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Pumpkin seed oil protects SH-SY5Y cells against amyloid-beta 42-induced oxidative stress and neuronal cell death

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ABSTRACT

Amyloid-beta (Aβ) peptide causes neuronal oxidative stress and eventual neuronal cell death. Pumpkin seed oil (PSO) contains fatty acids with antioxidant properties. The present study aimed to investigate the effect of PSO on amyloid-beta 42 ($A\beta_{42}$) peptide-induced oxidative stress and neuronal cell death in human neuroblastoma SH-SY5Y cells. SH-SY5Y cells were pretreated with 0.001 and 10 µg/mL of PSO, following exposure to 1.25 µM of $A\beta_{42}$ peptide. $A\beta_{42}$-induced neuronal toxicity was characterized by decreased cell viability and Bcl-2 level and increased intracellular reactive oxygen species (ROS), Bax, and procaspase-3 levels. PSO pretreatment attenuated the $A\beta_{42}$-induced loss of cell viability and neuronal cell death. The mechanisms by which PSO protects neuronal cells from oxidative stress include reducing levels of intracellular ROS, Bax, and procaspase-3, and increasing Bcl-2 levels. Based on our findings, the beneficial properties of PSO could be explored in future studies on co-treatment with modern medicine to prevent or slow cognitive impairment progression in Alzheimer's disease.

Keywords: pumpkin seed oil; oxidative stress; neuronal cell death; antioxidant

1. INTRODUCTION

Alzheimer's disease (AD) is characterized by impaired cognitive function. Extracellular amyloid-beta (Aβ) plague accumulation and the neurofibrillary tangle (NFT) formation in the brain are critical neuropathological features of AD. It is recognized that Aβ plays a role in the progression of AD (Abubakar et al., 2022; Zhang et al., 2023). Aβ is a 40–42 amino acid peptide produced by the processing of β-secretase and γ-secretase cleavage of the larger Aβ precursor protein (Ma et al., 2022). The accumulation of Aβ in the brain is crucial for oxidative stress-induced neuronal cell death. Neuronal toxicity and injury have been reported in both *in vitro* and *in vivo* AD models through the activation of oxidative stress and neuronal cell apoptosis (Ohashi et al., 2022; She et al., 2023). Apoptosis, programmed cell death, is divided into intrinsic and extrinsic pathways. Normally, cell survival is regulated via anti-apoptotic proteins (Bcl-2 and Bcl-XL) and cell death effectors (Bax and Bak) (Czabotar et al., 2014). Numerous studies have reported that antioxidant

mechanisms prevent or delay neuronal cell death through apoptosis (Feng et al., 2021; Forman and Zhang, 2021). Additionally, the generation of exogenous or endogenous reactive oxygen species (ROS) promotes Bax expression through p53 and c-Jun N-terminal kinase signaling, inhibiting Bcl-2 functions (Czabotar et al., 2014; Topuridze et al., 2007). Furthermore, endogenous ROS generation induced by Aβ can activate mitochondrial dysfunction, which plays a vital role in apoptosis. During mitochondrial dysfunction, several essential factors of apoptosis are released into the cytosol, including procaspases-3 and -9, cytochrome C, apoptosis-inducing factor, and apoptotic protease-activating factor, forming an apoptosome that activates downstream caspase signaling (such as caspase-3 and -7), resulting in apoptotic cell death (Ashleigh et al., 2023). Thus, attenuating Aβ-promoted oxidative stress-mediated neurotoxicity and neuronal cell death may prevent or delay neurodegeneration.

Herbal medicines have gained attention as protective agents against neurodegenerative diseases, including AD. Accumulating evidence has demonstrated that plants, vegetables, and fruits are sources of antioxidant compounds. Compared to synthetic drugs, naturally active compounds exhibit minimal side effects and are cost-effective (Stevenson-Hoare et al., 2023; Kim et al., 2018; Chang et al., 2013). Pumpkin (*Cucurbita moschata* Decne.) is a fruit that belongs to the genus *Cucurbita* of the Cucurbitaceae family (Yadav et al., 2010). In this study, we focused on the neuroprotective effects of oil extracted from pumpkin seeds—commonly known as pumpkin seed oil (PSO). PSO is used in cooking and as a nutritional supplement. The main active compounds in PSO are fatty acids, flavonoids, and tocopherol (vitamin E), which exert antioxidant effects (Abou Seif, 2014; Bardaa et al., 2016; Eraslan et al., 2013). Previous studies have reported that fatty acids, particularly linoleic acid, exhibit antioxidant activity (Tofighi et al., 2021; Yaguchi et al., 2010). Additionally, linoleic acid has been shown to protect against Aβ-induced neuronal cell death by suppressing oxidative stress and regulating apoptosis. It has been demonstrated that linoleic acid treatment reduced Bax/Bcl-2, caspase-3, and caspase-9 expression (Tang, 2014). However, no studies have explored the neuroprotective effect of PSO. Therefore, this study aimed to examine the effects of PSO against oxidative stress and neuronal cell death in SH-SY5Y cells induced by the $A\beta_{42}$ peptide.

2. MATERIALS AND METHODS

2.1 Materials

The human neuroblastoma SH-SY5Y cell line, CRL-2266, was purchased from the American Type Culture Collection (Manassas, USA). Amyloid-beta 42 ($A\beta_{42}$) peptide, radioimmunoprecipitation assay (RIPA) buffer, protease inhibitor cocktails, and dimethyl sulfoxide (DMSO) were purchased from Merck (Billerica, MO, USA). Minimum essential medium (MEM), Nutrient mixture F12 medium (F12), fetal bovine serum (FBS), antibiotic-antimycotic, trypsin, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) dye, sodium pyruvate, and the fluorescent dye H_2DCF-DA were purchased from Invitrogen (Thermo Fisher Scientific, USA). A protein assay kit was obtained from Bio-Rad (Hercules, CA, USA). Antibodies against Bcl-2, Bax, and procaspase-3 were obtained from Abcam (Cambridge, UK). The β-actin antibody and horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody were obtained from Merck (Billerica, MO, USA).

2.2 Cell culture

Undifferentiated SH-SY5Y cells were cultured in a complete medium (1:1 v/v MEM: F12 medium supplemented with 10% FBS, 1 mM sodium pyruvate, and 1% antibiotic-antimycotic) under a humidified atmosphere containing 5% CO_2 at 37 °C.

2.3 $A\beta_{42}$ peptide preparation

The $A\beta_{42}$ peptide was initially dissolved in sterile water to prepare a 50–μM $A\beta_{42}$ stock solution. Afterward, various concentrations of $A\beta_{42}$ (ranging from 0.15625 to 2.5 μM) were prepared by diluting the stock solution using a complete medium containing 0.5% DMSO.

2.4 PSO extraction

Pumpkin seeds were purchased from a community agricultural enterprise in Santisuk district, Nan province, Thailand, where pumpkin is widely cultivated and utilized as a staple food source. Before starting the extraction procedures, plant samples were carefully identified and compared with depository plant material from the Faculty of Pharmacy, Srinakharinwirot University, Thailand, ensuring accurate botanical classification. All methods were conducted in strict adherence to Thailand's Plant Variety Protection Act B.E. 2542 (1999). PSO was extracted using the Soxhlet procedure (AOAC 1995, 920.85) as described by Srbinoska et al. (2012).

2.5 Determination of fatty acids and α-tocopherol contents in PSO

The analyses were performed at the Institute of Food Research and Product Development, Kasetsart University, Bangkok, Thailand. Two grams of PSO were mixed with a 50 mL mixture of $CHCl_3$: MeOH (2:1) and shaken on a shaker for 30 min. Next, the upper layer solution was separated and filtrated through a 70-mm Whatman filter paper with anhydrous sodium sulfate on top. A rotary evaporator was used to remove the solvent to reduce the volume and concentrate the esterified sample. Then, 5 mL of 0.5 N KOH in MeOH was added to the sample and mixed. The mixture sample was heated at 100±2 °C for 5 min and cooled at 25 °C. After cooling, 2 mL of 14% BF_3 in methanol was added, mixed, and heated at 100±2 °C for 15 min followed by cooling at 25 °C. Afterward, 10 mL of sodium chloride solution was added to the mixture, and extraction was performed 3 times using 5 mL of petroleum ether each time. The clear solution from the extraction was passed through a rotary evaporator to remove the solvent and the fatty acid was collected. The fatty acid was dissolved in 3.0 mL of n-heptane and filtrated through a syringe filter (0.45 μM, 13 mm,). The contents of fatty acid were analyzed using gas chromatography (Agilent Technology, USA). In addition, α-tocopherol content was evaluated using high-performance liquid chromatography (HPLC, Agilent Technology, USA). For sample preparation, 0.1–1 g of PSO was saponified with KOH and ethanol, boiled at 100±2 °C for 30 min, followed by a cooling in a cooling bath. The sample was then extracted with hexane and ethyl acetate, and a rotary evaporator was used to eliminate the solvent. Finally, the sample was dissolved in ethanol and analyzed using an HPLC.

2.6 PSO preparation for neuronal assays

The PSO stock at 0.2 mg/mL was dissolved in sterile water with 10% DMSO. For the neuronal assays, various concentrations of PSO (0.001–10 µg/mL) were prepared in a complete medium by diluting from the PSO stock. The final concentration of DMSO was set at 0.5%.

2.7 Determination of $A\beta_{42}$ toxicity on SH-SY5Y cells

SH-SY5Y cells (1×10^4 cells/well) were cultured as described above and incubated with 0.15–2.5 µM of $A\beta_{42}$ for 24 h. Untreated cells were cultured in a complete medium containing 0.5% DMSO. Following the incubation period, the MTT assay was used to assess cell viability. Fifty µL of MTT solution (0.5 mg/mL in MEM: F12 medium) was added to all wells and incubated at 37 °C for 4 h. After incubation, the absorbance was measured at 570 nm using a microplate spectrophotometer. The percentage of cell viability was calculated using the following equation:

$$\% \text{ cell viability} = \frac{\text{OD of treated cell}}{\text{OD of untreated cell}} \times 100.$$

Finally, the 50% inhibitory concentration (IC_{50}) of $A\beta_{42}$ was determined using GraphPad Prism software, employing the formula of the logarithmic curve of the inhibition-concentration.

2.8 Toxicity of PSO on SH-SY5Y cells

SH-SY5Y cells (1×10^4 cells/well) were cultured as described above and treated with 0.001–10 µg/mL of PSO, as detailed in section 2.6, for 24 h. Untreated cells were cultured in a complete medium containing 0.5% DMSO. Cell viability was determined using the MTT assay, as described above.

2.9 Protective effect of PSO on $A\beta_{42}$-induced toxicity in SH-SY5Y cells

The protective effect of PSO against $A\beta_{42}$-induced SH-SY5Y cell toxicity was assessed. The cells (1×10^4 cells/well) were cultured as described above. After 24 h, the cells were pretreated with 0.001 and 10 µg/mL of PSO for 24 h before being incubated with 1.25 µM $A\beta_{42}$ for another 24 h. The untreated cells were cultured in a complete medium containing 0.5% DMSO. Cell viability was determined using the MTT assay described above.

2.10 Effect of PSO on $A\beta_{42}$-induced intracellular ROS generation in SH-SY5Y cells

The intracellular ROS was detected using a fluorescent H_2DCF-DA probe, a sensitive ROS-detecting dye. SH-SY5Y cells (1×10^4 cells/well) were cultured as described above and pretreated with 0.001 and 10 µg/mL of PSO for 24 h, followed by incubation with 1.25 µM $A\beta_{42}$ for 24 h. The untreated cells were cultured in a complete medium containing 0.5% DMSO. The cells were then probed with 10 µM H_2DCF-DA for 30 min at 37 °C, and the fluorescence intensity was quantified using a fluorescence microplate reader.

2.11 Western blot analysis

SH-SY5Y cells (2×10^6 cells/well) were cultured in 35-mm plates and treated as described above for the intracellular ROS assay. After incubation, the cells were collected by centrifugation at 14,000 rpm for 5 min. Proteins were extracted by lysing the cells in RIPA buffer containing 1% protease inhibitor cocktail. A Bio-Rad protein assay was used to measure the total protein concentration. Afterward, 30 µg of protein were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were transferred onto polyvinylidene difluoride membranes. Then, the membranes were blocked with 5% skim milk in 0.1% Tween 20 in Tris buffer saline (TBST, pH 7.6) at 25 °C for 1 h. The membranes were then probed with antibodies against Bax (1:1,000), Bcl-2 (1:1,000), procaspase-3 (1:1,000), and β-actin (1:40,000) at 4 °C for 16 h, followed by incubation with horseradish peroxidase-conjugated anti-rabbit or mouse IgG secondary antibody (1:5,000) at 25 °C for 1 h. Primary and secondary antibodies were diluted in 1% skim milk in TBST and 1% bovine serum albumin in TBST, respectively. An enhanced chemiluminescence system with X-ray exposure was used to visualize the protein bands. Then, protein band densities were analyzed using Scion Image software.

2.12 Statistical analysis

The data are presented as the mean±standard error of the mean (SEM) and were analyzed using GraphPad Prism software. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test ($p < 0.05$) was used to determine differences between groups,

3. RESULTS

3.1 Fatty acid contents of PSO

The study showed that ethanol extraction yielded 30 g of PSO per 100 g of pumpkin seeds. The chemical composition of PSO, shown in Table 1, includes both unsaturated and saturated fatty acids. However, α-tocopherol was not detected in PSO.

Table 1. Fatty acids contents of PSO

Fatty acids	Percentage of total fatty acids (%)
Linoleic acid (C18:2n6c)	39.11
Cis-9-oleic acid (C18:1n:9c)	28.10
Palmitic acid (C16:0)	19.30
Stearic acid (C18:0)	8.02
Arachidic acid (C20:0)	0.37
Alpha-linoleic acid (C18:3n3c)	0.27

3.2 Toxicity of $A\beta_{42}$ on SH-SY5Y cells

Figure 1 shows the viability of SH-SY5Y cells after 24-h incubation with $A\beta_{42}$ (0.15625–2.5 µM). $A\beta_{42}$ exhibited toxicity to SH-SY5Y cells, with noticeable effects evident at 0.3125 µM. Increasing the concentration of β_{42} led to a significantly decrease in cell viability in a concentration-dependent manner. The IC_{50} value of $A\beta_{42}$, as calculated using GraphPad Prism software, was 1.25±0.21 µM.

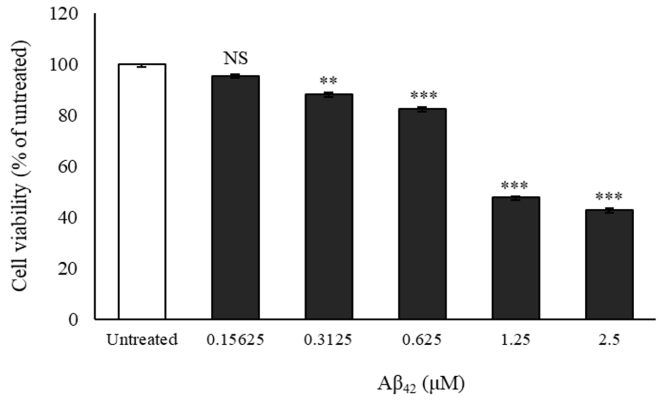

Figure 1. SH-SY5Y cell viability after incubation with $A\beta_{42}$ (0.15625–2.5 µM)
Note: The values are presented as the mean±SEM. NS represents no significant difference compared with untreated cells. Significant levels are indicated by ** for $p < 0.01$ and *** for $p < 0.001$ compared to untreated cells.

3.3 Toxicity of PSO on SH-SY5Y cells

Figure 2 shows the viability of SH-SY5Y cells after treatment with 0.001–10 µg/mL of PSO. All concentrations of PSO did not significantly reduce cell viability. Specifically, the viability of cells treated with PSO at 10, 1, 0.1, 0.01, and 0.001 µg/mL were 94.68±1.61, 101.90±0.92, 100.70±1.56, 100±0.74, and 100.80±0.79% of the untreated cell, respectively. These results demonstrated that PSO is not toxic to SH-SY5Y cells. Therefore, the lowest and highest doses of PSO (0.001 and 10 µg/mL) were selected for investigating their impact on oxidative stress and cell death induced by $A\beta_{42}$.

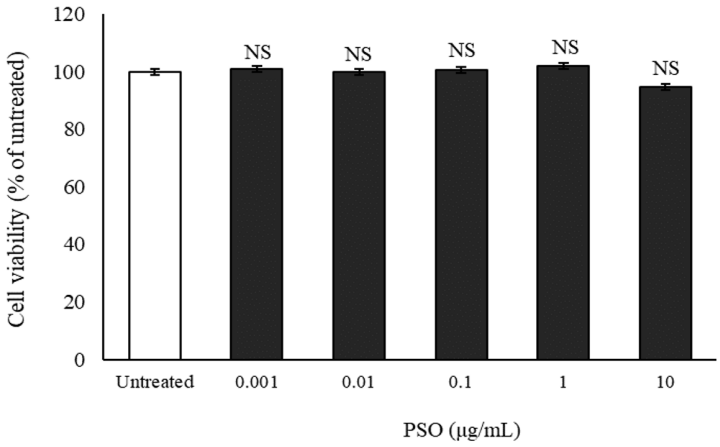

Figure 2. Viability of SH-SY5Y cells after treatment with PSO (0.001-10 µg/mL)
Note: The values are presented as the mean±SEM. NS indicates no significant difference compared to untreated cells.

3.4 Effect of PSO treatment on $A\beta_{42}$-induced toxicity in SH-SY5Y cells

Figures 3A and 3B show the morphology of SH-SY5Y cells and the percentage of viable cells under various conditions, respectively. Morphological observations revealed that when the cells were treated with 1.25 µM $A\beta_{42}$, noticeable reduction in viable cells was observed—the cell viability was decreased to 47.95±0.19% compared to untreated cells. In contrast, pretreatment with PSO increased the cell viability (Figure 3A). Pretreatment with 0.001 and 10 µg/mL PSO protected against $A\beta_{42}$-induced cell death. Specifically, viability was restored to 76.96±0.5% and 90.27±0.24% of untreated cells, respectively.

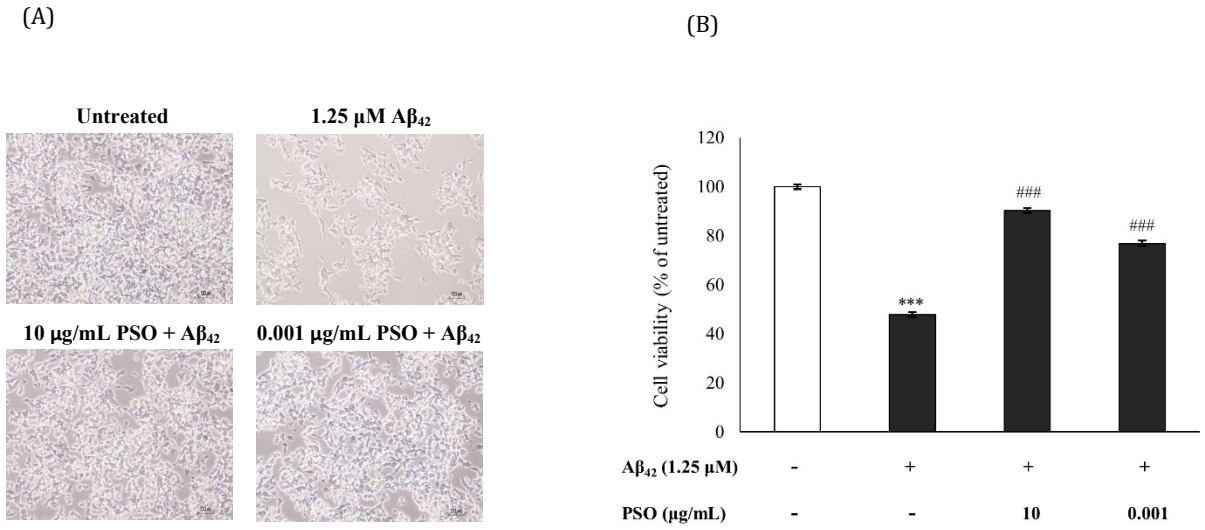

Figure 3. Effect of PSO on Aβ42-induced toxicity in SH-SY5Y cells; (A) morphology of SH-SY5Y cells and (B) percentage of viable cells after various treatments
Note: The values are presented as the mean±SEM. ***, ### signifies significant difference ($p < 0.001$) compared to untreated cells and cells treated with 1.25 µM Aβ42, respectively.

3.5 Effect of PSO on Aβ42-induced intracellular ROS generation in SH-SY5Y cells

SH-SY5Y cell labeled with H_2DCF-DA was used to determine intracellular ROS levels. The cells exposed to Aβ42 showed a significant increase in the fluorescence intensity (202.20±1.74% of the untreated, $p < 0.001$) compared to untreated cells. Compared to the cells treated with 1.25 µM Aβ42 alone, pretreatment with 0.001 and 10 µg/mL PSO significantly reduced ROS production (108.80±1.38 and 89.07±3.28% of the untreated, respectively), as shown in Figure 4B. DCF fluorescence, serving as an indicator of ROS formation, was visualized under a fluorescence microscope (Figure 4A). ROS production was increased in the cells treated with 1.25 µM of Aβ42, compared to the untreated cells. Furthermore, ROS production was significantly suppressed by pretreatment with 0.001 and 10 µg/mL of PSO. The fluorescence microscopy images were consistent with the intracellular ROS levels.

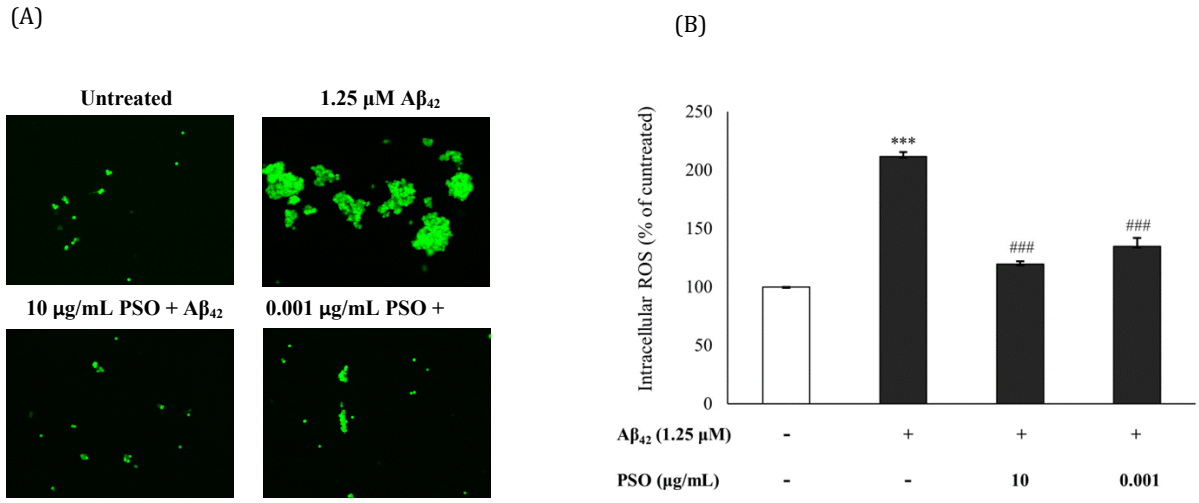

Figure 4. Effect of PSO on Aβ42-induced intracellular ROS production in SH-SY5Y cells; (A) fluorescence microscopy images of SH-SY5Y cells probed with H_2DCF-DA, (B) fluorescence density of the cells probed with H_2DCF-DA
Note: The values are presented as the mean±SEM. ***, ### signifies significant difference ($p < 0.001$) compared to untreated cells and cells treated with 1.25 µM Aβ42, respectively.

3.6 Effect of PSO on Aβ42- induced neuronal cell death in SH-SY5Y cells

The expressions of key proteins related to cell death, including Bax, Bcl-2, and procaspase-3, as determined by western blotting, are shown in Figure 5. Compared to the untreated cells, the cells treated with 1.25 µM Aβ42 exhibited significantly increased levels of Bax and procaspase-3, while the level of Bcl-2 was significantly decreased. Conversely, pretreatment the cells with 0.001 and 10 µg/mL PSO prior to Aβ42 exposure showed a significant reduction in Bax and procaspase-3 levels and concurrent increase in Bcl-2 levels. Our results suggested that pretreatment with PSO is associated with the prevention of cell death, characterized by increased Bcl-2 level and decreased Bax and procaspase-3 levels.

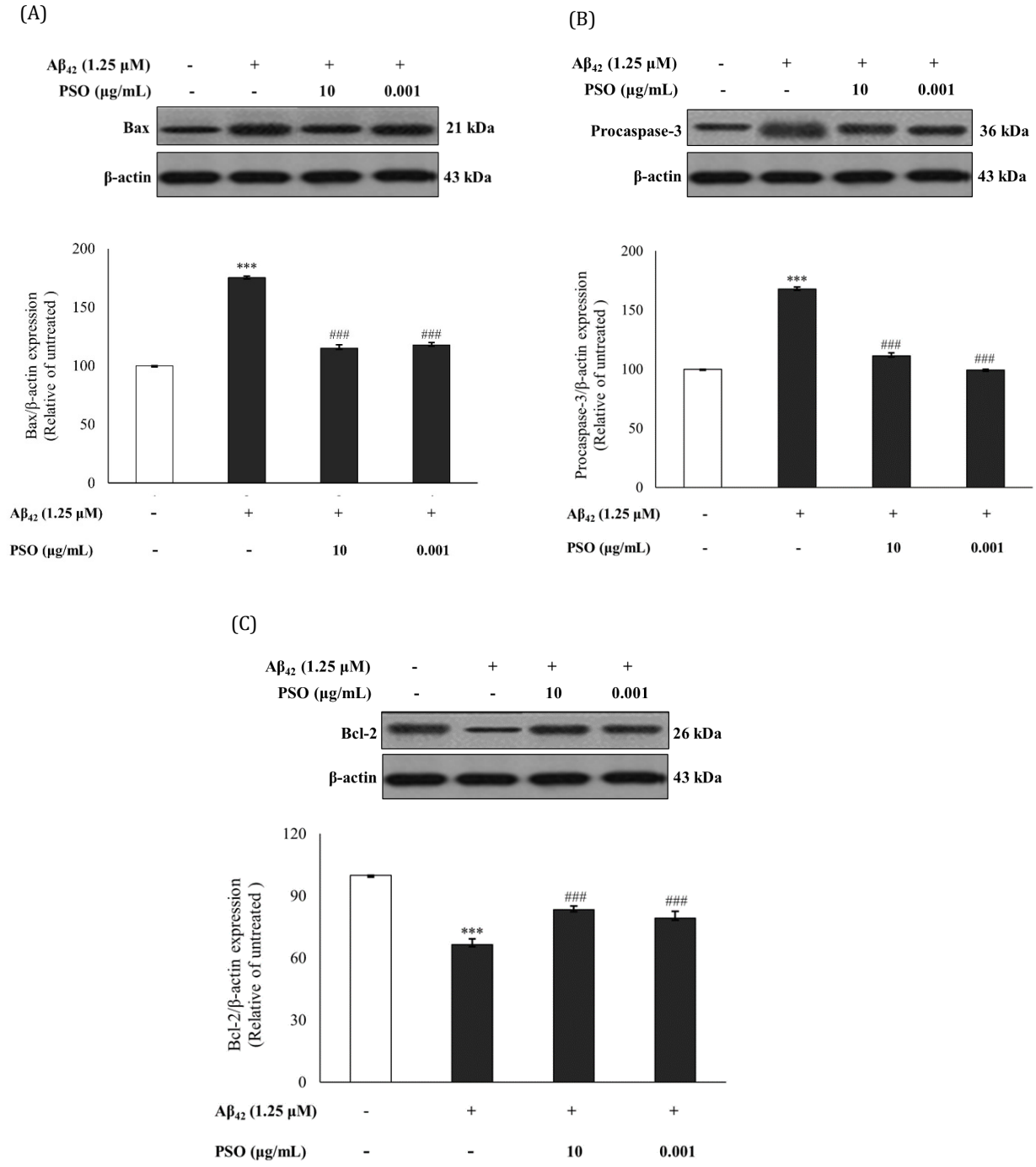

Figure 5. PSO prevented Aβ42-induced neuronal apoptosis in SH-SY5Y cells
Note: Western blots showing the effect of PSO treatment on Bax, procaspase-3, and Bcl-2 expression induced by the Aβ42 peptide, respectively. The values are presented as the mean±SEM. ***, ### signifies significant difference ($p < 0.001$) compared with untreated cells and cells treated to 1.25 µM Aβ42, respectively.

4. DISCUSSION

In the present study, we found that pretreatment with PSO provide protection against neuronal oxidative stress and apoptotic cell death induced by $A\beta_{42}$ peptide in SH-SY5Y cells. Exposing SH-SY5Y cells to $A\beta_{42}$ caused cell death, and a considerable increase in ROS generation, resulting in cell apoptosis. $A\beta_{42}$ caused a dose-dependent reduction in cell viability of SH-SY5Y compared with untreated cells (Figure 1). This finding is consistent with previous studies using $A\beta_{42}$ to induce neurotoxicity in cell culture (Deng et al., 2020; Feng et al., 2021). The calculated IC_{50} of the $A\beta_{42}$ peptide in this study was 1.25 μM; we then investigated the protective effects of PSO against $A\beta_{42}$-induced toxicity using this established dose.

The beneficial effects of PSO and its active compounds, including the antioxidant properties, have been reported previously (Abou Seif, 2014; Bardaa et al., 2016; Eraslan et al., 2013). In the present study, gas chromatography analysis revealed that the PSO was composed of several fatty acids, including linoleic (39.11%), oleic (28.10%), palmitic (19.30%), stearic (8.02%), and arachidic acids (0.37%) (Table 1). Previous studies have demonstrated that PSO has antioxidant properties and similar composition of fatty acids. (Abou Seif, 2014; Bardaa et al., 2016; Eraslan et al., 2013). Our study focused on evaluating the effects of PSO on neuronal oxidative stress and apoptotic cell death induced by $A\beta_{42}$ peptides in SH-SY5Y cells. All PSO concentrations (0.001–10 μg/mL) showed no significant reduction in SH-SY5Y cell viability, indicating non-neurotoxicity of PSO. Therefore, we determined the protective effects of PSO on $A\beta_{42}$-induced SH-SY5Y oxidative stress and apoptotic cell death at 0.001 and 10 μg/mL. Additionally, our study was the first to determine the protective effects of PSO on $A\beta_{42}$-induced SH-SY5Y cell death.

In this study, the antioxidant effects of PSO were examined by measuring intracellular ROS production using the H_2DCF-DA fluorescent probe, an ROS-sensitive dye. In addition, we also examined the mechanism of action of PSO on $A\beta_{42}$-induced SH-SY5Y cell death. We found that $A\beta_{42}$ treatment triggered ROS overproduction and the elevation of Bax and procaspase-3 expression, while significantly reducing the expression of Bcl-2 (Figure 5). Our findings were consistent with previous research suggesting that Aβ-induced neuronal cell death operates through the activation of apoptosis mechanisms, such as increasing the Bax/Bcl-2 ratio, inducing the expression of procaspase-3, activating caspase-3, and inhibiting Bcl-2 expression and function (Kalagatur et al., 2021; Lee et al., 2018; Lu et al., 2021; Song et al., 2022, Zatsepina et al., 2018). Our results demonstrated that $A\beta_{42}$ induced SH-SY5Y cell death through ROS/oxidative stress-mediated neuronal apoptosis. Pretreatment of SH-SY5Y cells with 0.001 and 10 μg/mL PSO significantly reduced intracellular ROS production (Figure 4), and significantly reduced neuronal cell death in SH-SY5Y cells exposed to $A\beta_{42}$ by increasing Bcl-2 and decreasing Bax and procaspase-3 (Figure 5). Louneva et al. (2008) demonstrated that the expression of procaspase-3 level was significantly higher in the hippocampal tissue of individuals with Alzheimer's disease. They reported elevated procaspase-3 level in blot cytosol and nuclear extract (Louneva et al., 2008). Consistent with their research, our result showed that procaspase-3 expression in $A\beta_{42}$-treated cells was significantly increased compared to untreated cells. In addition, activation of the apoptosis signaling pathway leads to the release of mitochondrial cytochrome C, which subsequently forms a complex with caspase-9 and Apaf1 to create apoptosome—a catalytic multiprotein that converts procaspase-3 into caspase-3. Therefore, procaspase-3 expression can support the apoptotic execution through the caspase-3 substrate (D'Amelio et al., 2012). Our results suggest that PSO can protect $A\beta_{42}$-induced neuronal apoptosis by reducing oxidative stress.

Additionally, there has no published research study investigated the effect of PSO on neuronal cells treated with $A\beta_{42}$. Our study was the first to demonstrate that PSO protects $A\beta_{42}$-induced SH-SY5Y cell apoptosis through oxidative stress. In our study, linoleic acid was a major composition in PSO, and the physiological effects were described in previous studies. Alarcon-Gil et al. (2022) reported that linoleic acid (15, 25, and 35 μM) could protect against the death of the SH-SY5Y cell induced by 6-hydroxydopamine, the *in vitro* Parkinson's disease model (Alarcon-Gil et al., 2022). In addition, Tang (2014) reported that 50 μM linoleic acid attenuated MPP^+-induced PC12 cell death (Tang, 2014). Lee et al. (2018) demonstrated that linoleic acid concentrations at 10, 50, and 100 μM protected Aβ-induced neuronal cell death by reducing ROS generation, activating of Bcl-2 expression, and suppressing Bax expression (Lee et al., 2018). Moreover, Kim et al. (2015) reported that oleic acid (10, 50, and 100 μM) attenuated cell death and intracellular accumulation of ROS in PC12 cells induced by Aβ (Kim et al., 2015). Compared to the studies mentioned, the concentrations of linoleic acid and oleic acid in PSO used in our study were lower. Specifically, the concentrations of linoleic acid in 0.001 and 10 μg/mL PSO were 1.39×10^{-6} and 1.39×10^{-2} μM, respectively. The concentration of oleic acid in 0.001 and 10 μg/mL PSO were 9.94×10^{-7} and 9.94×10^{-3} μM, respectively. Therefore, our results demonstrated that PSO protects against $A\beta_{42}$-induced SH-SY5Y cell death through ROS/oxidative stress-mediated neuronal apoptosis, attributable to a variety of fatty acids present in PSO. Additionally, our findings suggest that the use of PSO is more effective than using a single fatty acid due to the synergistic effect of several fatty acids contained in PSO.

5. CONCLUSION

The present study demonstrated that PSO showed antioxidant and anti-apoptotic properties on $A\beta_{42}$-induced neuronal oxidative stress and cell death. Pretreatment with PSO could reduce Aβ42-induced oxidative stress via inhibiting ROS production and attenuating apoptotic cell death via reducing apoptotic Bax, and procaspase-3, and increasing anti-apoptotic Bcl-2 in SH-SY5Y cells. Our findings suggested that the beneficial properties of PSO can be used in future studies on co-treatment with modern medicines to prevent or slow cognitive impairment progression in AD patients.

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