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Antioxidative and neuroprotective activities of peanut sprout extracts against oxidative stress in SK-N-SH cells

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ABSTRACT

Objective: To evaluate the protective effect of peanut sprout extract (PSE) against paraquat (PQ) induced SK-N-SH cells.

Methods: Three groups of cells were used in the experiment, together with a fourth, control group. One group was treated with PQ, the second group was treated with PSE, and the third group was pre-treated with PSE. The control group was untreated. Cell viability and toxicity were detected by MTT assay, cellular reactive oxygen species (ROS) was detected by Muse Cell Analyzer, quantitative RT-PCR was applied to investigate the expression of SIRT1 and α-synuclein genes, and Aβ42 was detected by western blot.

Results: The 50% effective concentration of PQ was 0.75 mmol/L. PSE had no significant cytotoxicity at a concentration of 1.5 mg/mL. In the group of cells pre-treated with PSE, cell death was significantly inhibited. In the PQ treated group, PQ was increased in the intracellular ROS in the cells. Intracellular ROS was significantly decreased in the cells treated with PSE and also those pre-treated with PSE. PSE significantly downregulated the expression of SIRT1 and α-syn genes, and it was found that PQ significantly increased β-amyloid 42 levels whereas this action was inhibited by PSE.

Conclusions: PSE has neuroprotective activities against oxidative stress in SK-N-SH cells induced by PQ, suggesting that PSE is a highly promising agent in the prevention of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease.

1. Introduction

Neurodegeneration is the progressive loss of structure or function of neurons, including the death of neurons. Many neurodegenerative diseases including Alzheimer's disease (AD) and Parkinson's disease (PD) occur as a result of neurodegenerative processes [1]. AD has been defined by the presence of extracellular amyloid-β (Aβ) containing plaques and cytoplasmic neurofibrillary tangles (NFT) consisting of abnormal microtubule associated protein tau. These proteinaceous aggregates are accompanied by synapse loss and neuronal cell death, which are thought to subserve the clinical syndrome of progressivecognitive impairment in AD [2,3]. PD is associated with progressive loss of dopaminergic neurons in the substantia nigra, as well as with more widespread neuronal changes that cause complex and variable motor and non-motor symptoms [4]. It is found that α-synuclein is a major component of Lewy bodies and Lewy neurites, the pathological hallmarks of PD, indicating its role in PD pathogenesis [5]. Environmental factors are important contributory factors in neurodegenerative disease [6].

Paraquat (PQ) (1,1-dimethyl-4,4′-bipyridinium dichloride) is a widely used herbicide. It has been suggested that PQ might be an environmental factor contributing to neurodegenerative disorder

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[7–9]. Studies using animal models have also indicated the neurotoxicity of PQ in nigrostriatal dopaminergic cells [10]. PQ reproduces the cardinal PD pathologies such as loss of dopaminergic neurons [11] and protein aggregation in dopaminergic neurons [12] as well as other pathologies that include oxidative stress [13], proteasome dysfunction [14], and mitochondrial dysfunction [15]. In addition, PQ in the presence of oxygen generates the superoxide radical [16,17], hydroxyl radical, and hydrogen peroxide (H_2O_2) leading to deleterious effects on cell function [8,18,19]. H_2O_2 induces SIRT1 overexpression [20]. SIRT1 has a dual effect on FOXO3 function by increasing FOXO3's ability to induce cell cycle arrest and to resist oxidative stress [21].

In recent years, nature has been a continuous source of pharmacologically active molecules and medicinal herbs [22]. Peanut sprouts have been noted for their antioxidant properties and the germinated peanut kernels have been used in the diet as a health food for several centuries. It has been reported that peanut sprouts are rich in flavonoids and phenolic compounds which may contribute to disease prevention and have health promoting properties [23,24]. They exhibit many biological functions such as anti-inflammatory activity attributed to inhibition of cyclooxygenase, estrogenic activity, and antiplatelet activity [25–27]. Moreover, it has been reported that flavonoids and phenolic compounds have a beneficial effect in the treatment of ischemia [28] and neurodegenerative disease [29]. Therefore, the purpose of the present study was to investigate the protective and antioxidative effects of peanut sprout extract (PSE) on PQ-induced SK-N-SH.

2. Materials and methods

2.1. Materials

Minimum essential medium, fetal bovine serum, 0.25% trypsin–ethylenediaminetetraacetic acid solution and 1% penicillin–streptomycin solution were purchased from Gibco (Invitrogen, Grand Island, NY, USA). Dimethyl sulfoxide and methyl viologen dichloride hydrate were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). MTT was purchased from Bio Basic Inc. (Markham, Canada). Muse® Oxidative Stress Kit was purchased from Merck Millipore Corporation (Germany). RiboZol RNA extraction reagent was purchased from Amresco (USA). DNase I and 50 mmol/L ethylenediaminetetraacetic acid were purchased from Fermentas (Thermo Fisher Scientific, USA). Tetro reverse transcriptase and SensiFAST™ SYBR® Kits were purchased from Bioline (Meridian Life Science, USA). Antibody for western blot was purchased from Merck Millipore Corporation (Germany) and 3,3′-diaminobenzidine reagent was purchased from Bio Basic Canada Inc.

2.2. Germination of peanut kernels and PSE

Mature peanut kernels (*Arachis hypogaea* cv. Tainan 9) were soaked in normal saline for 3 h, and then washed with sterile water three times and then soaked in normal saline for 30 min. The kernels were placed on a plastic net tray and germinated in a growth chamber for 3 days in the dark. After 1 day of incubation, the ungerminated kernels were discarded. After 3 days, the peanut sprouts were weighed and dried for 72 h at 60 °C.

The sprouted peanuts were ground and 100 g of the ground peanut powder was mixed with 100 mL of hexane and incubated overnight on a hot plate stirrer. The mixture was then passed through filter paper and 100 mL of 80% ethanol was added and the mixture was incubated overnight on a hot plate stirrer. The mixture was filtered with filter paper prior to rotary evaporation at 50 °C and 50 mmHg. The PSE was dried at 50 °C prior to use.

2.3. Cell culture

Human neuroblastoma cells (SK-N-SH) were obtained from the American Type Culture Collection (Manassas, VA, USA). These cells were cultured in 10% (v/v) fetal bovine serum and 1% penicillin–streptomycin solution and maintained at 37 °C in humidified incubator with 5% CO_2.

2.4. Cell viability/cytotoxicity assay

An MTT reduction assay was used to assess the viability of the cells. The cells were seeded in 96-well plate at a density of 3×10^4 cells per well and incubated overnight at 37 °C in 5% CO_2. The cells were then treated with PQ (0–1 mmol/L) and PSE (0.25–1.5 mg/mL) for 48 h. In one group, referred to as the prevention group, the cells were pre-treated with PSE for 4 h and then further treated with PQ (0.75 mmol/L) for 48 h. A solution of 1 mg/mL MTT was added to each well and the cells were further incubated for 4 h at 37 °C, 5% CO_2. The liquid in the wells was then removed. The reaction with the MTT had produced purple MTT formazan crystals which were then dissolved in dimethyl sulfoxide. The product was measured by a microplate reader at 540 nm. The percentage of cell viability was normalized to the control group.

2.5. Intracellular reactive oxygen species (ROS) determination

The cells undergoing oxidative stress defined by the presence of ROS, namely, superoxide, were determined by Muse® Oxidative Stress Kit. Briefly, after culturing and treatment, the cells were re-suspended at a concentration of 1×10^6 cells per mL in 1× assay buffer (Muse® Oxidative Stress Kit). After that, the samples were incubated for 30 min at 37 °C and then the ROS positive cells were examined using the Muse® Cell Analyzer.

2.6. RNA analysis

Total RNA was extracted from the cells produced in the cell culturing activity, with RiboZol RNA extraction reagent. To discard genomic DNA, the total RNA (500 ng) was treated with DNase I. First-strand cDNAs were synthesized from the total RNA (250 ng) by Tetro reverse transcriptase and oligo primer were incubated at 45 °C for 30 min. This reaction was terminated by incubating the treated total RNA at 85 °C for 5 min. The synthesized cDNAs were further utilized for quantitative PCR analysis.

The gene expression levels were determined by quantitative PCR using LightCycler® 96 (Roche Diagnostics) and