The Effect of Essential Oil of Lavandula Angustifolia on Amyloid Beta Polymerization: An In Vitro Study

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ABSTRACT: Alzheimer’s Disease (AD) is a progressive neurological disorder associated with cognitive and memory deficits. Accumulation of amyloid beta (Aβ) plaques is one of the major causes of AD. Therefore, inhibition of the plaque formation has been aimed to play a preventive role in the disease. Lavender, through some neuroprotective roles such as antioxidant effects, is known to be an effective candidate in the treatment of neurodegenerative disorders. In this study using Thioflavin T Measurement and Atomic Force Microscope (AFM) Imaging, we evaluated the effect of essential oil of lavender on Aβ polymerization. Thioflavin T Method showed that essential oil enhances the Aβ aggregation. The results of the AFM method also confirmed it. Our data antagonizes previous results indicating the clearing effect of aqueous extract of lavender on Aβ plaque. It seems that the different combination of essential oil and aqueous extract considerably determines if or not the aggregation occurs.

KEYWORDS: Alzheimer’s disease; Amyloid beta; Aggregation; Lavender; Essential oil.

INTRODUCTION
Alzheimer’s Disease (AD) is a devastating neurodegenerative disease that leads to behavioral, cognitive and memory deficits (Chang et al., 2016 [1]). Extracellular accumulation of amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles are the main causes of AD (Pascoal et al., 2017 [2]). Aβ, consisting of 36 to 43 amino acids, is a natural product of Amyloid Precursor Protein (APP) proteolysis catalyzed by...
secretase enzymes (Yaghmaei et al., 2014 [3]). APP is first cleaved by β-secretase and then the resulting C-terminal fragment undergoes gama-secretase cleavage that releases the amyloidogenic Aβ peptide (Park et al., 2012 [4]). The peptide is estimated to have a physiological production rate of 7.6% per hour and a clearance rate of 8.3% per hour in humans (Bateman et al., 2006 [5]).

The Aβ-42 and Aβ-40 isoforms have received the most attention in AD (Charkhakar et al., 2015 [6]). The Aβ-42 has two hydrophobic residues that increase its ability to aggregate (Gupta et al., 2016 [7]). Aβ is a potent mitochondrial poison that affects the key mitochondrial enzymes and synaptic function as well (Leal et al., 2016 [8]).

Binding Aβ oligomers to neurons leads to some important complications including neurotoxicity due to increased inward calcium current from NMDA receptors, increased synaptic glutamate release (Dansz & Parsons, 2012 [9]), apoptotic cell death (Sollvander et al. 2016 [10]), synaptic removal of the glutamate receptors (Xia et al., 2012 [11]), production of oxidative stressors (Sugandhthy et al., 2016 [12]), tau hyperphosphorylation (Takata & Kitamura, 2016 [13]) and inflammation (Cai et al., 2016 [14]). Depressed synaptic transmission is reported as an impact of Aβ on the hippocampal glutamatergic synapses (Malinov, 2012 [15]).

Disturbed astrocyte metabolism induced by Aβ oligomers might be involved in glial reactivity (Sanz-Blasco et al., 2016 [16]). Plentiful studies have concerned the behavioral and electrophysiological aspects of Aβ-42 action in the brain (Fujii et al., 2011 [17]). It is reported that icv injection of Aβ-42 impairs learning and memory in rats (Arefi et al., 2018 [18]). Our previous finding also showed that icv injection of Aβ deteriorates induction of synaptic plasticity in the hippocampus of rats (Soheili et al., 2015 [19]). Low concentration of even a single oligomer particle of Aβ generates Reactive Oxygen Species (ROS) in astrocytes (Angelova & Abramov, 2014 [20]). Astrocytes have a key role in the internalization of Aβ oligomers (Yamanoto et al., 2016 [21]). Another effect of Aβ is depression of glutamatergic transmission and induction of neuronal oxidative stress by activation of NMDA receptors (Garcia-Font et al., 2016 [22]).

Microglia, the resident macrophages of the central nervous system, act as the first and main form of active immune defense (Kim et al., 2014 [23]). Microglia cells express several receptors that have a key role in the recognition, internalization, and clearance of Aβ. Microglia produces several inflammatory agents that influence Aβ affected cells. Also, the glial cells have a potent phagocytic role in the prevention of early Aβ deposition (Doens & Fernández, 2014 [24]). It is reported that significant accumulation of Aβ, in turn, impair the phagocytic activity of microglia (Wes et al., 2016 [25]).

Lavender (Lavandula angustifolia), as a medicinal herb, known as "Ostokhodus" in Iran (Soheili et al., 2017 [26], Soheili et al. 2019 [27]). The lavender essential oil is famous in aromatherapy due to its delightful aroma (Zhao et al., 2017 [28]). The essential oil is prepared from leaves and flowers of lavender and consists of various components such as linalool, linalyl acetate and flavonoids (Lakusic et al., 2014 [29]). Several medicinal properties are attributed to the lavender oil. For example, it is an anti-inflammatory and antioxidant agent and can be effective in the treatment of neurodegenerative disorder such as AD (Giovannini et al., 2016 [30]). In previous studies, we proved that the lavender extract considerably restores weaken learning and memory (Kashani et al., 2011 [31]) and deteriorated hippocampal synaptic plasticity (Soheili, Rezaei Tavirany, 2015 [19]) in the Aβ treated animals. Accordingly, this in vitro study was designed to assess how essential oil of the herbal medicine influences Aβ aggregations.

**EXPERIMENTAL SECTION**

**Reagents**

Aβ proteins (1–42) and thioflavin T (ThT) were purchased from Sigma-Aldrich. All the reagents and drugs used were of analytical grade.

**Preparation of Medicinal Herb Essential Oil**

The leaves and flowers of lavender were dried and powdered. By using a Clevenger-type apparatus and hydro-distillation method volatile oil of lavender was isolated. For essential oil extraction, 50 g of the powder was hydro-distilled with 300 ml water in the Clevenger-type apparatus for 4 h. The extracted essential oil was stored in a dark glass and kept at -8 °C until use.

**Thioflavin T Measurement**

The Aβ monomers were dissolved in 1 mL dimethyl sulfoxide (DMSO) at a concentration of 1 µM, aliquoted in microtubes and kept in the freezer (-20 °C) until use.
The experiments were carried out on Aβ-DMSO in two different conditions. In one group the Aβ-DMSO was added to Tris buffer (PH=7.4) and the mixture was incubated for 24 hr at 37 °C under stirring (the control group, CON) (Fujiwara et al., 2006 [32]). In the second group, Aβ-DMSO was added to Tris buffer + essential oil and kept under the same condition (the test group, Test). The test group, in turn, was subdivided to three groups treated by different doses of the essential oil (1, 10 and 100 µg/mL), named Test1, Test10, and Test100, respectively. At the end of the incubation time, ThT (dissolved in deionized water at a concentration of 1mg/mL) was added to the mixtures (with a molar ratio of 1:100 for Amyloid:ThT).

Fluorescence of Thioflavin T bound to Aβ aggregates was measured with a microplate reader (Perkin Elmer LS55 Spectrofluorimeter) using 1cm quartz cell. Fluorescence was monitored at 440 nm (λex) and 485 nm (λem) with excitation and emission slits of 5 nm and 10 nm respectively. Optical Density (OD) of the different test groups was compared to that of the control one.

**Atomic Force Microscope (AFM) Imaging**

The samples were imaged with noncontact Veeco AFM imaging mode. In this method, a tip in the AFM scan sample and form an image of the three-dimensional shape (topography) of a sample surface at a high resolution. For AFM imaging, 5µL of samples from the reaction mixture of two groups including the CON and the Test 100 loaded on freshly mica plates. Then the mica plates were dried for about two minutes at ambient temperature. Using deionized water, buffer and salt components were washed and plates were dried again. This procedure was carried out to remain fibril and peptide molecules attached at the surface of mica, possibly due to the negative charge on the surface of mica plates.

**Data Analysis**

The acquisition data were analyzed by One-way analysis of variance (ANOVA) followed by LSD as a post hoc test. Differences considered significant when P<0.05. The data are reported as mean ± SEM.

**RESULTS**

**The effects of lavender essential oil on Aβ polymerization**

Incubation of the Aβ-DMSO with the herbal essential oil considerably developed the formation of Aβ aggregates. Analysis of variance indicated a general significant difference between the groups entered the experiments (F3,7= 2.859, P=0.014). Our results demonstrated that the effectiveness of the essential oil on Aβ aggregate formation is dose–dependent. The post hoc LSD test indicated no significant difference between the Test1 and CON group (P= 0.852). Although the increased concentration of the herbal medicine to 10 µg/mL promoted the formation of the Aβ fibrils, however, the change was not statistically significant (P= 0.181). Further increasing of the herbal medicine to 100 µg/mL gave rise to a real polymerization where the highest dose of the essential oil in the Test100 group induced considerable Aβ aggregates (P=0.037). Fig. 1 depicts how the lavender essential oil influences the Aβ fibrillation.

**AFM imaging**

In this study, the AFM microscope was used to visualize Aβ fibrils formation before and after addition of herbal medicine. In the CON group, there were obvious and visible paired helical fibrils of Aβ aggregates (131.63 nm in height, Fig. 2a). Incubation of Aβ-DMSO solution with the essential oil of lavender for 24 hours highly increased polymerization of Aβ monomers. Fig. 2b depicts the AFM image is taken from the Test100 group.

**DISCUSSION**

Accumulation of Aβ plaques followed by a series of neurotoxic events results in some neuronal dysfunction and death; a hypothesis known as "amyloid cascade"
Fig. 2: Atomic Force Microscopic imaging of Aβ fibrils. a: The CON group; the Aβ polymerizations are visible as aggregated fibrils (arrow). b: The Test100 group; essential oil of lavender highly developed formation of the Aβ aggregates.

(Sengupta et al., 2016 [33]). Hence, it is rational to think that the Aβ clearance could be beneficial to overcome the toxicity of aggregated plaques (Marr and Hafez, 2014 [34]). However, scant documents have considered the effect of herbal medicines on prevention of formation or remove of Aβ fibrils (Bradley et al., 2007 [35]). The aqueous extract of lavender has a potential role in the clearance of Aβ plaques from the brain of Alzheimeric animals (Soheili, Tavirani, 2012 [36]). Also, electrophysiological recordings from neuronal function (Soheili, Rezaei Tavirany, 2015 [19]) and behavioral performances in animal models of AD (Kashani, Tavirani, 2011 [31]) verify the histological findings. In this, in vitro study, we especially focused on the possible effectiveness of essential oil of the herbal medicine on the formation of Aβ plaques.

Using the florimetry and AFM imaging methods we found that, in contrast to the histological evidence of the aqueous extract, the essential oil of the herbal medicine proceed polymerization of the Aβ peptides. The discrepancy between the two forms of the application might be due to the different composition of the two extracts. While the essential oil of lavender consists of linalool and linalyl acetate, the aqueous extract is standardized based on rosmaric acid (Herman et al., 2016 [37], Soheili, Rezaei Tavirany, 2015 [19]). Findings of Ono et al. in that rosmarinic acid inhibit Aβ polymerization and destabilized Aβ fibrils confirm the anti-aggregative effect of aqueous extract of lavender (Ono et al., 2012 [38]). The Japanese researchers showed that Uncaria Rhynchophylla has Potent Anti-aggregation Effects on Alzheimer’s Aβ Peptides (Fujinawa, Iwasaki, 2006 [32]). In 2016, Lim and his coworkers revealed that a standardized herbal mixture of ginger and peony root prevent Aβ accumulation and memory impairment in transgenic mice (Lim et al., 2016 [39]). Snow et al showed that heparin sulfate induces aggregation of Aβ fibrils in the hippocampus of the rat brain (Snow et al., 1994 [40]). In another study, the effect of EMT-type zeolite nanoparticles on clot formation and degradation of Aβ-fibrinogen evaluated. The results showed that delay in clot dissolution was significantly reduced in the presence of EMT-type zeolite (Derakhshankhah et al., 2016 [41]).

In a recent study, Porter et al reported a discrepancy between the AFM and immunoblotting methods and the thioflavin T method where the latter method shows a reduction in Aβ aggregation while the two other methods demonstrate polymerization of the peptide (Porter et al., 2016 [42]). However, in the present study both the AFM and thioflavin-T techniques appeared fibrilarization of Aβ. If the used method itself considerably underlies Aβ polymerization requires further studies.

CONCLUSIONS
Taken together, we found that the oil essence of lavender promotes the formation of the Aβ fibrils. Therefore, vigilance must be considered when consuming the essential oil of the medicinal herb. According to present evidence the combination of essence or aqueous extract and the method examining the Aβ polymerization could determine if or not the aggregation occurs. Further investigation needs to evaluate the effectiveness of each of the factors.
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