at www.sciencedirect.com

ScienceDirect

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

The neuroprotective properties of a novel variety of passion fruit

Yael Tal [a], Sarit Anavi [a], Merav Reisman [a], Alon Samach [b], Oren Tirosh [a], Aron M. Troen [a,*]

[a] Institute of Biochemistry Food and Nutrition Science, The Robert H. Smith Faculty of Agriculture Food & Environment, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot, 76100, Israel

[b] Institute of Plant Sciences and Genetics, The Robert H. Smith Faculty of Agriculture Food & Environment, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot, 76100, Israel

ARTICLE INFO

Article history:
Received 17 November 2015
Received in revised form 20 February 2016
Accepted 22 February 2016
Available online 31 March 2016

Keywords:
Passiflora
Neuroprotection
MPTP
Glutamate-toxicity
Antioxidant

ABSTRACT

Passion fruit is a commercially important crop. The neuroprotective activity of fruit extracts from two hybrid lines of antioxidant ester thiol-rich *Passiflora edulis* Sims, the commercial "Passion Dream" and novel cultivar 428 ("Dena") line were studied. Crude extracts from line 428 displayed the strongest dose-dependent neuroprotective activity, preventing glutamate induced cell-death, mitochondrial depolarization and glutathione depletion, when added to the medium of cultured HT4 neurons ($p < 0.05$). Supplementing diet of mice with the 428 fruit-extract improved survival of dopaminergic neurons by 60% in mice injected with the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MTPT) compared to control-fed MPTP-injected mice ($p < 0.05$). The neuroprotection conferred by passion fruit extracts *in vivo* and *in vitro* shows promise for further research into their bioactive potential for medical exploitation.

1. Introduction

Neurodegenerative diseases, notably Alzheimer's Disease and Parkinson's Disease whose main risk-factor is ageing are increasingly prevalent (Joseph, Cole, Head, & Ingram, 2009; Mandel, Amit, Weinreb, & Youdim, 2011) due to increased life expectancy around the world. Symptomatic treatments and disease-modifying interventions that aim to target the specific hallmark pathologies in each disease (e.g. the plaques and tangles in Alzheimer's Disease or Lewy bodies in Parkinson's Disease) do not address the early age-related processes which initiate the disease and put the brain at risk. New preventive therapies are needed because the downstream neurodegenerative pathology is often irreversible by the time the clinical symptoms of the disease appears. Here, we present our

Chemical compounds: 3-Mercaptohexyl acetate (PubChem CID: 518810); 3-Mercaptohexyl butyrate (PubChem CID: 537754); 3-Mercaptohexyl hexanoate (PubChem CID: 518810).

* *Corresponding author.* The Nutrition and Brain Health Laboratory, Institute of Biochemistry Food and Nutrition Science, The Robert H. Smith Faculty of Agriculture Food & Environment, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot, 76100, Israel. Tel.: +972 8 9489476; fax: +972 9489822.

E-mail address: aron.troen@mail.huji.ac.il (A.M. Troen).

Abbreviations: 428, "Dena" line of passion fruit; CMFDA, chloromethylfluorescein diacetate; H2DCF, 2',7'-dichlorodihydrofluorescein diacetate; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Passion Dream; PF, passion fruit; PI, propidium iodide; ROS, reactive oxygen species; SOD, superoxide dismutase; TH, tyrosine hydroxylase

http://dx.doi.org/10.1016/j.jff.2016.02.039
1756-4646/

findings on a new variety of passion fruit that may serve this role.

A substantial body of evidence indicates that in these diseases, neurodegeneration is accelerated by mitochondrial dysfunction and oxidative stress (Emerit, Edeas, & Bricaire, 2004; Rao & Balachandran, 2002) and that under conditions of heightened oxidative stress, reactive oxygen species (ROS) can induce cellular dysfunction and even death. The brain is generally susceptible to such oxidative damage due to its high metabolic activity in a lipid rich environment with relatively low levels of endogenous antioxidant defenses. However, certain neuronal populations may be particularly vulnerable due to their specific metabolic and neurochemical profiles. For example, an excess of the neurotransmitter dopamine can generate cytotoxic ROS due to dopamine's normal metabolism by monoamine oxidase (MAO) or through autoxidation. This phenomenon has been linked to the vulnerability of dopaminergic neurons in the substantia nigra in Parkinson's Disease (Blesa, Trigo-Damas, Quiroga-Varela, & Jackson-Lewis, 2015; Zucca et al., 2014). Pathologic conditions can also elicit ROS production and excitotoxicity as a result of hyper-stimulation of glutamatergic neurons. (Coyle & Puttfarcken, 1993; Gilgun-Sherki, Melamed, & Offen, 2001). This process in striatal neurons has also been implicated in Parkinson's Disease (Ambrosi, Cerri, & Blandini, 2014; Van Laar et al., 2015).

Chronic oxidative stress in the brain can overwhelm the endogenous antioxidant defenses including the enzymes CuZn- and Mn-superoxide dismutase (SOD), glutathione (GSH) peroxidase and catalase, and deplete antioxidant small molecules such as glutathione (Schulz, Lindenau, Seyfried, & Dichgans, 2000) (Halliwell & Gutteridge, 1985). Under such conditions, delivery of antioxidant to the CNS by increasing the intake of dietary antioxidants may be beneficial (Gilgun-Sherki et al., 2001). Thus, the identification of novel antioxidants as potential therapeutics is a challenging area of neuroscience research (Kelsey, Wilkins, & Linseman, 2010) and a variety of plant-derived compounds have shown neuroprotective benefit in cell and animal models of Parkinson's Disease (Kim et al., 2010; Lu et al., 2014; McGuire et al., 2006; Nataraj, Manivasagam, Justin Thenmozhi, & Essa, 2015; Rajeswari, 2006; Strathearn et al., 2014; Tseng, Chang, & Lo, 2014). Recent research suggests that consumption of botanical extracts or of the whole fruit, which comprises multiple compounds, may be more beneficial than individual compounds, due to synergistic effects (Joseph et al., 2010). Moreover, superior therapeutic benefit may result if the combination of compounds in a whole fruit targets multiple pathological pathways (polypharmacology) (Mandel et al., 2011). Fruits and especially berries are of special interest for neuroprotection because they contain a wide variety of antioxidants, are palatable and habitually consumed in the diet (Habib & Ibrahim, 2011; Holt et al., 2009; Scalbert, Johnson, & Saltmarsh, 2005).

The present study examined the neuroprotective effects, *in vitro* and *in vivo*, of a novel cultivar of passion fruit (*Passiflora edulis* Sims), a member of genus Passiflora, whose vines yield juicy fruit with a distinctive aroma and flavor. There are two described forma within the species: *flavicarpa* (yellow peel) and *edulis* (purple peel). The majority of varieties grown commercially are of the *flavicarpa* forma, with the fruit used primarily as a source for concentrate juice. Hybrids between the two forma are also grown commercially, mostly for fresh fruit consumption. One such hybrid, 'Passion Dream' (PD), is commercially grown in Israel. Ripening attributes and chemical composition of fruit from this cultivar, and from an additional cultivar termed 'Ripens during summer' (previously named 428, cultivar name 'Dena'), an F2 progeny from self-fertilized PD (F1), were recently described (Goldenberg, Feygenberg, Samach, & Pesis, 2012). They are of interest here because edible pulp from both cultivars contains relatively high levels of the ester thiol volatiles 3-mercaptohexyl acetate, 3-mercaptohexyl butanoate and 3-mercaptohexyl hexanoate (Goldenberg, 2012; Goldenberg et al., 2012). These thiols are potential antioxidants, scavengers of many ROS species that may interact with cysteine residues in proteins to help keep them in the reduced state. In addition, given their size and structure (low molecular weight, small surface area, hydrophobic compounds with little capacity to form hydrogen bonds) these thiol esters have the ideal traits to cross the blood brain barrier (Atlas, Melamed, & Ofen, 1999; Bahat-Stroomza et al., 2005; Serlin, Shelef, Knyazer, & Friedman, 2015; Talcott, Percival, Pittet-Moore, & Celoria, 2003).

Extracts prepared from flowers of a different species, *Passiflora incarnata*, are reported to be anxiolytic (Miroddi, Calapai, Navarra, Minciullo, & Gangemi, 2013) (Sampath, Holbik, Krenn, & Butterweck, 2011). Since most of the commercial *P. edulis* fruit is used for juice production, factories collect huge quantities of fruit peel (rind, the fruits' pericarp) (Canteri et al., 2010). The potential neuroprotective properties of the fruit pulp, that is normally consumed, have not yet been explored, however, several studies suggest that in addition to being a source for industrial pectin extraction, rind extracts have significant anti-inflammatory and antioxidant health benefits (Chilakapati, Serasanambati, Manikonda, Chilakapati, & Watson, 2014). The current study explored the neuroprotective potential of the commercial PD and line 428 fruit extracts using a well-established cell culture model in which oxidative stress and mitochondrial dysfunction are induced by glutamate toxicity. Because the line 428 extract gave the most promising results in cultured neurons, we then tested its capacity to protect against neuronal-cell death in a well-established animal model of neurodegeneration that mimics Parkinson's disease by selective death of dopaminergic neurons in the brain substantia nigra due to acute administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MTPT).

2. Materials and methods

2.1. *Passion fruit extracts*

Passion Dream and PF428 fruit were grown on the experimental farm of the Robert H. Smith Faculty of Agriculture, Food and Environment of the Hebrew University of Jerusalem. The juice, pulp and seeds were removed from the ripe fruits, freeze-dried, powdered and stored at –80 °C until used in cell culture and *in vivo* studies. For cell culture experiments, stock extracts were prepared by suspending the freeze-dried powder in phosphate buffered saline (PBS) at an initial concentration of 20% by weight. The suspension was centrifuged at 3400g for 20 min at room temperature (RT) and the supernatant was