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Açaí (*Euterpe oleraceae Mart.*) berry extract exerts neuroprotective effects against β-amyloid exposure in vitro

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HIGHLIGHTS

- Açaí extract inhibited neurotoxicity following $A\beta_{1-42}$ exposure in PC12 cells.
- Açaí extract inhibited $A\beta_{1-42}$ fibril and aggregate formation in vitro.
- Açaí extract did not provide significant protection against oxidative stress.
- Açaí may have neuroprotective potential in Alzheimer's disease.

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ABSTRACT

The native South American palm açaí berry (*Euterpe oleraceae Mart.*) has high polyphenolic and antioxidant levels. This study examined whether açaí berry extract afforded protection against β-amyloid (Aβ)-mediated loss of cell viability and oxidative stress associated with anti-fibrillar effects. PC12 cells were exposed to either $A\beta_{1-42}$, $A\beta_{25-35}$ or *tert* butyl hydroperoxide (*t*-BHP), alone or in the presence of açaí extract (0.5–50 μg/ml). Thioflavin T (ThT) binding assay and transmission electron microscopy were used to determine effects of açaí extract on $A\beta_{1-42}$ fibril morphology and compared to açaí phenolics gallic acid, cyanidin rutinoside and cyanidin glucoside. Exposure to $A\beta_{1-42}$, $A\beta_{25-35}$ or *t*-BHP decreased PC12 cell viability. Pretreatment with açaí extract significantly improved cell viability following $A\beta_{1-42}$ exposure, however $A\beta_{25-35}$ or *t*-BHP-mediated viability loss was unaltered. Açaí extract inhibited ThT fluorescence and disrupted $A\beta_{1-42}$ fibril and aggregate morphology. In comparison with other phenolics, açaí was most effective at inhibiting $A\beta_{1-42}$ aggregation. Inhibition of β-amyloid aggregation may underlie a neuroprotective effect of açaí.

1. Introduction

Neurotoxicity in Alzheimer's disease (AD) is associated with increased levels of β-amyloid (Aβ) peptide in the brain, manifest via Aβ oligomer and fibril formation [17,25]. Many naturally occurring compounds ascribed neuroprotective properties may act at multiple pathways in mitigating Aβ-evoked neurotoxicity, in particular the flavonoids [34]. Their bioactivity may extend beyond antioxidant capacity to include influencing cell signalling pathways and direct anti-amyloid actions, exemplified by the green tea flavanol epigallocatechin gallate (EGCG) [1,34].

The berry from the native South American palm açaí (*Euterpe oleraceae Mart.*) has been ascribed high anti-oxidant capacity [15,23,29]. Açaí has been shown to prevent hydrogen peroxide-induced cell damage [31], lower oxidative stress in neutrophils [29] and reduce inflammatory signalling in microglial cells [27]. Collectively, the beneficial effects of açaí are mainly attributed to antioxidant capacity; however, potential neuroprotection against β-amyloid toxicity and any direct effects on β-amyloid fibrillisation are unknown.

This study characterised the capacity of açaí berry extract to increase neuronal cell viability following exposure to two β amyloid species, $A\beta_{1-42}$ and $A\beta_{25-35}$, in addition to the pro-oxidant *tert*-butyl hydroperoxide (*t*-BHP). Açaí's capacity to alter β-amyloid fibrillisation was also compared with major polyphenolics in the açaí berry extract including cyanidin glucoside, cyanidin rutinoside and gallic acid [15,20,28].

2. Materials and methods

2.1. Materials

Human amyloid-β protein 1–42 ($A\beta_{1-42}$) and 25–35 ($A\beta_{25-35}$) were obtained from Abcam (Cambridge, MA, USA). Cyanidin

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glucoside, (-)-epigallocatechin-3-gallate (EGCG), gallic acid, Folin-Ciocalteu reagent, thiazolyl blue tetrazolium bromide (MTT), trypan blue, *tert* butyl hydroperoxide (*t*-BHP), trolox, quercetin, thioflavin T (ThT) and uranyl acetate were obtained from Sigma–Aldrich (Castle Hill, NSW, Australia). RPMI-1640 medium, foetal calf serum (FCS), L-glutamine and penicillin/streptomycin were obtained from Life Technologies (Mulgrave, VIC, Australia). Cyanidin rutinoside was provided by Dr Graham Jones (Discipline of Oenology, University of Adelaide).

2.2. PC12 cell culture

Rat phaeochromocytoma cells (Ordway PC12) cells displaying a semi-differentiated phenotype with neuronal projections [6] were provided by Professor John Piletz (Loyola University Medical Centre, IL, USA) and maintained in RPMI-1640 media with 5% FCS, 1% L-glutamine, 1% non-essential amino acids and 1% penicillin/streptomycin. Cells were seeded at 2×10^4 cells per well in RPMI-1640 and 10% FCS. PC12 cells were equilibrated for 24 h before treatment with açaí extract and either of $A\beta_{1-42}$, $A\beta_{25-35}$ or *t*-BHP.

2.3. Açaí extract preparation

Fresh açaí aqueous extract was prepared by diluting freeze-dried commercially available açaí fruit pulp and skin powder (Riolife NSW, Australia) in phosphate-buffered saline (PBS) [29]. This solution was vortexed, centrifuged at 400 rpm and 0.20 μm syringe-filtered. The extract was further diluted in PBS for cell viability experiments (0.5–50 μg/ml).

2.4. Aβ and açaí treatment in PC12 cells

Both $A\beta_{1-42}$ and $A\beta_{25-35}$ were prepared as per established protocols [14]. $A\beta_{25-35}$ was pre-fibrillised at 37 °C for 24 h prior to application to PC12 cells, to ensure it was in a fibrillar state upon application to cells. Conversely, native $A\beta_{1-42}$ was used directly in cell incubations as it readily fibrillises rapidly at 37 °C, so that over 48 h of incubation PC12 cells were predominantly exposed to fibrils and aggregates. PC12 cells were treated with açaí extract (0.5–50 μg/ml) for 15 min prior to exposure to either $A\beta_{1-42}$ or $A\beta_{25-35}$ (each at 0–5 μM for 48 h) or the pro-oxidant *tert* butyl hydroperoxide (*t*-BHP; 0–100 μM for 24 h). PC12 cells were also incubated with *t*-BHP plus the vitamin E analogue trolox (50–200 μM).

2.5. Cell viability measurements

Cell viability was determined using the thiazolyl blue tetrazolium bromide (MTT) assay. Following PC12 cell pre-treatments (2.4), media was replaced with 0.25 mg/ml MTT and further incubated for 2 h, MTT solution removed and cells lysed with DMSO. Absorbance was read at 570 nm using a PolarStar Galaxy microplate reader (BMG Labtech, Durham, NC, USA).

2.6. Biochemical assessment of Aβ fibril formation

Thioflavin T (ThT) emits fluorescence when bound to β-amyloid fibrils [21] and was used to analyse the effects of açaí on $A\beta_{1-42}$ fibril and aggregate formation in a cell-free system. Representative constituent phenolics and polyphenolics of açaí extract were also compared, including gallic acid, cyanidin glucoside, cyanidin rutinoside and the anti-fibrillar flavanol (-)-epigallocatechin-3-gallate (EGCG) [13]. ThT (10 μM in PBS) was added to wells together with non-fibrillar $A\beta_{1-42}$ (10 μM) and açaí extract (50 μg/ml), gallic acid or polyphenolic (each at 100 μM). Fluorescence was measured every 30 min for 24 h at 37 °C using a Synergy MX microplate reader (Bio-Tek, Bedfordshire, UK) with excitation and emission wavelengths at 446 nm and 490 nm respectively. ThT output from all treatment groups was normalised to blank values (ThT in PBS).

2.7. Transmission electron microscopy of Aβ fibril formation (TEM)

10 μl of açaí extract (50 μg/ml), constituent flavonoids or gallic acid (each at 100 μM) was incubated with 10 μl of $A\beta_{1-42}$ (10 μM) at 37 °C for 24 h. Samples of fibrillar $A\beta_{25-35}$ (10 μM) were also prepared to confirm aggregation at 24 h and investigate effects of açaí on fibril morphology. Following incubation, 5 μl of sample was placed onto a carbon-coated nickel electron microscopy grid, with 10 μl of contrast dye containing 2% uranyl acetate and blotted dry. Grids were loaded onto a specimen holder and air-lock of a Philips CM100 80 kV transmission electron microscope (Philips Research, The Netherlands) and viewed at a magnification of 34,000–96,000×.

2.8. Determination of total phenolic, flavonoid and anthocyanin content of açaí extract

The total phenolic concentration of açaí extract was measured using the Folin-Ciocalteu (FC) reagent and expressed as mg gallic acid equivalents per 100 g of açaí powder [10]. Total flavonoid content was determined by the aluminium chloride colorimetric assay [15] and expressed as mg quercetin equivalents. Total anthocyanin content was quantified via the pH differential absorbance method [15] and expressed as mg of cyanidin rutinoside equivalents.

2.9. Statistical analysis

Data from the MTT assay was analysed via a two-way ANOVA with Bonferroni's post hoc test to assess the effects of açaí against $A\beta_{1-42}$, $A\beta_{25-35}$ or *t*-BHP-mediated cell viability loss versus vehicle control. Thioflavin T fluorescence area under the curve (AUC) analysis and effects of açaí extract, anthocyanidin or gallic acid treatments were compared against $A\beta_{1-42}$ via a one-way ANOVA with Dunnett's post hoc test. A significance value of $P < 0.05$ was used for all experiments. Data analysis and graph production was performed in GraphPad Prism 6 (GraphPad Software, San Diego, USA).

3. Results

3.1. Açaí extract protected neuronal cells against $A\beta_{1-42}$-evoked PC12 loss of cell viability but not $A\beta_{25-35}$ or pro-oxidant exposure

A 48 h incubation with $A\beta_{1-42}$ resulted in a significant reduction in PC12 cell viability over the concentration range of 1–5 μM, with a % viability of 52.8 ± 2.5% at 5 μM $A\beta_{1-42}$ (Fig. 1a). Pretreatment of PC12 cells with açaí extract significantly inhibited the loss of cell viability at the highest $A\beta_{1-42}$ concentration (5 μM), at both 5 μg/ml (67.0 ± 2.7%) and 50 μg/ml (69.7 ± 4.7%) açaí concentrations (Fig. 1a). Incubation of PC12 cells with fibrillar $A\beta_{25-35}$ resulted in a significant decrease in cell viability over 0.1–5 μM (Fig. 1b); however toxicity was comparatively less than for equivalent concentrations of $A\beta_{1-42}$ (73.1 ± 4.0% $A\beta_{25-35}$ vs. 52.8 ± 2.5% $A\beta_{1-42}$ at 5 μM). Pretreatment of PC12 cells with açaí extract did not provide any significant degree of protection against $A\beta_{25-35}$-mediated cell loss of viability (Fig. 1b).

PC12 cell viability significantly diminished following exposure to increasing concentrations of *tert* butyl hydroperoxide (*t*-BHP; 60–100 μM) (Fig. 2). *t*-BHP caused a higher degree of maximal cell toxicity than $A\beta_{1-42}$ (16.2 ± 2.8% at 100 μM). However, neither