Populus tomentiglandulosa protects against amyloid-beta$_{25\text{-}35}$-induced neuronal damage in SH-SY5Y cells

Yu Ri Kwon[1] (ID) · **Ji-Hyun Kim**[1] (ID) · **Sanghyun Lee**[2,3] (ID) · **Hyun Young Kim**[4] (ID) · **Eun Ju Cho**[1] (ID)

Abstract Alzheimer's disease constitutes a large proportion of all neurodegenerative diseases and is mainly caused by excess aggregation of amyloid beta (Aβ), which results in oxidative stress, inflammation, and apoptosis in the neurons. *Populus tomentiglandulosa* belongs to the Salicaceae family and is widely distributed in Korea; the antioxidant activities of the extract and fractions from *P. tomentiglandulosa* have been demonstrated in previous studies. Specifically, the ethyl acetate (EtOAc) fraction of *P. tomentiglandulosa* (EtOAc-PT) shows the most powerful antioxidative activity. Therefore, the present study investigates the protective effects of EtOAc-PT against neuronal damage in Aβ$_{25\text{-}35}$-stimulated SH-SY5Y cells. EtOAc-PT restored cell viability significantly as well as inhibited the levels of reactive oxygen species and lactate dehydrogenase release compared to the Aβ$_{25\text{-}35}$-induced control group. Furthermore, the inflammation- and apoptosis-related protein expressions were investigated to demonstrate its neuroprotective mechanism. EtOAc-PT down-modulated the expressions of inducible nitric oxide synthase, cyclooxygenase-2, B-cell lymphoma 2 associated X, and B-cell lymphoma 2. Thus, the findings show that EtOAc-PT has protective effects against Aβ$_{25\text{-}35}$ by suppressing oxidative stress, inflammation, and apoptosis.

Keywords Alzheimer disease · Amyloid beta · Neurons · Neuroprotective agents · *Populus*

YR Kwon and J-H Kim contributed equally to this work.

Hyun Young Kim (✉)
E-mail: hyunyoung.kim@gnu.ac.kr

Eun Ju Cho (✉)
E-mail: ejcho@pusan.ac.kr

[1]Department of Food Science and Nutrition, Pusan National University, Busan 46241, Republic of Korea

[2]Department of Plant Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea

[3]Natural Product Institute of Science and Technology, Anseong 17546, Republic of Korea

[4]Department of Food Science and Nutrition, Gyeongsang National University, Jinju 52725, Republic of Korea

Introduction

Alzheimer's disease (AD) is an age-related neurodegenerative disorder that accounts for more than 60% of the cases of dementia [1]. The AD-related association reported in the "2023 Alzheimer's disease facts and figures" that AD prevalence in the United States was 10.8% for ages 65 years and above, escalating rapidly with age such that the prevalence rate increased to 46.4% over the age of 75 years [2]. The pathology of AD is diverse and complex, but the beginning of pathogenesis is known to be excess accumulation of extracellular amyloid beta (Aβ) plaques [3]. Accumulation of Aβ damages the neuronal cells, causing symptoms such as memory loss, language disorders, judgment degradation, and visual disabilities in AD patients [4]. "Amyloid hypothesis" is a representative hypothesis related to the manifestation of AD and indicates that proteolysis of the Aβ precursor protein produces Aβ, whose accumulation then stimulates oxidative stress in the neurons [5]. Oxidative stress induced by Aβ causes expression of inflammation-related proteins, including inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), which result in apoptosis. Through this series of processes, Aβ directly contributes to the pathogenesis of AD.

The SH-SY5Y cells used in the present study are relatively homogeneous human neuroblast-like cells; owing to these properties,

SH-SY5Y cells have been used in neuronal research on neuro-degenerative diseases and neurotoxicity analyses [6]. In particular, previous studies have demonstrated the pharmacological effects of herbal medicines using SH-SY5Y cells that were damaged by Aβ in the nervous system. Thus, in the present study, the SH-SY5Y cell line was chosen as the AD cell model.

The *Populus* species contains various phenolic compounds and flavonoids with biological and pharmaceutical activities, such as antioxidant, anti-inflammation, hepatoprotection, and vascular relaxation effects [7,8]. Of these, *Populus tomentiglandulosa* is grown naturally in Korea and is an artificial hybrid between *P. davidiana* and *P. alba* [9]. According to previous studies, *P. tomentiglandulosa* has several beneficial biological activities, such as antioxidant and antioxidative enzyme upregulation effects [10,11]. In addition, it has been reported that *P. tomentiglandulosa* has protective effects against hydrogen peroxide (H_2O_2)-induced neuronal damage and cerebrovascular disorders [12,13]. However, the protective effects of *P. tomentiglandulosa* against Aβ-induced neuronal damage have not been studied. In a previous study by the authors, the ethyl acetate fraction (EtOAc) of *P. tomentiglandulosa* (EtOAc-PT) showed the strongest *in vitro* radical scavenging activity and cellular neuroprotective effects among ethanol (EtOH) extract and four fractions including *n*-butanol, EtOAc, chloroform, and *n*-hexane. Hence, the present study is focused on identifying the protective effects and mechanisms of EtOAc-PT against Aβ$_{25\text{-}35}$-induced neuronal damage using the human neuron-like SH-SY5Y cells.

Materials and Methods

Reagents

Dulbecco's modified eagle's medium (DMEM), trypsin-EDTA solution, penicillin-streptomycin solution, and fetal bovine serum (FBS) were purchased from Welgene Inc. (Daegu, Korea). Aβ fragment 25-35 (Aβ$_{25\text{-}35}$), dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and 2′,7′-dichloro-fluorescein diacetate (DCF-DA) were acquired from Sigma-Aldrich Inc. (St. Louis, MO, USA). The radio-immunoprecipitation assay (RIPA) buffer was procured from Elpis Biotech (Daejeon, Korea) and protease inhibitor cocktail was supplied by Calbiochem (Cambridge, MA, USA). Polyvinylidene fluoride (PVDF) membranes were obtained from Millipore (Billerica, MA, USA) and the enhanced chemiluminescence substrate solution was acquired from Bio-Rad Laboratories (Hercules, CA, USA). The antibodies were purchased from Cell Signaling Technology, Inc. (Beverly, MA, USA).

Sample preparation

The dried leaves of *P. tomentiglandulosa* (1442.6 g) were extracted using EtOH three times (86 °C, 3 h), and the extracts were combined and evaporated to obtain a brown residue (278.3 g). Then, the residue was divided using EtOAc (36.0 g), and the EtOAc fractions were dissolved in DMSO for use in the experiments.

Cell cultures

SH-SY5Y neuronal cells were purchased from the Korea Cell Line Bank (Seoul, Korea). These cells were then cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin and maintained in an atmosphere of 5% CO_2 at 37 °C.

Cell viability measurements

Cell viabilities were measured according to the method of the MTT assay [14]. The SH-SY5Y cells were seeded in 96-well plate at a density of 2.5×10^5 cells/mL and cultured for 24 h; the cells were pretreated with EtOAc-PT at various concentrations (1, 5, and 10 μg/mL). After incubation for 2 h, the cells were treated with Aβ$_{25\text{-}35}$ (25 μM) and further incubated for 24 h. Aβ$_{25\text{-}35}$ dissolved in double distilled water was incubated at 37 °C for 72 h, and the concentration of Aβ$_{25\text{-}35}$ was 1 mM. Prior to use, the Aβ$_{25\text{-}35}$ was diluted with the cell culture medium. Then, 200 μL of 5 mg/mL MTT solution was added to the cells to form formazan crystals, followed by incubation for 4 h. Next, the medium was removed and 200 μL of DMSO was added to each well to dissolve the formazan crystals for 30 min. The plate was finally read with a microplate reader at an absorbance wavelength of 540 nm.

Reactive oxygen species (ROS) measurement

ROS production was measured by the DCFH-DA assay [15]. The SH-SY5Y cells were seeded (2.5×10^5 cells/mL) in 96-well black plate. After 24 h of incubation, the wells were treated with EtOAc-PT at various concentrations (1, 5, and 10 μg/mL) for 2 h followed by Aβ$_{25\text{-}35}$ (25 μM) treatment for 24 h. DCF-DA was then mixed into the plate and incubated for 30 min. Subsequently, the plate was evaluated at an excitation wavelength of 480 nm and emission wavelength of 535 nm using a fluorescence spectro-photometer (FLUOstar OPTIMA, BMG Labtech, Ortenberg, Germany).

Lactate dehydrogenase (LDH) release assay

LDH release was performed using a commercial kit (Takara Bio, Shiga, Japan). The SH-SY5Y cells were seeded in a 96-well plate (2.5×10^5 cells/mL) for 24 h, following which the plate was treated with EtOAc-PT (1, 5, and 10 μg/mL) for 2 h. After that Aβ$_{25\text{-}35}$ (25 μM) was treated for 24 h. Consequently, the supernatant (100 μL) was combined with the reaction mixture (100 μL) at room temperature for 30 min, and the absorbance was measured using a microplate reader at 490 nm.

Western blotting analysis

The SH-SY5Y cells were seeded in a dish (90×20 mm) at a

density of 1×10^6 cells/mL and incubated for 24 h. The cells were pretreated with EtOAc-PT (1, 5, and 10 µg/mL) for 2 h, followed by treatment with $A\beta_{25-35}$ (25 µM). After 24 h, the cells were harvested and lysed with the RIPA buffer. The samples were next electrophoresed by sodium dodecyl sulfate polyacrylamide gels electrophoresis and transferred to PVDF membranes. The membranes were then blocked with skim milk in PBS-T and immersed in the primary antibody, followed by overnight incubation at 4 °C. The primary antibodies (dilution ratios) used in this experiment were as follows: β-actin (1:1000), iNOS (1:1000; Santa Cruz Biotechnology, Dallas, TX, USA), COX-2 (1:500; Santa Cruz), B-cell lymphoma 2 associated X (Bax, 1:1000; Santa Cruz), B-cell lymphoma 2 (Bcl-2, 1:500; Santa Cruz). After the overnight incubation at 4 °C, each membrane was immersed in an appropriate secondary antibody for 1 h at room temperature (1:1000; Cell Signaling). Protein expressions were then measured using the Davinci chemiluminescent imaging system (CoreBio, Seoul, Korea).

Statistical analysis

SPSS 23.0 (IBM Corporation, Armonk, NY, USA) was used to analyze the significance of the data among the experiment groups. The results of the present study are showed as mean ± standard deviation. Statistical significance was confirmed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple-range test. P values less than 0.05 were considered significant.

Results

EtOAc-PT reverses cell viability in $A\beta_{25-35}$-induced SH-SY5Y cells

To investigate the neuroprotective effects of EtOAc-PT, the cell viability was evaluated using MTT assay in $A\beta_{25-35}$-treated SH-SY5Y cells. As seen in Fig. 1, the $A\beta_{25-35}$-treated Control group showed decreased cell viability of 62.43% compared with the untreated Normal group (100%). In contrast, pretreatment with EtOAc-PT significantly increased the cell viability compared to the Control cells. These data demonstrate that treatment with EtOAc-PT may protect against $A\beta_{25-35}$-induced neuronal damage.

EtOAc-PT inhibits ROS production in $A\beta_{25-35}$-induced SH-SY5Y cells

The ROS production inhibitory effects of EtOAc-PT were detected for $A\beta_{25-35}$-stimulated SH-SY5Y cells using the DCFH-DA assay. The $A\beta_{25-35}$-induced Control group showed higher levels of ROS release than the untreated Normal group (Fig. 2A). In addition, as seen in Fig. 2B, the level of ROS production was significantly elevated in the Control group than the Normal group at 60 min. These results indicate that $A\beta_{25-35}$ stimulated ROS generation in the SH-SY5Y cells. On the other hand, pretreatment

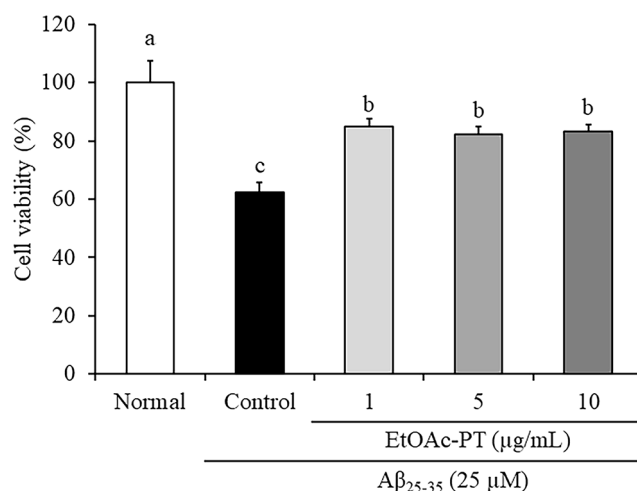**Fig. 1** Effects of the ethyl acetate fraction of *P. tomentiglandulosa* (EtOAc-PT) on cell viability in $A\beta_{25-35}$-treated SH-SY5Y cells. The results are expressed as mean ± SD. The different letters (a-c) among the groups indicate significant differences (p <0.05) by Duncan's multiple-range test

with EtOAc-PT at a concentration of 1 µg/mL significantly decreased ROS production. These finding suggest that $A\beta_{25-35}$ treatment increases ROS production, which can be suppressed by EtOAc-PT.

EtOAc-PT inhibits LDH release in $A\beta_{25-35}$-induced SH-SY5Y cells

The LDH release levels were measured in $A\beta_{25-35}$-induced SH-SY5Y cells to assess the inhibitory effects of EtOAc-PT to LDH release. As seen in Fig. 3, the LDH release level increased in the $A\beta_{25-35}$-treated cell group compared with the untreated cell group. Contrarily, treatment with EtOAc-PT significantly reduced the level of LDH release compared with the $A\beta_{25-35}$-stimulated Control group. In particular, at the concentration of 1 µg/mL, the level of LDH release was 89.72%, which matches with that of the Normal group. The data thus show that $A\beta_{25-35}$ induces LDH release, whose increase is inhibited by EtOAc-PT.

EtOAc-PT inhibits inflammation-related proteins in $A\beta_{25-35}$-induced SH-SY5Y cells

Western blotting was conducted on the $A\beta_{25-35}$-induced SH-SY5Y cells to investigate the protective effects of EtOAc-PT against inflammation. The expressions of iNOS and COX-2 proteins were higher in the $A\beta_{25-35}$-stimulated group than the untreated Normal group (Fig. 4). However, EtOAc-PT-pretreated cells relevantly decreased the expressions of iNOS and COX-2 compared with those of the untreated Normal cells. These findings indicate that $A\beta_{25-35}$ increases inflammation-related protein expression but that EtOAc-PT effectively decreases such expressions in the SH-SY5Y cells.
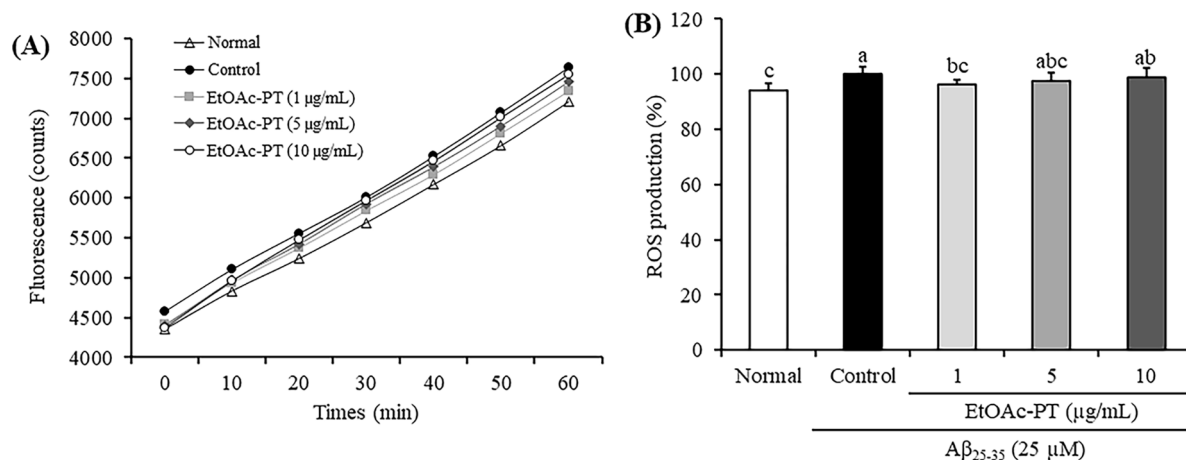
Fig. 2 Effects of the ethyl acetate fraction of *P. tomentiglandulosa* (EtOAc-PT) on ROS production in $A\beta_{25\text{-}35}$-treated SH-SY5Y cells. (A) Time-course changes of DCFH fluorescence intensities and (B) percentage ROS production at 60 min. The results are expressed as mean ± SD. The different letters (a-c) among the groups indicate significant differences (p <0.05) by Duncan's multiple-range test

Fig. 3 Effects of the ethyl acetate fraction of *P. tomentiglandulosa* (EtOAc-PT) on LDH release in $A\beta_{25\text{-}35}$-treated SH-SY5Y cells. The results are expressed as mean ± SD. The different letters (a-d) among the groups indicate significant differences (p <0.05) by Duncan's multiple-range test
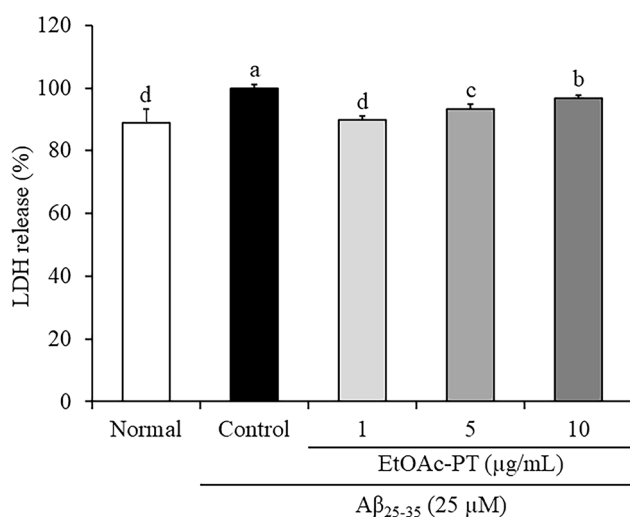
EtOAc-PT inhibits apoptosis-related proteins in $A\beta_{25\text{-}35}$-induced SH-SY5Y cells

The levels of apoptosis-related protein expressions, including Bax and Bcl-2, were determined to investigate whether EtOAc-PT has neuroprotective effects against $A\beta_{25\text{-}35}$-stimulated apoptosis. As seen in Fig. 5, the EtOAc-PT-induced group significantly reduced the Bax/Bcl-2 expression ratio at 5 and 10 μg/mL concentrations in comparison with the $A\beta_{25\text{-}35}$-treated Control group. These results indicate that $A\beta_{25\text{-}35}$ treatment increases the apoptosis-related protein expressions but that EtOAc-PT suppresses upregulation of these proteins.

Discussion

$A\beta_{25\text{-}35}$ exhibits the same level of neurotoxicity as the full-length Aβ peptide in cells [16]. Additionally, it has aggregation features and shows a more critical toxicity of Aβ, which is strongly related to AD pathology [17]. Accumulated evidence has been used to investigate the protective effects of extracts, products, and bioactive compounds from natural sources using SH-SY5Y cells. Wang et al. [18] demonstrated the protective effects of paeoniflorin on Aβ-induced SH-SY5Y cell injury, and Mairuae et al. [19] revealed that *Orocylum indicum* extract prevented neurotoxicity induced by Aβ in SH-SY5Y cells. Therefore, $A\beta_{25\text{-}35}$-induced SH-SY5Y cells were used as the AD cell model in the present study. *P. tomentiglandulosa* has been demonstrated that phenolic compounds such as catechin, caffeic acid, *p*-coumaric acid, chlorogenic acid, and gallic acid are the main components which exert various pharmacological effects [20]. Lee et al. [21] reported that EtOH extract of *P. tomentiglandulosa* prevents the death of neurons, astrogliosis, and leakage for blood-brain barrier in gerbil hippocampus against ischemia-reperfusion injury. Lee et al. [22] demonstrated that EtOH extract of *P. tomentiglandulosa* represents hepato- and nephro-protective effects depending on antioxidant activity. Therefore, interest has been focused on the application as dietary supplement using *P. tomentiglandulos*. In addition, our previous study reported the main component of EtOAc-PT used in present study is salicin comfirmed by HPLC analysis [23]. Salicin is a phenolic glycoside derived from plants including *Populus* genus, and has been used as medicine for its potential biological activities such as anti-inflammatory, anticancer, antiaging, and neuroprotective effects [24-27].

Meanwhile, the authors previously reported that EtOAc-PT showed the strongest antioxidant activity among EtOH extract and four fractions, including *n*-butanol, EtOAc, chloroform, and *n*-
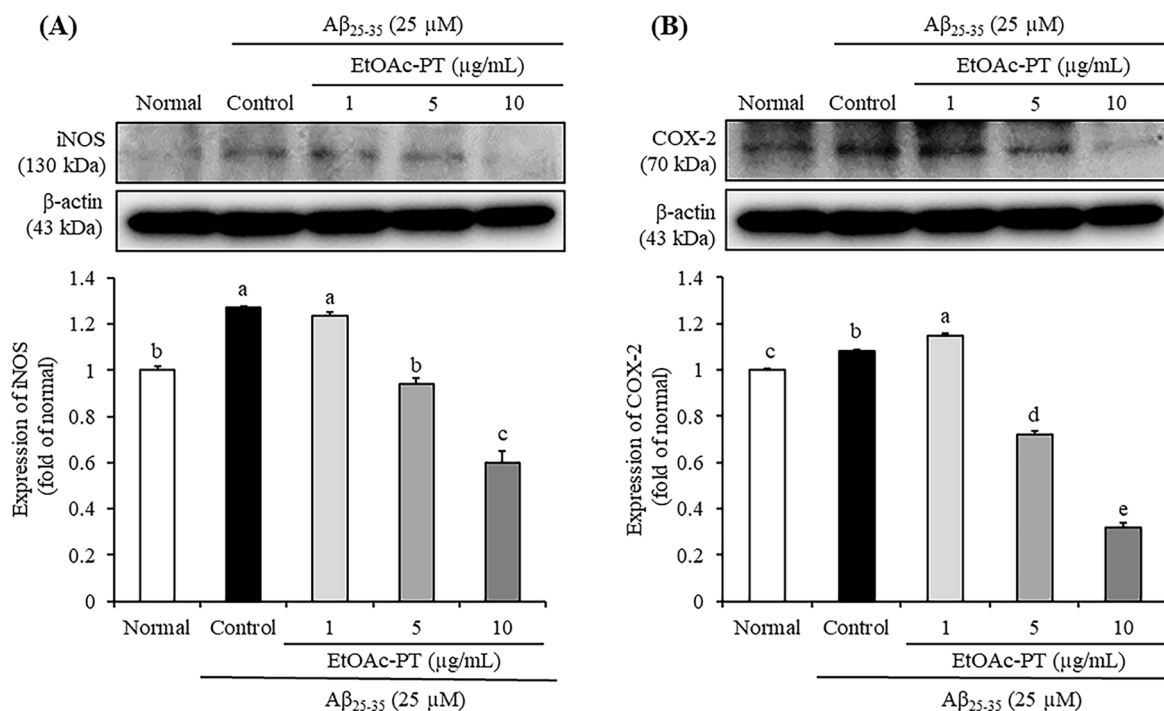
Fig. 4 Effects of the ethyl acetate fraction of *P. tomentiglandulosa* (EtOAc-PT) on protein expressions of iNOS (A) and COX-2 (B) in Aβ$_{25-35}$-treated SH-SY5Y cells. The results are expressed as mean ± SD. The different letters (a-e) among the groups indicate significant differences ($p < 0.05$) by Duncan's multiple-range test

hexane [12]. Moreover, EtOAc-PT protected against neuronal damage in H$_2$O$_2$-treated SH-SY5Y cells by controlling oxidative stress. Based on these reported results, the present study evaluated the neuroprotective effects of EtOAc-PT in Aβ$_{25-35}$-induced SH-SY5Y cells.

Accumulation of Aβ can induce cytotoxicity, which directly attacks cells or produces other materials such as ROS and free radicals to induce cell damage indirectly. Meanwhile, the exact mechanism by which Aβ causes cytotoxicity remains unknown to date [28]. One of the hypotheses of Aβ cytotoxicity is that a β-sheet-rich structure of Aβ interacts with the cell surface receptors, resulting in abnormal cell signaling. When activation by Aβ is applied continuously, it causes neuronal apoptosis [29]. Another hypothesis is that Aβ causes pores in the cell membrane, which allow influx of calcium, resulting in destruction of calcium homeostasis [30]. Through these mechanisms, Aβ cytotoxicity inhibits viability of neuronal cells. Oguchi et al. [31] reported that Aβ stimulation induced critical damage in the dendrite morphology, thereby declining cell viability in SH-SY5Y cells in a dose-dependent manner. In the present study, cell viability was investigated by the MTT assay, which has been reported in various studies as a very sensitive indicator of Aβ$_{25-35}$-induced cytotoxicity. Accordingly, the data in the present study show that Aβ treatment decreases cell viability in SH-SY5Y cells. However, pretreatment with EtOAc-PT efficaciously restored cell viability, indicating that *P. tomenti-glandulosa* could attenuate cytotoxicity induced by Aβ. Meanwhile,

we investigated the dose level through the toxicity test of EtOAc-PT to the SH-SY5Y cells. In the absence of Aβ$_{25-35}$, there was no significant toxicity at SH-SY5Y cells by only treatment of EtOAc-PT.

Aβ causes oxidative stress in neuronal cells, inducing increased ROS accumulation. Aβ-mediated oxidative stress impairs the proteins and DNA, thereby activating neuronal apoptotic cell death and subsequently playing an important role in AD [32]. Therefore, the ROS level in the neurons is considered as a marker of AD pathogenesis [33]. DCFH-DA is a nonfluorescent substance that is converted to its fluorescent form dichlorofluorescein (DCF) upon reaction with ROS [34]. The DCFH-DA assay can be used to estimate the level of intracellular ROS based on this principle. Aβ$_{25-35}$ treatment significantly upregulates the ROS levels in SH-SY5Y cells [35], suggesting that Aβ induces oxidative stress in the neuronal cells. The results of the present study show that pretreatment with EtOAc-PT decreases the ROS level compared with that of the Control group. In addition, previous studies have demonstrated that *P. tomentiglandulosa* extract has antioxidative effects under both *in vitro* and *in vivo* conditions [10,11]. Choi et al. [10] reported that the *P. tomentiglandulosa* EtOH extract significantly increased the levels of antioxidant enzymes and protected the neurons *in vivo* against induction of ischemia-reperfusion injury. Lee et al. [11] also demonstrated that oral administration of the *P. tomentiglandulosa* EtOH extract significantly upregulated antioxidant enzymes in the rat liver and
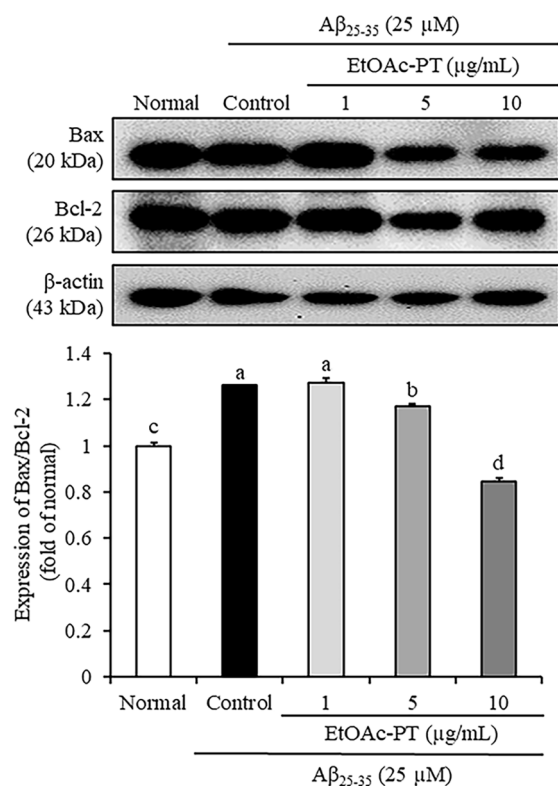
Fig. 5 Effect of the ethyl acetate fraction of *P. tomentiglandulosa* (EtOAc-PT) on protein expression ratio of Bax/Bcl-2 in Aβ$_{25-35}$-treated SH-SY5Y cells. The results were expressed as mean ± SD. The different letters (a-d) among groups indicate significant differences (p <0.05) by Duncan's multiple range test

kidney. In relation with these studies, the present findings demonstrate that EtOAc-PT shows neuroprotective effects against ROS production by suppressing Aβ$_{25-35}$-stimulated oxidative stress.

LDH is a well-known enzyme present in most tissues and organs, and the level of LDH can be used as an indicator to evaluate cellular damage [36]. Previous studies have demonstrated that Aβ stimulates neuronal cells and leads to cell injuries, thereby increasing LDH release [37]. Moreover, Mei et al. [38] reported that Aβ-exposed SH-SY5Y cells showed increased LDH release compared to Aβ-non-exposed cells, whose results are in agreement with those of the present study. Here, LDH levels were significantly reduced in the EtOAc-PT-treated cells, indicating that EtOAc-PT can regulate LDH exflux by protecting against Aβ$_{25-35}$-stimulated cell damage.

Inflammatory responses were initiated by glial cell stimulation, such as astrocytes and microglia, which are typically present around the Aβ plaques in an AD patient [39]. It was observed that activation of the astrocytes and microglia upregulated the expressions of inflammation-related factors (i.e., iNOS and COX-2) [40]. The expression of iNOS protein increases NO production,

and NO combines with superoxide radicals to produce peroxynitrite that plays a role in the pathogenesis of AD [39]. Moreover, NO produced by iNOS is also a free radical that induces oxidative stress, thereby contributing to the pathogenesis of AD [41]. COX-2 is related to the generation of prostaglandin E2 (PGE2) and thromboxane from arachidonic acid, and PGE2 can stimulate apoptotic pathways, leading to neuronal cell death [42]. Guan et al. [43] reported that increased COX-2 and PGE2 expressions were closely connected with AD pathogenesis. Therefore, Aβ-induced inflammation is well-known as a stimulator of AD characteristics [39]. Xu et al. [44] also demonstrated that Aβ$_{25-35}$ treatment increased the expressions of inflammatory factors, including tumor necrosis factor-α, COX-2, interleukin-1β, and PGE2, in SH-SY5Y cells. Similarly, the present data show that treatment of Aβ$_{25-35}$ prompted the degree of inflammatory factor expressions. Contrastively, administration of EtOAc-PT inhibited the expression levels of iNOS and COX-2, suggesting that EtOAc-PT can downregulate the inflammatory process induced by Aβ$_{25-35}$.

The Bcl-2 family critically regulates the apoptotic cell signaling pathway and contains approximately 20 protein members [45]. The Bcl-2 family can be widely categorized into three groups depending on their roles in apoptosis: (1) anti-apoptotic group, including Bcl-2, Bcl-xL, and Bcl-w; (2) pro-apoptotic group, including Bax, Bak, and Bok; (3) only BH3-containing group, including Noxa, Bfm, and Bik [45]. When apoptotic-related stimulation was applied, BH3-containing proteins were upregulated, leading to pro-apoptotic protein activation, such as Bax and Bak. The pro-apoptotic proteins flow into the mitochondrial membrane and form pores there, thereby releasing cytochrome c and caspase into the cytosol, which causes apoptosis in a short timeframe [46,47]. Bcl-2 binds to the pro-apoptotic proteins, consequently controlling the release of cytochrome c, thereby protecting from apoptotic cell death [40]. Therefore, the Bax/Bcl-2 ratio may be considered as an indicator of apoptosis, and Aβ induces cell apoptosis by stimulation of Bax/Bcl-2 expression [48]. In a previous study, the Bax/Bcl-2 expression ratio was significantly increased in Aβ$_{25-35}$-induced SH-SY5Y cells [49]. Similarly, the present results show that exposure to Aβ$_{25-35}$ induces a higher ratio of Bax/Bcl-2 protein expression, demonstrating that Aβ$_{25-35}$ promotes apoptosis. However, EtOAc-PT effectively reduces the Bax/Bcl-2 ratio; therefore, the present findings confirm that EtOAc-PT might inhibit Aβ$_{25-35}$-induced apoptotic cell death.

In conclusion, the present study demonstrated that EtOAc-PT has neuroprotective effects on Aβ$_{25-35}$-induced cell damage caused by oxidative stress, inflammation, and apoptosis. Specifically, the inflammation-related protein expressions and ratio of apoptosis-related protein expressions were significantly regulated by treatment with EtOAc-PT. Therefore, *P. tomentiglandulosa* could be considered as a natural agent for the prevention and treatment of AD.

Declarations

Data availability statement   The data used to support the findings of this study are included in this article.

References

1. Cao Q, Tan C-C, Xu W, Hu H, Cao X-P, Dong Q, Tan L, Yu J-T (2020) The prevalence of dementia: a systematic review and meta-analysis. J Alzheimers Dis 73(3): 1157–1166. doi: 10.3233/JAD-191092

2. Association As (2023) 2023 Alzheimer's disease facts and figures. Alzheimer's Dement 19(4): 1598–1695. doi: 10.1002/alz.13016

3. Edwards FA (2019) A unifying hypothesis for Alzheimer's disease: from plaques to neurodegeneration. Trends Neurosci 42(5): 310–322. doi: 10.1016/j.tins.2019.03.003

4. Castellani RJ, Rolston RK, Smith MA (2010) Alzheimer disease. Dis Mon 56(9): 484–546. doi: 10.1016/j.disamonth.2010.06.001

5. Selkoe DJ, Hardy J (2016) The amyloid hypothesis of Alzheimer's disease at 25 years. EMBO Mol Med 8: 595–608. doi: 10.15252/ emmm.201606210

6. Xie H-r, Hu L-s, Li G-y (2010) SH-SY5Y human neuroblastoma cell line: *in vitro* cell model of dopaminergic neurons in Parkinson's disease. Chin Med J 123(8): 1086–1092. doi: 10.3760/cma.j.issn.0366-6999. 2010.08.021

7. Debbache-Benaida N, Atmani-Kilani D, Schini-Keirth VB, Djebbli N, Atmani D (2013) Pharmacological potential of *Populus nigra* extract as antioxidant, anti-inflammatory, cardiovascular and hepatoprotective agent. Asian Pac J Trop Biomed 3(9): 697–704. doi: 10.1016/S2221-1691(13)60141-0

8. Dudonné S, Poupard P, Coutière P, Woillez M, Richard T, Mérillon J-M, Vitrac X (2011) Phenolic composition and antioxidant properties of Poplar bud (*Populus nigra*) extract: individual antioxidant contribution of phenolics and transcriptional effect on skin aging. J Agric Food Chem 59(9): 4527–4536. doi: 10.1021/jf104791t

9. Kwak J, Oh Y (2003) Evaluation of *populus alba × glandulosa* as raw material of particleboard. J Korean For Soc 92: 140–144

10. Choi S-I, Hwang S-J, Lee O-H, Kim JD (2020) Antioxidant activity and component analysis of *Populus tomentiglandulosa* extract. Korean J Food Sci Technol 52(2): 119–124. doi: 10.9721/KJFST.2020.52.2.119

11. Lee T-K, Park JH, Ahn JH, Kim H, Song M, Lee J-C, Kim JD, Jeon YH, Choi JH, Lee CH, Hwang IK, YAN B-C, Won M-H, Kang IJ (2019) Pretreatment of *Populus tomentiglandulosa* protects hippocampal CA1 pyramidal neurons from ischemia-reperfusion injury in gerbils via increasing SODs expressions and maintaining BDNF and IGF-I expressions. Chin J Nat Med 17(6): 424–434. doi: 10.1016/S1875-5364 (19)30050-0

12. Kwon YR, Kim JH, Lee S, Cho EJ, Kim HY (2020) Protective role of *Populus tomentiglandulosa* against hydrogen peroxide-induced oxidative stress in SH-SY5Y neuronal cells. J Appl Biol Chem 63(4): 357–363. doi: 10.3839/jabc.2020.047

13. Park JH, Lee TK, Ahn JH, Shin BN, Cho JH, Kim IH, Lee JC, Kim JD, Lee YJ, Kang IJ, Hong S, Kim YH, Jeon YH, Lee YL, Won M-H (2017)

14. Pre-treated *Populus tomentiglandulosa* extract inhibits neuronal loss and alleviates gliosis in the gerbil hippocampal CA1 area induced by transient global cerebral ischemia. Anat Cell Biol 50(4): 284–292. doi: 10.5115/acb.2017.50.4.284

14. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65(1-2): 55–63. doi: 10.1016/0022-1759(83)90303-4

15. Cathcart R, Schwiers E, Ames BN (1983) Detection of picomole levels of hydroperoxides using a fluorescent dichlorofluorescein assay. Anal Biochem 134(1): 111–116. doi: 10.1016/0003-2697(83)90270-1

16. Shanmugam G, Polavarapu PL (2004) Structure of Aβ (25–35) peptide in different environments. Biophy J 87(1): 622–630. doi: 10.1529/biophysj. 104.040907

17. Millucci L, Ghezzi L, Bernardini G, Santucci A (2010) Conformations and biological activities of amyloid beta peptide 25–35. Curr Protein Pept Sci 11(1): 54–67. doi: 10.2174/138920310790274626

18. Wang K, Zhu L, Zhu X, Zhang K, Huang B, Zhang J, Zhang Y, Zhu L, Zhou B, Zhou F (2014) Protective effect of paeoniflorin on Aβ$_{25-35}$-induced SH-SY5Y cell injury by preventing mitochondrial dysfunction. Cell Mol Neurobiol 34: 227–234. doi: 10.1007/s10571-013-0006-9

19. Mairuae N, Connor JR, Buranrat B, Lee SY (2019) *Oroxylum indicum* (L.) extract protects human neuroblastoma SH-SY5Y cells against β-amyloid-induced cell injury. Mol Med Rep 20(2): 1933–1942. doi: 10.3892/mmr.2019.10411

20. Choi SI, Hwang SJ, Lee OH, Kim JD (2020) Antioxidant activity and component analysis of *Populus tomentiglandulosa* extract. Korean J Food Sci Technol 52: 119–124. doi: 10.9721/KJFST.2020.52.2.119

21. Lee T-K, Lee J-C, Kim J-D, Kim D-W, Ahn J-H, Park J-H, Kim H-I, Cho J-H, Choi S-Y, Won M-H, Kang I-J (2021) *Populus tomentiglandulosa* extract is rich in polyphenols and protects neurons, astrocytes, and the blood-brain barrier in gerbil striatum following ischemia-reperfusion injury. Molecules 26: 5430. doi: 10.3390/molecules26185430

22. Lee C-H, Park JH, Ahn JH, Kim JD, Cho JH, Lee T-K, Won M-H (2019) Stronger antioxidant enzyme immunoreactivity of *Populus tomentiglandulosa* extract than ascorbic acid in rat liver and kidney. Iran J Basic Med Sci 22: 963–967. doi: 10.22038/ijbms.2019.34926.8296

23. Kwon YR, Kim J-H, Lee S, Kim HY, Cho EJ (2022) Protective effects of *Populus tomentiglandulosa* against cognitive impairment by regulating oxidative stress in an amyloid beta25-35-induced Alzheimer's disease mouse model. Nutr Res Pract 16(2): 173–193. doi: 10.4162/nrp.2022.16. 2.173

24. Kong CS, Kim KH, Choi JS, Kim JE, Park C, Jeong JW (2014) Salicin, an extract from white willow bark, inhibits angiogenesis by blocking the ROS-ERK pathways. Phytother Res 28(8): 1246−1251. doi: 10.1002/ ptr.5126

25. Gao F, Zhang S (2019) Salicin inhibits AGE-induced degradation of type II collagen and aggrecan in human SW1353 chondrocytes: therapeutic potential in osteoarthritis. Artif Cells Nanomed Biotechnol 47(1): 1043−1049. doi: 10.1080/21691401.2019.1591427

26. Sabaa M, ELFayoumi HM, Elshazly S, Youns M, Barakat W (2017) Anticancer activity of salicin and fenofibrate. N Schmied Arch Pharmacol 390: 1061−1071. doi: 10.1007/s00210-017-1407-y

27. Park J-H, Lee T-K, Kim D-W, Sim H, Lee J-C, Kim J-D, Ahn J-H, Lee C-H, Kim Y-M, Won M-H, Choi S-Y (2021) Neuroprotective effects of salicin in a gerbil model of transient forebrain ischemia by attenuating oxidative stress and activating PI3K/Akt/GSK3beta pathway. Antioxidants 10(1): 62. doi: 10.3390/antiox10010062

28. Awasthi A, Matsunaga Y, Yamada T (2005) Amyloid-beta causes apoptosis of neuronal cells via caspase cascade, which can be prevented by amyloid-beta-derived short peptides. Exp Neurol 196(2): 282–289. doi: 10.1016/j.expneurol.2005.08.001

29. Walsh DM, Teplow DB (2012) Alzheimer's disease and the amyloid β-protein. Prog Mol Biol Transl Sci 107: 101–124. doi: 10.1016/B978-0-12-385883-2.00012-6

30. Green KN, LaFerla FM (2008) Linking calcium to Aβ and Alzheimer's

disease. Neuron 59(2): 190–194. doi: 10.1016/j.neuron.2008.07.013

31. Oguchi T, Ono R, Tsuji M, Shozawa H, Somei M, Inagaki M, Mori Y, Yasumoto T, Ono K, Kiuchi Y (2017) Cilostazol suppresses Aβ-induced neurotoxicity in SH-SY5Y cells through inhibition of oxidative stress and MAPK signaling pathway. Front Aging Neurosci 9: 337. doi: 10.3389/fnagi.2017.00337

32. Wang Y, Fu X-t, Li D-w, Wang K, Wang X-z, Li Y, Sun B-l, Yang X-y, Zheng Z-c, Cho NC (2016) Cyanidin suppresses amyloid beta-induced neurotoxicity by inhibiting reactive oxygen species-mediated DNA damage and apoptosis in PC12 cells. Neural Regen Res 11(5): 795–800. doi: 10.4103/1673-5374.182707

33. Jiang S, Zhao Y, Zhang T, Lan J, Yang J, Yuan L, Zhang Q, Pan K, Zhang K (2018) Galantamine inhibits β-amyloid-induced cytostatic autophagy in PC 12 cells through decreasing ROS production. Cell Prolif 51(3): e12427. doi: 10.1111/cpr.12427

34. Pathak J, Chatterjee A, Singh SP, Sinha RP (2017) Detection of reactive oxygen species (ROS) in cyanobacteria using the oxidant-sensing probe 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA). Bio Protoc 7(17): e2545. doi: 10.21769/BioProtoc.2545

35. Zhang L, Yu H, Zhao X, Lin X, Tan C, Cao G, Wang Z (2010) Neuroprotective effects of salidroside against beta-amyloid-induced oxidative stress in SH-SY5Y human neuroblastoma cells. Neurochem Int 57(5): 547–555. doi: 10.1016/j.neuint.2010.06.021

36. El-Hussein A, Mfouo-Tynga I, Abdel-Harith M, Abrahamse H (2015) Comparative study between the photodynamic ability of gold and silver nanoparticles in mediating cell death in breast and lung cancer cell lines. J Photochem Photobiol B 153: 67–75. doi: 10.1016/j.jphotobiol.2015.08.028

37. Kimura M, Komatsu H, Ogura H, Sawada K (2005) Comparison of donepezil and memantine for protective effect against amyloid-beta (1–42) toxicity in rat septal neurons. Neurosci Lett 391(1-2): 17–21. doi: 10.1016/j.neulet.2005.08.036

38. Mei Z, Yan P, Situ B, Mou Y, Liu P (2012) Cryptotanshinione inhibits β-amyloid aggregation and protects damage from β-amyloid in SH-SY5Y cells. Neurochem Res 37(3):622–628. doi: 10.1007/s11064-011-0652-6

39. Ayasolla K, Khan M, Singh AK, Singh I (2004) Inflammatory mediator and β-amyloid (25–35)-induced ceramide generation and iNOS expression are inhibited by vitamin E. Free Radic Biol Med 37(3): 325–338. doi: 10.1016/j.freeradbiomed.2004.04.007

40. Kaur D, Sharma V, Deshmukh R (2019) Activation of microglia and astrocytes: a roadway to neuroinflammation and Alzheimer's disease. Inflammopharmacology 27(4): 663–677. doi: 10.1007/s10787-019-00580-x

41. Law A, Gauthier S, Quirion R (2001) Say NO to Alzheimer's disease: the putative links between nitric oxide and dementia of the Alzheimer's type. Brain Res Rev 35(1): 73–96. doi: 10.1016/S0165-0173(00)00051-5

42. Kotilinek LA, Westerman MA, Wang Q, Panizzon K, Lim GP, Simonyi A, Lesne S, Falinska A, Younkin LH, Younkin SG (2008) Cyclooxygenase-2 inhibition improves amyloid-β-mediated suppression of memory and synaptic plasticity. Brain 131(3): 651–664. doi: 10.1093/brain/awn008

43. Guan P-P, Liang Y-Y, Cao L-L, Yu X, Wang P (2019) Cyclooxygenase-2 induced the β-amyloid protein deposition and neuronal apoptosis via upregulating the synthesis of prostaglandin E2 and 15-deoxy-Δ12, 14-prostaglandin J2. Neurotherapeutics 16(4): 1255–1268. doi: 10.1007/s13311-019-00770-z

44. Xu J, Wu W, Zhang H, Yang L (2018) Berberine alleviates amyloid β25-35-induced inflammatory response in human neuroblastoma cells by inhibiting proinflammatory factors. Exp Ther Med 16(6): 4865–4872. doi: 10.3892/etm.2018.6749

45. Garcia-Saez A (2012) The secrets of the Bcl-2 family. Cell Death Differ 19(11): 1733–1740. doi: 10.1038/cdd.2012.105

46. Jiang Z, Zhang H, Böckmann RA (2016) Allostery in BAX protein activation. J Biomol Struct Dyn 34(11): 2469–2480. doi: 10.1080/07391102.2015.1119731

47. Peña-Blanco A, García-Sáez AJ (2018) Bax, Bak and beyond—mitochondrial performance in apoptosis. FEBS J 285(3): 416–431. doi: 10.1111/febs.14186

48. Clementi M, Pezzotti M, Orsini F, Sampaolese B, Mezzogori D, Grassi C, Giardina B, Misiti F (2006) Alzheimer's amyloid β-peptide (1–42) induces cell death in human neuroblastoma via bax/bcl-2 ratio increase: an intriguing role for methionine 35. Biochem Biophys Res Commun 342(1): 206–213. doi: 10.1016/j.bbrc.2006.01.137

49. Zhang Y, Jiao G, Song C, Gu S, Brown RE, Zhang J, Zhang P, Gagnon J, Locke S, Stefanova R, Pelletier C, Zhang Y, Lu H (2017) An extract from shrimp processing by-products protects SH-SY5Y cells from neurotoxicity induced by Aβ25–35. Mar Drugs 15(3): 83. doi: 10.3390/md15030083