Archives of Epilepsy

Original Article

Protective Effects of Allantoin on Neural Cells of Hippocampal Region and Cognitive Function in a Mouse Model of Temporal Lobe Epilepsy

Mohsen Eslami Farsani[1], Ahmad Hatami Shahmir[2], Reihaneh Seyedebrahimi[1], Shima Ababzadeh[3], Azam Moslehi[4], Javad Tafaroji[5]

[1]Qom University of Medical Sciences Faculty of Medicine, Department of Anatomy, Qom, Iran

[2]Qom University of Medical Sciences Faculty of Medicine, Medical Student, Student Research Committee, Qom, Iran

[3]Qom University of Medical Sciences Faculty of Medicine, Department of Tissue Engineering and Applied Cell Sciences, Qom, Iran

[4]Qom University of Medical Sciences, Cellular and Molecular Research Center, Qom, Iran

[5]Qom University of Medical Sciences Faculty of Medicine, Hazrat-e-Masoumeh Hospital, Clinic of Pediatrics, Qom, Iran

Shima Ababzadeh PhD

Cite this article as: Farsani ME, Shahmir AH, Seyedebrahimi R, Ababzadeh S, Moslehi A, Tafaroji J. Protective Effects of Allantoin on Neural Cells of Hippocampal Region and Cognitive Function in a Mouse Model of Temporal Lobe Epilepsy. *Arch Epilepsy.* 2024;30(1):1-6

Corresponding Author: Shima Ababzadeh MD, E-mail: shimaababzadeh@gmail.com

Received: 20.09.2022 **Accepted:** 07.08.2023 **Publication Date:** 13.03.2024

DOI: 10.4274/ArchEpilepsy.2023.23058

Abstract

Objective: Excitotoxic damage results in cell death in several human neurodegenerative diseases. Epilepsy is one of the most common neurological disorders and it causes complications. To this end, we examined the protective role of allantoin (AL) in a model of excitotoxic neuronal death induced by intraperitoneal injection of kainic acid (KA) in mice.

Methods: Two-month-old C57 male mice (n=35) were divided into five groups: control (received 0.9% saline), AL (received 10 mg/kg of AL), KA (received a single dose of 10 mg/kg of KA), KA+AL10 and KA+AL15 groups (received a single dose of 10 mg/kg of KA then were treated with 10 mg/kg of AL or 15 mg/kg respectively). On the 14th day of the experiment, learning and memory were tested using a shuttle-box. The number of intact neurons and damaged neural cells in hippocampal CA1 and CA3 were assessed by Nissl staining and Flour-J B immunohistochemistry, respectively.

Results: The results showed that injection of KA decreased the latency time while administration of 10 mg/kg AL improved memory. In addition, there was a significant difference between KA and KA+AL10 mg/kg groups ($p<0.01$). The histological results revealed that the use of 10 mg/kg AL significantly increased the number of intact pyramidal cells in the hippocampal CA1 and CA3 regions compared with the KA group ($p<0.01$). Moreover, neurodegeneration of the hippocampal region was significantly decreased in the groups treated with AL compared with the KA group ($p<0.05$).

Conclusion: This study indicated that AL at a concentration of 10 mg/kg/day improved neurodegenerative complications following temporal lobe epilepsy.

Keywords: Neurotoxicity, kainic acid, allantoin, learning and memory, hippocampus

INTRODUCTION

Excitotoxicity is one of the most important causes of neuronal cell death that occurs in many human neurodegenerative diseases such as Alzheimer's disease, multiple sclerosis, Parkinson's disease, epilepsy, and ischemia.[1]

Epilepsy is a neurological disorder that is determined by loss of consciousness and seizure.[2] Seizures result in neuronal death by overactivating glutamate receptors in the brain. 8Glutamate is activated as a chief excitatory neurotransmitter in the mammalian central nervous system (CNS), and it plays many roles in neural progress, synaptic plasticity, learning, and memory.[3] However, the high concentrations of this neurotransmitter and overactivation of its receptors interrupt calcium homeostasis in neural cells, so it can increase production of nitric oxide and free radicals.[4] In addition, high activation of glutamate excites some death signaling pathways through stimulation of metabotropic and ionotropic receptors, and it, finally, causes extensive neuronal complications.[5]

Kainic acid (KA)-induced model is one of the experimental models of epileptic status that KA is widely used in temporal lobe epilepsy (TLE). KA can induce neuronal injuries through the activation of ionotropic glutamate receptor.[6,7] Moreover, KA is a glutamate analog

that can act similar to patients with TLE.[8] Although the pyramidal neurons of the hippocampus region are damaged in TLE, GABAergic interneurons of the hippocampus and granule cells of the dentate gyrus are persistent to seizure.[9]

Despite the progress of many antiepileptic drugs, a number of patients do not respond to treatment. In addition, antiepileptic drugs have severe side effects.[10]

Allantoin (AL) (5-ureidohydantoin) is a heterocyclic derivative of purine that is found in many plants such as Melaleuca nodosa roots, comfrey, yam, sugar beet, and leguminous.[11,12] In addition, it is safe to stimulate new tissue development and has a positive effect on neuronal cell proliferation.[13] Moreover, anti-inflammatory, anti-hypertensive, anti-nociceptive, anti-ulcerogenic, and anti-asthmatic properties of AL have been AL.[14]

Therefore, we decided to assess the neuroprotective effects of AL on cell death and neurogenesis following KA-induced excitotoxicity damage in mice.

METHODS

AL (Sigma Aldrich-05670, 25 g), KA (TOCRIS-0222, 10 mg), and cresyl violet acetate were purchased from Sigma. Fluoro-Jade B (FJB) (Millipore-AG310) was obtained from Abcam. In addition, phosphate-buffered saline, paraformaldehyde, xylazine, and ketamine were supplied by Invitrogen.

Animals

Two-month-old C57 male mice (weight 20-25 g) were purchased from the Animals Care Center of Qom University of Medical Sciences. The animals were housed under stable conditions with a suitable temperature (22±1 °C) and a 12/12 h light/dark cycle. Mice had free access to water and food.[15]

All mice were randomly divided into five groups (n=7 for each group) including control group (receive 0.9% normal saline), AL group (injection of 10 mg/kg AL), KA group (injection a single dose of 10 mg/kg KA), treatment groups including injection a single dose of 10 mg/kg KA and treatment with 10 mg/kg (KA + AL10) or 15 mg/kg AL (KA + AL15). To induce the epilepsy model, mice received a single dose of 10 mg/kg KA intraperitoneally (IP) in which KA was dissolved in 0.9% normal saline. Seizures were evaluated and graded according to the Racine scale after administration of KA.[16,17] Seizures were evaluated and divided into 6 grades according to the Racine scale.[16] The mice were evaluated approximately 24 h after administration of KA for status epilepticus (SE). Only animals that reached the fourth stage of the Racine scale were selected for this study. Each seizure lasted greater than 1-2 min when mice were in the fourth stage and terminated abruptly following the stopping of abnormal movements. In addition, the animals were monitored for behavioral progression of seizure 5 h/day during the treatment period.

MAIN POINTS

- The injection of kainic acid decreased the latency time and induced a model of epilepsy in mice.
- The administration of allantoin (AL) at a concentration of 10 mg/kg improved neuronal degeneration in an epilepsy model.
- The use of 15 mg/kg AL has a low effect on neuronal injuries.

To treatment, two groups of mice were administered AL in concentrations of 10 or 15 mg/kg as IP on the next day of induction of epilepsy during 14 days. In addition, AL was solved in 0.9% normal saline. All procedures of this study were approved by the Ethics Committee of the Qom University of Medical Sciences (ethical no: IR.MUQ.REC. 1395.161, date: 14.03.2017).

Shuttle-box Test (Passive Avoidance) for Learning and Memory

The shuttle-box test is used to evaluate CNS disorders in rodent models. This test was performed 14th days after the start of treatment for evaluation of the short and long memories based on previous studies.[18] The passive avoidance apparatus has two lighted and darkened compartments with 20×20×20 cm dimensions (Borj Sanat Company, Tehran, Iran). A guillotine door is located between the two compartments. The lighted compartment contained a 40 W bulb, whereas the darkened compartment was composed of 2 mm stainless steel rods spaced 1 cm apart. The avoidance test was performed in two trials: a training trial and a test trial 24 h later. Each animal was placed in the lighted compartment and could move freely to the dark compartment. When animals entered the dark compartment, an electric shock was received (0.5 mA, 1 s). One day after the training trial, a testing trial was performed in which the animals were again placed in the lighted compartment and the experiment was repeated. To evaluate memory and learning, the time taken by the animal to enter the dark chamber was recorded as the step-through latency.

Histological Study of the Hippocampus Region by Nissl Staining

Evaluation of neurogenesis and survival of hippocampal cells was performed using Nissl staining.[19] The animals were anesthetized following intraperitoneal injection of xylazine (10 mg/kg) and ketamine (100 mg/kg). Perfusion was then performed using 4% paraformaldehyde in 0.1 mol/L phosphate buffer solution (pH: 7.4). Following tissue processing and embedding, coronal sections (4 µm thickness) were cut using a microtome rotary (LEICA RM 2235, Germany) and stained with Nissl (cresyl violet acetate: 0.01%). Sections were visualized under an optical microscope (Eclipse E200-LED, Tokyo, Japan). Then, the four sections of each sample at 400×magnification were selected to count pyramidal neurons and measure granular layer thickness in the CA1 and CA3 regions of the hippocampus. Finally, three fields in each section were evaluated using ImageJ analysis software.

Evaluation of Degeneration of Neurons Using Fluoro-Jade B Staining

To detect neuronal degeneration, FJB staining was performed according to previously reported methods. First, the 4 µm sections were deparaffinize and dehydrated. The samples were immersed in descending alcohols and 1% sodium hydroxide solution. Then, they were stained with FJB (0.002%). Finally, the samples were examined using a fluorescence microscope with blue light and a FITC filter (450-490 nm).

Statistical Analysis

The data were presented as mean±standard deviation and statistically analyzed using one-way analysis of variance and Tukey's post-hoc test using Statistical Package for the Social Sciences (SPSS) 26 software (SPSS Inc., Chicago, IL, USA). Statistical significance was determined at $p<0.05$.

RESULTS

Seizure Activity by Behavior Observation

The animal's behavior was assessed during the 24 h after KA administration according to Racine's standard classification. There was no seizure status in the control and AL groups, whereas spontaneous seizures with 4 and 5 stages were observed in 100% of the mice treated with KA.

The control and AL groups showed no signs of seizure behavior, whereas 98% of mice had several seizures with 4 and 5 classes based on the Racine scale during treatment. However, administration of AL at doses of 10 and 15 mg/kg significantly decreased the intensity of seizure compared with the AL group during treatment ($p<0.05$) (Table 1).

Effect of Kainic Acid and Allantoin on Mouse Weight

The results showed that there was a significant increase in the final weight compared with the initial weight in the control group, while a decrease was observed in the final weight of mice in the KA group ($p\leq0.05$) (Table 2).

Passive Avoidance in the Evaluation of Memory

There was a significant increase in latency time in the control group compared with the KA group ($p<0.01$), whereas there was no significant difference between the group treated with 15 mg/kg of AL and the KA group ($p\geq0.05$). Also, treatment with 10 mg/kg of AL resulted in an increase in latency time compared with the KA group ($p<0.01$) (Figure 1).

Preservation of Neuronal Cells in the Seizure-induced Hippocampal Region by Allantoin Treatment

Nissl staining results indicated that KA decreased the number of neuronal cells in the CA1 region (Figure 2). The mean number of intact cells was significantly increased following injection of 10 mg/kg of AL compared with the KA group ($p<0.01$). There were no significant differences between the group treated with AL and the control group ($p\geq0.05$). In addition, treatment with 10 mg/kg of AL increased the thickness of the granular layer compared with the KA group ($p<0.01$), whereas there was no significant difference between mice receiving 15 mg/kg AL and the KA group ($p\geq0.05$).

Next, the histological results of the CA3 region are shown in Figure 3A. There was a significant reduction in the mean number of intact cells in the KA group compared with the other groups ($p<0.05$) (Figure 3B). Moreover, the mean of degenerated neurons significantly increased after induction of epilepsy in the KA group compared with other groups ($p<0.01$) (Figure 3C). However, administration of AL decreased the mean number of degenerated cells compared with the KA group ($p<0.05$). The average thickness of the granular layer of the CA3 region also significantly increased in mice treated with AL compared with the KA group ($p<0.05$) (Figure 3D).

Allantoin Reduces Seizure-induced Hippocampal Neuronal Death

Degeneration of neurons was evaluated by FJB staining (Figure 4A). In the control and AL groups, no neurons were stained with FJB in hippocampal CA1. However, the number of degenerated neurons increased in the CA1 region in mice induced with KA. The number of FJB-positive cells (degenerated neurons) was rarely detected in the group treated with 10 mg/kg of AL, whereas there was more neuronal damage in the group treated with 15 mg/kg of

Table 1. Numbers and percentages of epileptic status in each group

Groups	% of mice that reached the 4th stage in 24 h	Number of seizures in 24 h	% of mice that reached the 4th stage during treatment	Number of seizures during treatment
Control	0	0	0	0
AL	0	0	0	0
KA	100	3.8±1.32	98.2	6.8±2.4
KA+AL10	100	4±0.63	41.6*	5.2±0.74*
KA+AL15	100	3.4±1.01	52.32*	3.8±1.6*

*P<0.05 compared with the KA group.
KA: Kainic acid, AL: Allantoin

Table 2. Comparison of average initial and final weight mice in different groups

Groups	Initial weight	Final weight	p value
Control	19.87±0.72	21.52±0.86	p=0.042
AL	20.82±1.24	21.29±1.16	p=0.3
KA	19.79±0.73	18.71±0.77	p=0.002
KA+AL10	21.95±0.94	21.62±0.39	p=0.124
KA+AL15	22.84±0.77	22.36±0.58	p=0.7

KA: Kainic acid, AL: Allantoin

AL (Figure 4B). Also, there was a significant difference between the KA group and other groups ($p<0.01$), except for the group treated with 15 mg/kg AL ($p\geq0.05$).

DISCUSSION

Epilepsy is a neurological disease with numerous unpredicted seizures. Due to extensive neuronal damage, continued SE can be life-threatening.[20]

KA acts similar to major excitatory neurotransmitters in the CNS and increases neuronal excitability[21]; thus, KA probably causes brain inflammation by increasing the expression levels of inflammatory cytokines and oxidative stress.[22] Additionally, administration of KA promotes the release of large amounts of neurotoxic substances, leading to the influx of $Ca2^{+}$ and Na^{+} in the neurons in brain tissue.[23] Thus, administration of KA can induce SE, inducing neuronal death, especially in the hippocampal CA1 and CA3 regions.[24] In this study, KA induced a model of epileptic status in mice, and a significant decrease of memory and destruction of CA1 and CA3 regions was observed after injection of a single dose of KA.

Because regeneration of neuronal cells is extremely limited[25] and there is drug resistance during treatment for traumatic and degenerative brain injuries, the use of neuroprotective agents can prevent neuronal damage during the seizure process.[26] AL as a

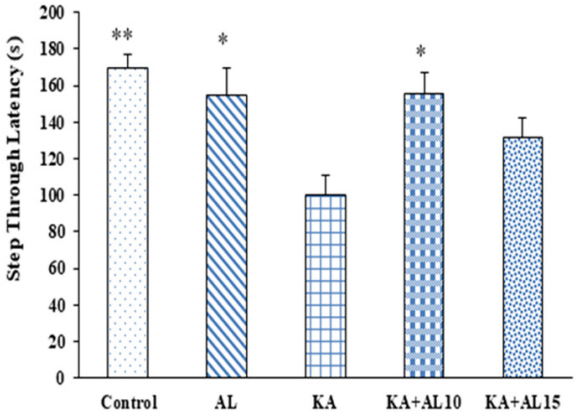

Figure 1. The comparison between the mean latency time to dark room in different groups (*p<0.05, **p<0.01 compared with KA group)
KA: Kainic acid, AL: Allantoin

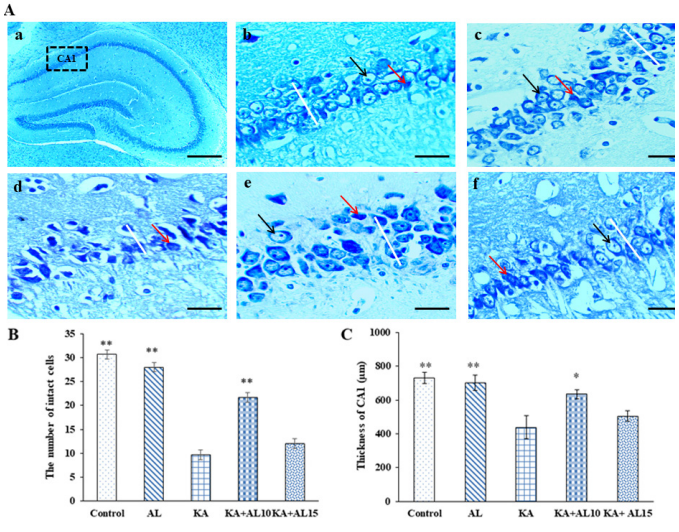

Figure 2. (A) Photomicrograph show CA1 area of hippocampus in control group with ×4 magnification; scale bar is 500 μm in Figure A. (a), control (b), AL (c), KA (d), KA+AL10 (e), KA+AL15 (f) groups with ×40 magnification; scale bar is 50 μm in Figures b-f. Intact neurons (black arrow), degenerated neurons (red arrow) and thickness of granular layer of CA1 (white line). (B) Mean of the number of intact granular cells of CA1 region of hippocampus in different groups. (C) The comparison of thickness of granular layer of CA1 among different groups (*p<0.05, **p<0.01 compared with KA group)
KA: Kainic acid, AL: Allantoin

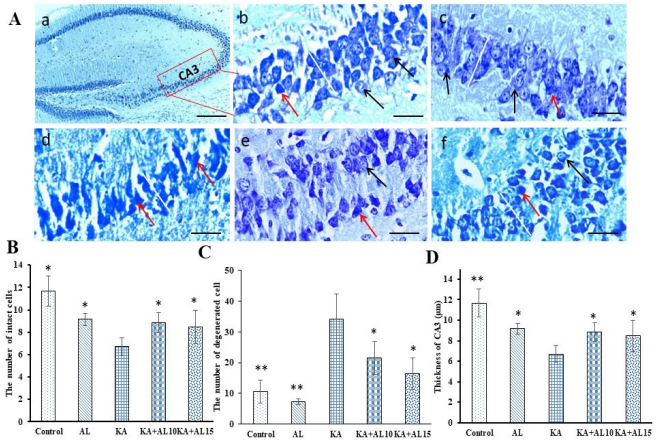

Figure 3. (A) Photomicrograph show CA3 area of hippocampus in control group with ×4 magnification; scale bar is 500 μm in Figure A. (a), control (b), AL (c), KA (d), KA+AL10 (e), KA+AL15 (f) groups with ×40 magnification; scale bar is 50 μm in Figures b-f. Intact neurons (black arrow), degenerated neurons (red arrow) and thickness of granular layer of CA3 (white line). (B) Mean of the number of intact cells of CA3 region of hippocampus in different groups. (C) Mean of the number of degenerated cells of CA3 region of hippocampus in different groups. (D) The comparison of thickness of granular layer of CA3 among different groups (*p<0.05, **p<0.01 compared with KA group)
KA: Kainic acid, AL: Allantoin

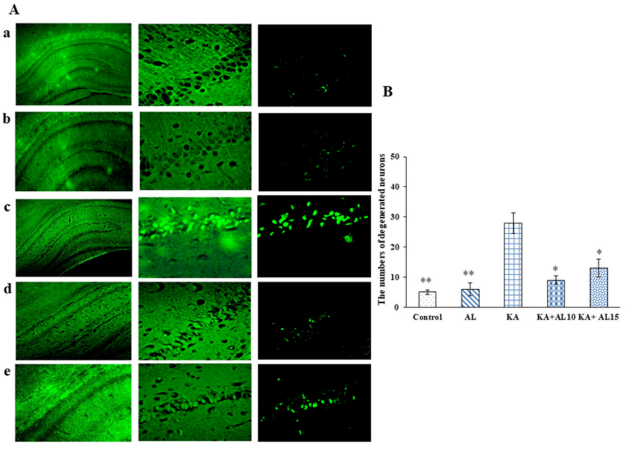

Figure 4. (A) Effect of AL on neurodegeneration in the CA1 area of hippocampus in different groups: control (a), AL (b), KA (c), KA+AL10 (d), KA+AL15 (e). The FJB positive cells exposed with green color. (B) The mean of the number of the degenerated neurons of CA1 region of hippocampus in different groups (*p<0.05, **p<0.01 compared with KA group)
KA: Kainic acid, AL: Allantoin

neuroprotective agent may recover faults of motor nerve conduction velocity and can defend against cisplatin-induced neuropathy.[27]

Many studies have indicated that the level of uric acid significantly increases in epilepsy patients.[28-30] Untreated epilepsy may chronically raise the level of uric acid in patients. In addition, it was proved that administration of KA can increase the level of uric acid in the brain in animal models for epilepsy.[28] Therefore, uric acid can be used as a biomarker in the prognosis and treatment of epilepsy. In fact, a reduction in uric acid levels can be valuable in suppressing clinical symptoms of epilepsy, such as seizure severity and frequency.[31] However, AL acts as a mediator in uric acid metabolism. It can pass through the blood-brain barrier and plays an essential role in the treatment of different neurodegenerative diseases.[32] Also, it may decrease the epileptic attacks through a decrease in uric acid levels.

A study showed that AL suppresses neuronal apoptosis by inhibiting the mitochondrial apoptotic pathway and decreasing the levels of oxidative stress and inflammatory cytokines. Therefore, it plays a high therapeutic role in brain injury.[33] AL has a high affinity to bind to the imidazoline receptors, and this property appears to prevent KA-induced seizures by preventing neuronal cell apoptosis.[34] In addition, another study showed that imidazoline agonists can be a valuable tool in the treatment of neurodegenerative disorders.[35]

This study showed that injection of 10 mg/kg AL improved memory and the thickness of hippocampus regions (CA1 and CA3), whereas the use of 15 mg/kg AL had a low effect on neuronal injuries. In addition, a study demonstrated the effect of different concentrations of AL on gastritis and determined that 12.5 mg/kg of AL provides better results on treatment.[36] Another study showed that intraperitoneal injection of AL may reduce Tau protein phosphorylation by activating the PI3K/Akt/GSK-3β signaling pathway in a rat model of Alzheimer disease. In addition, it was reported that administration of AL at 10 mg/kg concentration had a beneficial effect on improving memory impairment compared with other concentrations.[37] Therefore, the function of AL is probably dose-dependent.

Generally, 10 mg/kg AL presented better results compared with 15 mg/kg concentration, and this is a main issue that low dose has fewer side effects.

Study Limitations

The aim of this study was to investigate the effects of AL on an epilepsy model in mice that accessed the memory and histopathology of the hippocampal region. However, due to lack of budget, the limitations of this study were the failure to investigate the many genes involved in epilepsy in real time and the inability to stain many protein markers in the tissue by immunohistochemistry.

CONCLUSION

This study demonstrated that injection of KA can induce a model of TLE and that administration of AL at a specified dose had positive effects on memorial functions and the improvement of neurodegeneration in the hippocampal region following neuronal injury.

Acknowledgment

The authors are grateful to Qom University of Medical Sciences for their financial support.

Ethics

Ethic Committee Approval: The ethical approval of the study was taken from the Qom University of Medical Sciences, Ethics Committee in Biomedical Research (decision no: IR.MUQ.REC.1395.161, 1395.161, date: 14.03.2017).

Informed Consent: Animal experiment.

Authorship Contributions

Concept: M.E.F., S.A., Design: M.E.F., S.A., Data Collection or Processing: A.H.S., R.S., A.M., Analysis or Interpretation: A.H.S., R.S., A. M., Literature Search: A.M., S.A., J.T., Writing: M.E.F., R.S., S.A.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study is financially supported by Qom University of Medical Sciences.

REFERENCES

1. Prentice H, Modi JP, Wu JY. Mechanisms of Neuronal Protection against Excitotoxicity, Endoplasmic Reticulum Stress, and Mitochondrial Dysfunction in Stroke and Neurodegenerative Diseases. *Oxid Med Cell Longev.* 2015;2015:964518. [Crossref]
2. Laxer KD, Trinka E, Hirsch LJ, et al. The consequences of refractory epilepsy and its treatment. *Epilepsy Behav.* 2014;37:59-70. [Crossref]
3. Wang Y, Qin ZH. Molecular and cellular mechanisms of excitotoxic neuronal death. *Apoptosis*. 2010;15(11):1382-1402. [Crossref]
4. Dong XX, Wang Y, Qin ZH. Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacol Sin.* 2009;30(4):379-387. [Crossref]
5. Mehta A, Prabhakar M, Kumar P, Deshmukh R, Sharma PL. Excitotoxicity: bridge to various triggers in neurodegenerative disorders. *Eur J Pharmacol.* 2013;698(1-3):6-18. [Crossref]
6. Zhang XM, Zhu J. Kainic acid-induced neurotoxicity: targeting glial responses and glia-derived cytokines. *Curr Neuropharmacol.* 2011;9(2):388-398. [Crossref]
7. Kienzler-Norwood F, Costard L, Sadangi C, et al. A novel animal model of acquired human temporal lobe epilepsy based on the simultaneous administration of kainic acid and lorazepam. *Epilepsia*. 2017;58(2):222-230. [Crossref]
8. Lévesque M, Avoli M. The kainic acid model of temporal lobe epilepsy. *Neurosci Biobehav Rev.* 2013;37(10):2887-2899. [Crossref]
9. Grooms SY, Opitz T, Bennett MV, Zukin RS. Status epilepticus decreases glutamate receptor 2 mRNA and protein expression in hippocampal pyramidal cells before neuronal death. *Proc Natl Acad. Sci.* 2000;97(7):3631-3636. [Crossref]
10. Wahab A. Difficulties in treatment and management of epilepsy and challenges in new drug development. *Pharmaceuticals.* 2010;3(7):2090-2110. [Crossref]
11. Chen MF, Tsai JT, Chen LJ, et al. Antihypertensive action of allantoin in animals. *Biomed Res Int.* 2014;690135. [Crossref]
12. da Silva DM, Martins JLR, de Oliveira DR, et al. Effect of allantoin on experimentally induced gastric ulcers: pathways of gastroprotection. *Eur J Pharmacol.* 2018;821:68-78. [Crossref]
13. Ahn YJ, Park SJ, Woo H, et al. Effects of allantoin on cognitive function and hippocampal neurogenesis. *Food Chem Toxicol*. 2014;64:210-216. [Crossref]
14. Florentino IF, Silva DP, Galdino PM, Lino RC, Martins JL, Silva DM, et al. Antinociceptive and anti-inflammatory effects of Memora nodosa and allantoin in mice. *J Ethnopharmacol.* 2016;186:298-304. [Crossref]

15. McGrath J, Drummond G, McLachlan E, Kilkenny C, Wainwright C. Guidelines for reporting experiments involving animals: the ARRIVE guidelines. *Br J Pharmacol.* 2010;160(7):1573-1576. **[Crossref]**
16. Bertoglio D, Amhaoul H, Van Eetveldt A, et al. Kainic acid-induced post-status epilepticus models of temporal lobe epilepsy with diverging seizure phenotype and neuropathology. *Front Neurol.* 2017;8:588 **[Crossref]**
17. Pan G, Chen Z, Zheng H, et al. Compensatory mechanisms modulate the neuronal excitability in a kainic acid-induced epilepsy mouse model. *Front Neural Circuits.* 2018;12:48. **[Crossref]**
18. Esmaeilpour K, Sheibani V, Shabani M, Mirnajafi-Zadeh J, Akbarnejad Z. Low frequency stimulation reverses the kindling-induced impairment of learning and memory in the rat passive-avoidance test. *Basic Clin Neurosci*. 2018;9(1):51-58. **[Crossref]**
19. Hamidizad Z, Ababzadeh S, Heidari F, Haeri N, Eslami Farsani M, Sadegh M. Cobalamin modulate neurotoxic effects of trimethyltin chloride on hippocampus neural cells and cognitive function. *Physiol Pharmacol*. 2019;23:82-90. **[Crossref]**
20. Legriel S. Hypothermia as a treatment in status epilepticus: A narrative review. *Epilepsy Behav*. 2019;101:106298. **[Crossref]**
21. Pan G, Chen Z, Zheng H, et al. Compensatory mechanisms modulate the neuronal excitability in a kainic acid-induced epilepsy mouse model. *Front Neural Circuits.* 2018;12:48. **[Crossref]**
22. Huang W, Zhu L. MiR-134 expression and changes in inflammatory cytokines of rats with epileptic seizures. *Eur Rev Med Pharmacol Sci.* 2018;22(11):3479-3484. **[Crossref]**
23. Hasanvand A, Hosseinzadeh A, Saeedavi M, Goudarzi M, Basir Z, Mehrzadi S. Neuroprotective effects of tannic acid against kainic acid-induced seizures in mice. *Hum Exp Toxicol.* 2022;1;41:9603271221093989. **[Crossref]**
24. Tan KN, Simmons D, Carrasco-Pozo C, Borges K. Triheptanoin protects against status epilepticus-induced hippocampal mitochondrial dysfunctions, oxidative stress and neuronal degeneration. *J Neurochem.* 2018;144(4):431-442. **[Crossref]**
25. Nocera G, Jacob C. Mechanisms of Schwann cell plasticity involved in peripheral nerve repair after injury. *Cell Mol Life Sci.* 2020;77(20):3977-3989. **[Crossref]**
26. Luft JG, Steffens L, Morás AM,et al. Rosmarinic acid improves oxidative stress parameters and mitochondrial respiratory chain activity following 4-aminopyridine and picrotoxin-induced seizure in mice. *Naunyn Schmiedebergs Arch Pharmacol.* 2019;392(11):1347-1358. **[Crossref]**
27. NS V, Mohamad A, Razdan R. Allantoin attenuates deficits of behavioural and motor nerve conduction in an animal model of cisplatin-induced neurotoxicity in rats. *AMEM.* 2019;2(2):114-120. **[Crossref]**
28. Thyrion L, Raedt R, Portelli J,et al. Uric acid is released in the brain during seizure activity and increases severity of seizures in a mouse model for acute limbic seizures. *Exp neurol.* 2016;277:244-251. **[Crossref]**
29. Wang D, Hu B, Dai Y, Sun J, Liu Z, Feng Y, Cheng F, Zhang X. Serum Uric Acid Is Highly Associated with Epilepsy Secondary to Cerebral Infarction. *Neurotox Res.* 2019;35(1):63-70. **[Crossref]**
30. Patel C, Wilson CP, Ahmed N, Hattab Y. Acute Uric Acid Nephropathy following Epileptic Seizures: Case Report and Review. *Case Rep Nephrol*. 2019;2019:4890287. **[Crossref]**
31. Ismail MH, Kamel AE, Sobhy E, Shehta N. The Relation between Uric Acid and Epilepsy. *ZUMJ.* 2022;28(5):1127-1134. **[Crossref]**
32. Terpstra BT, Sortwell CE. Allantoin administration for the treatment of neurodegenerative disease and neurotrauma. *Patents*. 2012:11. **[Crossref]**
33. Wang X, Lan YL, Xing JS, Lan XQ, Wang LT, Zhang B. Alantolactone plays neuroprotective roles in traumatic brain injury in rats via anti-inflammatory, anti-oxidative and anti-apoptosis pathways. *Am J Transl Res*. 2018;10(2):368-380. **[Crossref]**
34. Tsai CC, Chen LJ, Niu HS, Chung KM, Cheng JT, Lin KC. Allantoin activates imidazoline I-3 receptors to enhance insulin secretion in pancreatic β-cells. *Nutrition & Metabolism.* 2014;11(1):1-7. **[Crossref]**
35. Keller B, García-Sevilla JA. Inhibitory effects of imidazoline receptor ligands on basal and kainic acid-induced neurotoxic signalling in mice. *J Psychopharmacol.* 2016;30(9):875-886. **[Crossref]**
36. Eslami-Farsani M, Moslehi A, Hatami-Shahmir A. Allantoin improves histopathological evaluations in a rat model of gastritis. Physiol Int. 2018;105(4):325-334. **[Crossref]**
37. Tzeng CY, Lee WS, Liu KF,et al. Allantoin ameliorates amyloid β-peptide-induced memory impairment by regulating the PI3K/Akt/GSK-3β signaling pathway in rats. *Biomed Pharmacother.* 2022; 153:113389. **[Crossref]**