The Effect of Alpha-Lipoic Acid on Learning and Memory Deficit in a Rat Model of Temporal Lobe Epilepsy

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ABSTRACT

Introduction: Epilepsy is a chronic neurological disorder in which patients experience spontaneous recurrent seizures and deficiency in learning and memory. Although the most commonly recommended therapy is drug treatment, some patients do not achieve adequate control of their seizures on existing drugs. New medications with novel mechanisms of action are needed to help those patients whose seizures are resistant to currently-available drugs. While alpha-lipoic acid as a antioxidant has some neuroprotective properties, but this action has not been investigated in models of epilepsy. Therefore, the protective effect of pretreatment with alpha-lipoic acid was evaluated in experimental model of temporal lobe epilepsy in male rats.

Methods: In the present study, Wistar male rats were injected intrahippocampally with 0.9% saline(Sham-operated group), kainic acid(4 μg) alone, or α-lipoic acid (25mg and 50mg/kg) in association with kainic acid(4μg). We performed behavior monitoring(spontaneous seizure, learning and memory by Y-maze and passive avoidance test), intracranial electroencephalography (iEEG) recording, histological analysis, to evaluate the anti- epilepsy effect of α-lipoic acid in kainate-induced epileptic rats.

Results: Behavior data showed that the kainate rats exhibit spontaneous seizures, lower spontaneous alternation score inY-maze tasks ($p<0.01$), impaired retention and recall capability in the passive avoidance test ($p<0.05$). Administration of alpha-lipoic acid, in both doses, significantly decrease the number of spontaneous seizures, improved alternation score in Y-maze task ($p<0.005$) and impaired retention and recall capability in the passive avoidance test ($p<0.01$) in kainite rats. Moreover, lipoic acid could improve the lipid peroxidation and nitrite level and superoxid dismutase activity.

Discussion: This study indicates that lipoic acid pretreatment attenuates kainic acid-induced impairment of short-term spatial memory in rats probably due to its antioxidant activity.

Key Words:
Kainic Acid,
Lipoic Acid,
Passive Avoidance,
Y Maze,
Oxidative Stress,
Rat.

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1. Introduction

Epilepsy is the most common neurodegenerative disease after stroke. It afflicts more than 50 million people worldwide (Strine et al., 2005), of whom as many as 40% may have complex partial epilepsy, in particular, temporal lobe epilepsy (Shorvon, 1996; Jallon, 1997). Temporal lobe epilepsy (TLE) is characterized by spontaneous seizures that lead to brain injury and neuronal cell death. Kainic acid(KA)-induced epilepsy model in rats shows the most clinical characteristics of human TLE (Ben-Ari, Tremblay, Riche, Ghilini &Naquet,1981; Turski et al.,1983). In adult rats, the injection of this drug leads to a status epilepticus followed by a chronic period of spontaneous convulsive seizures(Goffin, Nissinen, Van Laere, Pitka¨nen, 2007; Williams et al., 2009; Cavalheiro et al., 1991).

Activation of KA receptors results in intracellular signaling cascades that include nitric oxide synthase (NOS) activation, free radical formation, and mitochondrial dysfunction, which in turn, result in inflammatory responses, cytokine expression and oxidative stress via reactive oxygen or nitrogen species(Carrasco, Penkowa, Hadberg, Molinero, & Hidalgo 2000; Lehtimäki , Peltola, Koskikallio, Keränen, & Honkaniemi, 2003).

The central nervous system is highly susceptible to free-radical-mediated damage because of their high lipid content. Moreover, inflammation is closely related to the overproduction of the reactive oxygen species (ROS) and plays an important role in the neuronal death processes that occur in a number of pathological settings, including Alzheimer's disease (AD)and Parkinson disease(PD), amyotrophic lateral sclerosis, and epilepsy (Perry et al., 2002; Migliore et al., 2005; Ashrafi et al., 2007). According to recent findings, it seems that when excitatory amino acid receptors are over-stimulated, the development of radical scavengers and the maintenance of low ROS levels for neuroprotection is unavoidable (Sumanont et al., 2006). Thus, agents with antioxidant and anti-inflammatory properties are suggested to be useful in this conditions.

Alpha-lipoic acid (α-LA) play principle role in antioxidant defense in the brain because of their roles as biologic antioxidants. Also, it was previously shown that α-LA has neuroprotective effects in experimental brain injury caused by trauma and subarachnoid hemorrhage (Toklu et al., 2009; Ersahin et al., 2010). Some reports support this speculation that lipoic acid may have a neuroprotective role by reducing apoptosis in both caspase-dependent and –independent manner(Santos et al., 2011). Additionally, LA could reduce spinal cord injury-induced oxidative stress and exerts neuroprotection by inhibiting lipid peroxidation, glutathione depletion, and DNA fragmentation (Toklu et al., 2010).

Accordingly, this study was designed to determine the possible protective effect of LA against kainic acid – induced oxidative stress in rats by determining some biochemical parameters and behavioral examination.

2. Methods

2.1. Animals

Adult male Wistar rats (n = 60) (Pasteur's Institute, Tehran), weighing 300-350 g at the start of the experiment were housed three per cage in a temperature-controlled colony room under natural light/dark cycle. Animals were given free access to tap water and standard rat chow. Procedures involving animals were made to minimize animal suffering.

2.2. Experimental Procedure

Rats were randomly divided into the following equal groups: Sham-operated (SH); vehicle-treated SH; α-LA -treated SH; Kainate; α-LA(25 mg/Kg) -treated kainate and α-LA(50 mg/Kg) -treated kainate rats. For stereotaxic surgery, rats were anesthetized with a combination of ketamin (100 mg/Kg, i.p.) and xylazine (5 mg/Kg, i.p.), placed in a Stoelting stereotaxic apparatus (incisor bar -3.3 mm, ear bars positioned symmetrically). The scalp was cleaned with iodine solution and incised on the midline, and a burr hole was drilled through the skull. Animals in the kainate group were unilaterally injected in the dorsal hippocampus with 10 µl of normal salin containing 0.4 µg/µl kainic acid (Sigma Chemicals, USA). α-LA (Sigma Chemicals, USA) was dissolved in propylene glycol and administered daily(25 and 50 mg/kg body weight; i.p) for one week before surgery.

The vehicle-treated SH rats were infused with an equivalent volume of normal salin in the same stereotaxic coordinates and received daily propylene glycol (i.p.) for one week.

In the first 24 hours after the first surgery, all rats were monitored under a video capture system for 8 hours to record the spontaneous seizures.

The progression of kainate-induced seizures was scored according to Racine's standard classification: 0, no reaction; 1, stereotype mounting, eye blinking, and/

or mild facial clonus; 2, head nodding and/or several facial clonus; 3, myoclonic jerks in the forelimbs; 4, clonic convulsions in the forelimbs with rearing; and 5, generalized clonic convulsions associated with loss of balance (Racine, Okujava , & Chipashvili, 1972).

2.3. Y-maze Task

Spatial recognition memory was assessed by recording spontaneous alternation behavior in a single-session Y-maze on the 14th day post-surgery, as described elsewhere (Rasoolijazi, Joghataei, Roghani, & Nobakht, 2007). The maze was made of black Plexiglas. Each arm was 40 cm long, 30 cm high and 15 cm wide. The arms converged in an equilateral triangular central area that was 15 cm at its longest axis. The procedure was as follows: each rat, naive to the maze, was placed at the end of one arm and was allowed to move freely through the maze during an 8-min session. The series of arm entries were recorded visually. Entry was considered to be complete when the base of the animal's tail was entirely within the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The maximum number of possible spontaneous alternations was determined as the total number of arms entered - 2, and the percentage was calculated as the ratio of actual to possible alternations × 100.

2.4. Single-Trial Passive Avoidance Test

This test was conducted 2 days after Y-maze and was done according to a previous study (Baluchnejadmojarad, & Roghani, 2006). The apparatus (40 cm long - 20 cm wide - 30 cm high) consisted of an illuminated chamber connected to a dark chamber by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator. On the first and second days of testing, each rat was placed in the apparatus for 15 min to habituate. On the third day, an acquisition trial was performed. Rats were placed individually in the illuminated chamber. After a habituation period (5 min), the guillotine door was lifted, and, after the rat had entered the dark chamber, the door was lowered and an inescapable scrambled single electric shock (1 mA, 1 s) was delivered. In this trial, the initial latency (IL) of entrance into the dark chamber was recorded and rats had ILs greater than 60 s were excluded from the study. Twenty-four hours later, each rat was placed in the illuminated chamber for retention trial. The interval between placement in the illuminated chamber and entry into the dark chamber was measured as step-through latency (STL, up to a maximum of 300 s).

2.5. Determination of hippocampal MDA concentration

The rats were anesthetized with ketamine (100 mg/kg) and decapitated. Hippocampi were isolated and blotted dry, and then weighed and prepared as a 5% tissue homogenate in ice-cold 0.9% saline solution. After centrifugation (1000g, 4 °C, 10 min), the supernatant was aliquoted and stored at _80 _C until assayed. The concentration of malondialdehyde (MDA), used as a marker of lipid peroxidation index, was calculated by measuring thiobarbituric acid reactive substances (TBARS) in the supernatant as described previously (Roghani & Baluchnejadmojarad, 2009).

Briefly, Trichloroacetic Acid and TBARS Reagent Were Added to Aliquots of the supernatant, which were subsequently mixed and incubated at 100 _C for 80 min. After cooling on ice, the samples were centrifuged at 1000g for 10 min, and the absorbance of the supernatant was read at 532 nm. The results of TBARS measurements were expressed as MDA equivalents, using tetraethoxypropane as standard.

2.6. Measurement of Hippocampal SOD Activity

The supernatant of hippocampal homogenate was obtained as described above. Superoxide dismutase (SOD) activity was measured as previously reported (Roghani & Baluchnejadmojarad, 2009). Briefly, supernatant was incubated with xanthine and xanthine oxidase in potassium phosphate buffer (pH 7.8, 37 °C) for 40 min, and then nitroblue tetrazolium (NBT) was added. Thereafter, blue formazan was monitored spectrophotometrically at 550 nm. The amount of protein that inhibited NBT reduction to 50% maximum was defined as 1 nitrite unit (NU) of SOD activity.

2.7. Assay of Hippocampal Nitrite Concentration

Supernatant nitrite (NO_2^-) content was assayed by the Griess Method. The compound NO has a short half-life and is rapidly converted to the stable end products nitrate (NO_2^- and NO_3^-). In the assay used here NO_3^- is converted to NO_2^- by cadmium, and this is followed by color development with Griess reagent (sulfanilamide and N-naphthyl ethylenediamine) in acidic medium. The absorbance was determined using a spectrophotometer at 540 nm.

2.8. Protein Assay

The protein content of the supernatant was measured by the Bradford method, using bovine serum albumin (Sigma Chemical, St. Louis, MO) as the standard (Bradford, 1976).

2.9. Statistical Analysis

All results were expressed as mean ± SEM. The non-parametric Kruskal–Wallis test was used to analyze the behavioral tests, and if a difference was found to be significant, pair-wise comparison was done using the Mann–Whitney U-test, except the percentage of rats with spontaneous seizure, which is examined by X^2 test. Parametric one-way ANOVA was used to assess the biochemical tests. In all calculations, a difference at $p < 0.05$ was regarded as significant.

3. Results

3.1. Behavior Observation

According to Racine's standard classification, the class 5 and 4 seizures were observed during the acute period (24 hours after kainic acid administration) in 66.6% and 83.3% of rats treated with kainite respectively. Behavioral data showed that α-LA could significantly decrease the number of spontaneous seizures (Table 1). The vehicle-treated SH rats showed no spontaneous seizures.

3.2. Spatial Recognition Memory in Y-Maze

The short-term spatial recognition memory can be examined as alternation behavior in Y-maze task. In this respect, the alternation score of the kainate injected rats was found to be significantly lower (43.22 ± 11.34%) than that of the sham-operated group(79.58 ± 6.95%) (P<0.01). In addition, α-LA -treated kainic acid injected rats at a dose of 25 and 50 mg/kg showed a higher alternation score respectively(71.58 ± 4.80 and 81.88 ± 5.67%) as compared to kainate group (P < 0.01-0.005). To assess compounding effect of locomotor activity on memory processes in experimental groups, total number of arms entered was considered as an index of locomotor activity. In this regard, there was no statistically significant difference between the kainic acid injected rats (20.5 ± 7.64) compared to the sham-operated group (15.6 ± 2.01). Moreover, administration of α-LA caused no considerable change in total number of entered arms (Fig. 1).

3.3. Passive Avoidance Test

Fig. 2 shows the performance of rats in passive avoidance paradigm as indicated by IL and STL. Regarding initial latency, there was no significant difference among the groups. In addition, kainic acid injected rats developed a significant impairment in retention and recall in passive avoidance test (P < 0.05), as it is evident by a lower STL. α-LA treatment at doses of 25 and 50 mg/kg did produce an improvement in this respect (P < 0.05-0.01).

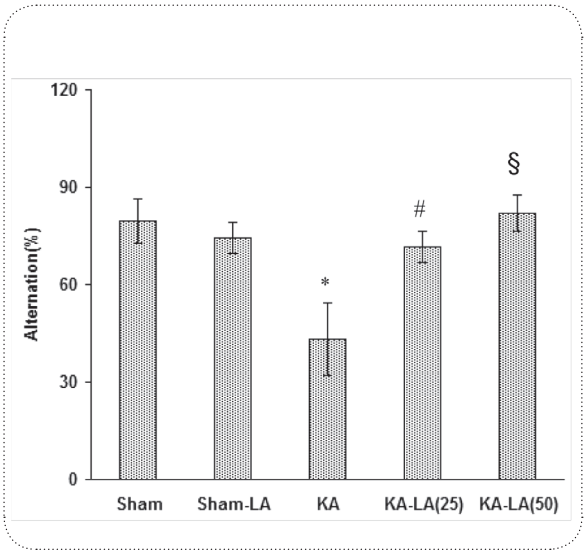

Figure 1. Alternation behavior displayed in the Y-maze by rats. Values are means ± SEM

*P< 0.01 (vs. sham); # P < 0.01, § P < 0.005 (vs. KA).

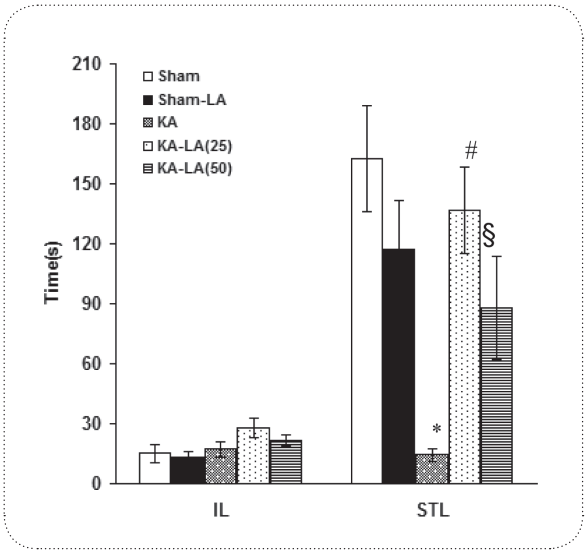

Figure 2. Initial latency (IL) and step-through latency (STL) recorded in a single-trial passive avoidance test for rats. Values are means ± SEM

*P< 0.05 (vs. sham); # P < 0.01, § P < 0.05 (vs. KA).

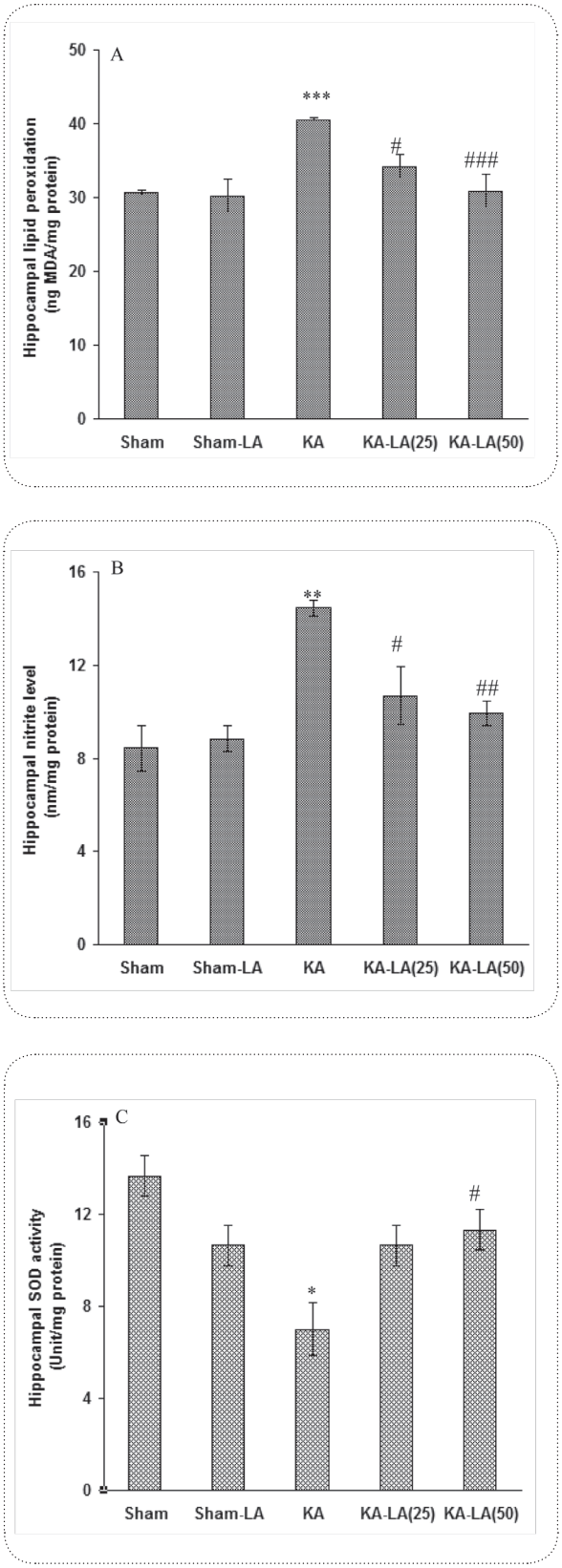

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Figure 3. (A) MDA concentration, (B) nitrite content, and (C) SOD activity in hippocampal homogenate from different groups.

*p < 0.01, **p < 0.005, ***p < 0.0001 (vs. sham); # P < 0.05, ## P < 0.01, ### P < 0.005(vs. KA).

3.4. Markers of Oxidative Stress

Kainic acid injected rats exhibited significantly elevated levels of MDA (40.49 ± 0.40 ng/mg protein; p < 0.0001) and nitrite (14.45 ± 0.35 nmol/ mg protein; p < 0.005), and a significant reduction in SOD activity (7 ± 1.15 unit/mg protein; p < 0.01) in hippocampal tissue compared to the sham-operated group (MDA, 30.61 ± 0.39 ng/mg protein; nitrite, 8.43 ± 0.96 nmol/mg protein; SOD, 13.66 ± 0.88 unit/mg protein). Pretreatment of kainic acid injected rats with 25 and 50 mg/kg α-LA significantly attenuated the increased MDA (34.12 ± 1.65 and 30.87 ± 2.36 ; p < 0.05-0.01) and nitrite (10.69 ± 1.22 and 9.92 ± 0.54 ; p < 0.05-0.005) content and increased SOD activity (10.66 ± 0.88 and 11.33 ± 0.88 ; p < 0.05) respectively relative to kainic acid rats (Figs. 3A, B, C).

4. Discussion

The main findings of this study were three-fold. First, intrahippocampal administration of kainic acid was accompanied with acute spontaneous seizures, disturbances in animal performance in Y-maze and passive avoidance as were evident by a lower alternation score and STL respectively. Second, pretreatment of kainite rats with α-LA at two doses of 25 and 50 mg/kg for one week improved spontaneous seizures, short-term spatial recognition memory performance in Y-maze and prevented retention and recall abnormality in passive avoidance test. Third, part of beneficial effect of α-LA in this research could be attributed to attenuation of oxidative stress in those brain structures involved in learning and memory processes.

The KA- induced seizure model is a useful method for the study of the Pathological mechanisms of epileptic discharge in the limbic system. Neuropathology pattern of activation of KA ionotropic glutamate receptors is comparable to human TLE (Ben-Ari, 1985; Ben-Ari & Cossart, 2000). This model has some criteria of human TLE such as the hippocampus, amygdala, and other limbic structures play a central role in its symptomatology and spontaneous seizures occur consistently following intracerebral applying of KA.

Some of evidence indicate that at acute periods (i.e., 4and 24 h) post-KA, protein oxidation and lipid peroxidation elevate in the hippocampus (Kim et al., 1997). Also, Tang et al. (1998) found that 8-hydroxy-2-deoxyguanosine (8-OHdG), an oxidative marker for DNA damage, may be elevated in the hippocampus and cerebral cortex 8 h after KA treatment. In present study,

Table 1. Numbers and rates of spontaneous seizures in each group

Group	Class 4 Seizure Number	Class 5 Seizure Number	Class 4 Seizure Rate%	Class 5 Seizure
Sham (n=12)	0	0	0	0
Sham-LA (n=12)	0	0	0	0
KA (n=12)	10	8	83.3*	66.6
KA-LA(25) (n=12)	2	1	16.6*	8.3*
KA-LA(50) (n=12)	1	1	8.3*	8.3*

*X*2 test, * P<0.05 compared with Kainate group

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we also observed that two weeks after intracerebral KA administration, some oxidative stress markers including hippocampal MDA and nitrite levels were intensified and SOD activity lowered. Moreover, Activation of ionotropic glutamate receptors by KA causes an increase in the intracellular calcium and deplorization of neuronal mitochondrial membrane potential in many regions of the brain, mainly in the hippocampal regions of CA1 and CA3 and in the dentate hilus. These effects lead to O2 consumption deficiency, diminished ATP production, excessive production of ROS, NO, and peroxynitrite, and resulting impairment of cell component including lipids, proteins, and DNA. In addition to the increase in lipid peroxidation, systemic administration of KA also caused a decrease in reduced form of glutathione (GSH) levels in the hippocampus (Shin et al., 2008). Intravenous GSH administration protected against KA-induced neuronal loss in the hippocampus and subsequent development of edema (Saija et al., 1994). Thus, mitochondrial malfunction, lipid peroxidation and decreased GSH related to seizure activity may be in front of neuronal death in susceptible brain regions (Frantseva et al., 2000). It seems that extreme activation of glutamate receptors and oxidative stress represents factors that come together for neuronal vulnerability (Coyle & Puttfarcken, 1993). Since, oxidative damage is associated with cognitive dysfunction (Kucukatay, Agar, Gumuslu, & Yargicoglu, 2007; Fukui et al., 2002), therefore, treatment with antioxidants could be a therapeutic approach in various types of neurodegenerative diseases.

Alpha-lipoic acid is a disulphide derivative of octanoic acid and has been used due to the presence of a prosthetic group of various cellular enzymatic complexes (Tardif, 2008). LA has some attributes which make it an conspicuous antioxidant (Packer, Tritschler, & Wessel, 1997; Roy & Packer 1998). It avidly crosses the blood–brain barrier and is readily received by human cells and is reduced to DHLA. Therefore, unlike ascorbic acid, DHLA not only is not destroyed by free radicals, but also can be recycled from LA. Moreover, LA and DHLA are amphipathic molecules and may act as antioxidants in hydrophilic and lipophilic environments. Also, it shows synergistic action with other antioxidants (Suzuki, Tsuchiya, & Packer, 1993). It has been shown that LA and DHLA may act as a strong direct chain-breaking antioxidant and intensify the antioxidant strength of other antioxidants (ascorbate and vitamin E) in the hydrophilic and the hydrophobic membranous phases (Kagan , 1992). Additionally, administration of lipoic acid has considerable effects on tissue thiol status, raising glutathione levels by reducing extracellular cystine to cysteine, which bypasses the cystine transporter (Han, 1997). Since α-lipoic acid has an effective antioxidant activity, it is a potential curative factor in prevention of different pathologic conditions that may be related to an imbalance of the oxido-reductive cellular status (Moini, Packer, & Saris, 2002; Ferreira, Militao, & Freitas , 2009; Holmquist et al., 2007; Militão, Ferreira, & de Freitas, 2010).). LA has been shown to be beneficial as a therapeutic agent in ischemia and reperfusion injury and diabetic complications (Glantzounis et al., 2006; Biewenga, Haenen, & Bast, 1997; Sehirli, 2008; Bilska, & Wlodek, 2005; Foster, 2007) as well as various neurologic disorders related to oxidative stress (Manda, Ueno, Moritake, & Anzai, 2007; Salinthone, Yadav, Bourdette, & Carr, 2008). Also, it was previously shown that LA has neuroprotective effects in experimental brain injury caused by trauma and subarachnoid hemorrhage (Toklu, 2009; Erşahin, 2010). It has been shown that α-lipoic acid exerts protective effects in neurodegeneration, ischemia-reperfusion, polyneuropathy, diabetes, AIDS, seizures and hepatic disorder status (Maczurek et al., 2008). Lipoic acid pretreatment can reverse cognitive dysfunction in pilocarpine –epileptic rats through increase the ChAT and AChE activities in

hippocampus (de Freitas, 2010). Moreover, Lipoic acid has an ameliorative potential on Memory impairment, oxidative damage and apoptosis induced by space radiation (Manda, Ueno, & Anzai K, 2008).

α-lipoic acid inhibit microglial activation and caspase-related apoptotic pathways (Bustamante, Slater, Orrenius, 1995). It was reported that the nuclear translocation of apoptosis-inducing factor (AIF), is involved in protective effect of lipoic acid (Selvakumar, Prahalathan, Sudharsan, &Varalakshmi, 2006).

In conclusion, our results suggest that LA pretreatment could prevent kainic acid-induced impairment of short-term spatial recognition memory in a Y-maze and learning and memory in the passive avoidance test partially via its antioxidant activity.

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