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Elderberry Diet Improves Memory Function and Prevents Cell Death in Rat Models of Alzheimer's Disease Induced by Amyloid Beta Injection

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Abstract

Background: Alzheimer's disease (AD) is a neurodegenerative disease which adversely affects memory and learning skills. Currently, there is no disease-modifying therapeutic approach for AD.

Aim: A growing body of literature suggests elderberry as a promising remedy for neurological disorders. This study investigates the therapeutic effects of the elderberry diet on amyloid beta-induced (Aβ) rat model.

Methods: Initially, Alzheimer's model was generated by Aβ administration followed by the treatment of elderberry diet. Then, short term spatial memory was assessed. Stereology was also performed for the evaluation of density of neurons and dark neuron in the hippocampus.

Results: The findings showed that the administration of the elderberry diet in an Aβ model of Alzheimer' disease improved memory and learning function and prevented the degeneration of hippocampal neurons.

Conclusion: Overall, the findings imply that the elderberry diet attenuates the Aβ-induced degeneration and improves memory performance. As such, the elderberry diet could be used as a therapeutic procedure for Alzheimer due to its neuroprotective effects.

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Introduction

Alzheimer's disease (AD) is the most common cause of dementia (1). The first symptom of AD as a neurodegenerative disease is having difficulties remembering recent events, which worsens over time (2, 3). With the continuous decline in patients' conditions, communication with others and bodily functions will be gravely hindered by AD. The possible causes of AD haven't been intimately understood yet; however genetic factors, head injury, and high blood pressure have been reported as possible risk factors (3). On molecular levels, AD can be characterized by β-amyloid (Aβ)-containing extracellular plaques and tau-containing intracellular neurofibrillary tangles (2). On cellular levels, however, it's characterized by loss of neurons and synapses in the cerebral cortex and certain subcortical regions, such as the hippocampus (4). Until now, no treatment has been able to successfully stop or reverse the progression of AD (5). With over 50 million people -mostly over 65- affected by the disease, AD puts a heavy burden on societies (6).

Elderberry, a flowering plant native to Europe and many other regions, has long been known

as a plant with special medical usages (7). More than 30 species of elderberry have been detected, the most famous one being *Sambucus nigra*. Hence, all parts of the plant can be used for different purposes; elderberry is considered a valuable plant (8). The fruit of *Sambucus nigra* contains several components that may contribute to its pharmacological activity. Large amounts of anthocyanins are present in fresh fruits; the main constituents have been identified as cyanidin-3-glucoside and cyanidin-3-sambubioside in addition to small amounts of other types of anthocyanins and flavonols (9, 10, 11). Vitamins B_2 and C are the most prominently found vitamins in elderberry fruit (11). Elderberry can also be considered a source of fibers. Multiple antioxidants present in elderberry can reduce damages caused by inflammation and oxidative stress (12, 13).

The principal objective of this study is to find correlations between the treatment by elderberry diet and the severity of symptoms of AD in rats.

Methods

Animals, diets and Aβ toxicity model

In this study, 36 adult male rats (Sprague–Dawley, 200–220 g) were obtained from the Laboratory Animal Center of Shahid Beheshti University of Medical Sciences, Tehran, Iran. The Ethics Committee of the University approved this animal experiment (IR.SBMU.RETECH.REC.1400.1228). Rats were housed at 22 ◦C, under 12 hrs light/12 hrs dark cycles with ad libitum access to water and food. The EB fruits were harvested from west Mazandaran (Iran) and frozen in zippered plastic freezer bags. Berries were then de-stemmed and cleaned, lyophilized and ground into fine powder before being added to diets. In this study, rats were divided into three experimental groups: (a) control group with a control diet (n = 12), (b) Aβ group with a control diet (n = 12), (c) Aβ group with an oral diet containing 2% EB for 8 consecutive weeks (n = 12). For induction of Aβ toxicity models, rats were anesthetized using ketamine and xylazine (100 and 2.5 mg/kg, respectively), and their skull was fixed in a stereotaxic device. A longitudinal incision was made along the sagittal line on the scalp. $A\beta_{1-42}$ was prepared freshly and administered with a Hamilton microsyringe. Two micrograms of $A\beta_{1-42}$ solution in 4 μl PBS was administered over 2 min into the dorsal hippocampus bilaterally at coordinates 3.6 mm posterior and ±2 mm lateral to bregma, and 3.2 mm ventral to the skull surface. The needle was remained in position for an additional 2 min after injection. The needle was slowly removed from the brain and the scalp was sutured. The animals were returned to their cages to recover.

Measurement of short term spatial memory by T-maze test

T-maze (each arm 30 × 15 × 7 cm and 7 × 7 cm center piece) was constructed using black acrylic plastic. After 30 min habitation, the trial was started by placing mice into the start arm facing away from the goal arms. The mouse was allowed to freely explore and to choose the left or right goal arm. After tail has cleared goal arm and then close the goal arm using the center piece. After 30 seconds, the centerpiece was eliminated, and the mouse was moved back into the start arm, letting it choose again between the 2 open goal arms. T-maze test was performed through 2 trials per day with a 1 h interval for 3 consecutive days. Analysis was done by scoring. If mouse chose the same goal arm repeatedly in the same trial score of 0, if mouse chose different goal arms in the same trial score of 1 was given.

Estimation of the Number of the Neurons

The total number of neurons and dark neuron was determined using the optical dissector method. Microcator was used for measurement of Z-axis movement of the microscope stage. Numerical density (Nv) was calculated with the following formula:

$$Nv = (\Sigma Q- / (h \times x\ a/f \times \Sigma p)) \times (t / BA)$$

where "ΣQ-" is the number of the nuclei, "h" is the height of the dissector, "a/f" is the frame area, "ΣP" is the total number of the unbiased counting frame in all fields, "t" is the real section thickness measured in every field using the microcator, and BA is the block advance of the microtome.

Data analysis

Results were analyzed using the Graph Pad Prism 8 software (Graph Pad Software Inc., La Jolla, CA, USA). Statistical analysis was performed using the one-way analysis of variance (ANOVA) protocols followed by multiple comparison tests using Tukey's method to analyze the differences. Moreover, the P-value of less than 0.05 was considered statistically significant.

Results

EB diet attenuates the cognitive impairment in rat's model of Aβ toxicity

To measure the effect of EB diet on the short term spatial memory, the T maze was performed (Figure 1a). Our results alternation rate decreased significantly in the Aβ group as opposed to the control group (P <0.001). Also, following the treatment by EB diet, alternation rate increased compared to the Aβ group (P< 0.01) (Figure 1b). On the other hand, following Aβ injection, latency showed a considerable increment compared to the control (P <0.001). However, latency showed a considerable decline in the EB diet group compared to the Aβ group (P <0.001) (Figure 1c). Increase of alternation rate and decrease of latency in the EB diet group can be attributed to improve of short term spatial memory.

EB diet protects pyramidal neurons of hippocampus against Aβ toxicity

For stereological examination, hematoxylin and eosin (H&E) staining of the hippocampus was conducted (Figure 2a). The stereological counting of neurons in the hippocampus revealed a considerable decline in the density of neurons in the Aβ group compared to the control (P <0.001).
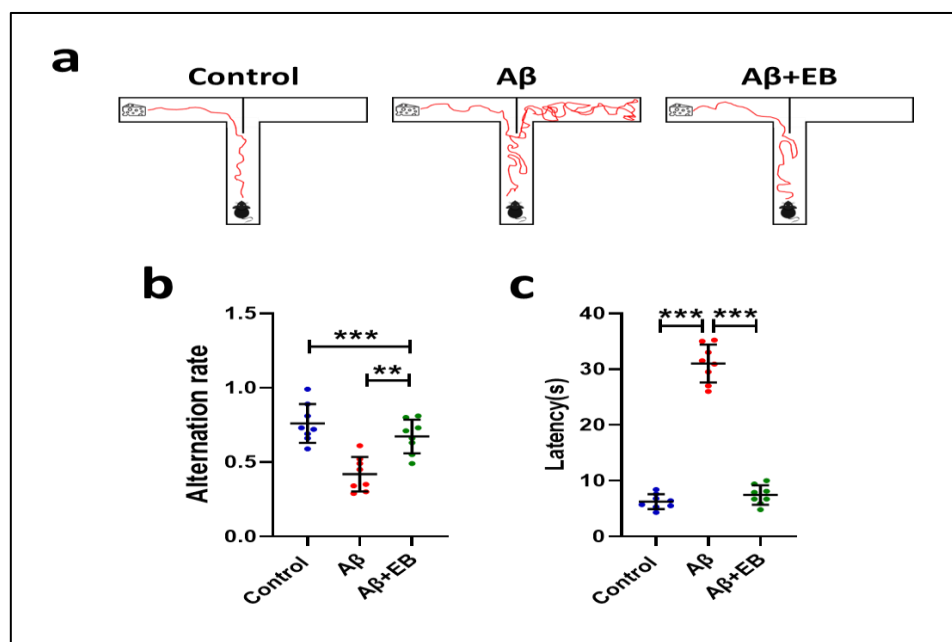

Figure.1. Assessment of short term spatial memory. The trace to reach the target in the T maze (a). Alternation rate in the Aβ group decreased significantly (b). Also, the latency was significantly longer in the Aβ group than in the EB diet group (c). The values are expressed as means± SEM. **P<0.01, ***P<0.001 indicates significant differences between groups.

However, in the EB diet group, density of neuron was protected in comparison to those in the Aβ group (P< 0.001) (Figure 2b). Our results showed that the mean number of dark neurons increased in the Aβ group compared to the control group (P <0.001). However, the mean number of dark neurons decreased significantly in the EB diet group compared to the Aβ group (P <0.001) (Figure 2c).
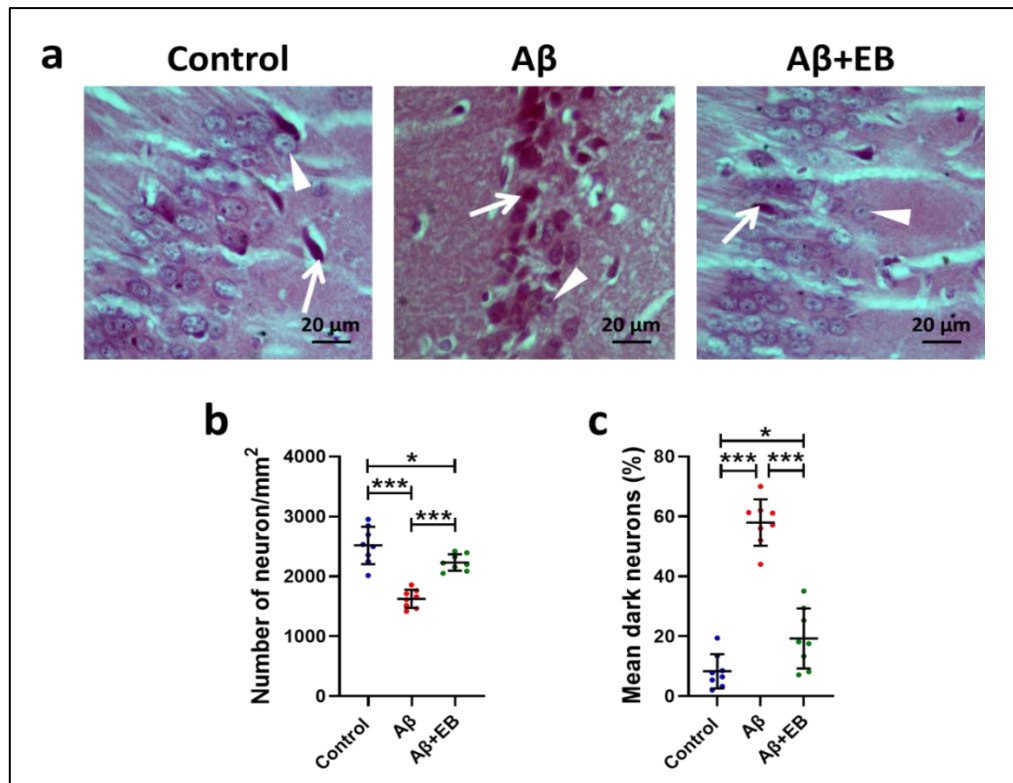

Figure.2. Stereological assessment of hippocampus in the study groups. Hematoxylin and eosin (H&E)-stained brain section for the measurement of the cells (a). Examination of the number of neurons showed that the density of neurons in the EB diet group increased significantly in comparison to the Aβ group (b) and also mean number of dark neurons were downregulated in the EB diet group (c). White triangles showed healthy pyramidal neurons and white arrows showed dark /degenerated neurons. The values are expressed as means± SEM. *P<0.05, **P<0.01 and ***P<0.001 indicates significant differences between groups.

Discussion

In our study, three groups of rats were included in behavioral and histopathological studies. Some were treated with β-amyloid, some received β-amyloid while on a diet of elderberry, with a third group being used as control.

In rats that received β-amyloid, clear behavioral signs of memory dysfunctions were visible. In the T-maze test, which is used principally for evaluating short-term spatial memory, they performed slower and less precisely in comparison to the control group. Yet, in rats receiving β-amyloid which also had a diet consisting of elderberry, a difference with the control group was minuscule to nonexistent.

This observation demonstrates that by having elderberry as a part of the diet, the effects of β-amyloid plaques, one of the major chemicals increased in AD, can be eliminated; resulting in the prevention of memory loss caused by AD in rats which were made prone to it. The

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effect of elderberry on rats is attributable to some of its constituents, chiefly antioxidants such as anthocyanins. Not only does elderberry fruit contain high levels of antioxidants, but it also is proven to have some of the most potent ones among fruits (10, 14).

Oxidative stress can lead to neuroinflammation and the initiation of apoptosis pathways in neurons of the central nervous system (CNS) (15). This process can cause degeneration in the CNS, embarking on the development of AD and other forms of dementia.

In histological studies, a plunge in the number of neurons in rats that received β-amyloid was evident, meaning many neurons underwent apoptosis. In rats having elderberry as a part of their diet though, this number was much less. Multiple mechanisms can lead to apoptosis. Neuroinflammation and immune responses to trauma can cause living cells to be forced to undergo apoptosis (15, 16). It is understood that elderberry can interfere with processes linked to apoptosis, hence slowing the degeneration of neurons and preventing dementia.

A curious phenomenon that has been exceedingly studied recently, "dark neuron" was observed in this research. A sound scientific base for explaining the reason behind the presence of these neurons is not yet available. However, it is possible to assume these dark neurons are dead or going through their terminal stages (17). The number of dark neurons in rats that received β-amyloid was noticeably increased in comparison with the control subjects, while in rats on a diet of elderberry; much fewer dark neurons were present.

Conclusion

Based on our study, elderberry diet can lessen the risk of developing AD by reducing the effects of β-amyloid on apoptosis and neuroinflammation in neurons of the CNS.

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