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Research Paper

Ferulago angulata Methanolic Extract Protects PC12 Cells Against Beta-amyloid-induced Toxicity

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ABSTRACT

Introduction: Alzheimer's disease (AD) is an age-dependent neurodegenerative disease. Beta-amyloid (Aβ)-induced neurotoxicity has a pivotal role in AD pathogenesis; therefore, the modulation of Aβ toxicity is the promising therapeutic approach to control the disease progression. Medicinal plants because of their multiple active ingredients are effective in complex diseases, such as AD. Therefore, several studies have studied medicinal plants to find an effective treatment for AD. *Ferulago angulata* is a medicinal plant with antioxidant and neuroprotective activity. The present study was done to assess the protective effect of the methanolic extract of Ferulago angulate on Aβ-induced toxicity and oxidative stress in PC12 cells.

Methods: The methanolic extract of aerial parts of the plant was prepared by the maceration method. PC12 cells were cultured according to a standard protocol. PC12 cells were incubated for 24 hours with Aβ alone, and Aβ in combination with various concentrations of the *F. angulata* extract. Cell viability was determined by the methyl thiazole tetrazolium (MTT) assay. Also, reactive oxygen species (ROS) production and the activity of acetylcholine esterase (AChE), glutathione peroxidase (GPx), and caspase-3 enzymes were measured.

Results: The extract dose-dependently protected PC12 cells against Aβ-induced cell death. Also, Aβ increased ROS production, AChE, and caspase-3 activity, and decreased the GPx activity, which all were ameliorated by *F. angulata* extract.

Conclusion: *F. angulata* extract protects against Aβ-induced oxidative stress and apoptosis. These effects may be due to the antioxidant and anticholinesterase activity of the extract. It is recommended to assess *F. angulata* extract as an anti-AD agent.

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Highlights

- *Ferulago angulata* extract dose-dependently ameliorates Aβ-induced cytotoxicity in PC12 cells.
- Aβ induced oxidative stress in PC12 cells, which was attenuated by the *F. angulata* extract.
- Aβ increased acetylcholinesterase activity in PC12 cells, which was prevented by the *F. angulata* extract.

Plain Language Summary

Alzheimer's disease (AD) is a common form of dementia in the elderly with a complex pathophysiology. Beta-amyloid (Aβ)- 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-AD effect of *F. angulata* extract was investigated by assessing its protective effect against Aβ-induced toxicity in PC12 cells *F. angulata* extract improved Aβ-induced toxicity by diminishing oxidative stress and apoptosis. Therefore, *F. angulata* extract merits further studies for use in the treatment of AD.

1. Introduction

Alzheimer's disease (AD), the most common form of dementia and age-related neurodegenerative disease, is characterized by progressive cognitive decline and memory loss. Behavioral disorders and an inability to perform daily tasks are other symptoms of AD (Castellani et al., 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 increase in population and life expectancy (Hebert et al., 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 the explanation of AD pathogenesis, including "the cholinergic deficit" and "the beta-amyloid (Aβ) cascade" (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 et al., 2008). The main component of extracellular senile plaques is Aβ peptide (Aβ). Aβ 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, Aβ more produced and accumulated. The accumulated Aβ is extracellularly deposited and forms the central core of extracellular Aβ plaques. According to the Aβ hypothesis, it is postulated that abnormal accumulation of Aβ plaques is the primary event that causes AD (Cappai & Barnham, 2008; Femminella et al., 2018). Several studies have indicated that aggregated Aβ is toxic to neural cells and triggers various mechanisms, such as oxidative stress, inflammation, mitochondrial dysfunction, and apoptosis, which leads to neural cell death (Sun et al., 2015). Based on the Aβ hypothesis, the disease-modifying drugs, which modulate Aβ production, clearance, and toxicity, are developed to control AD progression (Carrillo-Mora et al., 2014).

Oxidative stress has a key pathogenic role in the progression of AD. Several studies have reported oxidative damage in the AD brain. Reactive oxygen species (ROS) oxidize the cellular biomolecules, such as proteins, lipids, and nucleic acids, leading to the dysfunction of proteins, alteration of membrane integrity, and production of toxic metabolites in neurons and finally, cell death (Dumont & Beal, 2011; Wang et al., 2014). There are many indications that Aβ increases oxidative stress. In vivo and in vitro studies have shown that Aβ in aggregated form causes increased ROS production, decreased antioxidant enzyme activity, and mitochondrial dysfunction, which activate the apoptosis pathways and lead to cell death. (Cheignon et al., 2018; Jang & Surh, 2003). Antioxidant compounds improve Aβ-induced oxidative stress, which are considered therapeutic targets for AD (Ono et al., 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 degen-

eration of the septohippocampal cholinergic pathway in the brain of AD patients. The septohippocampal cholinergic neurons are responsible for learning and memory function, and the hypofunction or loss of these neurons in AD results in memory loss (Francis et al., 1999). According to this hypothesis, augmentation of the cholinergic system can improve memory function in AD patients (Ferreira-Vieira et al., 2016). The acetylcholinesterase inhibitors (AChEIs), 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 et al., 2011).

Due to the complex pathophysiology of AD, new therapies focus on compounds that can improve the symptoms and progression of the disease through several mechanisms (Batool et al., 2018). In recent years, plants 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 plants with have antioxidant effects that strengthen the cholinergic system have significant effects on the improvement of this disease (Ambure et al., 2019) *Ferulago angulata (Schlecht.) Boiss.* (Apiaceae) is a medicinal plant found in Western Asia and Iran. Its local name is Chovir, which is used in traditional medicine to treat a wide range of disorders, such as headaches, digestive problems, snakebites, hemorrhoids, 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 et al., 2014; Heidari et al., 2014; Zareii et al., 2014). Its extract consists of a mixture of various polyphenols with antioxidant and neuroprotective effects (Hosseini et al., 2012; Lorigooini et al., 2019). *F. angulata* essential oil improves scopolamine-induced learning and memory deficit; therefore, it is recommended for the treatment of dementia (Hritcu et al., 2015).

Considering the importance of prevention of the development and progression of AD, and the antioxidant, AChE inhibitory, and neuroprotective effects of this plant, in the present study, the protective effect of the methanolic extract of *F. angulata* was investigated on Aβ-induced toxicity and oxidative stress in PC12 cells.

2. Material and Methods

Rat pheochromocytoma (PC12) cell was purchased from the Pasteur Institute of Iran. Aβ25–35 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

F. angulata was collected from the Kohgiluyeh and Boyer-Ahmad Province of Iran. It was identified by the botanists of the Traditional Medicine and 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 the maceration method. The aerial parts of the plant were dried in shade and ground. Then, 10 g of the plant powder was mixed with 100 mL of methanol (80:20). Every 24 h, 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 phenolic contents using Gallic acid as the standard. The methanolic solution of gallic acid or extract was prepared and mixed with Folin-Ciocalteu 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 grams per 100 g dried extract (Singleton et al., 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% CO_2. The culture medium was replaced every three days, and culture was passaged after reaching 70% confluency.

Protective effect of *F. angulata* against Aβ-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 24 h, the cell culture medium was removed and replaced with a new medium that contained different concentrations of the extract ranging from 10 to 200 µg/mL. Subsequently, after one-hour incubation with different extract concentrations, aggregated Aβ peptide was added to each well, and cells were further incubated for 24 h. The final concentration of Aβ in the medium was 0.5 µM. Aβ 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 the extract and Aβ peptide, and cells in some wells were incubated with Aβ peptide alone. Cell viability was measured by methyl thiazole tetrazolium (MTT) reduction assay at the end of incubation time (Sepand et al., 2013).

MTT assay

MTT assay is a standard method to measure 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, and then, the intensity of the purple color was measured by a spectrophotometer. At the end of incubation time, the medium was removed and replaced with a fresh medium containing MTT solution at a final concentration of 0.5 mg/mL. Cell cultures were incubated with MTT containing the medium for 4 h at 37˚C. Subsequently, the medium was removed, 100 µL 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′-Dichlorodihydrofluorescin Diacetate (DCFH-DA) as a prob. DCFH-DA readily crosses the cell membrane and is converted to Dichlorodihydrofluorescin (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 a PDL-coated 24-well plate (4×10^5 cells/well). Cells were incubated with 0.5 µM of Aβ alone, and 0.5 µM of Aβ in combination with the extract (200 µg/mL) for 24 h, and then, the cells were harvested by trypsinization and washed with PBS. After that, the cells were resuspended in 1 mL assay buffer containing NaCl (140 mM), KCl (5 mM), $MgCl_2$ (1 mM), $CaCl_2$ (1.5 mM), glucose (5.6 mM), HEPES-Na (20 mM) at pH=7.4, and 1 µL of DCFH-DA (10 mM) was added to this solution, and the cell suspension was incubated for 45 at 37˚C in a CO_2 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 cytometry 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 and its activity was measured by the colorimetric kits (BioVision). PC12 cells were plated onto PDL-coated 6-well plates (1×10^6 cell/well) and then incubated with Aβ (10 µM) alone and along with the extract (200 µg/mL) for 24 h. After incubation, the cells were rinsed with phosphate-buffered saline (PBS) and harvested, and then, 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 the kit instruction. Data were expressed as mU/mg protein (Dashti et al., 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 was mentioned in the previous section. The AChE enzyme activity was measured in cell lysate by the Ellman method, as described previously (Ellman et al., 1961). Briefly, after adding cell lysate to the Ellman reagent containing phosphate buffer (0.1 M pH=8), thiocholine, and 5,5'-dithiol-bis (2-nitro-benzoic acid) , the absorbance was monitored at 405 nm for 10 min 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 and measured at 405 nm. PC12 cells were cultured, treated, and harvested, as it was mentioned in the previous section. The harvested cells were resuspended in 1 mL of cell lysis buffer containing 50 mM tris-HCl and 1.0 mM DTT (pH=7.5), and then sonicated for 20 s and centrifuged at 1000 g for 10 min. The supernatant was collected for the caspase-3 assay. Then, 10 µL of supernatant was mixed with 0.2 mM AC-DEVD-pNA solution and incubated at 37°C for at least 1 h. The absorbance was measured by a microplate reader at 405 nm. The results were expressed as a percent of control (Soodi et al., 2017).

Statistical analysis

Data are presented as Mean±SEM of three separate experiments. The statistical analysis was performed by GraphPad Prism software, version 8. Statistical differences were estimated by one-way ANOVA followed by Tukey's multiple comparison test.

3. Results

The content of total phenolic compounds

The amount of total phenolic compounds in the extracts was calculated as the equivalent of the gallic acid (g) in 100 g 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 100 g dried extract that is equal to 2.11%.

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

The results of the cell viability assay by the MTT method are shown in Figure 1. One-way ANOVA results indicated significant differences between groups ($F_{(6,14)}=185.2$, $P<0.001$). Treatment of PC12 cells with Aβ (0.5 µM) for 24 h significantly decreased the cell viability. However, pre-incubation of cells with the extract significantly protected the PC12 cells against the Aβ-induced cytotoxicity. This protective effect was dose-dependent, and extract at 200 µg/mL completely attenuated the Aβ-induced cytotoxicity.

Effect of *F. angulate* 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 Aβ peptide for 24 h, whereas 1 h of pretreatment with 200 µg/mL of the *F. angulate* extract before the addition of Aβ peptide inhibited the production of ROS by the Aβ peptide (Figure 2).

Effect of *F. angulata* extract on GPx activity

As shown in Figure 3, significant differences were observed between groups ($F_{(3, 8)}=8.48$, $P<0.01$). After incubation of PC12 cells with Aβ peptide, GPx activity significantly decreased compared to the control group, whereas, pretreatment of PC12 cells with *F. angulata* extract (200 µg/mL) significantly prevented Aβ-induced GPx activity reduction.

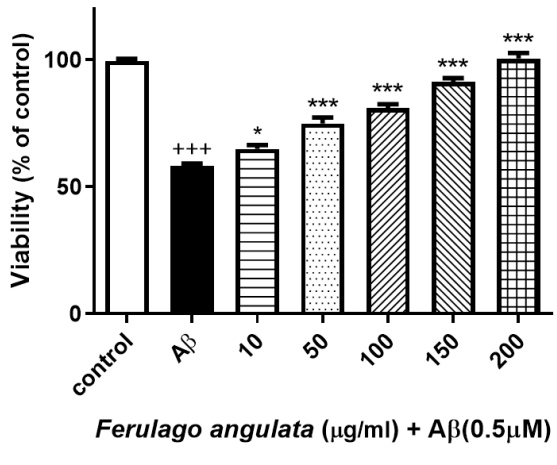

Figure 1. Effect of the *F. angulata* extract on beta-amyloid (Aβ)-induced cytotoxicity in PC12 cells

+++P<0.001 vs. the control group, *P<0.05, ***P<0.001 vs. the Aβ-treated group.

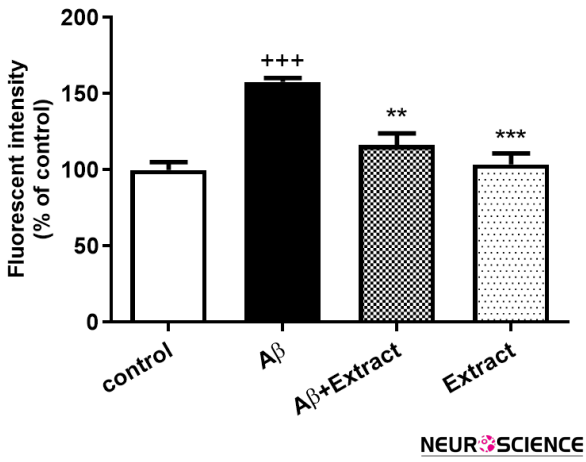

Figure 2. ROS production in PC12 cells after treatment with beta-amyloid (Aβ) alone and in combination with the *F. angulata* extract

+++P<0.001 vs. the control group,**P<0.01, ***P<0.001 vs. the Aβ-treated group.

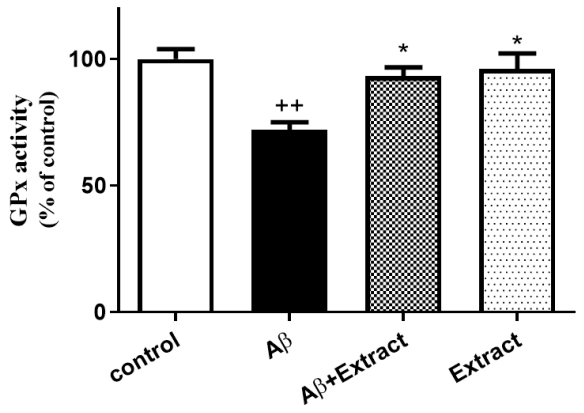

Figure 3. GPx activity in PC12 cells after treatment with beta-amyloid (Aβ) alone and in combination with the *F. angulata* extract pre

$^{++}$P<0.001 vs. the control group, *P<0.05 vs. the Aβ-treated group.

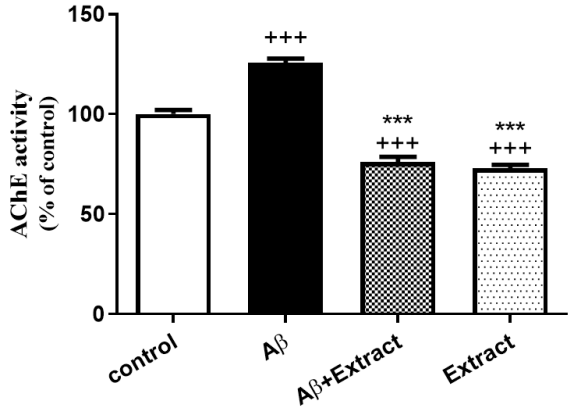

Figure 4. AChE activity in PC12 cells after treatment with beta-amyloid (Aβ)alone and in combination with the *F. angulata* extract

$^{+++}$P<0.001 vs. the control group and vs. the Aβ-treated group.

Effect of *F. angulate* extract on AChE activity

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

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

Significant differences were observed in caspase-3 activity between the studied groups ($F_{(3, 8)}$=10.28, P<0.01) The increased caspase-3 activity was observed in PC12 cells following exposure to 0.5 µM Aβ for 24 h, whereas, significantly diminished caspase-3 activity was detected in PC12 cells pretreated with the *F. angulate* extract (Figure 5).

4. Discussion

Based on pathological studies on AD patients at various stages of the disease, in the brain tissue of all these patients, accumulated Aβ plaques have been observed. Aβ peptide accumulation is involved in developing and progressing AD by several mechanisms, including oxidative stress (Murpy & LeVine III, 2010). Aβ interacts with mitochondrial proteins, enhancing ROS production and inducing structural and functional destruction. Mitochondrial malfunction causes loss of normal activity and death of nerve cells (Murpy & LeVine III, 2010; Pagani & Eckert, 2011).

According to the tests carried out in this study, the extract of *F. angulata* can prevent the oxidative stress caused by the Aβ by decreasing ROS production and increasing the GPx activity. Thus, it can play an important role in cellular defense pathways against oxidative stress during the neurodegenerative process in AD. This finding is in line with previous studies indicating the antioxidant and neuroprotective activity of this extract (Rafieian-Kopaei et al, 2014; Sharifi et al., 2015).

Oxidative stress is one of the major mechanisms of Aβ toxicity, which occurs due to the depletion of antioxidant enzymes and an increase in ROS. It plays an important role in the development and progression of neurodegenerative diseases, including AD. One of the important antioxidant enzymes is GPx, which has an important role in providing the equilibrium conditions of oxidant and antioxidant agents in the body. After 24 h of cellular exposure to Aβ, a significant decrease in the GPx activity was observed, which corresponded with the previous studies (Kim et al., 2003). However, in cells treated with the extract, increased GPx enzyme activity was observed, leading to improved antioxidant conditions. Recent studies have shown that *F. angulata* contains phenolic and polyphenolic compounds with antioxidant effects and neuroprotective effects; thus, we can assume that these compounds can be responsible for the high antioxidant properties of this plant (Hosseini et al., 2012; Sodeifian et al., 2011).

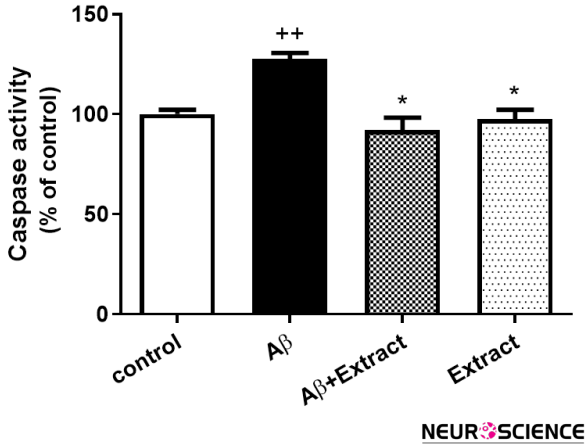

Figure 5. Caspase-3 activity in PC12 cells after treatment with beta-amyloid (Aβ)alone and in combination with the *F. angulata* extract

$^{++}$P<0.05 vs. the control group and *P<0.01 vs. the Aβ-treated group.

The results of a study on the hydro-alcoholic extract of *F. angulata* in an 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 *F. 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 the extract.

F. angulata is rich in flavonoids and polyphenolic compounds with 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, antioxidant effects, and metal ion chelating (Spencer, 2007). Consistent with our findings, another study reported that flavonoids act as direct free radical scavengers and stabilize ROS with their hydroxyl groups (Ebrahimi & Schluesener, 2012).

Acetylcholine was the first neurotransmitter disorder detected in AD. Proper performance of acetylcholine is essential for short-term memory. Cholinergic disorder in AD is believed to be responsible for short-term memory impairment (Watanabe et al., 2009). Acetylcholine precursors, muscarinic agonists, nicotine agonists, and acetylcholinesterase inhibitors are used to treat this disorder, of which acetylcholinesterase inhibitors have been the most advanced and prosperous. Treatment of AD is a serious clinical challenge. With the development of acetylcholinesterase inhibitors, a good perspective has been created to control the symptoms of AD (Ferreira-Vieira et al., 2016). Acetylcholinesterase enzyme interaction with Aβ peptide in the AD brain increases the AChE activity, resulting in a more decrease in acetylcholine levels and deterioration of the AD symptoms and increases the Aβ toxicity on neurons resulting in more neuronal loss and progression of the disease (Alvarez et al., 1998; Dinamarca et al., 2008). Aβ increases acetylcholinesterase activity in vivo and in vitro (Soodi et al., 2017; Soodi et al., 2016). This study showed that the extract of *F. angulata* reduced the activity of acetylcholinesterase increased by Aβ 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 improved cognitive function, including learning and memory (Ebrahimi & Schluesener, 2012; Roseiro et al., 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 *F. angulata* extract on the activity of the caspase-3 enzyme (apoptosis marker), it was found that the extract reduced the activity of this enzyme, which implies its anti-apoptotic effect. Apoptosis plays an important role in the destruction of neurons in AD. The mechanism of induction of Aβ-induced apoptosis has not been clearly elucidated, but induction of apoptosis through various pathways, such as intracellular calcium homeostasis, impairment of mitochondrial structure and activity, increasing free radicals and oxidative stress, and production of more autophagic vacuoles have been more observed in the presence of Aβ (Ghavami et al., 2014). As an example, in an experiment on PC12 cells in the presence of Aβ, decreased ATP levels, decreased mitochondrial respiratory chain activity, and depolarized mitochondrial membrane were observed in these cells, causing mitochondrial

swelling and consequently, the release of cytochrome C, which also activated caspase-3 and eventually caused apoptosis 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 polyphenols regulate cellular pathways and are involved in cell survival (Ebrahimi & Schluesener, 2012). Polyphenolic compounds in the *F. angulata* extract may also be responsible for these anti-apoptotic effects.

5. Conclusion

Overall, this study showed that the treatment of PC12 cells with Aβ peptide decreased cell viability and increased oxidative stress and acetylcholinesterase activity. *F. angulata* extract has a protective effect against Aβ toxicity and its induced oxidative stress and apoptosis on PC12 cells. This plant acts as a direct scavenger of ROS and an antioxidant due to its phenolic compounds and can protect cells by removing ROS. Part of these protective effects may also be due to the acetylcholinesterase inhibitory activity of this extract. Thus, it is recommended to assess the anti-AD effect of this extract in future studies in an AD animal model.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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The paper was extracted from the MSc thesis of Leila Hashemi, approved by Department of Toxicology, Tarbiat Modares University.

Authors' contributions

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

Conflict of interest

The authors declared no conflict of interest.

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