Tissue and Cell 95 (2025) 102889

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

The antioxidant and anticonvulsant effects of ellagic acid in kainic acid-induced temporal lobe epilepsy in mice

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ARTICLE INFO

Keywords:
Epilepsy
Kainic acid
Ellagic acid
Mice
Oxidative stress

ABSTRACT

Oxidative stress (OS) resulting from high levels of free radicals contributes to the initiation and progression of epilepsy. Temporal lobe epilepsy (TLE) is associated with alteration in the structure and function of the hippocampus and is modeled in mice using kainic acid (KA). In this study, the neuroprotective effect of ellagic acid (EA) on KA-induced epilepsy in mice was evaluated. Sixty male Swiss albino mice were assigned to six groups: I received normal saline (NS; 10 ml/kg, intraperitoneally (i.p.)); II, received KA (15 mg/kg, i.p.); III, received diazepam (20 mg/kg, i.p.) and KA (15 mg/kg, i.p.); IV-VI, received EA (10, 20 and 40 mg/kg, i.p.), and KA (15 mg/kg, i.p.). Treatments were done 30 min before KA injection. Seizure (latency, duration, activity) and mortality were monitored for 2 h post-injection. OS was evaluated by measuring MDA, NO, and GSH levels and CAT, SOD, and GPx activities. Levels of TNF-α in the brain tissue were measured. Furthermore, a histological examination of the hippocampus was carried out.

Results: showed that EA pretreatment caused a decline in seizure activity score and duration compared to the KA-treated group. EA pretreatment reduced mortality in KA-treated mice. EA suppressed the generation of MDA and NO; whereas it preserved GSH and the activity of GPx, SOD, and CAT. Additionally, EA exerted anti-inflammatory effects by reducing TNF-α level. Histopathologically, EA reduced KA-induced neuronal damage. EA demonstrated protective effects against KA-related epilepsy and brain damage. Due to its anti-inflammatory and radical scavenging properties, EA may be considered a potential therapeutic option in epilepsy.

1. Background

Oxidative stress (OS), along with metabolic and redox status disturbances, is a hallmark of epileptic seizure and neuronal injury progression; hence, prevention of oxidative stress using antioxidant agents has neuroprotective effects and improves epileptic seizures (Pearson-Smith and Patel, 2017; Shekh-Ahmad et al., 2019). Epilepsy is a major neurological disorder with an annual prevalence of about 50 per 100,000 of the general population, and a lifetime probability of about 3 % (Nassiri-Asl et al., 2013). There are different etiologies for epilepsy, among which OS is the main possible mechanism. Also, proinflammation is involved in epilepsy development. Kainic acid (KA) is an analog of the excitotoxin glutamate inducing seizure and hippocampal cell death by stimulating glutamate receptors. It is widely used to model seizure in rodents. KA is reported to heighten glutaminergic activity and increase reactive oxygen species (ROS) levels. OS is a potential molecular mechanism of KA-related neurotoxicity causing hippocampal cell death (Shin et al., 2007). Glutamate, the main excitatory neurotransmitter in the central nervous system, plays a critical role in epileptic seizures (Lévesque and Avoli, 2013). Elevated levels of glutamate have been linked to epilepsy in humans and experimental models (Zheng et al., 2011); therefore, glutamate receptor antagonism and glutamate release reduction by antiepileptic agents such as gabapentin and Phenobarbital show anti-epipleptic effects. Hence, affecting glutamate

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https://doi.org/10.1016/j.tice.2025.102889
Received 25 November 2024; Received in revised form 21 March 2025; Accepted 22 March 2025
Available online 28 March 2025

neurotransmission by blocking glutamate receptor function or glutamate release at nerve terminals can be a promising treatment for epilepsy (Hakami, 2021).

In about 70 % of cases, current antiepileptic drugs are effective in controlling seizures; however, the drug side effects, limited aqueous solubility, drug resistance, and inadequate gastrointestinal absorption have narrowed the use (Bialer, 2012; Margineanu and Klitgaard, 2009; Porter and Meldrum, 2001). With the rise of alternative treatments, finding natural therapeutic compounds has become essential for managing epileptic disorders (Dhingra and Jangra, 2014; El-Missiry et al., 2020).

Antioxidant compounds can potentially protect cells against OS damage resulting from free radicals. Ellagic acid (EA) (C14H6O8) is a natural phenolic component of many species of flowering plant families such as grapes, raspberries, blackberries, strawberries, and walnuts (Farbood et al., 2015; Whitley et al., 2003).

Phenolic acids and polyphenols are natural antioxidants that protect against oxidative stress and support overall health (Javaid et al., 2021; Sabbagh et al., 2023).

During the last decades, numerous investigations have been done on the antioxidant, anti-mutagen, and anti-carcinogen properties of EA (Evtyugin et al., 2020). It has a metal chelating capacity and it can directly react with free radicals and activate the cellular antioxidant enzyme system (Maas et al., 1991). Another study showed that EA reduced DNA and cell damage by protecting DNA from ROS (Vattem and Shetty, 2005). In addition to its endogenous antioxidant effect, studies have shown the exogenous antioxidant activity of EA as inhibitory potential against lipid peroxidation (LPO) in the cytochrome P450-dependent monooxygenase system, mitochondria, and microsomes (Maas et al., 1991). Following traumatic brain injury, EA has been found to have anti-inflammatory properties, as well as the ability to prevent cognitive, learning, and memory impairment. Indeed, EA exerts its anti-inflammatory properties via modulation of IL-1β and IL-6, which increase during inflammation (Farbood et al., 2015). Furthermore, EA treatment rebalanced cellular anti-oxidants and pro-oxidants in the rats' brains improving Alzheimer-associated dementia (Gupta et al., 2016).

Therefore, the purpose of this study was to investigate the effects of EA on seizure latency and duration, as well as oxidative stress and inflammation induced by KA in the brains of mice.

2. Materials and methods

2.1. Chemical agents

Trichloroacetic acid (TCA), 1,1,3,3-Tetraethoxypropane (TEP), 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB), Coomassie Blue G, Bovine Serum Albumin (BSA), Reduced glutathione (GSH), Thiobarbituric acid (TBA), Ellagic acid (EA) and KA were purchased from Sigma–Aldrich, USA. All chemical agents and reagents were of analytical grade.

2.2. Animals

Male Swiss albino mice (20–25 g; 6–8 weeks) were obtained from the animal house of Ahvaz Jundishapur University of Medical Sciences (AJUMS) and maintained in polypropylene cages at 20 ± 2℃ with a 12 h light: 12 h dark cycle. They had free access to drinking water and standard rat chow. The animals were adapted to the habitat for seven days before the experiments. The research followed the Animal Ethics Committee Guidelines, AJUMS (IR.AJUMS.ABHC.REC.1398.051).

2.3. study design

Animals were randomly allocated to six groups (n = 10 per group); (I) normal saline group (NS; 10 ml/kg, intraperitoneally (i.p.)), (II) KA (15 mg/kg in saline, i.p.) (Park et al., 2013), (III) KA (15 mg/kg in saline, i.p.) and diazepam (DZ; 20 mg/kg, i.p.), and (IV-VI) KA (15 mg/kg in saline, i.p.) and EA (10, 20 and 40 mg/kg, i.p.) groups. Treatments were performed 30 min before KA injection (Chiu et al., 2015; Lin TzuYu et al., 2015). Following the KA injection, mice were continuously monitored for mortality and seizure activity for 2 h.

2.4. Behavioral monitoring and mortality

Animals were observed in their plexiglass boxes for 2 h based on the Racine's scale: 0: no behavior arrest or convulsions; 1: facial or chewing clonus; 2: head nodding related to more severe facial clonus; 3: one-sided forelimb clonus; 4: two-sided forelimb clonus with rearing; 5: uncontrollable jumping, continuous generalized seizures, death and falling during 2 h (Kim et al., 2014). Seizure duration and onset time were recorded for all animals. Cassation of heartbeat and breathing for at least 3 minutes was considered as death in this experiment.

2.5. Sample collection

Following behavioral monitoring, the mice were sacrificed after anesthetization by ketamine/xylazine (100/10 mg/kg; i.p.), and spinal cord dislocation, and their brains were quickly removed, weighed and then homogenized (1/10 w/v) in ice-cold Tris-HCl buffer (0.1 M, pH 7.4) (Hilgenberg and Smith, 2007). The homogenates' protein content was assessed using the Bradford technique (Bradford, 1976), with crystalline BSA as standard.

2.6. Biochemical parameters assay

2.6.1. Glutathione reduced (GSH) assay

The Elman protocol was followed to measure the GSH levels in the tissue homogenate according to forming a complex turning yellow as GSH reacts with Ellman's Reagent (DTNB) (Ellman, 1959). In brief, Tris–EDTA buffer (2 ml; pH = 8.6) was mixed with 40 μL of homogenate, it was then treated with 40 μL DTNB reagent (10 mM in methanol). The reaction was continued for 20 min at room temperature and the absorption measurement was done at 412 nm by a spectrophotometer (UV-1650 PC, Japan). The resulting reads were used to extrapolate the levels of GSH from a standard curve plotted. The findings were reported as nmol/mg protein.

2.6.2. Malondialdehyde (MDA) assay

MDA was assayed according to the protocol by Buege and Aust (1978), based on the TBA color reaction. A mixture with tissue homogenate (0.5 ml) was mixed with TCA (10 %, w/v; 2.5 ml) and centrifuged for 10 min at 3000 rpm. Afterward, the supernatant (2 ml) was intermingled using TBA solution (1 ml; 0.67 %, w/v). The reaction was continued at 100 ℃ for 10 min until observing a pink color. The temperature of reaction was decreased quickly and absorbance was measured at 532 nm by spectrophotometer. A standard curve ranging from 1–10 μM of TEP was plotted and the results were extrapolated and reported as nmol/mg of

2.6.3. Enzyme activity assay

The activities of superoxide dismutase (SOD), catalase (CAT) and Glutathione peroxidase (GPx) were assessed in brain samples' supernatant as instructed (Teb Pazhouhan Razi (TPR), IRAN). SOD activity was measured by utilizing tetrazolium salt, which produces a water-soluble formazan dye upon reduction with superoxide anion. The formazan formation rate is inhibited by the presence of SOD in environments and is measurable photometrically and CAT activity was measured via the reaction of the CAT present in the sample with methanol in the presence of an optimal concentration of H_2O_2 to produce formaldehyde. The formation of formaldehyde is colorimetrically determined by using a chromogen that turns aldehydes purple. In the GPx activity kit, oxidized glutathione, produced upon reduction of peroxide substrate by glutathione peroxidase, is then recycled back to

glutathione using glutathione reductase and NADPH. In this assay, cumene hydroperoxide is used as the peroxide substrate, and so the total GPx (selenium and non-selenium containing) activity in a variety of samples can be measured.

2.6.4. Nitric oxide (NO) and TNF-α assay

NO was measured by the nitric oxide assay kit (ZellBio, Germany). In this method, the amount of nitric oxide was assessed indirectly by measuring the concentration of nitrate and nitrite that reacted to the colorant and produced an azo-pink composition. The TNF-α levels were evaluated in brain tissues by mouse TNF-α ELISA kit (IBL company; Catalog No.: IB49688).

2.6.5. Histopathological study of the hippocampus

The brains of the animals were fixed using 10 % neutral buffered formalin solution. The specimens were sectioned (5 μm) and embedded in paraffin.

The slides were stained with Hematoxylin and Eosin (H & E) and Nissl staining (Bancroft and Gamble, 2008).

Under a microscope, the prepared tissue slides were inspected in a random order. The total number of pyramidal neurons and degenerated neurons in the hippocampal cornu ammonis 1 (CA1) and dentate gyrus (DG) regions of hippocampus was counted in six microscopic fields per slide using DinoCapture imaging software (version 2.0) and Dino-Lite Digital camera at a magnification of × 400. The percentage of degenerated neurons was calculated across different groups (Tchekalarova et al., 2014).

2.7. Statistical analysis

Data analysis was done by GraphPad Prism 5 using a one-way ANOVA test followed by Tukey's post hoc test, and data were reported as Mean±SD. A P-value < 0.05 was considered significant.

3. Results

3.1. Behavioral observations

In mice treated with DZ and EA (20 and 40 mg/kg), the seizure latency significantly increased ($p < 0.05$) and seizure duration significantly decreased in DZ and all doses of EA than the KA-administrated group ($p < 0.05$). Seizure activity showed a significant reduction in DZ and EA-treated groups (20 and 40 mg/kg) compared to the KA-administrated group ($p < 0.05$). Treatment with DZ and different doses of EA increased the survival percentage compared to the KA-injected group (Fig. 1).

3.2. Markers of OS

Results in Fig. 2 showed that KA significantly elevated the NO and MDA levels in the brains of mice compared to the NS group ($p < 0.05$). EA (20 and 40 mg/kg) significantly decreased the MDA and NO levels compared to the KA-administrated group ($p < 0.05$).

The KA group showed a significant decrease in GSH levels and GPx, SOD, and CAT activity than the NS group ($p < 0.05$). EA significantly decreased KA-related decrease in GSH levels (20 and 40 mg/kg; $p < 0.05$) and CAT ((20 and 40 mg/kg; $p < 0.05$), SOD (40 mg/kg; $p < 0.05$) and GPx (20 and 40 mg/kg; $p < 0.05$) activity in the brains of

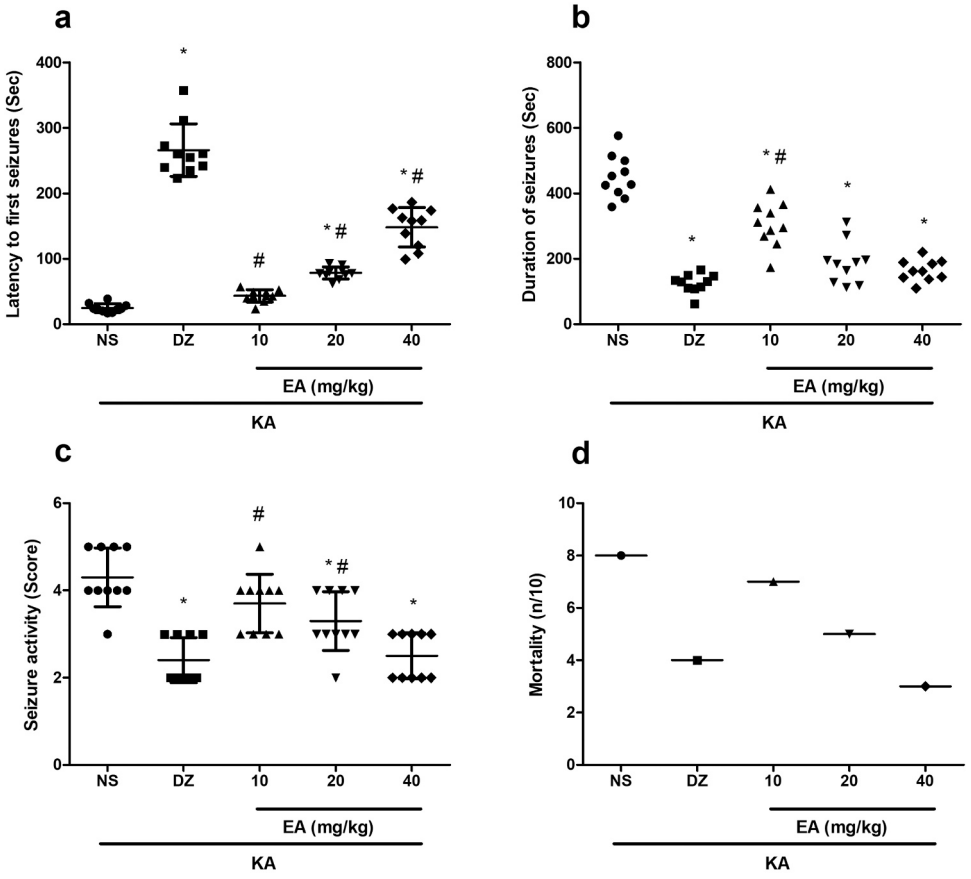

Fig. 1. The effect of EA on the latency, activity and duration of seizures and death induced by KA. Data are expressed as mean ± SD (n = 10). * Significant difference with NS group ($p < 0.05$). # Significant difference with KA+DZ group ($p < 0.05$).

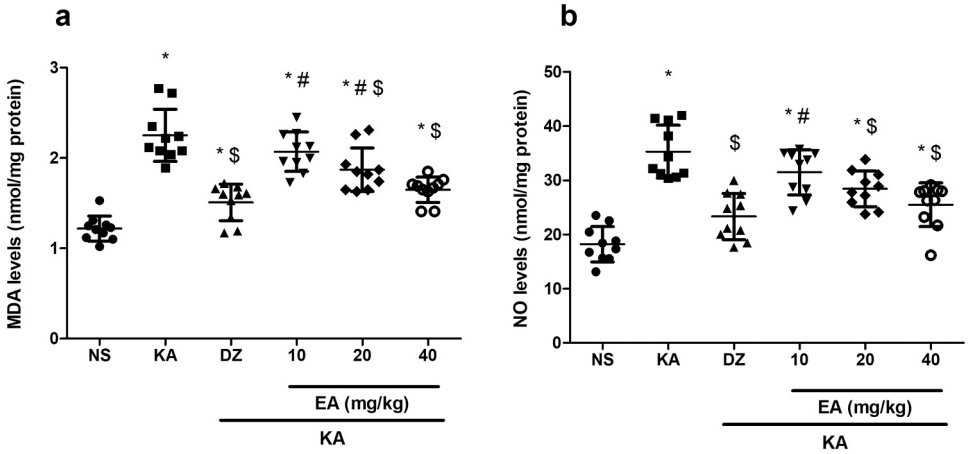

Fig. 2. The effect of EA on MDA and NO level in the brain tissues of mice injected with KA. Data are expressed as mean ± SD (n = 10). * Significant difference with NS group ($p < 0.05$). # Significant difference with KA+DZ group. $ Significant difference with KA group ($p < 0.05$).

mice (Fig. 3).

KA significantly enhanced TNF-α level compared to the NS group ($p < 0.05$). EA at all doses significantly inhibited KA-related elevation in TNF-α levels ($p < 0.05$) in the brains of mice (Fig. 4).

3.3. Histopathology of hippocampus

Histopathological analysis of the CA1 region of the hippocampus in H & E staining showed that KA significantly increased the percentage of degenerated neurons compared to the control group ($p < 0.05$).

Administration of EA at different doses reduced the percentage of degenerated neurons compared to the KA group, with significant reductions observed at all three doses ($p < 0.05$).

In Nissl staining, the DG region was examined, and the results showed a significant increase in the percentage of degenerated pyramidal neurons in the KA group compared to the control group ($p < 0.05$).

EA administration reduced the percentage of degenerated neurons compared to the KA group, with a significant reduction only at the 40 mg/kg dose ($p < 0.05$).

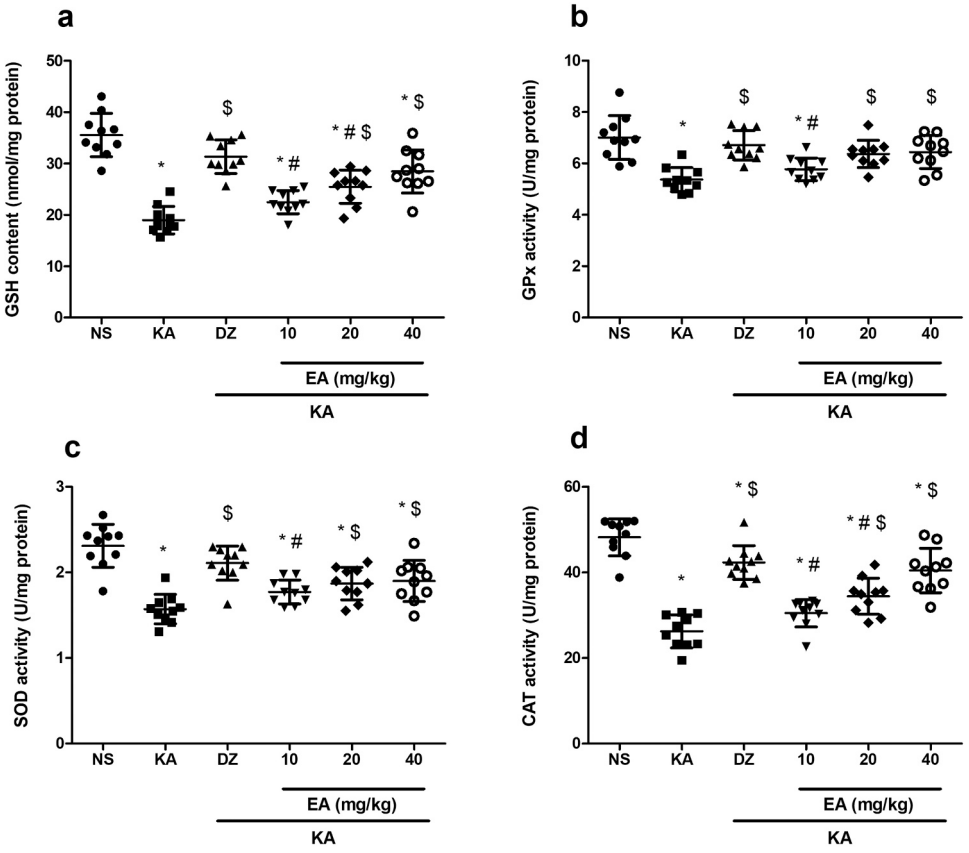

Fig. 3. The effect of EA on GSH level and CAT, SOD and GPx activities in the brain tissues of mice injected with KA. Data are expressed as mean ± SD (n = 10). * Significant difference with NS group ($p < 0.05$). # Significant difference with KA+DZ group ($p < 0.05$). $ Significant difference with KA group ($p < 0.05$).

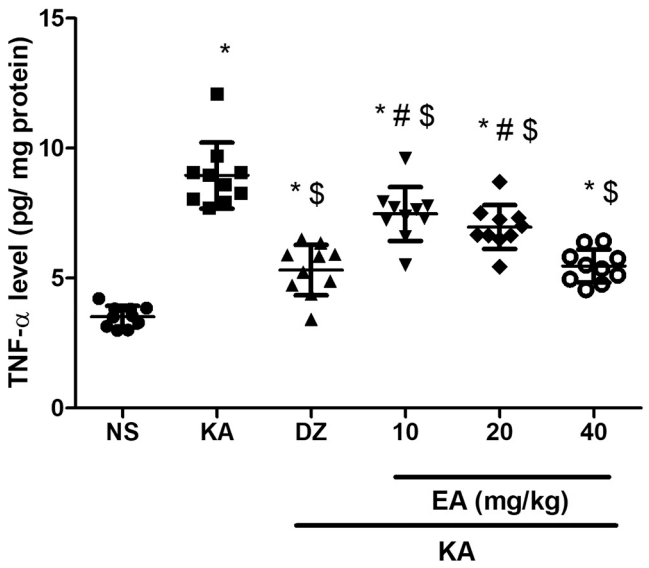

Fig. 4. The effect of EA on TNF-α level in the brain tissues of mice injected with KA. Data are expressed as mean ± SD (n = 10). * Significant difference with NS group ($p < 0.05$). # Significant difference with KA+DZ group ($p < 0.05$). $ Significant difference with KA group ($p < 0.05$).

4. Discussion

The present study found that EA significantly decreased mortality and duration of KA-induced seizures while increasing the latency to the first seizure (Fig. 1). Also, the protective effect of EA on KA-related seizure was associated with changes in anti-oxidative status. EA pretreatment could mitigate MDA and NO levels (Fig. 2) and preserve GPx, SOD, and CAT activity (Fig. 3). The obtained data indicated the cerebral anti-inflammatory properties of EA by modulating TNF-α production in brain tissue.

KA is a glutamate-related and excitotoxic chemical frequently used to induce seizures in rodent models. The underlying mechanism of the effect of KA involves the activation of glutamate receptors, which enhances glutamatergic activity. It induces neuronal excitability and lipid peroxidation due to ROS production in neurons, which in turn enhances inflammation, oxidative stress, and cell death. It can also trigger neuronal membrane depolarization through calcium release involved in nerve impulses (Hsieh et al., 2011; Lin et al., 2015).

The majority of KA-subtype receptors are found in the glial fibers and astrocytic end feet surrounding hippocampal capillaries; thus, the non-degradable excitotoxic glutamate analog can induce seizure through hippocampal cell death (Matute et al., 1994; Vargas et al., 2013). When KA activates kainate receptors, it promotes the release of potentially neurotoxic levels of glutamate, leading to an influx of Ca^{2+} and Na^{+} ions into neurons. Although mitochondria are capable of buffering considerable amounts of Ca^{2+}, excessive accumulation of Ca^{2+} can disrupt the mitochondrial oxidative phosphorylation (OXPHOS) process and lead to the overproduction of ROS (Dabbeni-Sala et al., 2001).

Although KA is 30-fold more potent than glutamate as a neuronal excitant in inducing seizures, it has been extensively applied for researching the propagation, evolution, and pathological outcomes of epileptic discharge in the limbic system (Zhang and Zhu, 2011). Our findings indicate that administering KA at a dosage of 15 mg/kg triggered epileptic seizures in mice, which aligns with earlier research using a similar status epilepticus induction procedure (Lin et al., 2015).

The brain is highly sensitive to oxidative stress. Compared to other organs in the body, it consumes the most oxygen and contains high levels of polyunsaturated fatty acids that are prone to lipid peroxidation. Additionally, the brain has low catalase activity, which is only 10 % of that found in the liver, and is rich in iron that can catalyze the generation of hydroxyl radicals (Mariani et al., 2005).

The administration of KA in mice was associated with a higher level of oxidative stress, indicated by increased lipid peroxidation as evidenced by elevated MDA and 4-hydroxy-alkenes, as well as increased NO production, alongside a decrease in GSH levels and the activities of CAT, SOD, and GPx in the brain tissue of the mice (Dabbeni-Sala et al., 2001; Shin et al., 2008). Furthermore, antiepileptic drugs can exacerbate or induce oxidative injury in epileptic patients. The decrease in GSH content and SOD, CAT, and GPx activities in the brain, liver, and kidneys of KA-injected animals suggests that oxidative stress is a significant factor in the pathophysiology of KA (Akcay et al., 2005).

KA triggers the activation of glial cells along with the production of pro-inflammatory cytokines (such as IL-1β, IL-6, and TNF-α), which contribute to neurodegeneration progression (Lin et al., 2015). It has been noted that pro-inflammatory cytokines IL-1β and TNF-α can suppress the production of the glutamate transporter protein, leading to dysfunctional glutamate uptake. This impairment in glutamate uptake causes an increase in glutamate levels, which in turn activates both NMDA and non-NMDA receptors (Oprica et al., 2003). Moreover, the increased presence of pro-inflammatory cytokines in chronic epilepsy patients indicates that neuroinflammation is involved in the pathogenesis of epileptic seizures (Wang et al., 2019). Evidence suggests that increased glutamate levels enhance the release of TNF-α, thereby regulating the excitatory effect by promoting glutamatergic transmission. Moreover, TNF-α elevates calcium influx, upregulates glutamate receptors, and stimulates the endocytosis of the GABA receptor, leading to significant neuronal excitation (Rana and Musto, 2018).

Thus, natural compounds that have anti-inflammatory and antioxidant qualities may be crucial in preventing damage to neurons prompted by seizures (Gerzson et al., 2020).

In the present study, our results showed that pretreatment with EA has neuroprotective effect through enhancing the level of GSH and the

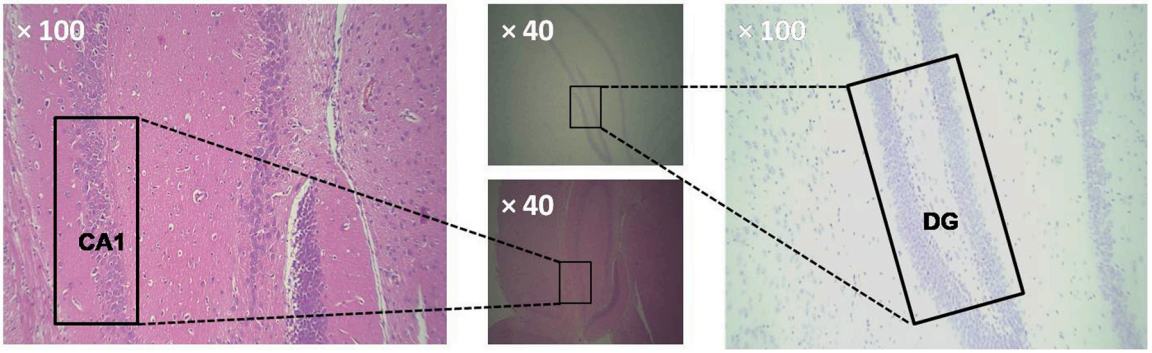

Fig. 5. Photomicrograph of examined regions in two staining methods: hippocampal cornu ammonis 1 (CA1) region in H & E staining and hippocampal dentate gyrus (DG) region in Nissl staining.

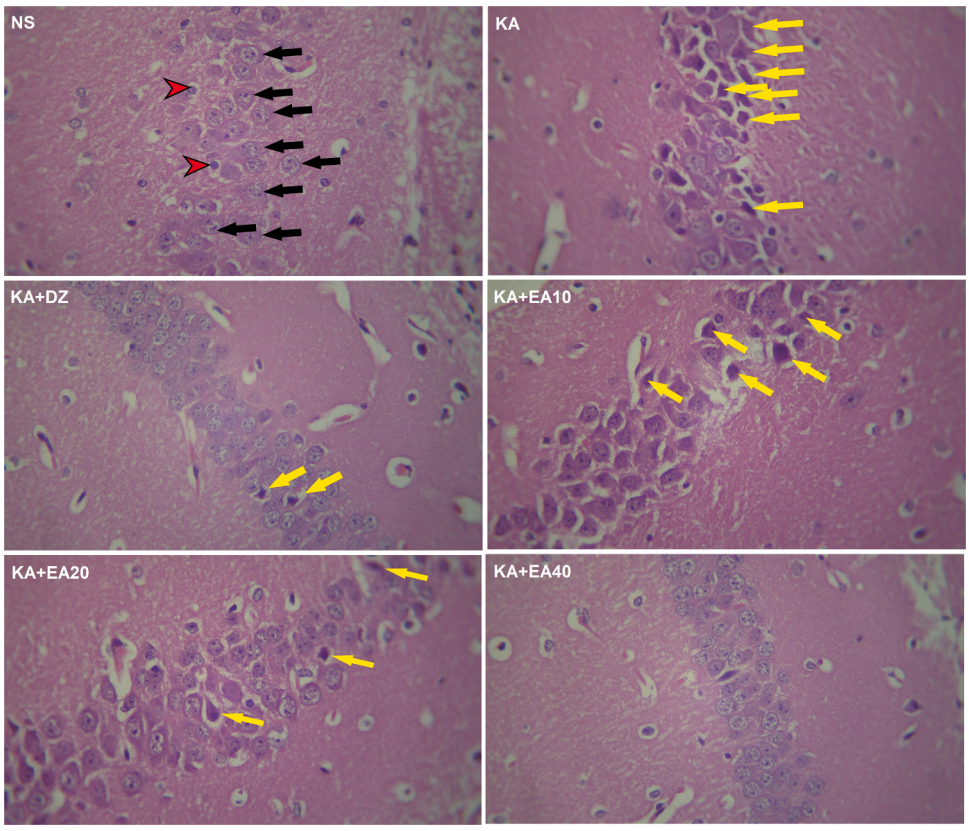

Fig. 6. Photomicrographs of hippocampal cornu ammonis 1 (CA1) area of the different groups stained with Hematoxylin and Eosin (H & E). (Magnification ×400). Black arrows shows nucleus of normal pyramidal cells, notice to vesicular form of nucleus and prominent nucleolus. Red arrowheads indicate cross section of axons. Yellow arrows shows pyknotic form of nucleus of degenerated pyramidal neurons.

activity of CAT, SOD and GPx in mice injected with EA, which was associated with the reduction of lipid peroxidation and NO and TNF-α levels. These results are consistent with previous research demonstrating the neuroprotective effect of EA. In our past investigations, we demonstrated that EA could diminish oxidative stress by lowering MDA and NO levels while boosting the efficiency of the endogenous antioxidant systems, including CAT and SOD activities, as well as GPx levels, to defend against neurotoxicity induced by acrylamide and sodium arsenate (Goudarzi et al., 2018; Goudarzi et al., 2019).

The neuroprotective effects of EA against oxidative stress can be attributed to several mechanisms, including DNA binding, reduction of ROS production, radical scavenging, and inhibition of lipid peroxidation (Sakthivel et al., 2008). Studies have shown that EA alleviates brain damage and oxidative stress by mediating Nrf2 signaling pathways. Therefore, it can be suggested that the potential mechanism for maintaining antioxidant enzyme levels is through mediating Nrf2 (Aslan et al., 2020; Xiao et al., 2022).

Our results are consistent with previous studies reporting that the anti-inflammatory effects of EA occur through the reduction of NO, MDA, and TNF-α levels and the promotion of GSH production (El-Shitany et al., 2014). EA can inhibit PGE2 and COX-2 production, as well as reduce TNF-α levels (Mansouri et al., 2015). Furthermore, other findings have demonstrated that EA can stimulate gamma-glutamylcysteine synthetase and modulate GSH levels within the brain tissue of mice (Carlsen et al., 2003). In agreement with our findings, previous studies have reported that EA exhibits anticonvulsant effects in animal models of seizures induced by pentylenetetrazole (PTZ). Studies have shown that EA reduces seizure severity and increases neuronal density in the hippocampus following PTZ administration, suggesting its neuroprotective potential (Khazaei et al., 2019; Khodabandeh et al., 2017; Rahimi-Madiseh et al., 2022). Neuronal cell death, observed in both experimental models and clinical neuroimaging studies of epilepsy patients, contributes to cognitive function impairment; (Gao et al., 2019) this damage may result from glutamate's excitotoxic effects. Excessive glutamatergic activity results in intracellular calcium overload, which subsequently activates apoptotic factors, including caspases, p53, and pro-apoptotic Bcl-2 proteins (Ono and Galanopoulou, 2012). Our histopathological results indicated that KA administration significantly increased the number of damaged neurons in the hippocampal CA1 region. Interestingly, treatment of KA-injected rats with different doses of EA significantly decreased the number of damaged neurons in the hippocampal CA1 region. This is in line with a previously published report showing that EA can reduce histopathological alterations such as encephalomalacia and focal cerebral hemorrhage induced by doxorubicin (Rizk et al., 2017).

In the present study, KA caused a two-fold increase in TNF-α level in brain tissue at the end of the evaluation. However, pretreatment with EA was found to be effective in lowering the levels to at least the same extent as DZ.

5. Conclusion

The use of natural and potent antioxidants has considerable benefits, including inhibiting the generation of ROS or at least scavenging them and increasing endogenous antioxidant defenses. EA prevents neuronal death by neutralizing ROS, reducing MDA, NO, and enhancing antioxidant defenses (GSH, SOD, CAT, GPx) via Nrf2 activation. ROS induce neuronal apoptosis by triggering mitochondrial dysfunction, cytochrome c release, and caspase-3 activation. EA counteracts this by stabilizing mitochondria, inhibiting inflammatory mediators (TNF-α, IL-1β), and preventing apoptosis, thereby protecting against KA-induced seizure and neuronal damage.

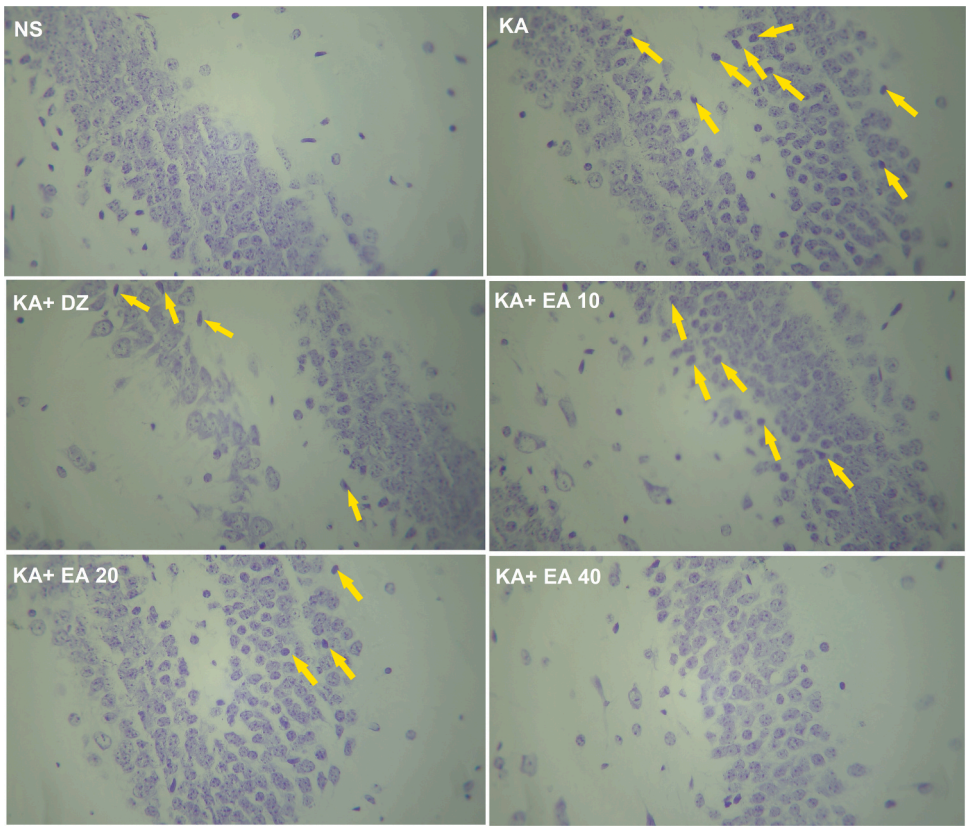

Fig. 7. Photomicrographs of hippocampal dentate gyrus (DG) area of the different groups stained with Nissl. (Magnification ×400). Yellow arrows shows pyknotic form of nucleus of degenerated pyramidal neurons.

Table 1
Percentage of degenerated neurons in six microscopic fields per slide in the CA1 and DG regions of the hippocampus across different groups. Data are expressed as mean ± SD (n = 10). * Significant difference with NS group ($p < 0.05$). # Significant difference with KA+DZ group ($p < 0.05$). $ Significant difference with KA group ($p < 0.05$).

Group	NS	KA	KA+DZ	KA+EA10	KA+EA20	KA+EA40
Percent of degenerated neurons in CA1	2.19 ± 1.04	8.80 ± 1.49*	1.41 ± 1.53$	5.45 ± 2.08*$#	3.00 ± 2.65*$	2.05 ± 3.84$
Percent of degenerated neurons in DG	0.91 ± 0.79	2.78 ± 0.78*	0.58 ± 0.20$	2.11 ± 1.27*#	1.70 ± 0.91	1.46 ± 0.95$

Ethical compliance

Ethical approval has been obtained for studies involving animal subjects, and this has been stated in the manuscript.

CRediT authorship contribution statement

Goudarzi Mehdi: Software, Formal analysis, Data curation. **Mardani Erfan:** Methodology, Investigation, Funding acquisition. **Nesari Ali:** Writing – review & editing, Writing – original draft, Validation, Software, Project administration, Methodology, Investigation, Conceptualization. **Malayeri Alireza:** Visualization, Supervision, Resources, Conceptualization. **Bakhtiari Nima:** Methodology, Formal analysis, Data curation. **Nooshabadi Mohammadreza Rashidi:** Visualization, Validation, Resources. **Sabbagh Susan:** Validation, Software, Project administration, Methodology.

Declaration of Competing Interest

There are no conflicts of interest that may affect the integrity of this work. Any potential conflicts have been disclosed.

Acknowledgement

This work was supported and funded at the grant number MPRC-9820 provided by the Deputy of Research of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Proper citation

All sources of information, data, and ideas from other works have been properly cited.

Data availability

Data will be made available on request.

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