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# Ellagic acid attenuates enhanced acetylcholinesterase reactivity in an experimental model of Alzheimer′s disease induced by beta amyloid$_{25-35}$ in the rat

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## ABSTRACT

**Background and Objective:** Alzheimer's disease (AD) is a multifactorial disease with debilitating consequences and few therapeutic strategies exist for it. With regard to antioxidant capacity and anti-β-amyloid polymerization potential of ellagic acid, this study was conducted to evaluate the effect of this substance on enhanced acetylcholinesterase reactivity in an experimental model of Alzheimer′s disease induced by beta amyloid25-35 in the rat.

**Materials and Methods:** In this experimental study, 32 male Wistar rats were divided into 4 equal groups, i.e. sham, treated-sham, Alzheimeric, and treated-Alzheimeric. For induction of AD, 2 µl of β-amyloid 25-35 (10 µg/2 µl) was microinjected bilaterally into CA1 of the dorsal hippocampus. The daily treatment with ellagic acid was done for one week at a dose of 100 mg/kg (i.p.) till 1 h pre-surgery. At third week after surgery, histochemistry for acetylcholinesterase reactivity was performed.

**Results:** Alzheimeric group showed enhanced reactivity for acetylcholinesterase in dorsal hippocampus that was significant as compared to sham group ($p<0.005$) and treatment of Alzheimeric rats with ellagic acid significantly prevented this abnormal change versus Alzheimeric group ($p<0.05$). There was also no significant change in ellagic acid-treated sham group versus sham regarding acetylcholinesterase reactivity.

**Conclusion:** Pretreatment of beta amyloid-induced-Alzheimeric rats by ellagic acid could ameliorate acetylcholinesterase reactivity in the dorsal hippocampus and this could possibly improve memory deterioration in AD.

## 1. Introduction

Alzheimer's disease (AD) is regarded as the most prevalent kind of dementia which affects more than 35 million worldwide and 5.5 million in the United States (1). AD is known a debilitating condition of learning, memory and cognition skills especially those involving medial temporal lobe region including the hippocampus. AD could lead to death within 3 to 9 years post-medical diagnosis (1, 2). The disease imposes various emotional and economic burdens to the family and to the society due to its debilitating nature (3). Apart from deranged mechanisms such as mitochondrial dysfunction and genetic components, many molecular defects have also been found in the course of the disease including amyloid cascade, inflammatory mediators, cholinergic system impairment and glutamate-induced excitotoxicity (1, 3). Despite significant achievements in AD pathophysiology and therapy, some cholinesterase inhibitors and memantine are the sole palliative therapies for it (3).

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Ellagic acid is a polyphenol, found in certain fruits and nuts including grapes, strawberries, raspberries, pomegranate and walnut. This phenol is one of the most promising chemopreventive agents. Medical findings have shown that ellagic acid may reduce the incidence of birth defects, promote wound healing, reduce and reverse chemically induced liver fibrosis and may help in the fight against heart disease. It also has antibacterial and antiviral properties (4). Ellagic acid has also exhibited neuroprotective effects against oxidative damage in diabetic rats (5). Since soluble oligomers of beta-amyloid (A beta) play a critical role in the pathogenesis of AD, ellagic acid could significantly reduce Ab-induced neurotoxicity toward SH-SY5Y cells (6). Therefore, we designed this study to investigate the effect of ellagic acid on acetylcholinesterase reactivity in an intrahippocampal amyloid beta 25-35 rat model of AD using histochemical methods.

## 2. Materials and Methods

In this study, 32 male Wistar rats (n=32) weighing 200-240 g were divided into equal-sized groups and kept four to five per cage at animal facility with free access to standard chow and tap water at 21 ± 2ºC, relative humidity of 45±15% and 12 hour light/dark cycle. Behavioral tests were accomplished from 8 a.m. till 4 p.m. All procedures for care and use of animals were conducted in accordance with Shahed University regulations and those specified by National Institutes of Health (NIH).

Rats were equally divided into 4 groups of sham, ellagic acid-pretreated sham, Aβ, and ellagic acid-pretreated Aβ (10 mg/kg; *i.p.*). Stereotaxic surgery was done under general anesthesia upon intraperitoneal ketamine (100 mg/kg) and xylazine (5 mg/kg) mixture administration. After anesthesia induction, the animal head was symmetrically held in Stoelting stereotaxic instrument to achieve skull flat position. The scalp skin was clean shaved and scrubbed with solution of 10% iodine. A midline incision was made. Two bur holes were symmetrically made with a microdrill over the skull at coordinates of -3.5 mm posterior to the bregma, ± 2 mm lateral to the sagittal suture and 2.8 mm ventral to dura matter, based on the rat brain in stereotaxic coordinates (7) for bilateral amyloid beta$_{25\text{-}35}$ fragment, vehicle or saline microinjections. Ellagic acid (10 mg/kg, SigmaAldrich, USA) was administered once a day for one week till 1 h pre-surgery. In Aβ microinjected animals, 2 µl of A$\beta_{25\text{-}35}$ (5 µg/µl) solution prepared in normal saline (pH = 8, preincubated at 37ºC for 72 hours), bilaterally microinjected into dorsal hippocampus. In sham operated rats, normal saline was microinjected accordingly. After 2 weeks post-surgery, acetylcholinesterase reactivity assessment was done as follows:

The animals were anesthetized with a high dose of ketamine (150 mg/kg, i.p.) and then perfused transcardially with 100 ml of saline followed by 200 ml of 4% paraformaldehyde solution prepared in phosphate buffer. The brains were then removed, postfixed in the same fixative for 2-3 h at 4 °C and then placed overnight in PBS containing 30% sucrose at 4 °C. Tissue sections were cut with a microtome (a thickness of 20 µm). The sections were incubated for 1 h at room temperature in 0.1 M sodium hydrogen phosphate buffer containing 0.1 M sodium citrate and 30 mM copper sulfate anhydrous, 5 mM potassium ferricyanide and acetylcholine iodide. When incubation was complete, the processed tissue was rinsed with the buffer and mounted from a separate dish of buffer.

### 2.1.Statistical analysis

Results were presented as means ± SEM. Data analysis was performed using Sigmastat 3.5 statistical software using one-way ANOVA with the Tukey's *post hoc* test. In all statistical analysis, $p<0.05$ was considered significant.

## 3. Results

As shown in Fig. 1, acetylcholinesterase reactivity in the CA1 area of the hippocampus of ellagic acid-pretreated sham group was not markedly different versus sham group and it was markedly higher in Aβ group as compared to sham group. Statistical analysis showed that this reactivity was significantly lower in ellagic-acid pretreated group versus Aβ one ($p<0.05$) (Figures 1 and 2). However, acetylcholinesterase reactivity was still higher in ellagic-acid pretreated group as compared to sham group.

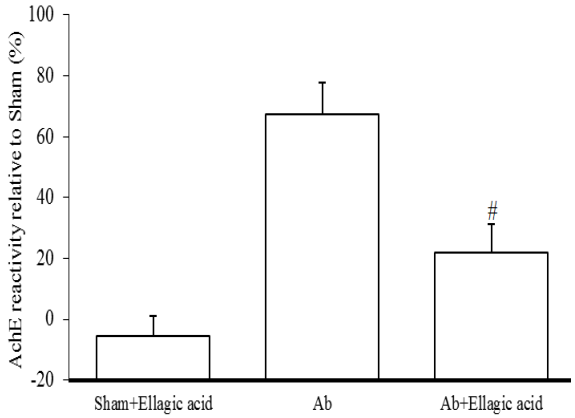


**Fig. 1.** Acetylcholinesterase reactivity in the hippocampus of different groups versus sham.
* p<0.05 as compared to Aβ group.

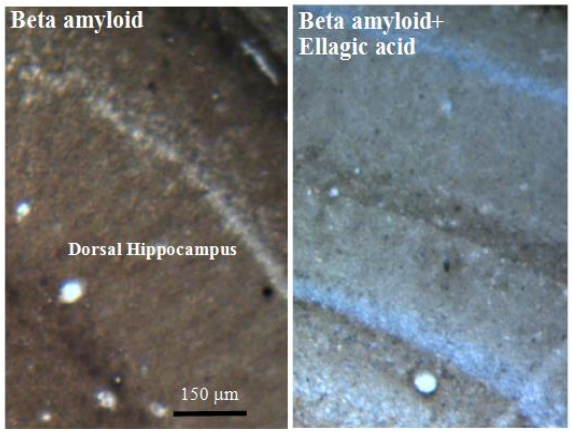


**Fig. 2.** The photomicrograph of acetylcholinesterase reactivity in the hippocampus. Scale bar = 150 micrometer.

## 4. Discussion

The mechanisms of *in vivo* neurotoxicity of beta amyloid have not been thoroughly understood, however there is evidence indicating that rats microinjected with Aβ peptide into the basal forebrain undergo a decrease in release of acetylcholine from the hippocampus (8). Other researchers have shown that intracerebral injections of Aβ1-40 in the rat brain led to neuronal cell loss in the cholinergic system, as well as an extracellular accumulation of acetylcholinesterase (9). Similar documents have shown that the intracerebral administration of Aβ1-42 produces neurotoxic effects in cholinergic neurons as indicated by a decrease in choline acetyltransferase immunoreactivity in the basal forebrain together with a reduction in its positive axons in the cerebral cortex (10) that such change in acetylcholinesterase reactivity was also observed in our study using a specific histochemical method.

Part of beneficial effect of ellagic acid in this study could be attributed to its anti-oxidative stress effect. In this respect, ina previous study, the possible effects of ellagic acid in brain and sciatic nerve tissues of diabetic rats were investigated and it was shown that ellagic acid could restore lipid peroxidation in the brain and sciatic nerve tissues in diabetic rats compared to control group, indicating its neuroprotective effects against oxidative damage in diabetic rats (5). In addition, it has been reported that soluble oligomers of beta amyloid play a critical role in the pathogenesis of Alzheimer's disease and selective inhibition of oligomer formation could provide an optimum target for AD therapy. Some polyphenols like ellagic acid have potent anti-amyloidogenic activities and protect against beta amyloid neurotoxicity (6). Ellagic acis could significantly reduce beta amyloid-induced neurotoxicity toward SH-SY5Y cells that may have therapeutic potential in AD (6).

In conclusion, pretreatment of beta amyloid-induced-Alzheimeric rats by ellagic acid could ameliorate acetylcholinesterase reactivity in the dorsal hippocampus and this could possibly improve memory deterioration in AD.

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## Conflict of interest

The authors declared no conflict of interest.

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