

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 (A β) 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 treatment of neurodegenerative disorders. In this study using Thioflavin T Measurement and Atomic Force Microscope (AFM) Imaging we evaluated effect of essential oil of lavender on A β polymerization. Thioflavin T Method showed that the essential oil enhances the A β aggregation. The results of AFM method also confirmed it. Our data antagonizes previous results indicating clearing effect of aqueous extract of lavender on A β 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.

1. Introduction

Alzheimer's disease (AD) is a devastating neurodegenerative disease that leads to behavioral, cognitive and memory deficits [1]. Extracellular accumulation of amyloid-beta (A β) plaques and intracellular neurofibrillary tangles are the main causes of AD [2]. A β , consisting 36 to 43 amino acids, is a natural product of amyloid precursor protein (APP) proteolysis catalyzed by secretase enzymes [3]. APP is first cleaved by β -secretase and then the resulting C-terminal fragment undergoes gamma-secretase cleavage that releases the amyloidogenic A β peptide [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 [5].

The A β -42 and A β -40 isoforms have received the most attention in AD [6]. The A β -42 has two hydrophobic residues that increase its ability to aggregate [7]. A β is a potent mitochondrial poison that affects the key mitochondrial enzymes and synaptic function as well [8].

Binding A β oligomers to neurons leads to some important complications including neurotoxicity due to increased inward calcium current from NMDA receptors, increased synaptic glutamate release [9], apoptotic cell death [10], synaptic removal of the glutamate receptors [11], production of oxidative stressors [12], tau hyperphosphorylation [13] and inflammation [14]. Depressed synaptic transmission is reported as an impact of A β on the hippocampal glutamatergic synapses [15].

Disturbed astrocyte metabolism induced by A β oligomers might be involved in glial reactivity [16]. Plentiful studies have concerned the behavioral and electrophysiological aspects of A β -42 action in the brain [17]. It is reported that icv injection of A β -42 impairs learning and memory in rats [18]. Our previous finding also showed that icv injection of A β deteriorates induction of synaptic plasticity in the hippocampus of rats [19]. Low concentration of even single oligomer particle of A β generates reactive oxygen species (ROS) in astrocytes [20]. Astrocytes have a key role in internalization of A β oligomers [21]. Another effect of A β is depression of glutamatergic transmission and induction of neuronal oxidative stress by activation of NMDA receptors [22].

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

Lavender (*Lavandula angustifolia*), as a medicinal herb, known as " Ostokhoddus " in Iran [26]. The lavender essential oil is famous in aromatherapy due to its delightful aroma [27]. The essential oil is prepared from leaves and flowers of lavender and consists of various components such as linalool, linalyl acetate and flavonoids [28]. Several medicinal properties are attributed to the lavender oil. For example it is anti-inflammatory and antioxidant agent and can be effective in treatment of neurodegenerative disorder such as AD [29]. In previous studies we proved that the lavender extracts considerably restores weaken learning and memory [30] and deteriorated hippocampal synaptic plasticity [19] in the A β treated animals. Accordingly, this in vitro study was designed to assess how essential oil of the herbal medicine influences A β aggregations.

2. Materials and Methods

2.1 Reagents

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

2.2 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 were 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.

2.3 Thioflavin T Measurements

The A β monomers were dissolved in dimethyl sulfoxide (DMSO) and kept in freezer (-20 °C) until use (A β -DMSO). The experiments were carried out on A β -DMSO in two different conditions. In one group the A β -DMSO was added to Tris buffer (PH=7.4) and the mixture was incubated for 24 hr at 37 °C (the control group, CON). In the second group A β -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 group treated by different doses of the essential oil (1, 10 and 100 μ g/ml), named Test1, Test10 and Test100, respectively. At the end of the incubation time, 1 mg thioflavin T was dissolved in 1 ml deionized water and added to the mixtures. Fluorescence of Thioflavin T bound to A β aggregates was measured with a microplate reader (Spectramax Gemini XS; Molecular Devices, Sunnyvale, CA) with a filter set (excitation at 442 nm and emission at 485 nm). Optical density (OD) of the different test groups was compared to that of the control one.

2.4 Atomic Force Microscope (AFM) Imaging

The samples were imaged with noncontact Veeco AFM imaging mode. In this method, a tip in AFM scan sample and form an image of the three-dimensional shape (topography) of a sample surface at a high resolution. For AFM imaging, 5 μ L 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.

2.5 Data Analysis

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

3. Results

3.1 The effects of lavender essential oil on A β polymerization

Incubation of the A β -DMSO with the herbal essential oil considerably developed the formation of A β aggregates. Analysis of variance indicated a general significant difference between the groups entered the experiments ($F_{3, 7} = 2.859$, $P = 0.114$). Our results demonstrated that the effectiveness of the essential oil on A β aggregate formation is dose-dependent. The post hoc

LSD test indicated no significant difference between the Test1 and CON group ($P=0.852$). Although increased concentration of the herbal medicine to 10 $\mu\text{g/ml}$ promoted formation of the $\text{A}\beta$ fibrils, however, the change was not statistically significant ($P=0.181$). Further increasing of the

herbal medicine to 100 $\mu\text{g/ml}$ gave rise to a real polymerization where the highest dose of the essential oil in the Test100 group induced considerable $\text{A}\beta$ aggregates ($P=0.037$). Figure 1 depicts how the lavender essential oil influences the $\text{A}\beta$ fibrillation.

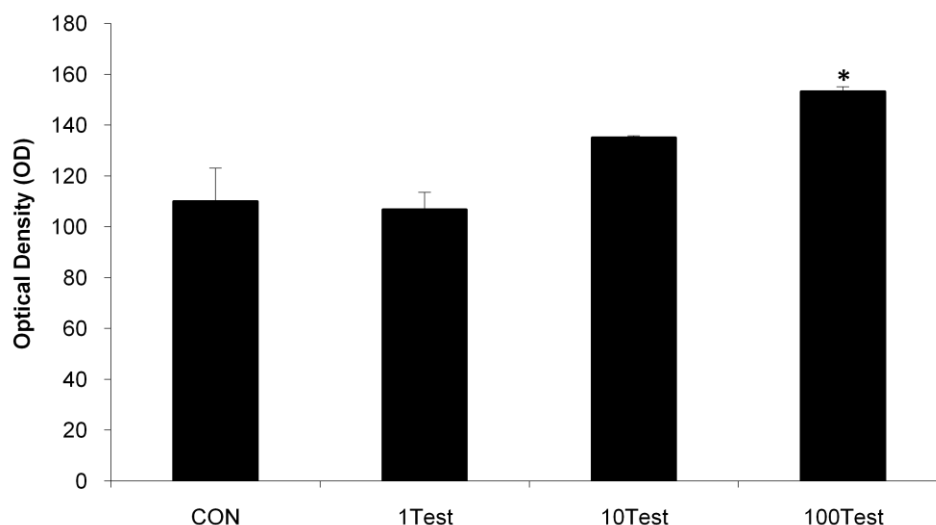


Figure 1: Effect of different doses of the essential oil of lavender on $\text{A}\beta$ polymerization. The Test100 group treated by 100 $\mu\text{g/ml}$ of the essential oil significantly increased the $\text{A}\beta$ polymerization ($P=0.037$).

3.2 AFM imaging

In this study the AFM microscope was used to visualize $\text{A}\beta$ fibrils formation before and after addition of herbal medicine. In the CON group, there were obvious and visible paired helical fibrils of $\text{A}\beta$ aggregates (131.63 nm,

Fig. 2a). Incubation of $\text{A}\beta$ -DMSO solution with the essential oil of lavender for 24 hours highly increased polymerization of $\text{A}\beta$ monomers. Figure 2b depicts the AFM image taken from the Test100 group.

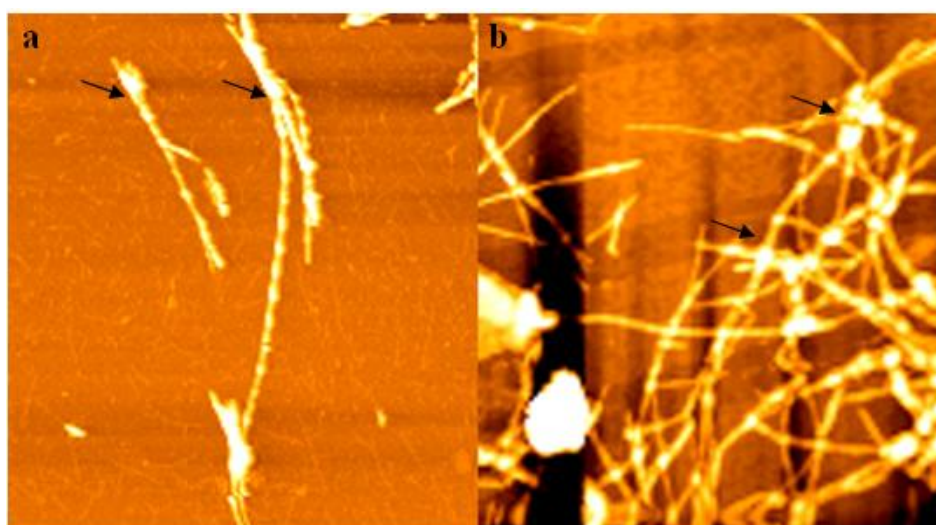


Figure 2: Atomic Force Microscopic imaging of $\text{A}\beta$ fibrils.

a: The CON group; the $\text{A}\beta$ polymerizations are visible as aggregated fibrils (arrow).

b: The Test100 group; essential oil of lavender highly developed formation of the $\text{A}\beta$ aggregates.

4. Discussion

Accumulation of A β plaques followed by a series of neurotoxic events results in some neuronal dysfunction and death; a hypothesis known as "amyloid cascade" [31]. Hence, it is rational to think that the A β clearance could be beneficial to overcome toxicity of aggregated plaques [32]. However, scant documents have considered effect of herbal medicines on prevention of formation or remove of A β fibrils [33]. The aqueous extract of lavender has a potential role in clearance of A β plaques from brain of Alzheimeric animals [26]. Also electrophysiological recordings from neuronal function [19] and behavioral performances in animal models of AD [30] verify the histological findings. In this in vitro study we especially focused on possible effectiveness of essential oil of the herbal medicine on formation of A β 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 proceeded polymerization of the A β peptides. The discrepancy between the two forms of application might be due to 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 rosmarinic acid [19, 34]. Findings of Ono *et al.* in that rosmarinic acid inhibit A β polymerization and destabilized A β fibrils confirm the anti-aggregative effect of aqueous extract of lavender [35].

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 show a reduction in A β aggregation while the two other methods demonstrate polymerization of the peptide [36]. However, in the present study both the AFM and thioflavin-T techniques appeared fibrillarization of A β . If the used method itself considerably underlies A β polymerization requires further studies.

Snow *et al* showed that heparin sulfate induce aggregation of A β fibrils in the hippocampus of rat brain [37].

5. Conclusion

Taken together, we found that the oil essence of lavender promotes formation of the A β 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 A β polymerization could determine if or not the aggregation occurs. Further investigation needs to evaluate effectiveness of each of factors.

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