Saudi Journal of Biological Sciences 28 (2021) 4247–4254

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Amomum tsaoko fruit extract exerts anticonvulsant effects through suppression of oxidative stress and neuroinflammation in a pentylenetetrazol kindling model of epilepsy in mice

Kaina Wang [a], Yani Liu [b], Yan Shi [c], Mingzhu Yan [c], Thamaraiselvan Rengarajan [d], Xin Feng [b,*]

[a] Department of Neurology, Xi'an Hospital of Traditional Chinese Medicine, Xi'an 710021, China
[b] Department of Neurology, Xi'an Yanliang District People's Hospital, Xi'an 710089, China
[c] Department of Neurology, Xijing Hospital, Air Force Medical University,Xi'an, Shaanxi 710032, China
[d] Scigen Research and Innovation Pvt. Ltd., Periyar Technology Business Incubator, Thanjavur, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 14 May 2021
Revised 27 May 2021
Accepted 2 June 2021
Available online 9 June 2021

Keywords:
Amomum tsaoko
Pentylenetetrazol
Na+K+ATPase
Seizure
Epilepsy

ABSTRACT

Background: Chronic epilepsy is a multifaceted common brain disorder with manifold underlying factors. Epilepsy affects around 70 million peoples worldwide. *Amomum tsaoko* is a perennial herbaceous plant that is extensively cultivated in many provinces of China reported to exert immense biological activities.
Objective: This research work was aimed to reveal the therapeutic actions of ethanolic extract of *A.tsaoko* fruits (EE-ATF) against the pentylenetetrazol (PTZ)-provoked convulsive seizures in the mice.
Methodology: The convulsive seizures were provoked to the animals via administering 70 mg/kg of PTZ through intraperitoneally to trigger the convulsive seizures then treated with the EE-ATF at 50, 75, and 100 mg/kg orally 30 min prior to PTZ challenge. After the 30 min of PTZ challenge, animals closely monitored for signs of convulsion, generalized clonic and tonic convulsion durations, and mortality. A subconvulsive dose 35 mg/kg of PTZ was used to provoke the kindling and seizure stages were examined using standard method. The levels of dopamine, GABA, glutamate, and Na + K + ATPase and Ca + ATPase activities in the brain tissues were studied using marker specific assay kits. The oxidative stress and antioxidant markers studied using standard methods. The mRNA expressions of COX-2, TNF-α, NF-κB, TLR-4, and IL-1β in the brain tissues were studied using RT-PCR analysis. The brain tissues were examined histologically.
Results: EE-ATF treatment remarkably decreased the onset and duration of convulsion and suppressed the seizure severity and mortality in the PTZ animals. EE-ATF treatment appreciably ameliorated the PTZ triggered modifications in the GABA, glutamate, dopamine levels and Ca + 2ATPase and Na + K + A TPase activities in the brain tissues. EE-ATF suppressed the mRNA expressions of NF-κB, IL-1β, TLR-4, TNF-α, and COX-2. The status of antioxidants were elevated by the EE-ATF. Histological findings also demonstrated the curative actions of EE-ATF.
Conclusion: Our findings evidenced that the EE-ATF substantially ameliorated the PTZ-provoked convulsive seizures in the mice.

1. Introduction

Epilepsy is a common disorder of central nervous system affecting around 70 million peoples worldwide and categorized by aberration in the electrical excitability of neurons and recurring seizures (Paudel et al. (2020). It produces inhibitory and excitatory neurotransmission imbalances within the neuronal system that further directs to the psychiatric comorbidities and worsened life quality (Suleymanova et al. (2019). An unprompted, irregular, and hyper-synchronous neuronal function resulted in the irregular

* Corresponding author.
E-mail address: gjl811009@sina.com (X. Feng).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.06.007
1319-562X/

electrical releases and an disparity of neurotransmitter in the brain that directs to ictogenesis (Thijs et al. (2019).

Large number of evidences has recommended that numerous chemical-provoked convulsive and seizure models were regularly occupied to develop the numerous antiepileptic agents (Loscher, 2017). Pentylenetetrazol (PTZ) is a gamma-Aminobutyric acid (GABA) receptor antagonist accountable for the triggering of convulsion and it was well-known that the PTZ affects numerous neurotransmitters, for instance glutamate and GABA that replicates the pathological characteristics of tonic-clonic seizures. PTZ has the capacity to provoke the oxidative injury, blocking of membrane-bound enzyme functions, stimulation of nuclear factor-κB (NF-κB), and pro-inflammatory reactions, which further directs to the neuroinflammation and dysfunction of mitochondria that consecutively leads to the DNA injury and neural apoptosis (Shimada and Yamagata, 2018).

Kindling is a well-known animal model of epilepsy that was broadly executed to investigate the principal mechanisms of epileptogenesis. Additionally, kindling is a circumstance where a convulsive stimuli whichever electrical and/or chemical is applied frequently and sporadically, will eventually result in the appearance of convulsions (Dhir, 2012). Various studies unveiled the basics of epileptogenesis, which demonstrated that the chronic epilepsy occurred with recurring seizures. It was reported that the numerous proinflammatory mediators for instance tumor necrosis factor-α (TNF-α) and interleukins (ILs) were participated in the commencement and progression of epileptic seizures (Kwon et al. (2018).

The inflammatory reactions were experimentally as well as clinically reported to play an critical functions in the provocation of seizures (Friedman and Dingledine, 2011). It was already stated that the pro-inflammatory regulators were found upregulated in the seizures progression in the anima models. In the clinical condition of epilepsy, the augmentation of pro-inflammatory regulators like IL − 1β was identified in the brain tissues of patients (Mazarati et al. (2017). Raised oxidative stress improves the intracellular Ca2 + level and intensifies the seizure. A huge quantity of Ca2+ influx causes the excitatory amino acid poisoning and elevates the Ca2+ status in the cells, in that way triggering the neuronal injury.

The debilitated immune system is frequently thought to be an partner of the commencement of epilepsy with other provocation factors (Matin et al. (2015). Additionally, cytokines participated in the mediatory effects of the immunity has been found to contributed in the epilepsy progression. The most of this event is a inflammatory reaction, and alterations in the inflammatory factors denote that the incidence of epilepsy could also provoke the inflammatory reactions. The IL-1β, IL-6, and TNF-α was aberrantly expressed in the epilepsy patients that could lead to neuronal degeneration (Ravizza et al. (2006). Thus, the effective anti-inflammatory and antioxidants has thought to be of more salutary importance in the management and treatment of epilepsy (Taiwe et al. (2016).

Presently existing anti-epileptic drugs primarily administered to treat the epilepsy are merely lessen the seizure symptomatically, however do not avert the seizure-triggered neurodegeneration, additionally they are synthetic agents that often reported with the more side effects as well as their therapeutic actions are not satisfactory and other psychological reasons for instance fear of losing control, anxiety, depression, and recurrent seizure incidences are the vital contributors of poor success rate of epilepsy treatment (Bagheri et al. (2019). Consequently there is an great interest to explore the more potent novel sources for the development of complete therapeutic agent epilepsy treatment.

The foods with bioactive constituents afford treasured sources for the development of novel nutritional supplements for averting ailments. *Amomum tsaoko* is a perennial herbaceous plant belongs to the Zingiberaceae family broadly distributed in many provinces of China (Li et al. (2011). The dried fruits of *A.tsaoko* is a folklore Chinese spice utilized as both medicine and food ingredient (Feng et al. (2011). The dried fruits were broadly utilized to treat the many complications like abdominal pain, indigestion, and coughing (Feng et al. (2011). Also it has demonstrated the potent antioxidant, antifungal, antitumor, neuroprotective, and antibacterial activities (Zhang et al. (2014; Moon et al. (2004; Zhang et al. (2015; Guo et al. (2017). Thus, the curative actions of *A.tsaoko* fruits against the convulsive seizures were not assessed yet. Consequently, the current exploration was deliberated to understand the therapeutic actions of ethanolic extract of *A.tsaoko* fruits (EE-ATF) against the PTZ-activated convulsive seizures in the mice via the attenuation of oxidative stress and neuroinflammaion.

2. Materials and methods

2.1. Chemicals

PTZ, sodium chloride (NaCl), and other chemicals were acquired from the Sigma-Aldrich, USA. The assay kits for respective biomarkers and PCR was procured from the Biocompare, USA and Thermofisher Scientific, USA, respectively.

2.2. Collection of A.tsaoko fruits and extract preparation

The dried fruits of *A.tsaoko* was collected from the supermarket in the Qujing city, Yunnan province, China. Then the fruits were cleaned and coarsely powdered using mechanical blender. For the extract preparation, 250 g of fruit powder was extracted in a 70% of aqueous ethanol using soxhlet apparatus. The resultant extract was evaporated under reduced pressure and stored at −20 °C until used.

2.3. Experimental animals

Male Swiss albino mice weighing 25 ± 5 g and 60 days aged were employed in this investigation. Animals were attained from the central animal facility. All animals were caged in a infection free polyacrylic confines. Animals were sustained under the regular laboratory situations with temperature 22 ± 2 °C, humidity 55–65%, and 12 h dark/light cycle. Animals were permitted to access freely the regular rodent diet and purified drinking water throughout the study. All protocols done in this work were ethically approved by Xi'an Hospital of Traditional Chinese Medicine animal ethical committee, Approved No. XAHTCM210426

2.4. Experimental setup

The mice were arbitrarily separated into six groups with six animals each. Animals from group I (control) provided with normal saline (0.9% NaCl) only without treatments. Group II animals administered with 70 mg/kg of PTZ through intraperitoneal route (in 0.9% saline) to trigger the seizures (PTZ group). EE-ATF were administered at three diverse doses i.e. 50, 75, and 100 mg/kg orally 30 min prior to PTZ challenge (EE-ATF 50, 75, and 100 mg/kg). Diazepam (5 mg/kg) was given intraperitoneally 30 min before the PTZ challenge (Diazepam 5 mg/kg). All animals were monitored closely for 30 min after the PTZ challenge for the signs of commencement of convulsion, generalized clonic and tonic convulsion durations, number of animals displaying convulsions, and mortality. All the data were noted carefully and tabulated respectively.

2.5. Stimulation of kindling

A 35 mg/kg PTZ s a sub-convulsive dosage was administered intraperitoneally to the animals on sporadic days for 15 times. Animals were carefully monitored for 30 min after the every PTZ challenge. The duration of seizure from PTZ challenge (latency) and the duration of clonic-tonic seizures were noted. The stages of seizure were examined as per the scale suggested by Schroder et al. (1993) as follows: stage 0 indicates no response, stage 1 indicates facial and mouth jerks; stage 2 indicates axial convulsive waves through body, stage 3 indicates rearing and myoclonic jerks, stage 4 indicates clonic convulsions with mice falling on its side, and stage 5 indicates the tonic-clonic seizure seems once. The severity of the seizures during kindling stimulation was noted.

After cessation of seizures, the locomotor activity of the mice were examined with the aid of actophotometer. Shortly, mice was separately located on the actophotometer, and the total activity was observed for 5 min. The locomotor activity of the animals were depicted as the counts/5min. Subsequently mice were investigated by forced swimming test to detect the depressive behaviour of mice. For this, mice were located separately in the glass cylinder ($25 \times 12 \times 25$ cm^3) with water up to the 15 cm for 5 min and the total period of immobility was noted.

2.6. Preparation of tissue sample

After the behavioral assessments, mice anesthetized using ether and sacrificed via decapitation. The brain tissues of each animals were excised and processed with chilled saline then tissues were homogenated (10% w/v) using 0.1 M phosphate buffer (pH-7.4). The homogenate was then centrifuged at 3000 rpm for 15 min and the upper aqueous solution was utilized for the additional biochemical examinations. A portion of brain tissues were utilized for the histological examinations.

2.7. Estimation of dopamine, GABA, glutamate, and Na + K + ATPase and Ca + ATPase levels in the brain tissues

The levels of dopamine, GABA, and glutamate in the brain tissues of control and experimental mice was studied using the respective assay kits using manufacturer's guidelines (Biocompare, USA). The activity of Na + K + ATPase and Ca + ATPase in the brain tissues were detected using assay kits using assay kits (Thermofisher Scientific, USA).

2.8. Measurement of oxidative stress and antioxidant markers in the brain tissues

The status of malondialdehyde (MDA) in the brain tissues of treated mice was quantified using Niehius and Samuelson, (1968) approach. The absorbance taken at the 530 nm using spectrophotometer. The status of GSH was detected as per the Jollow et al. (1974) approach. The absorbance was taken 412 nm and the outcomes were displayed as GSH/μg/mg of protein. The activity of SOD was examined using Sun et al. (1988) technique and the outcomes were displayed as SOD/U/mg of protein. The status of NO was studied as per the Green et al. (1982) technique and the outcomes were displayed as mg/ml.

2.9. Real-time PCR analysis

The mRNA expression altitudes of COX-2, TNF-α, NF-κB, TLR-4, and IL-1β in the brain tissues of control and treated mice were examined using RT-PCR analysis. For this, the total mRNA was separated from the brain tissues using TRIzol reagent and purity of RNA was investigated spectrophotometrically. The extracted mRNA was consumed to cDNA construction using the PCR kits (Thermofisher Scientific, USA). The primers utilized for NF-κB upstream: 5′-CTGGTGGACACATACAGGAAGAC-3′, downstream: 5′-ATAGGCACTGTCTTCTTTCACCTC-3′; IL-1β upstream: 5′-CACCTCT CAAGCAGAGCACAG-3′, downstream: 5′-GGGTTCCATGGTGAAGT CAAC-3′; TLR-4 upstream: 5′-TTGCCTTCATTACAGGGACTT-3′, downstream: 5′- CAGAGCGGCTACTCAGAAACT-3′; TNF-α upstream: 5′-CCAGGAGAAAGTCAGCCTCCT-3′, and downstream: 5′-TCATAC CAGGGCTTGAGCTCA-3′

COX-2 upstream: 5′-CAAGCAGTGGCAAAGGCCTCCA-3′, downstream: 5′-GGCACTTGCATTGATGGTGGCT-3′. The relative mRNA expressions of target genes were determined using $2 - \Delta\Delta CT$ technique. β-actin was utilized as a internal housekeeping gene to standardize the expressions of target genes.

2.10. Histopathological study of brain tissues

The excised brain hippocampal tissues were fixed in the 10% of neutral formalin for 24 h for and then processed using xylene and isopropyl alcohol for 12 h. then tissues were embedded on the paraffin wax and sliced at 5 mm size. The sliced portions were stained using hematoxylin and eosin (H&E). Finally tissues were scrutinized under light microscope and the microphotographs were taken at 40× magnification.

2.11. Statistical analysis

The outcomes were presented as mean ± SD of triplicates (n = 6/-group). Data analysis was completed using one way ANOVA successively Tukey's post hoc assay. The significance level was set at $p < 0.05$.

3. Results

3.1. Effect of EE-ATF on the duration and onset of clonic-tonic convulsion and mortality in the PTZ-challenged animals

PTZ-administered animals demonstrated the increased onset of convulsions and durations of both clonic and tonic convulsions in the mice. PTZ also demonstrated the increased mortalities in the mice (Fig. 1). Alternatively, the treatment with the 5, 10, and 15 mg/kg of EE-ATF demonstrated the notable ameliorative effects. EE-ATF decreased the onset of convulsions and diminished the durations of clonic and tonic convulsions in the PTZ-challenged mice. The mortality rate in the PTZ-challenged mice were also suppressed by the EE-ATF treatment (Fig. 1). The standard drug Diazepam also attenuated the PTZ-triggered onset of clonic-tonic convulsions and prevented the mortality.

3.2. Effect of EE-ATF on the kindling, locomotor activity, and immobility period in the PTZ-challenged animals

As demonstrated in the Fig. 2, the PTZ-challenged animals demonstrated the augmented seizure severity as compared with control. Surprisingly, the administration of EE-ATF to the PTZ-provoked mice displayed the appreciably lessening in the seizure severity on day 10 and 15, which is in contrast to the PTZ alone challenged animals. PTZ-challenge also demonstrated the remarkable reduction in the locomotor activity and increased the immobility period of animals. Here, the 5, 10, and 15 mg/kg of EE-ATF treatment did not showed any major variations from the PTZ challenge. EE-ATF did not improved the locomotor activity and decreased immobility period in the PTZ animals (Fig. 2). Diazepam treatment also suppressed the seizure severity in the PTZ-challenged animals. As seen in the EE-ATF, Diazepam treatment

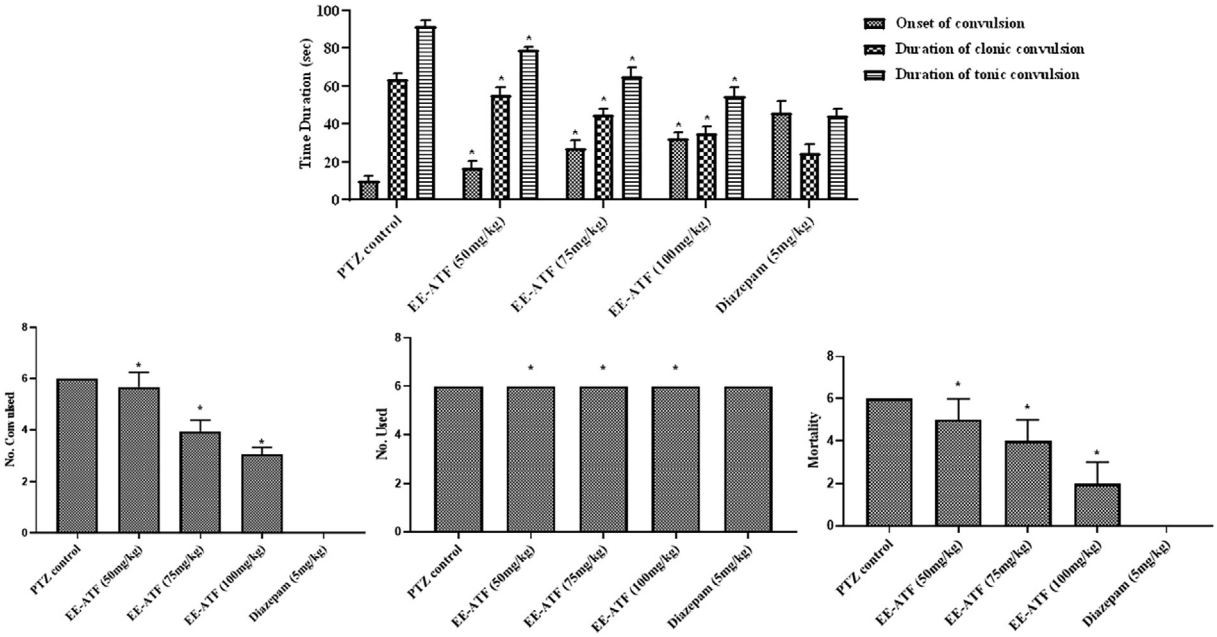

Fig. 1. Effect of EE-ATF on the duration and onset of clonic-tonic convulsion and mortality in the PTZ-challenged animals. Each bar signifies the mean ± SD of triplicate data from the respective assays. One-way ANOVA sequentially Tukey's post hoc assay was done to detect the statistical variations. '*' $p < 0.01$ compared with control and '#' $p < 0.05$ compared with PTZ-challenged group.

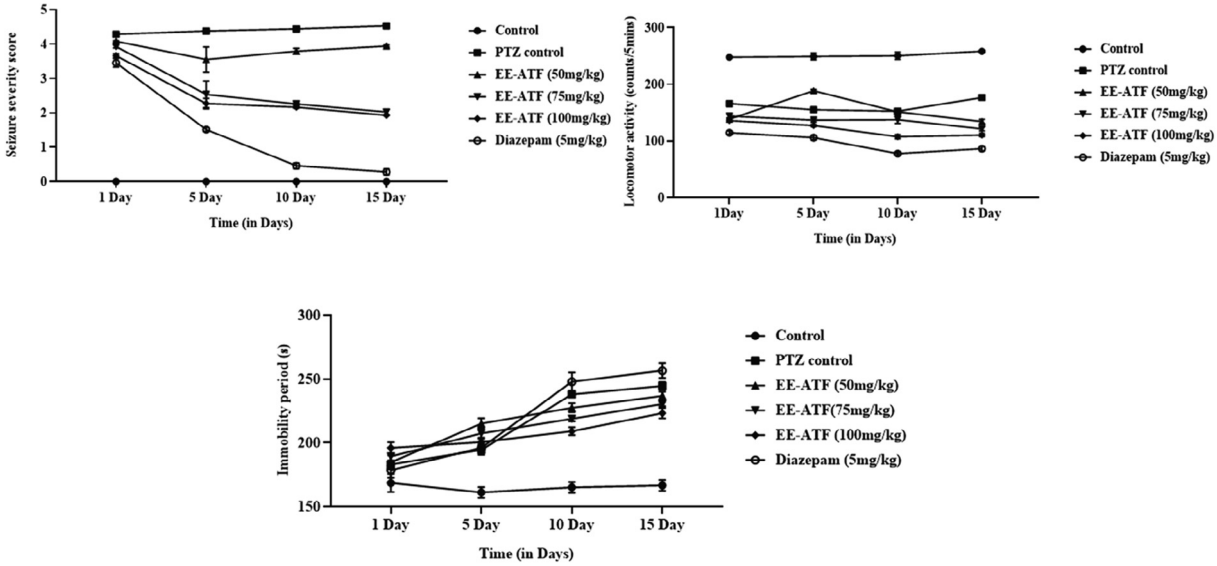

Fig. 2. Effect of EE-ATF on the kindling, locomotor activity, and immobility period in the PTZ-challenged animals. Each line signifies the mean ± SD of triplicate data from the respective assays. One-way ANOVA sequentially Tukey's post hoc assay was done to detect the statistical variations. '*' $p < 0.01$ compared with control and '#' $p < 0.05$ compared with PTZ-challenged group.

also showed no variations in the locomotor activity and immobility period of the PTZ-challenged animals.

3.3. Effect of EE-ATF on the dopamine, GABA, glutamate levels, and Ca + 2ATPase and Na + K + ATPase activities in the PTZ-challenged animals

The levels of GABA and dopamine status was found suppressed and glutamate status was rapidly elevated in the brain tissues of PTZ-challenged mice than control (Fig. 3). PTZ-challenge also notably decreased the Ca + 2ATPase and Na + K + ATPase activities in the brain tissues. The 5, 10, and 15 mg/kg of EE-ATF treatment to the PTZ-challenged animals substantially improved the dopamine and GABA levels and reduced the glutamate status in the brain tissues. The activities of Ca + 2ATPase and Na + K + ATPase was also improved considerably in the PTZ-challenged animals by the EE-ATF treatment (Fig. 3). The PTZ-animals administered with Diazepam also exhibited the appreciable improvement in the dopamine and GABA levels and reduction in the glutamate level. Diazepam also improved the Ca + 2ATPase and Na + K + ATPase activities in the PTZ animals.

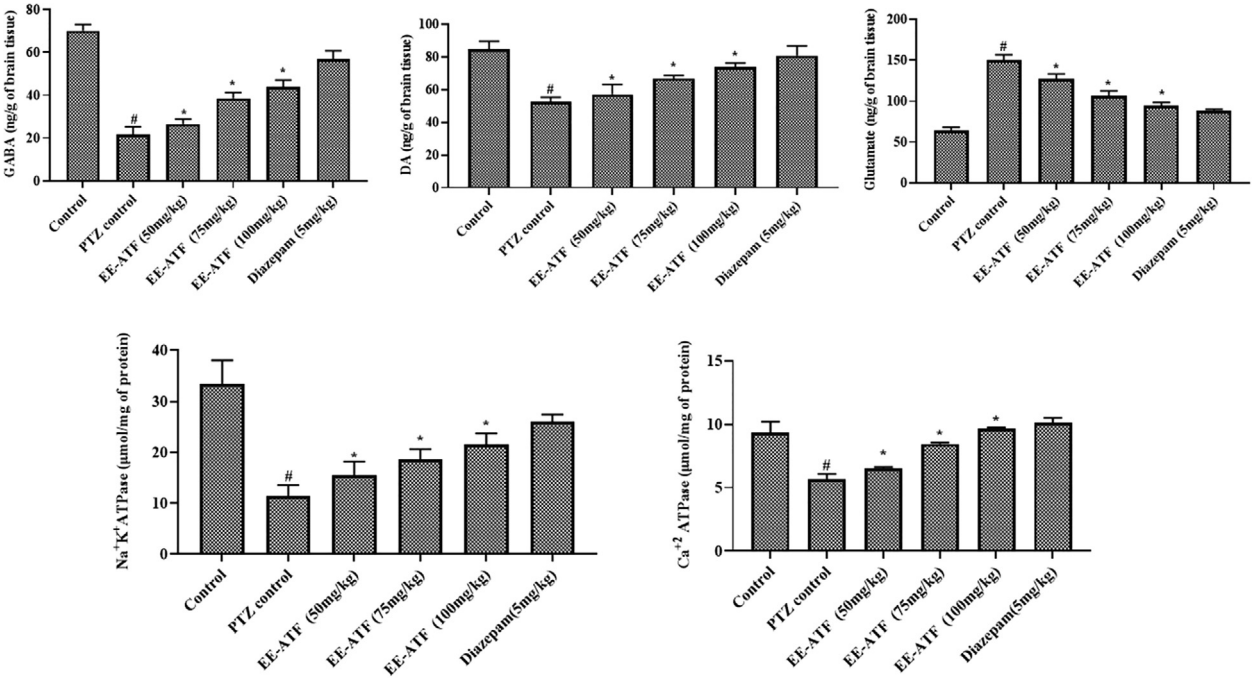

Fig. 3. Effect of EE-ATF on the dopamine, GABA, glutamate levels, and Ca + 2ATPase and Na + K + ATPase activities in the PTZ-challenged animals. Each bar signifies the mean ± SD of triplicate data from the respective assays. One-way ANOVA sequentially Tukey's post hoc assay was done to detect the statistical variations. '*' $p < 0.01$ compared with control and '#' $p < 0.05$ compared with PTZ-challenged group.

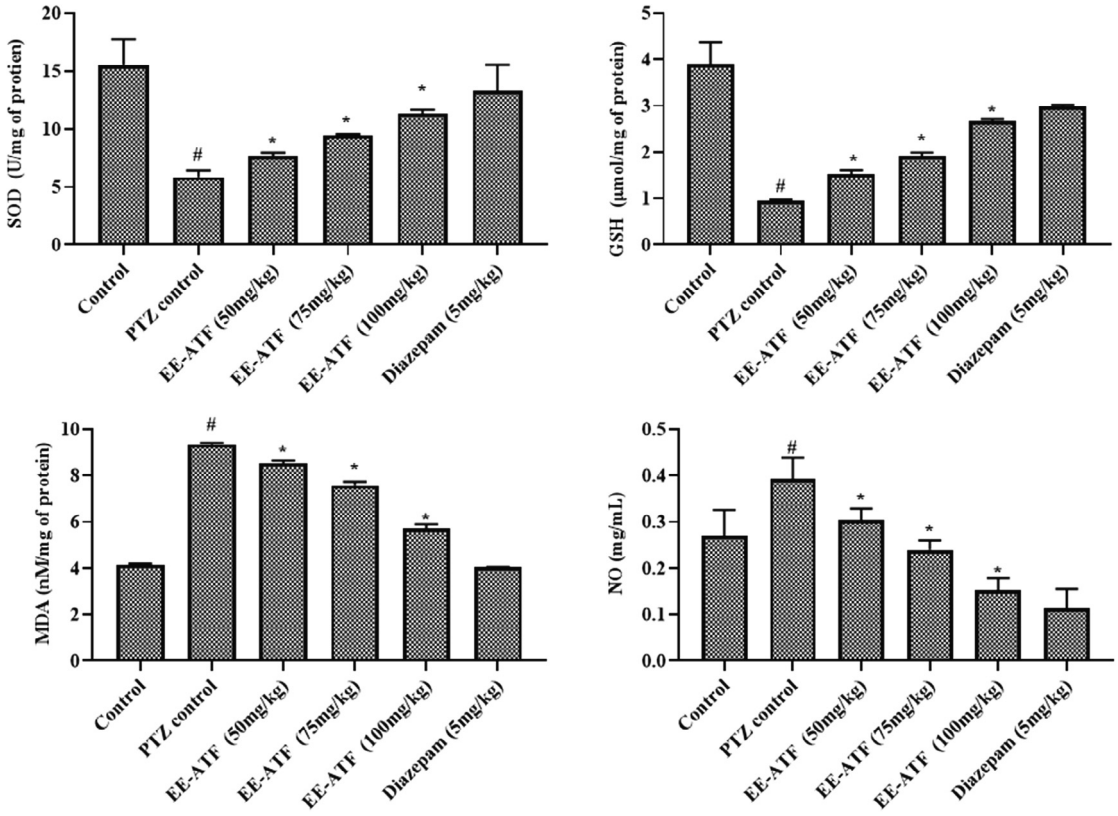

Fig. 4. Effect of EE-ATF on the oxidative stress and antioxidant markers in the brain tissues of PTZ-provoked mice. Each bar signifies the mean ± SD of triplicate data from the respective assays. One-way ANOVA sequentially Tukey's post hoc assay was done to detect the statistical variations. '*' $p < 0.01$ compared with control and '#' $p < 0.05$ compared with PTZ-challenged group.

3.4. Effect of EE-ATF on the oxidative stress and antioxidant markers in the brain tissues of PTZ-challenged animals

Fig. 4 reveals the effect of EE-ATF on the oxidative and antioxidant markers in the brain tissues of PTZ-triggered animals. PTZ mice displayed the remarkable lessening in the antioxidants SOD and GSH and increased the MDA and NO levels in the brain tissues. The PTZ-challenged animals administered with the 5, 10, and 15 mg/kg of EE-ATF was displayed the remarkable reduction in the oxidative stress markers NO and MDA in the brain. EE-ATF also improved the antioxidants SOD and GSH status (Fig. 4). Diazepam treatment also demonstrated the suppressed NO and MDA status and improved the SOD and GSH levels.

3.5. Effect of EE-ATF on the expressions of inflammatory markers in the brain tissues of PTZ-challenged mice

The inhibitory actions of EE-ATF on the mRNA expressions of inflammatory markers were studied using RT-PCR analysis and the outcomes were represented in the Fig. 5. As presented in the Fig. 5, the mRNA expressions of inflammatory markers i.e. NF-κB, IL-1β, TLR-4, TNF-α, and COX-2 was found up-regulated in the brain tissues of PTZ-challenged animals. However, NF-κB, IL-1β, TLR-4, TNF-α, and COX-2 expressions was effectively inhibited in the brain tissues of PTZ animals by the EE-ATF treatment. The NF-κB, IL-1β, TLR-4, TNF-α, and COX-2 expressions was also inhibited by the Diazepam treatment in the brain tissues of PTZ animals.

3.6. Effect of EE-ATF on the brain histopathology of the PTZ-challenged animals

Fig. 6 evidenced that the PTZ-challenged animals illustrating the major histological alterations in the brain tissues for instance, increased inflammatory cell penetrations, congestion, pyknosis, and neuronal necrosis as compared with control. The brain tissues of control animals displayed the normal architecture without any signs of inflammation and pyknosis. The PTZ stimulated histological alterations were appreciably attenuated by the EE-ATF treatment (Fig. 6). The brain tissues of PTZ-challenged animals treated with EE-ATF demonstrated the reduced inflammatory signs, congestion, pyknosis, and necrosis. Diazepam treatment also attenuated the PTZ triggered histological changes in the brain tissues.

4. Discussion

Chronic epilepsy is a multifaceted brain disorder demonstrating manifold underlying identified and unidentified factors with less recognized mechanisms (Staley, 2015). Neurotransmitters are the crucial players of facilitating neuronal excitation and upholding typical behaviour of the cognition (Moavero et al. 2017). The severe oxidative stress due to the over accumulation of free radicals were thought to play an imperative function in the epilepsy development. Besides, over oxidative stress participates to the neuronal degeneration in the epilepsy. Excessive ROS production lessens the effectiveness of antioxidants and also produces higher amounts of unsaturated fatty acids and lipids that further intensifies the lipid peroxidation (Eastman et al. 2020).

Behavioral deficits is normally fuelled through the cellular and molecular imbalances. The progressive brain injury in the PTZ triggered kindling is also convoyed with augmented oxidative stress. Indeed, oxidative stress is the most predominant reason that catalyze the origin and development of epileptic seizures and subsequently behavioral modifications. Kindling stimulates the array of biochemical reactions, which changes the membrane phospholipids metabolism and thus accelerates the accumulation of lipid peroxides (Tambe et al. 2016). PTZ triggered kindling was reported to augment the MDA status and improve the burden of oxidative stress in the brain (Hassanzadeh et al. 2017). As the same incident, we also found in the study that the MDA status was drastically elevated in the PTZ-triggered kindled mice that was convoyed with notable reduction in the SOD and GSH status. GSH and SOD are two most imperative antioxidants that normalize and maintain the ROS amount in the brain. GSH is the most copious antioxidant in the mammals that abolishes the most of the toxic hydroxyl radical (Borowicz-Reutt and Czuczwar, 2020). SOD effectively hunts and averts the accumulation of superoxide radicals (Weydert and Cullen, 2010). In this investigation, we noted that the PTZ-triggered mice demonstrated the severe reduction in the GSH and SOD and the same was improved appreciably by the EE-ATF treatment.

The inflammatory reactions in the brain are the vital players of the seizure commencement and progression (Maroso et al. 2010). Inflammatory regulators are the critical players of the inflammatory reactions and immune system. Pro-inflammatory regulators like IL-1β, IL-6, and TNF-α are participated in the pathological progression of epilepsy (Roseti et al. 2015). The stimulation of inflammatory regulators like NF-κB and COX-2 and the excessive generation of down-stream inflammatory mediators participated in the seizures development (Wang et al. 2018). The COX-2, IL-1β, TLR4, and TNF-α are the crucial pro-inflammatory regulators in the epilepsies that was investigated broadly to unveil their roles (Dey et al. 2016). TNF-α displays the double function in the pathological progression of epilepsy and seizure, which demonstrating pro-convulsive actions (Vezzani, 2020). IL-6 could trigger the COX-2 to generate the PGE2 via stimulating the NF-κB cascade as a same way to other inflammatory mediators and mediates the immune and inflammatory reactions (Mosili et al. 2020). The stimulation of IL-6 is frequently noted in the human as well as animal models of epilepsy. Many investigations decoded the active partic-

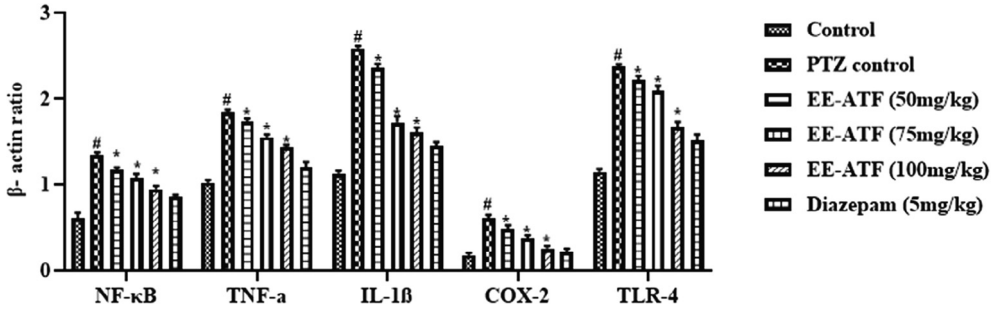

Fig. 5. Effect of EE-ATF on the expressions of inflammatory markers in the brain tissues of PTZ-challenged animals. Each bar signifies the mean ± SD of triplicate data from the respective assays. One-way ANOVA sequentially Tukey's post hoc assay was done to detect the statistical variations. '*' $p < 0.01$ compared with control and '#' $p < 0.05$ compared with PTZ-challenged group.

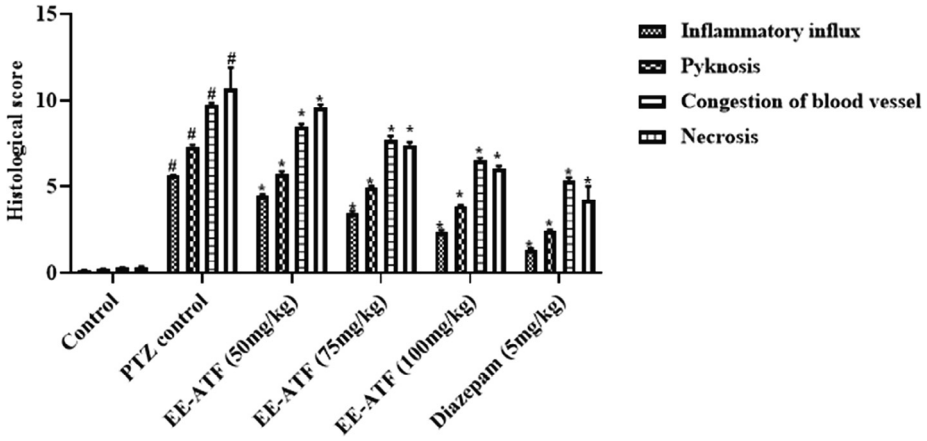

Fig. 6. Effect of EE-ATF on the brain histopathology of the PTZ-challenged animals. Control animals demonstrated the typical cellular arrangements with the inflammatory signs (Control). PTZ-challenged animals demonstrating the elevated inflammatory cell penetrations, congestion, pyknosis, and neuronal necrosis in the brain tissues (PTZ-control). EE-ATF treatment remarkably ameliorated the inflammatory signs, congestion, pyknosis, and necrosis (EE-ATF 50, 75, and 100 mg/kg). Diazepam treatment also ameliorated the PTZ-provoked histological changes in the brain tissues (Diazepam-5 mg/kg). Each bar signifies the mean ± SD of triplicate data from the respective assays. One-way ANOVA sequentially Tukey's post hoc assay was done to detect the statistical variations. '*' $p < 0.01$ compared with control and '#' $p < 0.05$ compared with PTZ-challenged group.

ipation of IL-6 in seizure commencement and progression (Sanz and Garcia-Gimeno, 2020).

TNF-α augments the amount of glutamate receptors and triggers the GABA ingestion that sequentially suppresses the inhibitory ambition and triggers neuronal excitation that further leads to the epilepsy progression (Liu et al. 2015). The TNF-α and IL-1β release is considered to trigger the synaptic pruning that leads to the neuroplasticity impairment and brain structural alterations, which negatively influence the cognition (Rosenblat et al. 2014). COX-2 is imperative pro-inflammatory enzyme that play a critical role in the epileptic seizures during kindling. Many researchers found the crosstalk between the COX-2 and the progression of epilepsy (Rawat et al. 2019). The participation of NF-κB in the seizure progression was well established and the inhibition of NF-κB expression effectively inhibits the seizure condition (Zhang et al. 2017). In this exploration, we detected that the mRNA expressions NF-κB, IL-1β, TLR-4, TNF-α, and COX-2 was enhanced PTZ animal brain tissues. Though, the EE-ATF treatment attenuated the NF-κB, IL-1β, TLR-4, TNF-α, and COX-2 expressions in the brain tissues of PTZ animals.

Epilepsy was thought to be related with the dissimilarity between the inhibitory (GABA) and excitatory (glutamate) amino acids, which plays the critical function in the cognitive function and neurodegeneration. Suppressed GABA or elevated glutamate levels escorts to the improved anxiety, reduced social behavior, and aggressive behaviors that results in the stimulation of high Ca2 + influx. Many studies on the human as well as animal epileptic studies models found the reduced GABA level (Ramanathan et al. 2012). Other investigations reported the appearance concerned in the epilepsy models that include the weakening in inorganic phosphate ion channels like Na^+/K^+-ATPase, impairments in receptor and voltage gated ion channels, cholinergic and glutamatergic transmissions (Kobylarek et al. 2019).

GABA is a imperative inhibitory neurotransmitter of the cerebral cortex, which sustains the inhibitory pressure to maintain the nerve excitation (Hirose, 2014). The seizure condition will arise if this equilibrium was troubled (Obata, 2013). The inhibition of the GABA neurotransmission was believed to be the fundamental reason of epilepsy. Many investigations has decoded that the improvement of GABA neurotransmission antagonize the seizures, while the hindering of its neurotransmission enhances the seizures. The protective effects of anticonvulsant drugs towards the PTZ-triggered seizures in animals is expected via improving the GABA neurotransmission (Mahomed & Ojewole, 2006). PTZ animals demonstrated the reduced GABA status in the brain tissues, which is potentially improved by the EE-ATF treatment.

The activated TLR-4 cascade is actively participated in the discharging of glutamate (Su et al. 2015). The over glutamate level augments the intracellular calcium flux, which directs to the neuronal apoptosis and necrosis. Besides, neuronal cell loss in the hippocampus region is convoyed with mossy fiber sprouting and reactive gliosis, which ultimately cause the serious impairments of cognition and memory deficiency (Russo et al. 2013). EE-ATF treatment effectively suppressed the glutamate status in the brain tissues of mice, which enhanced by the PTZ challenge.

The K+ channels resist the membrane potentials and allow the fast repolarization of the action latency via generating the outer K+ streams that restricts excitability of neurons. The imbalance of Ca2+ and the mitochondrial malfunctions develops the malicious cycles and results the insufficient brain organization of ATP generation and leads to the intracellular Ca2+ burden of the nerve cells that ultimately results in the nerve cell necrosis. Furthermore, the Ca2+ burden results in the extreme generations of NO in the neurons that could be united with super oxygenated constituents to generate ONOO– in the nervous cells. This phenomenon is extremely toxic to the membrane lipids, DNA, and white matter of the brains and ultimately results in the oxidative stress (Alexander et al. 2016). As well, mitochondrial dysfunction and oxidative stress result from prolonged seizure condition. The depolarization during the severe epileptic condition of the neurons as a result to the foreign stimuli directs to the accumulation of mitochondrial Ca2+ that provokes the mitochondrial apoptotic cascades and/or oxidative stress, which hasten the mitochondrial superoxide accumulation (Kudin et al. 2002). The brain tissues of PTZ-challenged animals demonstrated the suppressed Ca + 2ATPase and Na + K + ATPase activities. EE-ATF treatment appreciably enhanced the enzymatic activities of Ca + 2ATPase and Na + K + ATPase in the PTZ animals.

5. Conclusion

Herein, our findings demonstrated that EE-ATF lessens PTZ-triggered convulsive seizures in the mice. EE-ATF suppressed the onset and duration of convulsion and reduced the seizure severity

in the PTZ animals. The PTZ provoked alterations in the GABA, glutamate, dopamine levels and Ca + 2ATPase and Na + K + ATPase activities were effectively modulated by the EE-ATF. EE-ATF appreciably attenuated the oxidative stress markers and suppressed the NF-κB, IL-1β, TLR-4, TNF-α, and COX-2 expressions in the brain tissues of PTZ animals, which proved its antioxidant and anti-inflammatory roles. These outcomes evidenced that the EE-ATF effectively ameliorated the PTZ-activated convulsive seizures in the mice. Additionally, further investigations still required in the future to unveil the ameliorative effects of EE-ATF against the convulsive seizures and its molecular mechanisms.

Declaration of Competing 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.

References

Alexander, S.P., Catterall, W.A., Kelly, E., Marrion, N., Peters, J.A., Benson, H.E., et al., exander et al. (2016. CGTP collaborators, the concise guide to PHARMACOLOGY 2015/16: voltage-gated ion channels. Pharmacology 172, 5904–5941.

Bagheri, S., Heydari, A., Alinaghipour, A., Salami, M., gheri et al. (2019. Effect of probiotic supplementation on seizure activity and cognitive performance in PTZ-induced chemical kindling. Epilepsy Behav. 95, 43–50.

Borowicz-Reutt, K., Czuczwar, S.J., 2020. Role of oxidative stress in epileptogenesis and potential implications for therapy. Pharmacol. Rep. 72 (5), 1218–1226.

Dey, A., Kang, X., Qiu, J., Du, Y., Jiang, J., y et al. (2016. Anti-inflammatory small molecules to treat seizures and epilepsy: from bench to bedside. Trends Pharmacol. Sci. 37, 463–484.

Dhir, A., 2012. Pentylenetetrazol (PTZ) kindling model of epilepsy. Curr. Protoc. Neurosci. 58, 9.37.1–9.37.12.

Eastman, C.L., D'Ambrosio, R., Ganesh, T., stman et al. (2020. Modulating neuroinflammation and oxidative stress to prevent epilepsy and improve outcomes after traumatic brain injury. Neuropharmacology 172, 107907.

Feng, X., Jiang, Z.-T., Wang, Y., Li, R., ng et al. (2011. Composition comparison of essential oils extracted by hydrodistillation and microwave-assisted hydrodistillation from *Amomum tsao-ko* in China. J. Essent. Oil Res. 13, 286–291.

Friedman, A., Dingledine, R., 2011. Molecular cascades that mediate the influence of inflammation on epilepsy. Epilepsia 52 (Suppl 3), 33–39.

Green, L., Wagner, D., Glogowski, J., Skipper, P., Wishnok, J., Tannenbaum, S., een et al. (1982. Analysis of nitrate, nitrite, and nitrate in biological fluids. Anal. Biochem. 126, 131–138.

Guo, N., Zang, Y.-P., Cui, Q., Gai, Q.-Y., Jiao, J., Wang, W., Zu, Y.-G., Fu, Y.-J., o et al. (2017. The preservative potential of *Amomum tsaoko* essential oil against *E. coil*, its antibacterial property and mode of action. Food Control 75, 236–245.

Hassanzadeh, P., Arbabi, E., Atyabi, F., Dinarvand, R., ssanzadeh et al. (2017. Ferulic acid exhibits antiepileptogenic effect and prevents oxidative stress and cognitive impairment in the kindling model of epilepsy. Life Sci. 179, 9–14.

Hirose, S., 2014. Mutant GABA(A) receptor subunits in genetic (idiopathic) epilepsy. Prog. Brain Res. 213, 55–85.

Jollow, D.J., Mitchell, J.R., Zampaglione, N., Gillette, J.R., llow et al. (1974. Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 11 (3), 151–169.

Kobylarek, D., Iwanowski, P., Lewandowska, Z., et al., bylarek et al. (2019. Advances in the potential biomarkers of epilepsy. Front. Neurol. 10 (685), 685.

Kudin, A.P., Kudina, T.A., Seyfried, J., Vielhaber, S., Beck, H., Elger, C.E., et al., din et al. (2002. Seizure-dependent modulation of mitochondrial oxidative phosphorylation in rat hippocampus. Eur. J. Neurosci. 15, 1105–1114.

Kwon, A., Kwak, B.O., Kim, K., et al., on et al. (2018. Cytokine levels in febrile seizure patients: a systematic review and meta-analysis. Seizure 59, 5–10.

Li, W., Wang, P., Masami, S., Lu, Z., et al. (2011. Chemical composition and antimicrobial activity of essential oil from *Amomum tsao-ko* cultivated in Yunnan area. Adv. Mater. Res. 183–185, 910–914.

Liu, Y.Z., Xie, W., Ren, Z.J., Zhou, Y., Zheng, Y.H., u et al. (2015. Modernization of traditional Chinese medicine and materia medica. World Sci. Technol. 17, 850–855.

Loscher, W., 2017. Animal models of seizures and epilepsy: past, present, and future role for the discovery of antiseizure drugs. Neurochem. Res. 42 (7), 1873–1888.

Mahomed, I.M., Ojewole, J.A., 2006. Anticonvulsant activity of Harpagophytum procumbens DC. (Pedaliaceae) secondary root aqueous extract in mice. Brain Res. Bull. 69, 57–62.

Maroso, M., Balosso, S., Ravizza, T., Liu, J., Aronica, E., Iyer, A.M., Rossetti, C., Molteni, M., Casalgrandi, M., Manfredi, A.A., roso et al. (2010. Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures. Nat. Med. 16, 413.

Matin, N., Tabatabaie, O., Falsaperla, R., Lubrano, R., Pavone, P., Mahmood, F., tin et al. (2015. Epilepsy and innate immune system: a possible immunogenic predisposition and related therapeutic implications. Hum. Vaccines Immunother. 11, 2021–2029.

Mazarati, A.M., Lewis, M.L., Pittman, Q.J., zarati et al. (2017. Neurobehavioral comorbidities of epilepsy: role of inflammation. Epilepsia 58 (Suppl 3), 48–56.

Moavero, R., Santarone, M.E., Galasso, C., Curatolo, P., avero et al. (2017. Cognitive and behavioral effects of new antiepileptic drugs in pediatric epilepsy. Brain Dev. 39, 464–469.

Moon, S.-S., Lee, J.-Y., Cho, S.-C., on et al. (2004. Isotsaokoin, an antifungal agent from Amomum tsao-ko. J. Nat. Prod. 67, 889–891.

Mosili, P., Maikoo, S., Mabandla, V., Qulu, L., sili et al. (2020. The pathogenesis of fever-induced febrile seizures and its current state. Neurosci. Insights 15. 2633105520956973.

Niehius, W.G., Samuelson, B., 1968. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid per-oxidation. Eur. J. Biochem. 6, 126–130.

Obata, K., 2013. Synaptic inhibition and γ-aminobutyric acid in the mammalian central nervous system. Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 89, 139–156.

Paudel, Y.N., Angelopoulou, E., Piperi, C., Gnatkovsky, V., Othman, I., Shaikh, M.F., udel et al. (2020. From the molecular mechanism to pre-clinical results: anti-epileptic effects of fingolimod. Curr. Neuropharmacol. 18 (11), 1126–1137.

Ramanathan, M., Babu, C.S., Justin, A., Shanthakumari, S., manathan et al. (2012. Elucidation of neuroprotective role of endogenous GABA and energy metabolites in middle cerebral artery occluded model in rats. Indian J. Exp. Biol. 50, 391–397.

Ravizza, T., Boer, K., Redeker, S., Spliet, W.G.M., Rijen, P.C.V., Troost, D., vizza et al. (2006. The IL-1β system in epilepsy-associated malformations of cortical development. Neurobiol. Dis. 24, 128–143.

Rawat, C., Kukal, S., Dahiya, U.R., Kukreti, R., wat et al. (2019. Cyclooxygenase-2 (COX-2) inhibitors: future therapeutic strategies for epilepsy management. J. Neuroinflam. 16 (1), 197.

Rosenblat, J.D., Cha, D.S., Mansur, R.B., McIntyre, R.S., senblat et al. (2014. Inflamed moods: a review of the interactions between inflammation and mood disorders. Prog. Neuropsychopharmacol. Biol. Psychiatry 53, 23–34.

Roseti, C., van Vliet, E.A., Cifelli, P., Ruffolo, G., Baayen, J.C., Di Castro, M.A., Bertollini, C., Limatola, C., Aronica, E., Vezzani, A., seti et al. (2015. GABAA currents are decreased by IL-1β in epileptogenic tissue of patients with temporal lobe epilepsy: implications for ictogenesis. Neurobiol. Dis. 82, 311–320.

Russo, E., Chimirri, S., Aiello, R., De Frazio, S., Leo, A., Rispoli, V., Marra, R., Labate, A., De Fazio, P., Citraro, R., De Sarro, G., sso et al. (2013. Lamotrigine positively affects the development of psychiatric comorbidity in epileptic animals, while psychiatric comorbidity aggravates seizures. Epilepsy Behav. 28, 232–240.

Sanz, P., Garcia-Gimeno, M.A., 2020. Reactive glia inflammatory signaling pathways and epilepsy. Int. J. Mol. Sci. 21 (11), 4096.

Schroder, H., Becker, A., Lossner, B., hroder et al. (1993. Glutamate binding to brain membranes is increased in pentylenetetrazole-kindled rats. J. Neurochem. 60, 1007–1011.

Shimada, T., Yamagata, K., 2018. Pentylenetetrazole-induced kindling mouse model. J. Vis. Exp. 136, 56573.

Staley, K., 2015. Molecular mechanisms of epilepsy. Nat. Neurosci. 18, 367.

Su,J.,Yin,J.,Qin,W.,Sha,S.,Xu,J.,Jiang,C.,et al.(2015.Roleforpro-inflammatorycytokines inregulatingexpressionofGABAtransportertype1and3inspecificbrainregionsof kainicacid-inducedstatusepilepticus.Neurochem.Res.40,621–627.

Suleymanova, E.M., Borisova, M.A., Vinogradova, L.V., 2019. Early endocannabinoid system activation attenuates behavioral impairments induced by initial impact but does not prevent epileptogenesis in lithium–pilocarpine status epilepticus model. Epilepsy Behav. 92, 71–78.

Sun, Y., Oberley, L.W., Li, Y., n et al. (1988. A simple method for clinical assay of superoxide dismutase. Clin. Chem. 34 (3), 497–500.

Taiwe, G.S., Tchoya, T.B., Menanga, J.R., Dabole, B., De Waard, M., iwe et al. (2016. Anticonvulsant activity of an active fraction extracted from Crinum jagus L. (Amaryllidaceae), and its possible effects on fully kindled seizures, depression-like behaviour and oxidative stress in experimental rodent models. J. Ethnopharmac. 194, 421–433.

Tambe, R., Jain, P., Patil, S., Ghumatkar, P., Sathaye, S., mbe et al. (2016. Antiepileptogenic effects of borneol in pentylenetetrazole-induced kindling in mice. Naunyn-Schmiedeberg's Arch. Pharmacol. 389, 467–475.

Thijs, R.D., Surges, R., O'Brien, T.J., Sander, J.W., ijs et al. (2019. Epilepsy in adults. Lancet 393 (10172), 689–701.

Vezzani, A., 2020. Brain inflammation and seizures: evolving concepts and new findings in the last 2 decades. Epilepsy Curr. 20 (6), 40S–43S.

Wang, Z.-H., Mong, M.-C., Yang, Y.-C., Yin, M.-C., ng et al. (2018. Asiatic acid and maslinic acid attenuated kainic acid-induced seizure through decreasing hippocampal inflammatory and oxidative stress. Epilepsy Res. 139, 28–34.

Weydert, C.J., Cullen, J.J., 2010. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat. Protoc. 5 (51–66), 52.

Zhang, H., Chu, X., Fu, T., Lv, H., Kong, Q., Hao, Y., ang et al. (2017. Inhibitory effect of interleukin-1β antibody for NLRP3 inflammasome on epilepsy rat model. Int. J. Clin. Exp. Pathol. 10, 1847–1853.

Zhang, T.-T., Lu, C.-L., Jiang, J.-G., ang et al. (2014. Bioactivity evaluation of ingredients identified from the fruits of *Amomum tsaoko Crevost* et Lemaire, a Chinese spice. Food Funct. 5, 1747–1754.

Zhang, T.-T., Lu, C.-L., Jiang, J.-G., ang et al. (2015. Antioxidant and anti-tumour evaluation of compounds identified from fruit of *Amomum tsaoko Crevost* et Lemaire. J. Funct. Foods 18, 423–431.