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Title: Ferulago Angulata Methanolic Extract Protects PC12 Cells Against Beta Amyloid Peptide Induced Toxicity

Running title: Neuroprotective Effect of F.angulata

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Highlights

Ferulago angulata extract dose-dependently ameliorates A β -induced cytotoxicity in PC12 cells.

A β induces oxidative stress in PC12 cells that attenuated by *Ferulago angulata* extract.

A β increases acetylcholinesterase activity in PC12 cells that prevented by *Ferulago angulata* extract.

Plain Language Summary

Alzheimer's disease (AD) is a common form of dementia in the elderly with a complex pathophysiology. Beta-amyloid- induced neurotoxicity plays a pivotal role in AD progression. So far, there is no cure for AD. Medicinal plants contain various pharmacologically active compounds that make them suitable for the treatment of complex diseases. In this study, the anti-Alzheimer effect of *F. angulata* extract by assessing its protective effect against A β -induced toxicity in PC12 cells has been investigated. Results indicated that *F. angulata* extract improved A β -induced toxicity by diminishing oxidative stress and apoptosis. Therefore, *F. angulata* extract merits further studies for use in the treatment of AD.

Abstract

Alzheimer's disease (AD) is an age-dependent neurodegenerative disease. Beta-amyloid peptide (A β)- induce neurotoxicity has a pivotal role in AD pathogenesis, therefore modulation of A β toxicity is the promising therapeutic approach for control of disease progression. Medicinal plants for having multiple active ingredients are effective in complex diseases such as AD therefore; several studies have been focused on medicinal plants for finding an effective treatment for AD. *Ferulago angulata* is a medicinal plant with the antioxidant and neuroprotective activity. The present study aims to assess the protective effect of the methanolic extract of *Ferulago angulata* on A β -induced toxicity and oxidative stress in PC12 cells. Methanolic extract of aerial parts of the plant was prepared by maceration method. PC12 cells were cultured according to a standard protocol. PC12 cells were incubated for 24 hours with A β alone, and A β in combined with various concentrations of the *Ferulago angulata* extract. Cell viability was determined by the MTT assay. Also, ROS production and the activity of Acetylcholine esterase (AChE), glutathione peroxidase (GPx), and caspase-3 enzymes were measured. The extract dose-dependently protects PC12 cells against A β -induced cell death. Also, A β increases the ROS production, AChE, and caspase-3 activity and decreases the GPx activity, which all were ameliorated by *Ferulago angulata* extract. Results of the present study indicate that *Ferulago angulata* extract protects against A β -induced oxidative stress and apoptosis. These effects may be due to the antioxidant and anticholinesterase activity of the extract. It is recommended *Ferulago angulata* extract be investigated more as an anti-Alzheimer agent.

Keywords: Beta Amyloid, Oxidative Stress, *Ferulago Angulate*, Alzheimer's Disease

Introduction

Alzheimer's disease (AD), the most common form of dementia and age-related neurodegenerative disease, is diagnosed with progressive cognitive decline and memory loss. Behavioral disorders and an inability to perform daily tasks are other symptoms of AD (Castellani, Rolston, & Smith, 2010).

According to a global report in 2010, 35.6 million people live with AD and related disorders, and the number of people affected by the disease is expected to rise to 115 million by 2050 due to the growing population and life expectancy (Hebert, Weuve, Scherr, & Evans, 2013).

AD is a disease with complex pathophysiology, and the exact mechanism of AD pathogenesis is still unknown, but researchers have suggested two central hypotheses for explanation of AD pathogenesis includes "the cholinergic deficit" and "the Amyloid cascade" hypothesis (dos Santos et al., 2018; Masters & Selkoe, 2012).

The extracellular senile plaques, intracellular neurofibrillary tangles, and massive loss of neurons are the most important pathological findings in the AD brain (Castellani, Lee, Zhu, Perry, & Smith, 2008). The main component of extracellular senile plaques is a beta-amyloid peptide ($A\beta$). $A\beta$ is a peptide produced in neural cells by sequential cleavage of amyloid precursor protein (APP) by β secretase and γ secretase enzymes. In the pathological condition, $A\beta$ more produced and accumulated. The accumulated $A\beta$ is extracellularly deposited and forms the central core of extracellular amyloid plaques. According to the Amyloid hypothesis, it is postulated that abnormal accumulation of amyloid plaques is the primary event that causes AD (Cappai & Barnham, 2008; Femminella et al., 2018). Several studies have been indicated that aggregated $A\beta$ is toxic for neural cells and triggers various mechanisms such as oxidative stress, inflammation, mitochondrial dysfunction, and apoptosis, which leads to neural cell death (Sun, Chen, & Wang, 2015). Based on the amyloid hypothesis, the disease-modifying drugs which modulate $A\beta$ production, clearance, and toxicity, are developed to control the AD progression (Carrillo-Mora, Luna, & Colín-Barenque, 2014).

Oxidative stress has a key pathogenic role in the progression of AD. Several studies reported oxidative damages in the AD brain. Reactive oxygen species (ROS) oxidize the cellular biomolecules such as proteins, lipids, and nucleic acids, leading to dysfunction of proteins, alteration of membrane integrity, and production of toxic metabolites in neurons and finally, cell death occurs (Dumont & Beal, 2011; Wang et al., 2014). There are many indications that beta-amyloid increases oxidative stress. In vivo and in vitro studies have shown that $A\beta$ in aggregated form causes increased ROS production, decreased antioxidant enzyme activity, and mitochondrial dysfunction, which activates the apoptosis pathways and leads to cell death. (Cheignon et al.,

2018; Jang & Surh, 2003). Antioxidant compounds improve A β –induced oxidative stress, considered as Alzheimer's therapeutic goals (Ono, Hamaguchi, Naiki, & Yamada, 2006).

The cholinergic deficit hypothesis is the first and oldest hypothesis for AD pathogenesis. This hypothesis is based on findings indicating a decreased level of Acetylcholine (ACh), a brain's cholinergic neurotransmitter, and degeneration of septohippocampal's cholinergic neurons pathway in the brain of the AD patient. The septohippocampal cholinergic neurons are responsible for learning and memory function, and hypofunction or loss of these neurons in AD results in memory loss (Francis, Palmer, Snape, & Wilcock, 1999). According to this hypothesis, augmentation of the cholinergic system can improve memory function in afflicted patients with AD (H Ferreira-Vieira, M Guimaraes, R Silva, & M Ribeiro, 2016). Then the acetylcholinesterase inhibitors (AChEIs) medication such as rivastigmine, galantamine, donepezil, and tacrine have been approved for the treatment of AD. However, these medications only reduce the disease's symptoms and have been unsuccessful in preventing disease progression (Herrmann, Chau, Kircanski, & Lanctot, 2011).

Due to Alzheimer's disease's complex pathophysiology, new therapies focus on compounds that can improve the disease's symptoms and progression through several mechanisms (Batoool, Kamal, Rizvi, & Rashid, 2018). In the recent years, herbs have been the focus of researchers because of having many compounds that can be effective at different stages of the disease, and studies have shown that herbs which have antioxidant effects that strengthen the cholinergic system have had significant effects on the improvement of this disease (Ambure, Bhat, Puzyn, & Roy, 2019). *Ferulago angulata* (Schlecht.) Boiss. (Apiaceae) is a medicinal plant that has been found in Western Asia and Iran. This plant's local name is *Chovir*, which is used in traditional medicine to treat a wide range of disorders such as headache, digestive problems, snakebite, hemorrhoid, and chronic ulcers. (Lorigooini et al., 2019). Several studies have reported various effects of this plant, such as antibacterial, anti-cancer, and acetylcholinesterase enzyme (AChE) inhibitory effects (Hajimehdipoor, Shekarchi, Aghighi, & HAMZELOO, 2014; Heidari et al., 2014; Zareii, Seyfi, Movahedi, Cheraghi, & Ebrahimi, 2014). This plant extract consists of a mixture of various polyphenols with antioxidant and neuroprotective effects (Hosseini, Akbari, Ghafarzadegan, Changizi Ashtiyani, & Shahmohammadi, 2012; Lorigooini et al., 2019). It is reported that *F.angulata* essential oil improves scopolamine-induced learning and memory deficit therefore recommended for treatment of dementia (Hritcu, Bagci, Aydin, & Mihasan, 2015).

Considering the importance of a way to prevent the development and progression of Alzheimer's disease, and the antioxidant, AChE inhibitory and neuroprotective effects of this plant, in the

present study, the protective effect of the methanolic extract of *Ferulago angulata* on A β -induced toxicity and oxidative stress in PC12 cells have been investigated.

Material and Methods

Rat pheochromocytoma (PC12) cell was purchased from The Pasteur Institute of Iran. A β 25–35 was purchased from Enzo life sciences (USA). Dulbecco's modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin (10000 U/mL), and trypsin (0.25%) were purchased from Gibco (USA). Poly-D-lysine (PDL) was purchased from Santa Cruz Biotechnology (USA). All other materials were purchased from Sigma (USA).

Plant material

Ferulago angulata was collected from Kohgiluyeh Va Boyer Ahmad province of Iran. It was identified by the botanists of the Traditional Medicine & Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences, and its voucher specimen was deposited at TMRC Herbarium for future reference (No. 2800 TMRC).

Plant extraction

The total extract was prepared by maceration method. The aerial parts of the plant were dried in shade and ground. 10 g of the plant powder was mixed with 100 ml of methanol: water (80:20). Every 24h, the mixture was filtered, and the fresh solvent was added for three days. All extracts were combined and dried by rotary evaporator and freeze dryer (Seidel, 2012).

Measurement of total phenolic compounds

The Folin-Ciocalteu method was used to determine the extract's total phenolics contents by using Gallic acid as the standard. The methanolic solution of Gallic acid or extract was prepared and mixed with Folin- Ciocalteu's reagent and incubated for 5 minutes. Then, sodium carbonate (7%) solution was added, and the reaction mixture was further incubated for 90 minutes. Then, the absorbance of the product was measured at 725 nm. The total phenolics content was expressed as Gallic acid equivalent in gram per 100 g dried extract (Singleton, Orthofer, & Lamuela-Raventós, 1999).

Cell culture

PC12 cells were cultured on PDL coated flasks containing RPMI 1640, supplemented with 10% (v/v) heat-inactivated fetal bovine serum, and 1% (v/v) penicillin and streptomycin. Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. the culture medium was replaced every three days, and culture was passaged after reaching 70% confluency.

Evaluation of protective effect of *F.angulata* against A β -induced cytotoxicity

PC12 cells were seeded on PDL coated 96 well plates at a density of 1×10^4 cells/well, and incubated at the condition mentioned above. After 24h, the cell culture medium was removed and replaced with a new medium that contains different concentrations of extract ranging between 10-200 μ g/ml. Subsequently, After one-hour incubation with different extract concentrations, aggregated A β peptide was added to each well, and cells were further incubated for 24h. The final concentration of A β in the medium was 0.5 μ M. A β peptide was dissolved in distilled water and incubated at 37°C for three days to prepare the aggregated form. Cells in control wells were incubated with the culture medium without extract and A β peptide, and cells in some wells were incubated with A β peptide alone. Cell viability was measured by methyl tetrazolium (MTT) reduction assay at the end of incubation time (Sepand, Soodi, Hajimehdipour, Soleimani, & Sahraei, 2013).

MTT assay

MTT assay is a standard method for measuring cell viability (Mosmann, 1983). In this assay, yellow tetrazolium salt was reduced by mitochondrial succinate dehydrogenase enzyme in viable cells and converted to purple formazan salt, then the intensity of the purple color is measured by spectrophotometer. At the end of incubation time, the medium was removed and replaced with fresh medium containing MTT solution at a final concentration of 0.5mg/ml. Cell cultures were incubated with MTT containing medium for 4h at 37°C. Subsequently, the medium was removed, and 100 μ l DMSO was added to each well, and the plate was shaken until the formazan product was completely dissolved. Absorbance was measured at 540 nm in an automated plate reader (BIOTEK) against 670 nm as the reference wavelength. The results were presented as a percentage of the control group.

Measurement of Reactive Oxygen Species

Reactive Oxygen Species (ROS) produced during oxidative stress was measured by flow cytometry using 2',7'-Dichlorodihydrofluorescein Diacetate (DCFH-DA) as a prob. DCFH-DA readily crosses the cell membrane and is converted to Dichlorodihydrofluorescein (DCFH) by intracellular esterases. DCFH is a non-fluorescent compound and is converted to DCF- a highly fluorescent compound-in the presence of ROS. PC12 cells were plated onto PDL coated 24 well plate (4×10^5 cell/well). Cells were incubated with 0.5 μ M A β alone, and 0.5 μ M A β in combination with extract (200 μ g / ml) for 24h, then, the cells were harvested by trypsinization and washed with PBS; after that, cells resuspended in 1ml assay buffer containing NaCl 140mM, KCl 5 mM, MgCl₂ 1mM, CaCl₂ 1.5mM, glucose 5.6mM, HEPES-Na 20mM, pH= 7.4, 1 μ l DCFH-DA (10mM) was added to this solution, and the cell suspension was incubated for 45 at

37°C in a CO₂ incubator. Subsequently, the cell suspension was centrifuged, the supernatant was discarded, and the cell plate was suspended in 1 ml assay buffer, and fluorescent intensity was measured by flow cytometer at the excitation wavelength of 485 nm and the emission wavelength of 520 nm (Kiani-Esfahani et al., 2016).

Measurement of glutathione peroxidase enzyme activity

The activity of Glutathione peroxidase (GPx) as an antioxidant enzyme alters during oxidative stress then its activity was measured by the colorimetric kit (BioVision). PC12 cells were plated onto PDL coated 6-well plate (1 × 10⁶ cell/well), then incubated with Aβ (10 μM) alone and Aβ with extracts (200 μg / ml) for 24 hours. After the incubation, the cells were rinsed with PBS and harvested, after, homogenized in PBS with sonication. The homogenate was centrifuged at 1000 g for 10 min at 4°C, and the supernatant was used for enzyme activity and protein assay. GPx activity was measured according to kit instruction. Data were expressed as mU/ mg protein (Dashti, Soodi, & Amani, 2016). The protein content of the samples was measured by the Bradford method (Bradford, 1976).

Measurement of acetylcholinesterase enzyme activity

PC12 cells were cultured, treated, and homogenized, as it is mentioned in the previous section. The acetylcholinesterase enzyme (AChE) activity was measured in cell lysate by the Ellman method, as described previously (Ellman, Courtney, Andres Jr, & Featherstone, 1961). Briefly, after adding cell lysate to Ellman reagent containing phosphate buffer (0.1M, pH=8), thiocholine, and Di-thio-nitro-benzoic acid, the absorbance was monitored at 405nm for 10 minutes by the plate reader, and the reaction rate was calculated. The results were expressed as a percent of control.

Measurement of Caspase-3 enzyme activity

The activity of the Caspase-3 enzyme was measured by the colorimetric method using ACDEVD-pNA as a substrate. In this assay, the substrate is cleaved with activated caspase-3, and para nitro aniline (pNA) is released that is measured at 405 nm. PC12 cells were cultured, treated, and harvested, as it is mentioned in the previous section. The harvested cells were resuspended in 1 mL of cell lysis buffer containing 50 mM tris-HCl, pH=7.5, and 1.0 mM DTT, then sonicated for 20 seconds and centrifuged at 1000 g for 10 minutes. The supernatant was collected for the caspase-3 assay. 10 μl supernatant was mixed with 0.2 mM AC-DEVD-pNA solution and incubated at 37°C for at least one hour. The absorbance was measured by a microplate reader at

405 nm. The results were expressed as a percent of control (M. Soodi, Dashti, Hajimehdipoor, Akbari, & Ataei, 2017).

Statistical analysis

Each data is presented as the Mean $\pm$ SEM of three separate experiments. The statistical analysis was performed by Graph-Pad Prism 8 software. Statistical differences were estimated by using one-way ANOVA followed with Tukey's Multiple Comparison Test.

Results

Total phenolic compounds content

The amount of total phenolic compounds in the extracts was calculated as the equivalent of the Gallic acid (g) in 100 gr dry extract. The data were obtained according to calibration curves of Gallic acid ($y=2.6x-0.038$, $r^2=0.99$), and the amount of total phenolic compounds in *F. angulata* extract was 2.11 ± 0.08 g as Gallic acid equivalent in 100g dried extract that is equal to 2.11%.

Protective effects of *F. angulata* extract on A β - induced cytotoxicity

The results of the cell viability assay by the MTT method have been shown in figure 1. One way ANOVA analysis indicated significant differences between groups ($F_{6,14}=185.2$, $p<0.001$). Treatment of PC12 cells with A β (0.5 μ M) for 24 h significantly decreased the cell viability. However, pre-incubation of cells with extract significantly protected the PC12 cells against the A β - induced cytotoxicity. This protective effect was dose-dependent, and extract at 200 μ g/ml completely attenuated the A β -induced cytotoxicity.

Effect of *F. angulata* extract on ROS production

ROS production was significantly different between groups ($F_{3,8}=19.67$, $p<0.001$). The level of intracellular ROS significantly increased in cells incubated with A β peptide for 24h, whereas one hour pretreated with 200 μ g/mL of the *F. angulata* extract before the addition of A β peptide inhibited the production of ROS by the A β peptide (Figure 2)

Effect of *F. angulata* extract on GPx activity

GPx is an essential antioxidant enzyme in cells which activity alters during oxidative stress. As shown in figure 3, significant differences between groups were observed ($F_{3,8}=8.48$, $p<0.01$). After incubation of PC12 cells with A β peptide, GPx activity was significantly decreased compared to the control group, whereas, Pretreatment of PC 12 cells with *F. angulata* extract (200 μ g/ml) significantly prevented against A β -induced GPx activity reduction.

Effect of *F. angulate* extract on AChE activity

The AChE activity was significantly different in studied groups ($F_{3,8}=131.8$, $p<0.001$). After incubation of PC12 cells with A β (0.5 μ M) for 24 hours, AChE activity significantly increased compared to the control group. Pretreatment with *F. angulate* extract declined the A β -induced increase in AChE activity. Besides, the treatment of PC12 cells with extract alone decreased the AChE activity compared to the control group. (Figure 4)

Effect of *F. angulate* extract on caspase 3 activity

Significant differences between caspase3 activity of studied groups were observed ($F_{3,8}=10.28$, $p<0.01$) The increased caspase3 activity was observed in PC12 cells following exposure to 0.5 μ M A β for 24 hours whereas, significantly diminished caspase activity was detected in PC12 cells which pretreated with *F. angulate* extract (Figure 5)

Discussion

Based on the extensive pathological studies that have been performed on Alzheimer's patients at various stages of the disease progression, in the brain tissue of all these patients, accumulated beta-amyloid plaques have been observed. Beta-amyloid peptide accumulation is involved in developing and progressing Alzheimer's disease by several mechanisms, including oxidative stress (Murphy & LeVine III, 2010). There is plenty of evidence showing that beta-amyloid interacts with mitochondrial proteins, enhancing the ROS production and inducing structural and functional destruction. Mitochondrial malfunction causes loss of normal activity and death of nerve cells (Murphy & LeVine III, 2010; Pagani & Eckert, 2011).

According to the tests carried out in this study, the extract of *Ferulago angulata* can prevent the oxidative stress caused by the A β peptide by decreasing the reactive oxygen species' production and increasing the Glutathione peroxidase activity. So, it can play an important role in cellular defense pathways against oxidative stress during the neurodegenerative process in Alzheimer's disease. This finding is in line with previous studies that have indicated antioxidant and neuroprotective activity for this extract (Rafieian-Kopaei, Shahinfard, Rouhi-Boroujeni, Gharipour, & Darvishzadeh-Boroujeni, 2014; Sharifi, Rafieirad, & Sazegar, 2015).

Oxidative stress is one of the major mechanisms of A β toxicity, which occurs due to the depletion of antioxidant enzymes and an increase in reactive oxygen species. It plays an important role in the development and progression of neurodegenerative diseases, including AD. One of the important antioxidant enzymes is glutathione peroxidase, which has an important role in providing

the equilibrium conditions of oxidant and antioxidant agents in the body. After 24 h of cellular exposure with beta-amyloid peptide, a significant decrease in the Glutathione peroxidase activity was observed, which corresponded with the previous studies (Kim et al., 2003). However, in cells treated with the extract, increased glutathione peroxidase enzyme activity was observed, leading to improved antioxidant conditions. Since recent studies have shown that *Ferulago angulata* contains phenolic and polyphenolic compounds in which the antioxidant effects and the neuroprotective effects of these compounds have also been observed, we can assume that these compounds can be responsible for the high antioxidant properties of this plant (Hosseini et al., 2012; Sodeifian, Ansari, Bamoniri, & Mirjalili, 2011).

The results of a study on the hydro-alcoholic extract of *Ferulago angulata* in ischemic brain tissue model also showed that the extract improves behavioral disorders and oxidative stress in brain tissue since phenolic compounds have antioxidant and free radical scavenging effects, this improvement, and recovery might probably be due to the presence of these compounds, which confirms our findings (Alami-Rostami & Rafieirad, 2018).

Total phenolic compounds in *Ferulago angulata* extract were measured by Folin-Ciocalto reagent using gallic acid as standard. The total extract of this plant was reported as 2.11 ± 0.08 g/100g extract or 2.11%, which was significant, and these phenolic compounds are likely to be responsible for many biological effects, especially the antioxidant effects of this plant extract.

Other studies have reported that the plant is rich in flavonoids and polyphenolic compounds, have beneficial effects on the central nervous system (Ebrahimi & Schluesener, 2012). These compounds protect neurons against stress-induced damage and suppress neuronal inflammation and reduce age-related neuronal damage. Flavonoids appear to play a protective role in various ways, including regulating intracellular signaling pathways that control life, death, and differentiation of neurons, effects on gene expression, effects on mitochondria, and antioxidant effects, and metal ion chelating (Spencer, 2007). In confirming this study's findings, another study reported that flavonoids act as direct free radical scavengers and stabilize reactive oxygen species with their hydroxyl groups (Ebrahimi & Schluesener, 2012).

The first neurotransmitter disorder, which was detected in Alzheimer's disease, was acetylcholine. Proper performance of acetylcholine is essential for short-term memory. Cholinergic disorder in Alzheimer's disease is believed to be responsible for short-term memory impairment (Watanabe T, 2009 Jan 16). Designed drugs for this issue, including acetylcholine precursors, muscarinic agonists, nicotine agonists, and acetylcholinesterase inhibitors; among them, acetylcholinesterase

inhibitors have been the most advanced and prosperous. Treatment of Alzheimer's disease is a serious clinical challenge. With the development of acetylcholinesterase inhibitors, a good perspective has been created to control the symptoms of Alzheimer's disease (H Ferreira-Vieira et al., 2016). Acetylcholinesterase enzyme interaction with A β peptide in the AD brain increases the AChE activity, resulting in a more decrease in acetylcholine level and deterioration of the AD symptoms and increases the A β toxicity on neurons results in more neuron loss and progression of the disease (Alvarez et al., 1998; Dinamarca MC, 2008). According to the results of the studies done before, beta-amyloid increases acetylcholinesterase activity in vivo and in vitro (M. Soodi et al., 2017; Maliheh Soodi et al., 2016). This study showed that the extract of *Ferulago angulata* reduced the activity of acetylcholinesterase increased by A β peptide. This result is consistent with another study that showed the cholinesterase enzyme inhibitory activity of this plant extract (Hajimehdipoor et al., 2014). It should be noted that many polyphenols have shown the cholinesterase inhibitory effect, which has been associated with an improved cognitive function such as learning and memory (Ebrahimi & Schluesener, 2012; Roseiro, Rauter, & Serralheiro, 2012). Besides, the plants of the genus *Ferulago* are rich in sesquiterpene terpene coumarin compounds. These compounds may be responsible for the major inhibitory effects of this plant on the AChE enzyme. However, proving this requires the purification of compounds and their accurate evaluation.

Finally, by evaluating the effect of the studied plant extract on the activity of the caspase-3 enzyme (apoptosis marker), it was found that the *Ferulago angulata* extract reduced the activity of this enzyme that implies the anti-apoptotic effect of the extract. Apoptosis plays an important role in the destruction of neurons in Alzheimer's disease. The mechanism of induction of A β -induce apoptosis has not been clearly elucidated, but induction of apoptosis through various pathways such as intracellular calcium homeostasis, impairment of mitochondrial structure and activity, free radical enhancement and oxidative stress, and production of more autophagic vacuoles have been more observed in the presence of A β (Ghavami et al., 2014). As an example, in an experiment done with PC12 cells in the presence of A β , decreased ATP levels, decreased mitochondrial respiratory chain activity, and depolarized mitochondrial membrane were shown in these cells, causing mitochondrial swelling and consequently, the release of cytochrome C, which also activated caspase-3 and eventually causing apoptosis to occur in these cells (Gao & Tang, 2006). Many vital cellular activities, such as division, differentiation, and cell growth, are accomplished through the sequential process of message transmission and mediated by messenger molecules. Some of the polyphenols regulate cellular pathways that are involved in cell survival (Ebrahimi

& Schluesener, 2012). According to this information, polyphenolic compounds in the *F. angulata* extract may also be responsible for these anti-apoptotic effects.

Conclusion

Overall, this study showed that the treatment of PC12 cells with A β peptide decreased cell viability and increased oxidative stress and acetylcholinesterase activity. *Ferulago angulata* extract has a protective effect against beta-amyloid toxicity and its induced oxidative stress and apoptosis on PC12 cells. This plant acts as a direct scavenger of reactive oxygen species and an antioxidant due to its phenolic compounds and can protect cells by removing reactive oxygen species. Part of these protective effects may also be due to the acetylcholinesterase inhibitory activity by this extract. So, it is recommended the anti-Alzheimer effect of this extract further study in an AD animal model.

Conflict of interest

The authors declare no conflict of interest.

Authors contributions

All authors contributed equally in preparing all parts of the research.

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Figure 1

Figure 1: Effects of the *Ferulago angulata* extracts on A β -induced cytotoxicity in PC12 cells. +++p<0.001 vs. control, * p <0.05, *** p<0.001 vs. A β -treated group.

Figure 2

Figure 2: ROS production in PC12 cells after treatment with A β alone and A β with extract pre-incubation. +++p<0.001 vs. control, ** p<0.01, *** p <0.001 vs. A β -treated group.

Figure 3

Figure 3: Glutathion peroxidase (GPx) activity in PC12 cells after treatment with A β alone and A β with extract pre-incubation. ++p<0.001 vs. control, * p <0.05 vs. A β -treated group.

Figure 4

Figure 4: Acetylcholinesterase (AChE) activity in PC12 cells after treatment with A β alone and A β with extract pre-incubation. *** p<0.001 vs. control, +++ p <0.01 vs. A β -treated group.

Figure 5

Figure 5. Caspase activity in PC12 cells after treatment with A β alone and A β with extract pre-incubation. * $p < 0.05$ vs. control, ++ $p < 0.01$ vs. A β -treated group.