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Fermentation enhances the neuroprotective effect of shogaol-enriched ginger extract via an increase in 6-paradol content

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ABSTRACT

This study was designed to investigate the neuroprotective effect of fermented shogaol-enriched extract (FSE), which is converted to 6-paradol by *Aspergillus niger*. FSE was fermented for varying time points (FSE-0, FSE-30, and FSE-60). Optimal condition for 6-paradol produced by A. *niger* was determined as initial inoculated cell concentration (1.5×10^6 spores/mL) from shogaol-enriched extract (240 μg/mL) for 60 h. The ability of FSE to protect the rat primary hippocampal cells from beta-amyloid (Aβ) oligomer and Aβ plaque induced neurotoxicity and to inhibit acetylcholinesterase (AChE) activity was evaluated. Hippocampal cell viability was reduced to 70% when exposed to Aβ oligomer, and exposure to Aβ plaque for 18 h and 50 μg/mL of FSE-60 increased cell viability (92% and 95.3%, respectively). In addition AChE inhibitory activity of FSE-60 at 50 μg/mL was 53%, which was similar to 0.1 μM tacrine. Therefore, these results demonstrate that FSE-60 has potential as a neuroprotective agent.

1. Introduction

Various degenerative diseases associated with ageing and cardiovascular disorders have recently emerged as a major social problem. The major causative agents of such diseases have been identified as free radicals and reactive oxygen species (ROS) (Emerit, Edeas, & Bricaire, 2004). Oxidative stress-induced neuronal cell damages are closely related to neurodegenerative conditions such as Alzheimer's disease (AD) (Behl, 1999).

AD is a progressive disease leading to the degeneration of cholinergic neurons, loss of cholinergic neurotransmission, and the formation of abnormal aggregates of β-amyloid (Aβ) protein fragments throughout the brain. The recommended treatments for AD include acetylcholinesterase inhibitors, cholinesterase inhibitors, antioxidants, Aβ-targeted drugs, nerve growth factors, c-secretase inhibitors, and vaccines against Aβ (Modi, Pillay, & Choonara, 2010); however, most treatments have high risk factors and adverse effects. Therefore, there is an urgent need for agents with neuroprotective effect with proven efficacy, and natural compounds or functional health foods might be an important option (Aruoma, Halliwell, Aeschbach, & Loligers, 1992; Essa et al., 2012).

As a perennial herb in the Zingiberaceae family, ginger (*Zingiber officinale* Roscoe) grows in subtropical and tropical

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regions; its rhizome has a unique odour and pungent. It is commercially available in various forms such as fresh and dried ginger, oleoresin, and essential oil. Ginger has been reported to have physiologically active ingredients such as gingerol, shogaol, and paradol along with various nutrients such as free sugars, protein, fat, and minerals (Bellik & Omonhinmin, 2014). Gingerol, shogaol, and zingerone have distinctive odour and pungent flavour, explaining the widespread use of ginger as a spice (Connell & Sutherland, 1969). Gingerol and its several isomers have physiological activities; 6-gingerol for example, displays excellent anti-inflammatory, analgesic, anti-thrombotic, anti-cancer, and anti-oxidative properties (Chang, Chang, Wang, & Chang, 1995; Ippoushi, Azuma, Ito, Horie, & Higashio, 2003; Thomson et al., 2002). 6-Shogaol is produced from 6-gingerol by a dehydration reaction and have antibacterial and antioxidative properties. Recently, 6-shogaol was reported to have marked anti-inflammatory, antioxidative, and cancer-preventing effects compared to 6-gingerol (Altman & Marcussen, 2001; Surh, 2002). The conversion of 6-gingerol to 6-shogaol is accelerated under high temperature (100–180 °C) and acidic conditions (Ok & Jeong, 2012).

Microbial metabolism of 6-shogaol produces 6-paradol, which is stable at high temperature and pH (Nagendra chari, Manasa, Srinivas, & Sowbhagya, 2013). 6-Paradol is used as an ingredient for food and medicines because of its higher bioavailability than that of 6-shogaol. It has no pungent flavour and exhibits higher water solubility than gingerol and shogaol (Koh & Lee, 1983). Functional properties of 6-paradol were reported such as attenuating neuroinflammation (Gaire et al., 2015) and promoting antitumor (Chung, Jung, Surh, Lee, & Park, 2001).

This study is designed to evaluate the effect on Aβ-induced neuroprotective and AChE of fermented shogaol-enriched extract (FSE) by *Aspergillus niger* and to use the FSE as a potential ingredient for functional food.

2. Materials and methods

2.1. Materials

Ginger was purchased in Seosan in South Korea and washed to remove surface impurities. *A. niger* (KFRI01227) was obtained from the research group of gut microbiome in Korea Food Research Institute, neurobasal medium and B27 were obtained from Gibco (Carlsbad, CA, USA), and $A\beta_{1-42}$, glutamine, and dimethyl sulphoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The reagents used in extraction and analysis were all reagent grade, and 6-shogaol standard was purchased from Chromadex (Laguna Hills, CA, USA); chemically synthesized 6-paradol (Gaire et al., 2015) was kindly provided by Prof. Dong Yun Shin at Gachon University's College of Pharmacy.

2.2. Extract and FSE manufacturing

The extract concentrated with 6-shogaol, the substrate for fermentation, was prepared according to our previous study (Park et al., 2014). Briefly, ginger was heat-treated at 120 °C for 4 h, and then hot air dried. Ground ginger was mixed with 20× (w/v) 70% ethanol and extracted by reflux extraction at 80 °C for 3 h. The extract was filtered using Whatman No.1 paper, and the filtrate was evaporated using rotary vacuum evaporator (Eyela, Japan) and subsequently re-dissolved in water to yield freeze-dried powder for use as substrate. *A. niger* was incubated in potato dextrose agar (PDA) at 30 °C for 3 days, and spores on the plate surface were then collected using 0.85% saline; gauze-filtered suspension was used. The 6-paradol production was monitored at 30 °C for 72 h in a 100-rpm shaking incubator with initial inoculation concentrations of 0.7×10^6, 1.5×10^6, and 3.0×10^6 spores/mL and substrate concentrations of 140, 240, and 500 μg/mL. The same volume of ethyl acetate as culture medium was added and vortexed, and then centrifuged at 8000 rpm for 15 min; the supernatant was collected. The above procedures were repeated for re-extraction to collect the supernatant, which was then concentrated and dissolved in methanol to measure the yield of 6-paradol using HPLC analysis.

FSE was manufactured at different times (0, 30 and 60 h; FSE-0, FSE-30 and FSE-60, respectively) with initial bacterial concentration of 1.5×10^6 spores/mL and substrate concentration of 500 μg/mL in the entire culture media. Ethanol was added to the culture media to make the final concentration 70%, vortexed, sonicated, followed by centrifugation at 8000 rpm for 15 min, and the supernatant was filtered with a 0.2-μm syringe. This filtrate was concentrated and freeze-dried to make FSE.

2.3. 6-Shogaol and 6-paradol content determination

6-Shogaol and 6-paradol, the marker substances for FSE, were quantitatively analysed using high-performance liquid chromatography (HPLC, Jasco, Japan) (Wohlmuth, Leach, Smith, & Myers, 2005). The column used was Eclipse XDB-C_{18} (4.6 × 250 mm), and sample detection was done using UV detector to quantify the chromatogram generated at 225 nm. A 20 μL sample was injected, and the mobile phase was developed using HPLC distilled water and acetonitrile at 1.0 mL/min for 30 min. The initial acetonitrile content was 45%, which increased to 65 and 80% at 12 and 18 min, respectively, and remained stable up to 25 min, and then decreased to 45% at 27 min and remained stable for 30 min. Chromatogram (Fig. 1) shows the presence of 6-shogaol and 6-paradol at 15 and 22 min, respectively. For quantitative analysis, both 6-shogaol and 6-paradol were diluted to 10, 25, 50, 100, and 250 μM to generate the standard curve, and analysis values with correlation coefficient (R^2) of over 0.99 were selected.

2.4. Aβ oligomer and Aβ plaque preparation

Aβ oligomer was prepared by dissolving $A\beta_{1-42}$ in hexafluoroisopropanol to a final concentration of 1 mg/mL, and then stored at room temperature for 3 days; subsequently, it was vacuum-dried for 1 h. The sample stock (1 mM) was prepared in DMSO and reaction sample of 10 μM was dissolved in PBS and incubated at 4 °C for 24 h to yield Aβ oligomers. Aβ plaque was prepared by dissolving $A\beta_{1-42}$ in distilled water to make the concentration 500 μM, and then incubated at 37 °C for 3 days to induce Aβ aggregation.