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**Research Article**

# Edaravone Ameliorates Memory, Hippocampal Morphology, and Inflammation in a Rat Model of Alzheimer's Disease

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**Abstract:**

Oxidative stress and neural inflammation play a role in the pathogenesis of Alzheimer's disease (AD). Edaravone (EDA) has an antioxidant-free radical scavenger property. The purpose of this study to evaluate the effect of EDA on memory, hippocampal morphology, and inflammation in a streptozocin (STZ)-induced AD model. This study used 18 Wistar albino adult rats, weighing 200-220 g. Following general anesthesia, 3 mg/kg STZ was dissolved in 0.9% NaCl and administered intracerebroventricularly (ICV) in both lateral ventricles to 12 rats in a total of 5 µl. The animals were allowed to recover for two weeks and then divided into two groups. Six rats were given 0.9% NaCl i.p. for 15 days, and the other six were administered EDA 40 mg/kg i.p. for 15 days, once a day. The control group of six rats did not undergo any surgical procedures or medications. After treatment, a passive avoidance learning (PAL) test was used, followed by the removal of the brain tissue in all animals. Nissl staining was used to count neurons in the hippocampal CA1 and CA3 regions, and TNF-α and IL-6 levels in the brain were measured. In the STZ group, significantly shorter latency time, and decreased number of neurons in the CA1 and CA3 hippocampal regions compared to the control group were observed. EDA significantly prolonged the latency time and increased hippocampal CA1 and CA3 neuron counts compared to the STZ group. TNF-α and IL-6 levels were higher in the STZ+saline group than in the control group. Similarly, EDA treatment reduced TNF-α and IL-6 levels when compared to the STZ+saline and control groups. For the first time, we demonstrated the neuroprotective and anti-inflammatory potential of EDA in an experimental AD model. Our results may provide evidence for EDA therapy in addition to the standard regimen in patients with cognitive decline.

## 1. Introduction

Alzheimer's disease (AD), which is a neurodegenerative disease, is a common disease in the advanced age group [1]. The incidence of dementia increases with age. The incidence in men is 19-29% lower than in women [2]. Patients have progressive functional impairment, emotional distress, loss of independence, and behavioral deficits. The pathophysiology of the disease is complex and multifactorial. Neuronal loss, particularly in certain brain regions such as the medial temporal lobe and temporo-parietal junctional cortices; intraneuronal neurofibrillary tangles composed of aggregated hyperphosphorylated tau protein; and extracellular neuritic plaques formed by β -amyloid peptide accumulation are pathological findings in AD [3]. Oxidative stress (OS) and neuroinflammation are features of neurodegeneration and contribute to AD progression. OS is caused by an imbalance between reactive oxygen species (ROS) formed as a result of cellular metabolism and the systems that detoxify

them. Many studies have shown that OS, which occurs primarily in AD, plays a role in the pathogenesis and progression of the disease [4]. Risk factors such as advanced age [5], apolipoprotein E ε4 alleles [6] may lead to oxidative damage in AD. OS causes the release of inflammatory and neurotoxic factors that cause a chronic neuroinflammatory response [7]. In AD, accumulation of the Aβ peptide initiates a spectrum of cerebral neuroinflammation mediated by the activation of microglia, the resident immune cells of the brain. Activated microglia express proinflammatory cytokines such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) [8]. Developed in Japan, edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, EDA) has an antioxidant free radical scavenger property [9]. It can prevent motor neuron death caused by OS in Amyotrophic Lateral Sclerosis (ALS) patients [10]. In addition, this molecule, which has neuroprotective and anti-inflammatory effects, is also used in the treatment of acute ischemic stroke and acute brain infarction [11]. A recent study has shown that EDA exerts its antioxidant and anti-inflammatory effects in asthma and cerebral infarction via the nuclear factor erythroid 2-related factor transcription factor/hemoxygenase 1 (Nrf2/HO-1) signaling pathway [12]. In the study in cultured neuron cells, it was understood that the neuroprotective effect of EDA increased the expression of mature brain-derived neurotrophic factor (mBDNF) and Bcl-2, decreased caspase-3 activity, and stimulated the activation of extracellular signal-regulated kinases ½ ($ERK_{1/2}$) [13]. EDA protects neurons [14], and glia (astrocytes, oligodendrocytes, and microglia) [15]. It suppresses the inflammatory response of activated microglial cells [16]. Streptozotocin (STZ) is widely used in diabetes research to establish an animal model because it selectively damages pancreatic β-cells [17]. Studies have shown that injection of intracerebroventricular (ICV) STZ in rodents produces AD-like pathology. ICV STZ stimulates progressive neuroinflammation, OS, and mitochondrial dysfunction in the hippocampus, exerting effects associated with significant learning and cognitive impairments [18]. The hippocampus, a part of the brain important for learning and memory, is susceptible to damage in the early stages of AD.
Based on this information, we aimed to evaluate the effect of edaravone on memory and hippocampal morphology in rats with a streptozocin (STZ)-induced AD model. TNF-α and IL-6 levels in brain tissue were measured for neuroinflammation

## 2. Material and Methods

### 2.1. Animal origin, housing, breeding, and study approval

Eighteen adult male Wistar albino rats weighing 200-220 g included in the study. The animals were kept at a temperature of 23±1°C with a 12-hour light/dark cycle. The experimental procedures of the study were approved by the Tokat Gaziosmanpaşa University Animal Ethics Research Committee (2014 HADYEK-47).

### 2.2. Experimental procedures

Animals were given general anesthesia by intraperitoneal (i.p.) administration of ketamine hydrochloride (80 mg/kg, Alfamine®, Ege Vet, Alfasan International B.V. Holland) and xylazine hydrochloride (4 mg/kg, Alfazyne®, Ege Vet, Alfasan International B.V. Holland). Using a streotaxic device to generate the AD model, rats were given bilateral ICV into both lateral ventricles STZ (Sigma-Aldrich, St Louis, MO, 3 mg / kg). STZ was infused into the left (2.5 µL) and right lateral ventricle (2.5 µL) (ICV) (AP = −0.8mm, L = ±1.6 mm, DV = −4.2 mm) with a Hamilton syringe [19]. Edaravone (Radicava®) was purchased from Mitsubishi Tanabe Pharma America. After the ICV STZ injection, the rats were divided into 3 groups: control (n = 6), ICV-STZ plus 0.9% NaCl (n = 6), and ICV-STZ plus EDA (n = 6). STZ was dissolved in 0.9% NaCl at a dose of 3 mg/kg, and ICV was administered in a total of 5 µl in both lateral ventricles to 12 rats after anesthesia. Rats were divided into two groups. After STZ injection, six rats were given 0.9% NaCl i.p. for 15 days, and the other six were administered EDA 40 mg/kg i.p. for 15 days, once a day. No surgery or treatment was given to the controls (Fig 1).

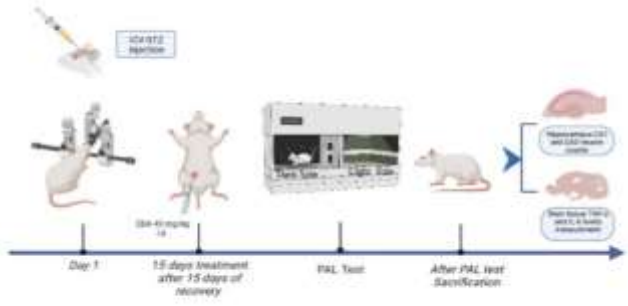


***Figure 1***. *Experimental Design*

### 2.3. Passive avoidance test

Two weeks after the application, a passive avoidance task that assesses learning and memory was carried out in all groups. Passive avoidance

learning (PAL) is a set of fear-guided tests classically used to assess negative reinforcement-based long-term memory in small laboratory animals. For this, a PAL box with a size of 20 x 20 x20 cm with dark and light sections was used. The rats were placed in the bright chamber of a two-compartment box. After a 10-second acclimatization time, the door between the light and dark chambers was opened. After the animal passed into the dark area, the door was closed, and an electric shock (FJ-919; 300 kV, 60 Hz, 1,5 mA) was applied to the animal for 3 seconds. The time it took for the animal to enter the dark zone was considered the latency period. Animals that did not enter the dark zone for more than 5 minutes were excluded from the study. The animals' latency time to the dark area was evaluated after 24 hours [20]. Then the rats were sacrificed, and their brain tissues were removed for histopathological and biochemical evaluation.

### 2.4. Histological Evaluation

Brain tissue samples from all animals were fixed in 10% formalin. After post-fixation in the same fixative solution for one week, the tissues were blocked with paraffin after routine histological procedures. Each paraffin block was sectioned into 5 µ thick sections, which were then deparaffinized in an oven at 60o C for 8 hours. The hippocampus cornu ammonis-1 (CA1), cornu ammonis-3 (CA3) neuron counts of brain sections stained with Nissl dye were examined using an Olympus BX51 microscope and an Olympus C-5050 digital camera. To quantify the surviving number of neurons, the image analysis system (Image-Pro Express 1.4.5, Media Cybernetics, Inc., USA) was used.

### 2.5. Detection of TNF-α and IL-6 levels in brain tissue

Brain tissue samples were stored in aliquots at -80 0C until the study day. ELISA was used to measure TNF-α (catalog no. E-EL-R2856) and IL-6 (catalog no. E-EL-R0015) using commercial kits (Elabscience, USA) and following the manufacturer's instructions. TNF-α and IL-6 levels per mg protein were determined using the Thermo ScientificTM PierceTM BCA Protein Assay Kit (Catalog no. 23225). TNF-α and IL-6 levels in the same homogenate were calculated in relation to protein concentration (ng/mg protein). The levels of TNF-α and IL-6 in brain homogenates were calculated in relation to protein concentration.

### 2.6. Statistical Analyzes

Statistical analyses were performed using the SPSS 26.00 program. To compare differences between groups, the one-way ANOVA' test was used, followed by post hoc LSD tests. The results were presented in the form of the mean (mean) and standard error (SEM). For TNF-α and IL-6, a one-way ANOVA was used. Tamhane's T2 was used post-hoc. A p value of <0.05 was considered statistically significant.

## 3. Results and Discussions

In the STZ-induced rat model, the latency period was shortened in the STZ+saline group, while the number of CA1 and CA3 neurons decreased (Fig 2). EDA improved all these harmful effects caused by STZ. The duration of latency increased, as did the number of CA1 and CA3 neurons. In the STZ group, significantly shorter latency time ($p<0.001$) and decreased number of neurons in the CA1 and CA3 hippocampal regions ($p<0.000$, $p<0.001$, respectively) compared to the control group were observed. EDA significantly prolonged the latency time ($p<0.05$) and increased hippocampal CA1 and CA3 neuron counts ($p<0.05$, $p<0.05$, respectively) compared to the STZ group. The histopathological and immunohistochemical findings are shown in Table 1. As can be seen Fig 3, when compared to the STZ and control groups, EDA administration reduced TNF-α and IL-6 levels. TNF-α and IL-6 levels in the STZ group were higher than in the control group ($p< 0.01$ and $p<0.05$, respectively). EDA treatment decreased TNF-α ($p <0.01$ and $p<0.001$, respectively) and IL-6 levels compared to STZ and control groups ($p<0.05$, $p<0.01$, respectively).

***Table 1.*** *Latency time and the number of neurons in hippocampal CA1 and CA3 regions of groups*

| Groups | Latency time (s) | Number of CA1 neurons | Number of CA3 neurons |
|---|---|---|---|
| **Control Group** | 229.2 ± 33.6 | 68.4 ± 5.3 | 65.8 ± 3.9 |
| **ICV-STZ+ Saline Group** | 40.4 ± 10.3 * | 45.9 ± 5.2 ** | 38.6 ± 1.8 * |
| **ICV-STZ+EDA Group** | 164.8 ± 59.3 # | 63.5 ± 7.2 # | 60.3 ± 4.8 # |

*Data expressed as mean ± SEM. * p < 0.001, ICV-STZ+Saline Group vs. Control Group; ** p < 0.000, ICV-STZ+Saline Group vs Control Group; # p < 0.05, ICV-STZ+EDA Group vs ICV-STZ+Saline Group.*

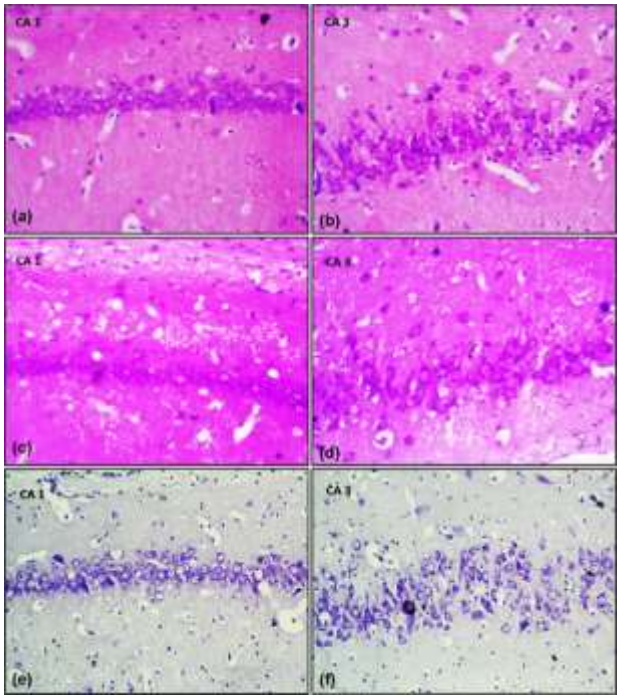

***Figure 2.*** *CA1 and CA3 hippocampal regions were stained with Cresyl violet stain (x 40 and x 100 magnification). a) Control Group CA1, b) Control Group CA3, c) ICV-STZ and Saline Group CA1, d) ICV-STZ and Saline Group CA3, e) ICV-STZ and EDA Group CA1, f) ICV-STZ and EDA Group CA3*

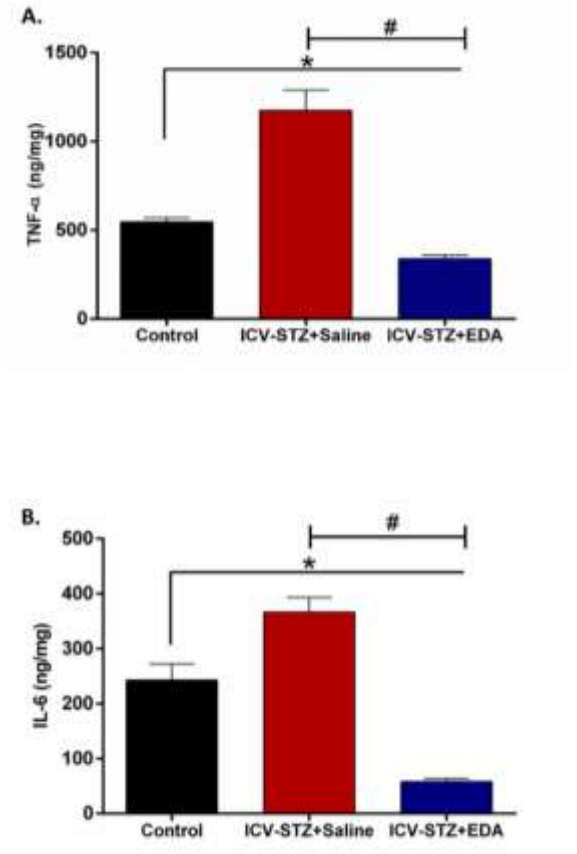


***Figure 3.*** *The concentration of TNF-α and IL-6 in the rat brain in the following groups: A) *Control group vs ICV-STZ+Saline and ICV-STZ+EDA group; p<0.01; #ICV-STZ+EDA group vs ICV-STZ+Saline group p<0.01. B) *Control group vs ICV-STZ+Saline and ICV-STZ+EDA group; p<0.05; # ICV-STZ+EDA group vs ICV-STZ+Saline group p<0.001.*

## Discussion

In neurodegenerative diseases, there is oxidative damage to the brain, and OS often appears to be a manifestation of these diseases. Because of the brain's very high dioxygen consumption, the brain may be particularly susceptible to oxidative damage to OS [21]. In addition, OS has been shown to induce intracellular Aβ accumulation and tau phosphorylation in cell cultures [22]. The presence of OS with high OS markers is noticed in the brains of AD patients and animal models of AD [23]. Studies have shown that inflammation [24], mitochondrial dysfunction [25], metal accumulation [26], hyperphosphorylated tau [27], and Aβ accumulation [28] are the basic mechanisms underlying the formation of OS. High levels of Aβ1–40 and Aβ1–42 have been shown to be associated with increased levels of oxidation products from proteins, lipids, and nucleic acids in the hippocampus and cortex in AD [29]. With proteomic analysis, potentially numerous oxidized proteins with key roles in vital cellular functions in the AD brain have been identified [30]. But the questions about how and where the OS is in the AD, as well as where it came from, remain unanswered.

AD is believed to affect neurons by disrupting synapses. However, astrocytes and microglia remain viable, acquire a reactive morphology, and mediate inflammatory-like responses [31]. Inflammatory mediators such as TNF-α and IL-6 are involved in the pathogenesis of AD. TNF-α, which contributes to the chronic inflammatory response, is expressed by neurons and glia in the brain [32]. TNF-α may aggravate phospho-tau pathologies in AD brains and contribute to amyloidogenesis via beta-secretase regulation. It also induces insulin resistance, chronic inflammation, glutamate excitotoxicity, OS, and mitochondrial dysfunction [33]. TNF-α has been implicated in disease progression, gliosis, demyelination, inflammation, disruption of the blood-brain barrier, and cell death in experiments induced by chronic neuroinflammation [34]. Tarkowski et al. found a 25-fold difference in TNF-α levels between AD patients and controls [35]. IL-6 is a cytokine that plays a critical role in the physiological homeostasis of neural tissue and inflammation [36]. However, prolonged and severe exposure of the brain to IL-6 has resulted in many

neuropathological findings. IL-6 overproduction in transgenic mice has been reported to reduce neurogenesis and the proliferation, survival and differentiation of neural progenitor cells in the dentate gyrus [37]. IL-6, a component of amyloid plaque formation in the early stage of AD brains, is associated with tau phosphorylation, synapse loss, and learning deficits in mice [38]. Although there are conflicting results, meta-analyses have shown that IL-6 is increased in the cerebrospinal fluid, in the plasma of patients with mild cognitive impairment, and in AD patients compared to controls [39, 40].

With a melting point of 129.7°C, EDA is freely soluble in acetic acid, methanol, or ethanol but sparingly soluble in water or diethyl ether [41]. EDA can protect body tissues by inhibiting cyclooxygenase activity (COX, including COX-1, and COX-2). COX-2, expressed mainly in hippocampal tissue and cortical neurons, is a key enzyme produced by prostaglandin E2 (PGE2) metabolism. PGE2 is associated with pathological events such as inflammation, OS, and apoptosis [42]. EDA has been shown to be effective in slowing the progression of symptoms and motor neuron degeneration in ALS model mice [43]. Due to the presence of upper and lower motor degeneration in ALS, muscle weakness that usually begins in the extremities leads to clinical symptoms [44]. The role of OS in ALS, AD, Parkinson's disease, and other neurodegenerative diseases has been demonstrated. ROS are produced by cellular enzymes that damage cell components and increase inflammation [45]. A review of experimental animal studies showed that EDA increased the functional and cognitive prognosis by 30.3% and 25.5%, respectively, in an animal model of focal cerebral ischemia [46]. Another review evaluating 18 randomized controlled trials reported that EDA can significantly reduce the mortality or morbidity rate of acute ischemic stroke [47]. Recently, a study reported that the therapeutic mechanisms of EDA in the treatment of cerebral ischemia may include OS, platelet activation and aggregation, intercellular adhesion, glycolysis and gluconeogenesis, iron metabolism, hypoxia, and the like [8]. The administration of EDA to rats for 5 weeks abolished rotenone-induced effects such as midbrain catalepsy, mitochondrial damage, and degeneration of dopamine neurons [48]. In vivo studies have shown that EDA can reduce microglial activation and suppress the production of proinflammatory mediators by activated microglia [16, 49]. Fujiwara et al. showed that there were elevations in plasma levels of some cytokines, including IL-6 in rats in acute ischemic conditions; these increases showed a decrease with EDA treatment [50].

In this study, we showed for the first time that EDA has neuroprotective and anti-inflammatory effects in the STZ-induced AD rat model. The role of the hippocampal region in semantic, learning, and episodic memory is well established. Therefore, the hippocampus is one of the first areas of the brain to be affected by pathological events in AD. In the STZ-induced rat model, the latency period was shortened in the STZ+saline group, while the number of CA1 and CA3 neurons decreased. EDA improved all these harmful effects caused by STZ. The duration of latency increased, as did the number of CA1 and CA3 neurons. When compared to the STZ and control groups, EDA administration reduced TNF-α and IL-6 levels.

## 4. Conclusions

Various neuropathological changes are seen in the brains of individuals with AD, which is the most common cause of dementia all over the world. Treatment of these neurodegenerative changes will be aimed at preventing memory loss and thinking and reasoning problems. For the first time, we demonstrated the neuroprotective and anti-inflammatory potential of EDA in an experimental AD model. Our study provides evidence for EDA treatment in addition to the standard regimen in patients with cognitive decline.

## Author Statements:

- **Ethical approval:** The conducted research is not related to either human or animal use.
- **Conflict of interest:** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper
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- **Data availability statement:** The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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