University of Jiroft

Biospecies Research

Journal homepage: bsr.ujiroft.ac.ir

Iranian Biology Society

Abscisic acid attenuates pentylenetetrazole-induced seizures and oxidative stress in adult male rats: evidence for GABAergic involvement

Hossein Akbari[1], Fatemeh Shahsavari[2,3], Mehdi Abbas nejad[4*], Saeed Esmaaeili Mahani[5]

https://doi.org/10.22034/bsr.2026.594736.1024

[1] *Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran.*

[2,3] *Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran; Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.*

[4] *Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran.*

[5] *Department of Biology, Faculty of Sciences, Shahid Bahonar University of Kerman, Kerman, Iran.*

ARTICLE INFO

Article Type
Original Article

Article History
Received: 31 July 2026
Accepted: 13 September 2026
Published: 31 August 2026

***Corresponding author**
mabbas@uk.ac.ir

ABSTRACT

Epilepsy is a common neurological disorder, and many patients remain resistant to available antiepileptic drugs. This study investigated the effects of intracerebroventricular (ICV) administration of abscisic acid (ABA), a plant-derived compound with antioxidant and anti-inflammatory properties, in a pentylenetetrazole (PTZ)-induced seizure model. Fifty-six adult male rats were divided into eight groups: Control, Sham + PTZ, PTZ, diazepam + PTZ, three ABA-treated groups (2.5, 5, and 10 µg/rat for five days), and a bicuculline + ABA + PTZ group to assess GABAergic involvement. Seizure severity was assessed using Racine's scale, anxiety-like behavior by the elevated plus maze (EPM) and open field tests (OFT), and hippocampal oxidative stress markers, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), using ELISA. PTZ induced severe seizures, increased anxiety-like behaviors, elevated MDA levels, and reduced SOD activity (all $p < 0.001$). ABA, particularly at 10 µg/rat, attenuated seizure severity, prolonged seizure latency, improved anxiety-like behaviors, decreased MDA levels, and restored SOD and CAT activities, with the greatest effects observed at 10 µg/rat. Bicuculline partially attenuated the anticonvulsant and antioxidant effects of ABA, indicating the involvement of GABAergic signaling. ABA exerts anticonvulsant, anxiolytic, and antioxidant effects in PTZ-induced seizure, at least partly through modulation of the GABAergic system, suggesting its potential as a therapeutic candidate for epilepsy.

Keywords: Abscisic Acid, Seizure, Pentylenetetrazole, Oxidative Stress, GABA

How to cite this paper

Akbari, H., Shahsavari, F., Abbas nejad, M., Esmaaeili Mahani, S., 2026. Abscisic acid attenuates pentylenetetrazole-induced seizures and oxidative stress in adult male rats: evidence for GABAergic involvement. Biospecies Research, 3, pp. 211-226.

Introduction

Epilepsy is a chronic neurological condition marked by recurrent, unprovoked seizures that arise from abnormal, excessive, and synchronized electrical discharges within the brain. Affecting nearly 50 million individuals across the globe, it represents one of the most common neurological disorders and continues to impose a substantial burden on public health systems worldwide (Kosenkov et al., 2025; Ji et al., 2025a).

In addition to recurrent seizures, individuals with epilepsy frequently experience psychiatric comorbidities, particularly anxiety and depression, which significantly affecting their quality of life (Lima et al., 2021, Mula et al., 2021, Kwong et al., 2016, Piazzini et al., 2008). Anxiety is one of the most common psychiatric comorbidities in patients with epilepsy, with a prevalence up to three times higher than that in the general population (Gavrilovic et al., 2024). The bidirectional relationship between epilepsy and anxiety is complex and is thought to involve several overlapping neurobiological mechanisms, including GABAergic dysfunction, dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis, chronic neuroinflammation, and oxidative stress (Basu et al., 2021, Suleymanova, 2021, Zhu et al., 2018, Peek et al., 2024).

Pentylenetetrazole (PTZ) is a widely used chemoconvulsant agent that reliably induces seizures by acting as a noncompetitive antagonist of GABA_A receptors, thereby disrupting inhibitory neurotransmission and increasing neuronal excitability (Monteiro et al., 2024). Accordingly, the PTZ model has become a well-established experimental platform for investigating seizure mechanisms and evaluating potential anticonvulsant therapies, as well as seizure-associated behavioral abnormalities.

Seizure activity induces mitochondrial dysfunction and excessive production of reactive oxygen species (ROS), leading to disruption of cellular homeostasis and neuronal damage (Pearson-Smith and Patel, 2017). Elevated MDA levels, together with the decreased activities of SOD, glutathione peroxidase (GPx), and reduced glutathione (GSH), have been consistently reported in both experimental models and patients with epilepsy. Furthermore, seizures stimulate the release of pro-inflammatory cytokines, creating a vicious cycle of oxidative stress and inflammation that further exacerbates seizure severity (Aguiar et al., 2012, Puttachary et al., 2015). Therefore, targeting oxidative stress and neuroinflammation represents a promising therapeutic strategy for epilepsy.

Given the central role of oxidative stress and inflammation in epileptogenesis, antioxidant and anti-inflammatory approaches have attracted increasing attention as potential adjunctive or alternative therapies. However, currently available antiepileptic drugs primarily modulate ion channels or synaptic transmission and often fail to prevent epileptogenesis or alleviate the cognitive and emotional comorbidities associated with epilepsy. Moreover, approximately one-third of patients remain resistant to pharmacological treatment (Löscher et al., 2020). These limitations highlight the need for novel therapeutic agents with multimodal mechanisms of action that target both seizure activity and its associated neuropsychiatric complications.

ABA is a plant-derived phytohormone traditionally recognized for its role in regulating plant stress responses. In both plant and

mammalian tissues, its biological effects are mediated, at least in part, through activation of Lanthionine synthetase C-like protein 2 (LANCL2) and peroxisome proliferator-activated receptors (PPARs) (Guri et al., 2010, Sturla et al., 2009). Recently, ABA has attracted considerable attention as a potential therapeutic agent for several diseases in mammalian systems (Gharib et al., 2024). ABA is also endogenously present in mammals and has demonstrated beneficial effects in experimental models of neuropsychiatric and neurodegenerative disorders. Central administration of ABA has been shown to alleviate anxiety- and depression-like behaviors in rodents exposed to stress (Shabani et al., 2022). In addition, ABA improves spatial learning, memory, and anxiety-like behaviors (Naderi et al., 2017). Several studies have reported that ABA enhances antioxidant defense by increasing the activities of antioxidant enzymes, including SOD and GSH, while reducing ROS production and lipid peroxidation in rats (Soti et al., 2019, Jiang and Zhang, 2001, Bellaire et al., 2000, Celik et al., 2007). Furthermore, accumulating evidence indicates that ABA exerts potent anti-inflammatory and analgesic effects (Ahmadi et al., 2025, Bassaganya-Riera et al., 2010).

Despite significant advances in understanding the pathophysiology of epilepsy and the development of numerous antiepileptic drugs, there remains an urgent need for novel therapeutic strategies with greater efficacy and fewer adverse effects. We hypothesized that central administration of ABA would protect against PTZ-induced seizures by reducing oxidative stress and improving anxiety-like behaviors. Therefore, this study evaluated the anticonvulsant effects of ABA by investigating: its efficacy against PTZ-induced seizures, effects on seizure-associated behavioral alterations, its antioxidant capacity in the hippocampus, and finally the involvement of GABAergic neurotransmission using pharmacological antagonism. Although the antioxidant and anti-inflammatory properties of ABA have been demonstrated in several neurological disorders, its interaction with GABAergic signaling during PTZ-induced seizures remains largely unexplored.

Materials and Methods

Animals and Housing

Fifty-six adult male Wistar rats (weighing 250–270 g) were obtained from the Animal House of Kerman University of Medical Sciences (KUMS), Iran. Animals were housed in groups of four per cage under controlled environmental conditions (22 ± 2 ˚C, 50–60% humidity, 12 h light/dark cycle), with free access to standard laboratory chow and tap water. All experimental protocols were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of Committee of Shahid Bahonar University of Kerman (IR.UK.VETMED.REC.1403.012).

Experimental Design and Grouping

Animals were randomly divided into eight experimental groups (n = 7/group). The sample size of seven animals per group was justified based on previous published studies and pilot experiments conducted in our laboratory evaluating anticonvulsant effects in acute PTZ-induced seizure models (Zamyad et al., 2019). The groups were designated as follows:

- Control group: Intact animals with no surgical intervention and no drug administration
- PTZ group: Animals receiving PTZ (50 mg/kg, i.p.) without any surgical intervention
- Sham + PTZ group: Animals undergoing complete stereotaxic surgery and ICV injection of vehicle (DMSO + saline, in a ratio of 2:1) followed by PTZ administration (50 mg/kg, i.p.)
- Diazepam + PTZ group: Animals receiving diazepam (2 mg/rat, i.p.) administered 20 minutes before PTZ
- ABA (2.5 µg/rat) + PTZ group: Animals receiving ICV ABA at 2.5 µg/rat once daily for 5 days, followed by PTZ.
- ABA (5 µg/rat) + PTZ group: Animals receiving ICV ABA at 5 µg/rat once daily for 5 days, followed by PTZ.
- ABA (10 µg/rat) + PTZ group: Animals receiving ICV ABA at 10 µg/rat once daily for 5 days, followed by PTZ.
- Bicuculline + ABA (10 µg/rat) + PTZ group: Animals receiving ICV bicuculline (6 µg/rat) 30 min prior to ABA (10 µg/rat, ICV), followed by PTZ

In addition, treatment administration was performed by a separate investigator not involved in outcome assessment. All behavioral assessments (seizure scoring, EPM, OFT) were performed by investigators blinded to treatment allocation. Biochemical sample processing and analysis were conducted by laboratory personnel unaware of group assignments. Unblinding occurred only after completion of all statistical analyses. A schematic representation of the experimental design is provided in Figure 1.

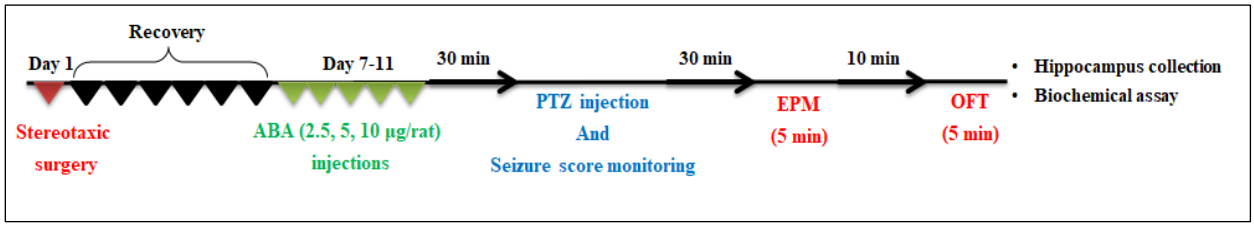

Figure 1. Schematic representation of experimental groups and treatment timeline.

Stereotaxic Surgery and Drug Administration

Rats were anesthetized with an intraperitoneal injection of ketamine (80 mg/kg) combined with xylazine (3 mg/kg) and mounted in a stereotaxic frame (Stoelting Co., USA). Bilateral 22-gauge guide cannulae were stereotaxically implanted into the lateral ventricles according to the Paxinos and Watson rat brain atlas, using the following coordinates relative to bregma: AP, −1.6 mm; ML, ±0.8 mm; and DV, −3.4 mm. The cannulae were fixed in place with stainless-steel screws and dental acrylic cement. Following surgery, the animals were allowed to recover for seven days before the experiments.

For intracerebroventricular administration, ABA was dissolved in DMSO and delivered in a total volume of 3 µL through a Hamilton microsyringe once daily for five consecutive days. Thirty minutes after the final administration of ABA or its vehicle (DMSO + saline, ratio 2:1), seizures were induced by intraperitoneal injection of pentylenetetrazol (PTZ; 50 mg/kg). Bicuculline was dissolved in normal saline and the final injection volume was considered 1 µL per side per rat. The injection volume for PTZ and diazepam were 1 mL/kg.

Seizure Induction and Behavioral Assessment

Seizures were induced with a single i.p. injection of PTZ (50 mg/kg), administered 30 minutes after the final treatment. Each rat was placed in an individual transparent Plexiglas chamber (30 × 30 × 30 cm), and behavior was video-recorded for 40 minutes. Seizure severity was scored using the Racine scale as follows: Stage 0: No response, Stage 1: Facial automatisms, Stage 2: Myoclonic jerks, Stage 3: Forelimb clonus, Stage 4: Rearing and Stage 5: Rearing and falling (generalized tonic–clonic seizures). In this study, latency to seizure onset and the duration of myoclonic, tonic, and tonic-clonic seizures were recorded for each animal.

Anxiety-Like Behavioral Tests

Elevated Plus Maze (EPM)

Anxiety-like behavior was assessed using the Elevated Plus Maze (EPM) test, conducted 30 minutes after PTZ administration. The EPM apparatus comprised two opposing open arms (50 × 10 cm) and two opposing closed arms (50 × 10 × 40 cm), positioned 54 cm above the floor. At the start of each trial, each rat was placed in the central platform facing one of the open arms and allowed to explore the maze freely for 5 minutes. Behavioral activity was recorded using an overhead video camera, and the time spent in the open arms and the number of open-arm entries were quantified during subsequent video analysis. To eliminate odor cues, the maze was thoroughly cleaned with 70% ethanol between successive trials (Shahsavari et al., 2020).

Open Field Test (OFT)

The OFT was conducted in a square arena (60 × 60 × 50 cm) with the floor divided into 16 equal squares, including a central zone. Each rat was placed in one corner and allowed to explore for 5 minutes. Time spent in the central area and the number of entries was measured using a video tracking system. Testing occurred under standardized light and noise conditions (Shahsavari et al., 2020).

Biochemical Analysis of Oxidative Stress Markers

At the end of behavioral testing, animals were euthanized using CO_2 inhalation. Brains were rapidly removed, and the hippocampi were dissected on ice and stored at −80 °C until analysis. Approximately 25 mg of tissue was homogenized in cold PBS and centrifuged at 1,200 ×g for 15 minutes at 4 °C. The supernatants were stored at −80 °C.

Oxidative stress parameters were evaluated using commercially available colorimetric assay kits from Carmania Pars Gene Company (Kerman, Iran). Hippocampal MDA, CAT, and SOD levels were measured according to the manufacturer's instructions using an ELISA reader. MDA levels were determined using the Lipid Peroxidation Assay Kit (Catalog No. KPG-Nob) based on the thiobarbituric acid reactive substances (TBARS) method. Serum samples (100 µL) were incubated with TBA reagent (200 µL) at 95°C for 60 minutes, centrifuged at 3000 rpm for 10 minutes, and the absorbance of the supernatant was measured at 532 nm. MDA concentration was calculated from a standard curve and expressed as µmol/L. CAT activity was measured using the Catalase Activity Assay Kit (Catalog No. KPG-CAT) based on colorimetric detection of residual H_2O_2 at 570 nm.

Absorbance was measured and catalase activity calculated using the formula. SOD activity was determined using the Superoxide Dismutase Assay Kit (Catalog No. KPG-SOD) based on the inhibition of pyrogallol auto-oxidation method at 420 nm. The inhibition percentage was calculated as: Inhibition (%) = [1 – (A2sample – A1sample) ÷ (A2standard – A1standard)] × 100, and SOD activity was expressed as: SOD activity (U/mL) = Inhibition (%) × 0.42.

Statistical Analysis

Data are presented as the mean ± standard error of the mean (SEM). Statistical analyses were conducted using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison post hoc test. All analyses were performed with GraphPad Prism software (version 10). A p-value < 0.05 was considered statistically significant.

Results

Effects of ABA on PTZ-Induced Seizure Parameters

Seizure Severity

Administration of PTZ induced a characteristic progression of seizure behaviors in adult male rats, encompassing myoclonic jerks through generalized tonic–clonic convulsions. One-way ANOVA demonstrated significant treatment effects on the duration of myoclonic ($F(2.299, 13.80) = 27.63$, $P<0.0001$), tonic ($F(2.669, 16.02) = 19.40$, $P<0.0001$), and tonic–clonic ($F(2.620, 15.72) = 24.91$, $P<0.0001$) seizure episodes. Post hoc analysis using Tukey's test revealed that pretreatment with ABA at the highest dose (10 µg/rat) attenuated significant anticonvulsant protection, as evidenced by substantial reductions in the duration of myoclonic ($P=0.001$), tonic ($P=0.01$), and tonic–clonic ($P=0.002$) episodes relative to the PTZ control group. Furthermore, the anticonvulsant efficacy of ABA at 10 µg/rat was dose-dependent, demonstrating significantly greater protective effects compared to lower doses of ABA (2.5 and 5 µg/rat) ($P<0.05$, $P<0.01$, and $P<0.001$). Notably, co-administration of bicuculline, a competitive GABA_A receptor antagonist, significantly attenuated the anticonvulsant effects of ABA (10 µg/rat), resulting in increased seizure behaviors compared to both ABA (10 µg/rat) and diazepam treatment groups ($P<0.05$, $P<0.01$) (Figure 2).

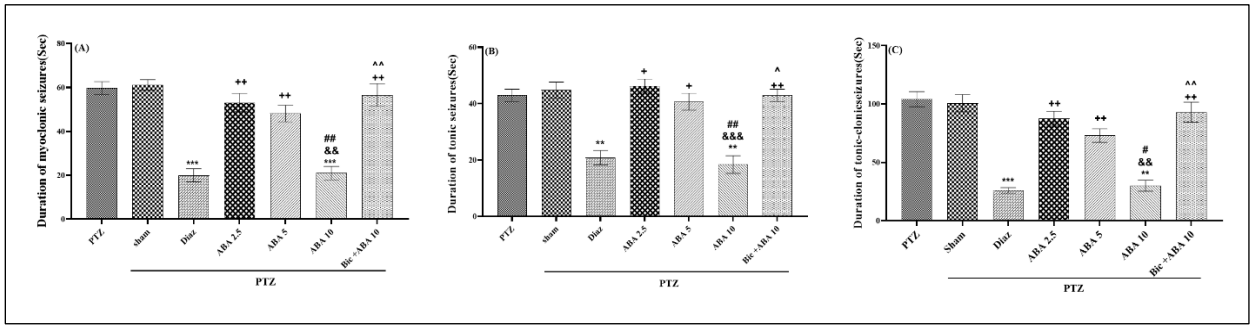

Figure 2. Duration of myoclonic (A), tonic (B), and tonic–clonic (C) seizures across treatment groups. Data are presented as mean ± SEM (n = 7). PTZ: pentylenetetrazole, Diaz: Diazepam, ABA: Abscisic acid, and Bic: Bicuculline. $^{**}p<0.01$ and $^{***}p<0.001$ *vs. Sham and PTZ groups,* $^{+}p<0.05$ and $^{++}p<0.01$ *vs. Diaz group,* $^{\hat{}}p<0.05$ and $^{\hat{}\hat{}}p<0.01$ *vs. ABA 10 group,* $^{\#}p<0.05$ and $^{\#\#}p<0.01$ *vs. ABA 5 group,* $^{\&\&}p<0.01$ and $^{\&\&\&}p<0.001$ *vs. ABA 2.5 group.*

Latency to Seizure Onset and Total Seizure Duration

One-way ANOVA indicated significant between-group differences in latency to first seizure ($F(2.312, 13.87) = 25.30$, $p < 0.001$) and cumulative seizure duration ($F(3.229, 19.38) = 19.36$, $p < 0.001$). Post hoc comparisons revealed that animals receiving PTZ alone exhibited rapid seizure onset with a mean latency of less than 200 seconds. In contrast, pretreatment with either diazepam or ABA at 10 µg/rat significantly prolonged seizure latency compared to the PTZ group ($p = 0.007$ and $p = 0.008$, respectively). This protective effect was reduced by bicuculline pretreatment, which significantly shortened seizure latency relative to both diazepam ($p = 0.004$) and ABA 10 µg/rat ($p = 0.005$) groups (Figure 3A).

Consistent with these findings, total seizure duration was significantly attenuated in animals treated with ABA 10 µg/rat or diazepam compared to PTZ controls ($p = 0.021$). Conversely, bicuculline pretreatment significantly prolonged total seizure duration relative to both diazepam ($p = 0.003$) and ABA 10 µg/rat ($p = 0.004$) treatments (Figure 3B).

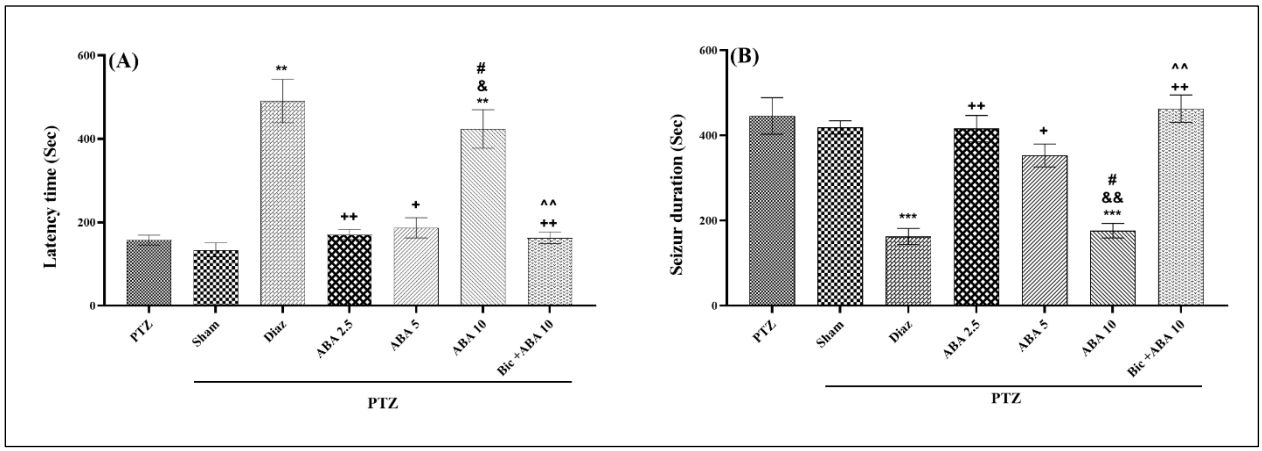

Figure 3. Seizure onset latency (A) and total seizure duration (B) across treatment groups. Data are mean ± SEM (n = 7). PTZ: pentylenetetrazole, Diaz: Diazepam, ABA: Abscisic acid and Bic: Bicuculline. $^{**}p < 0.01$ *and* $^{***}p < 0.001$ *vs. Sham and PTZ groups,* $^{+}p < 0.05$ *and* $^{++}p < 0.01$ *vs. Diaz group,* $^{\wedge\wedge}p < 0.01$ *vs. ABA 10 group,* $^{\#}p < 0.05$ *vs. ABA 5 group,* $^{\&}p < 0.05$ *and* $^{\&\&}p < 0.01$ *vs. ABA 2.5 group.*

Effects of ABA on Anxiety-Like Behavior in the Elevated Plus Maze

One-way ANOVA revealed significant treatment effects on time spent in open arms ($F(2.673, 16.04) = 14.42$, $p < 0.001$) and the frequency of open-arm entries ($F(3.376, 20.25) = 14.92$, $p < 0.001$). Post hoc analysis demonstrated that PTZ administration induced a pronounced anxiogenic response, characterized by a significant reduction in time spent exploring the open arms compared to control animals ($p = 0.004$). Although ABA at 10 µg/rat produced a notable increase in open-arm time, demonstrating an anxiolytic profile comparable to diazepam, this effect did not reach statistical significance when compared to either PTZ or sham-operated groups ($p = 0.561$). Bicuculline pretreatment reduced the anxiolytic tendency of ABA, significantly reducing open-arm time compared to both ABA 10 µg/rat and diazepam groups ($p < 0.05$) (Figure 4A).

A parallel pattern emerged in the analysis of open-arm entries. Post hoc testing indicated that PTZ significantly reduced the number of entries relative to controls ($p = 0.025$), whereas ABA at 10 µg/rat significantly increased entries compared to PTZ ($p = 0.0003$), ABA 2.5 µg/rat ($p = 0.0025$), and sham groups ($p = 0.017$). However, bicuculline administration significantly diminished open-arm entries relative to both control ($p = 0.011$) and ABA 10 µg/rat ($p = 0.0151$) groups (Figure 4B).

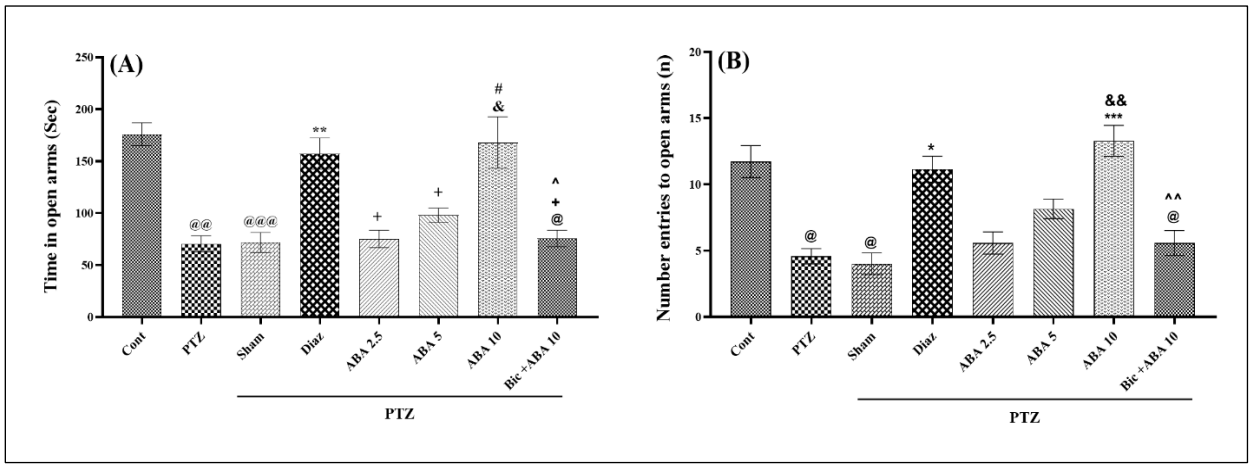

Figure 4. Time spent (A) and number of entries (B) into open arms in the EPM. Data are mean ± SEM (n = 7). Cont: Control, PTZ: pentylenetetrazole, Diaz: Diazepam, ABA: Abscisic acid and Bic: Bicuculline. *$^{@}p < 0.05$, $^{@@@}p < 0.001$ vs. Cont group, $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ vs. Sham and PTZ groups, $^{+}p < 0.05$ vs. Diaz group, $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$ vs. ABA 10 group, $^{\#}p < 0.05$ vs. ABA 5 group, $^{\&}p < 0.05$ and $^{\&\&}p < 0.01$ vs. ABA 2.5 group.*

Effects of ABA on Exploratory Behavior in the Open Field Test

In the OFT paradigm, one-way ANOVA demonstrated significant between-group differences in time spent in the central zone ($F(2.299, 13.80) = 27.63$, $p < 0.0001$) and the number of center entries ($F(2.978, 17.87) = 16.43$, $p < 0.0001$). Post hoc comparisons revealed that PTZ-treated animals exhibited significantly reduced central zone exploration time compared to controls ($p = 0.021$), indicative of heightened anxiety-like behavior. Conversely, ABA at 10 µg/rat significantly increased center zone duration relative to PTZ ($p = 0.001$), sham ($p = 0.003$), and ABA 5 µg/rat ($p = 0.027$) groups. This anxiolytic-like effect was reduced by bicuculline, which significantly decreased central zone time compared to both ABA 10 µg/rat ($p = 0.021$) and diazepam ($p = 0.010$) treatments (Figure 5A).

Similarly, analysis of center zone entries revealed that PTZ administration significantly reduced the frequency of entries compared to controls ($p = 0.021$). Both diazepam ($p = 0.006$) and ABA 10 µg/rat ($p = 0.001$) significantly increased center entries relative to the PTZ group. Bicuculline decreased this effect, significantly reducing center entries compared to control ($p = 0.046$), diazepam ($p = 0.01$), and ABA 10 µg/rat ($p = 0.021$) groups (Figure 5B).

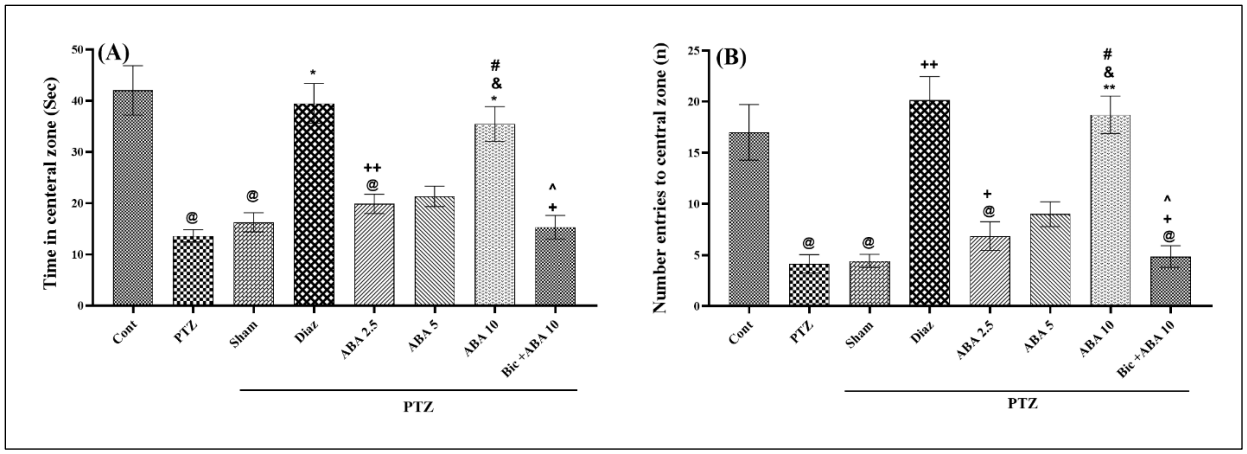

Figure 5. (A) Time spent and (B) entries into the central zone of the OFT. Data are mean ± SEM (n = 7). Cont: Control, PTZ: pentylenetetrazole, Diaz: Diazepam, ABA: Abscisic acid and Bic: Bicuculline. $^{@}p<0.05$ vs. Cont group, $^{*}p<0.05$ and $^{**}p<0.01$ vs. Sham and PTZ groups, $^{+}p<0.05$ and $^{++}p<0.01$ vs. Diaz group, $^{\wedge}p<0.05$ vs. ABA 10 group, $^{\#}p<0.05$ vs. ABA 5 group, $^{\&}p<0.05$ vs. ABA 2.5 group.

Effects of ABA on Hippocampal Oxidative Stress Markers

One-way ANOVA revealed significant treatment effects on hippocampal oxidative stress biomarkers, including CAT ($F(7, 32) = 9.623$, $P<0.0001$), SOD ($F(3.133, 12.53) = 29.73$, $P<0.0001$), and MDA ($F(2.618, 10.47) = 20.50$, $P<0.0001$). Post hoc analysis demonstrated that ABA treatment significantly modulated the hippocampal antioxidant defense system. Specifically, CAT activity was significantly elevated in animals receiving ABA at 10 µg/rat compared to both PTZ ($P=0.005$) and sham-operated ($P=0.030$) groups. This enhancement was decreased by bicuculline, which significantly reduced CAT activity relative to both ABA 10 µg/rat ($P=0.003$) and diazepam ($P=0.015$) groups (Figure 6A).

Analysis of SOD activity revealed significant reductions in PTZ ($P=0.011$) and ABA 2.5 µg/rat ($P=0.013$) groups compared to controls, potentially reflecting seizure-induced oxidative burden. ABA at 10 µg/rat significantly enhanced SOD activity compared to PTZ ($P=0.007$), sham ($P<0.0001$), and ABA 2.5 µg/rat ($P=0.014$) groups. However, bicuculline pretreatment significantly attenuated SOD activity relative to both diazepam ($p=0.013$) and ABA 10 µg/rat ($p=0.014$) (Figure 6B).

Evaluation of MDA levels, a well-established marker of lipid peroxidation and oxidative damage, demonstrated significant elevations in both PTZ ($P=0.010$) and sham ($P=0.007$) groups compared to untreated controls. ABA at 10 µg/rat significantly attenuated hippocampal MDA accumulation relative to both PTZ ($P=0.042$) and sham ($P=0.014$) groups. This antioxidant effect was reduced by bicuculline, which markedly increased MDA levels compared to ABA 10 µg/rat ($P=0.013$) and diazepam ($P=0.010$) treatments (Figure 6C).

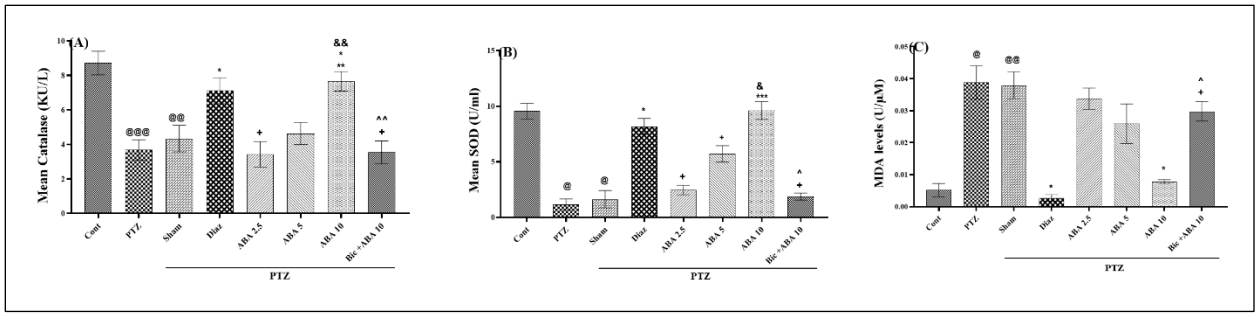

Figure 6. Hippocampal oxidative stress biomarkers: Catalase (CAT) (A), Superoxide dismutase (SOD) (B), and Malondialdehyde (MDA) (C). Data are expressed as mean ± SEM (n = 7). Cont: Control, PTZ: pentylenetetrazole, Diaz: Diazepam, ABA: Abscisic acid and Bic: Bicuculline. $^{@}p<0.05$, $^{@@}p<0.01$ and $^{@@@}p<0.001$ vs. Cont group, $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ vs. Sham and PTZ groups, $^{+}p<0.05$ vs. Diaz group, $^{\hat{}}p<0.05$ and $^{\hat{}\hat{}}p<0.01$ vs. ABA 10 group, $^{\&}p<0.05$ and $^{\&\&}p<0.01$ vs. ABA 2.5 group.

Discussion

The current study demonstrates that central administration of ABA significantly attenuated PTZ-induced seizure activity while simultaneously improving anxiety-like behavior and restoring hippocampal antioxidant status. Specifically, ABA at 10 µg/rat prolonged seizure latency, reduced seizure duration, improved behavioral performance in the EPM test, decreased MDA levels, and increased the activities of the endogenous antioxidant enzymes SOD and CAT. Furthermore, the partial attenuation of these protective effects by bicuculline suggests that activation of GABAergic signaling contributes, at least in part, to the potential neuroprotective actions of ABA. Collectively, these findings indicate that ABA at 10 µg/rat produced the most pronounced effects. In addition, ABA may exert anticonvulsant effects through multiple interacting mechanisms rather than through modulation of a single molecular pathway (Monteiro et al., 2024, Gharib et al., 2024).

Epileptic seizures are characterized by excessive and synchronous neuronal firing resulting from an imbalance between excitatory and inhibitory neurotransmission. In the PTZ model, seizure generation primarily occurs through antagonism of GABA_A receptors, leading to reduced inhibitory tone and increased neuronal excitability (Psarropoulou et al., 1994). Therefore, the significant prolongation of seizure latency together with the reduction in seizure duration observed in the present study suggests that ABA at 10 µg/rat effectively attenuated PTZ-induced neuronal hyperexcitability. Because bicuculline markedly attenuated the protective effects of ABA, our findings further support the hypothesis that enhancement of GABAergic neurotransmission is involved in the anticonvulsant action of ABA. Although the present study was not designed to determine whether ABA directly interacts with GABA_A receptors or indirectly regulates GABAergic signaling, previous investigations have demonstrated that modulation of inhibitory neurotransmission represents one of the principal mechanisms underlying seizure suppression (Tipton and Russek, 2022). The present findings therefore provide evidence suggesting that GABAergic signaling may contribute to the anticonvulsant effects of ABA.

Interestingly, bicuculline did not completely abolish the anticonvulsant effects of ABA. This

observation indicates that additional signaling pathways are likely involved. Increasing evidence suggests that epilepsy is a multifactorial process involving oxidative stress, neuroinflammation, mitochondrial dysfunction, altered calcium homeostasis, excitotoxic glutamate release, and apoptotic signaling (Sumadewi et al., 2023, Xu et al., 2025, Sun et al., 2025, Ji et al., 2025). Consequently, compounds capable of simultaneously targeting several pathological mechanisms may provide greater therapeutic efficacy than agents acting exclusively on neuronal excitability. The present findings support this concept and suggest that ABA may act as a potential neuroprotective agent rather than as a conventional anticonvulsant drug.

Oxidative stress is increasingly recognized as one of the major contributors to both seizure initiation and seizure-induced neuronal injury. During epileptic seizures, excessive neuronal activity dramatically increases cerebral metabolic demand, resulting in mitochondrial dysfunction and overproduction of reactive oxygen species (ROS). These reactive molecules subsequently induce lipid peroxidation, protein oxidation, DNA damage, and impairment of endogenous antioxidant defense systems, thereby establishing a vicious cycle that further lowers seizure threshold and exacerbates neuronal injury (Pearson-Smith and Patel, 2017, Ji et al., 2025, Borowicz-Reutt and Czuczwar, 2020). Consistent with this mechanism, the present study demonstrated a marked elevation of MDA together with significant reductions in CAT and SOD activities following PTZ administration. ABA effectively reversed these biochemical alterations, indicating restoration of redox homeostasis within the hippocampus.

The antioxidant effects observed in the present study are in agreement with previous investigations demonstrating that ABA activates endogenous antioxidant pathways through LANCL2-mediated signaling and subsequent activation of peroxisome proliferator-activated receptor gamma (PPARγ) and nuclear factor erythroid 2-related factor 2 (Nrf2)-dependent transcriptional mechanisms (Parajuli et al., 2024, Spinelli et al., 2021). However, while our study demonstrates significant antioxidant effects of ABA through direct measurement of oxidative stress markers (MDA, SOD, and CAT) and provides evidence for GABAergic pathway involvement via bicuculline antagonism experiments, we acknowledge that the underlying molecular mechanisms require cautious interpretation. Previous studies in other experimental models have suggested that ABA may exert its protective effects through activation of the LANCL2 receptor, PPARγ, and Nrf2 signaling pathways (Parajuli et al., 2024, Spinelli et al., 2021); however, these molecular targets were not directly measured in the present investigation. While existing literature suggests ABA may activate endogenous antioxidant pathways through these mechanisms, our findings remain hypothetical within our experimental model. Therefore, while the existing literature provides a theoretical framework suggesting that ABA may engage these pathways, the involvement of LANCL2, PPARγ, and Nrf2 in the anticonvulsant and antioxidant effects observed in the present study remains speculative and requires direct experimental validation in future studies. The GABAergic modulation demonstrated in our study through bicuculline antagonism represents the primary mechanistic insight directly

supported by our data, whereas the involvement of LANCL2, PPARγ, and Nrf2 pathways constitutes a plausible hypothesis derived from previous research that warrants future investigation. Further studies employing molecular techniques such as Western blotting, quantitative PCR, immunohistochemistry, or receptor binding assays are needed to directly investigate whether these proposed signaling cascades contribute to the neuroprotective and antioxidant effects of ABA observed in our PTZ-induced seizure model. We recognize this distinction between demonstrated findings and literature-based hypotheses as an important limitation that should guide the interpretation of our results and inform future mechanistic research.

Previous investigation also demonstrates activation of Nrf2 promotes transcription of numerous antioxidant enzymes, including SOD, CAT, glutathione peroxidase, and heme oxygenase-1, thereby enhancing cellular resistance against oxidative injury (Ngo and Duennwald, 2022, Sahu and Jain, 2025, Costa et al., 2026). Although these molecular pathways were not directly examined in the present study, the significant restoration of antioxidant enzyme activities strongly supports the hypothesis that activation of endogenous antioxidant defense mechanisms contributes substantially to the anticonvulsant properties of ABA.

Another important finding of the present study was the improvement in anxiety-like behavior following ABA administration. Moreover, significant effects were primarily observed with the 10 μg/rat dose. Anxiety is one of the most common neuropsychiatric comorbidities in patients with epilepsy and substantially reduces quality of life, even in individuals with adequate seizure control (Gavrilovic et al., 2024). The coexistence of epilepsy and anxiety is thought to reflect shared pathological mechanisms, including hippocampal dysfunction, oxidative stress, neuroinflammation, and impaired GABAergic neurotransmission (Espinosa-Garcia et al., 2021, Gulyaeva, 2021). Consequently, the behavioral improvement observed after ABA treatment is unlikely to be independent of its anticonvulsant activity. Instead, restoration of hippocampal redox balance together with normalization of inhibitory neurotransmission may contribute simultaneously to seizure suppression and anxiety reduction.

This study has several important limitations that should be considered. First, the acute PTZ-induced seizure model does not fully replicate the pathological complexity of chronic epilepsy; therefore, extrapolation of our findings to chronic epileptogenesis should be made cautiously. Future studies utilizing chronic seizure models or repeated PTZ administrations are necessary to assess whether ABA's antioxidant effects extend to sustained neuroinflammatory responses. Second, only male animals were included; sex-related differences in ABA's potential effects warrant investigation. Third, the absence of a bicuculline + PTZ control group limits our ability to distinguish between ABA's GABAergic antagonism and bicuculline's intrinsic proconvulsant properties. Finally, this study lacked direct assessment of neuroprotection through histological examination, apoptosis markers, or neuronal survival assays. Future studies incorporating morphological, electrophysiological, immunohistochemical, direct GABA quantification, inflammatory marker

measurements and biochemical measures of neuronal viability are necessary to substantiate the potential neuroprotective properties of ABA.

In conclusion, the present findings demonstrate that central administration of ABA significantly attenuates PTZ-induced seizures, improves anxiety-like behavior, and restores hippocampal antioxidant capacity. The partial attenuation of ABA's effects by bicuculline suggests potential GABAergic involvement in its neuroprotective actions, while restoration of oxidative balance may provide an additional mechanism underlying its anticonvulsant efficacy. Collectively, these findings support ABA as a multifunctional antioxidant agent that warrants further investigation in chronic seizure models.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare no conflicts of interest.

Funding

There is no financial support.

Author contributions

H.Akbari: Data curation and Formal analysis; F.Shahsavari: Writing–original draft, M. Abbasnejad: Conceptualization and Supervision, S. Esmaeili Mahani: Editing–original draft.

References

Aguiar, C.C.T., Almeida, A.B., Araújo, P.V.P., Abreu, R.N.D.C.D., Chaves, E.M.C., Vale, O.C.D., Macêdo, D.S., Woods, D.J., Fonteles, M.M.D.F. and Vasconcelos, S.M.M., 2012. Oxidative stress and epilepsy: literature review. Oxidative medicine and cellular longevity, 2012(1), p.795259.

Ahmadi, A., Soti, M., Zarei, M.R., Kohlmeier, K.A. and Shabani, M., 2025. Abscisic acid ameliorates cognitive dysfunctions, but not facial allodynia, in a male rat nitroglycerin migraine model. Archives of Oral Biology, 176, p.106280.

Bassaganya-Riera, J., Skoneczka, J., Kingston, D.G.J., Krishnan, A., Misyak, S.A., Guri, A.J., Pereira, A., Carter, A.B., Minorsky, P., Tumarkin, R. and Hontecillas, R., 2010. Mechanisms of action and medicinal applications of abscisic acid. Current medicinal chemistry, 17(5), pp.467-478.

Basu, T., Maguire, J. and Salpekar, J.A., 2021. Hypothalamic-pituitary-adrenal axis targets for the treatment of epilepsy. Neuroscience letters, 746, p.135618.

Bellaire, B.A., Carmody, J., Braud, J., Gossett, D.R., Banks, S.W., Cranlucas, M. and Fowler, T.E., 2000. Involvement of abscisic acid-dependent and—Independent pathways in the upregulation of antioxidant enzyme activity during NaCl stress in cotton callus tissue. Free Radical Research, 33(5), pp.531-545.

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

Celik, I., Turker, M. and Tuluce, Y., 2007. Abcisic acid and gibberellic acid cause increased lipid peroxidation and fluctuated antioxidant defense systems of various tissues in rats. Journal of hazardous materials, 148(3), pp.623-629.

Costa, V.B., de Matos, I.A., Nogueira, I.R., de Godoi, M.A., Leite, F.R. and Guimarães-Stabili, M.R., 2026. Nrf2 activation in inflammatory diseases:

a review of natural and synthetic modulators. Oxidative Medicine and Cellular Longevity, 2026(1), p.4538420.

Espinosa-Garcia, C., Zeleke, H. and Rojas, A., 2021. Impact of stress on epilepsy: Focus on neuroinflammation—a mini review. International journal of molecular sciences, 22(8), p.4061.

Gavrilovic, A., Gavrilovic, J., Ilic Zivojinovic, J., Jeličić, L., Radovanovic, S. and Vesic, K., 2024. Influence of epilepsy characteristics on the anxiety occurrence. Brain Sciences, 14(9), p.858.

Gharib, A., Marquez, C., Meseguer-Beltran, M., Sanchez-Sarasua, S. and Sanchez-Perez, A.M., 2024. Abscisic acid, an evolutionary conserved hormone: Biosynthesis, therapeutic and diagnostic applications in mammals. Biochemical Pharmacology, 229, p.116521.

Gulyaeva, N.V., 2021. Stress-associated molecular and cellular hippocampal mechanisms common for epilepsy and comorbid depressive disorders. Biochemistry (Moscow), 86(6), pp.641-656.

Guri, A.J., Misyak, S.A., Hontecillas, R., Hasty, A., Liu, D., Si, H. and Bassaganya-Riera, J., 2010. Abscisic acid ameliorates atherosclerosis by suppressing macrophage and CD4+ T cell recruitment into the aortic wall. The Journal of nutritional biochemistry, 21(12), pp.1178-1185.

Ji, D., Mylvaganam, S., Ravi Chander, P., Tarnopolsky, M., Murphy, K. and Carlen, P., 2025. Mitochondria and oxidative stress in epilepsy: advances in antioxidant therapy. Frontiers in Pharmacology, 15, p.1505867.

Jiang, M. and Zhang, J., 2001. Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. Plant and Cell Physiology, 42(11), pp.1265-1273.

Jiang, M.Y. and Zhang, J.H., 2004. Abscisic acid and antioxidant defense in plant cells. Acta Botanica Sinica-English Edition-, 46(1), pp.1-9.

Kosenkov, A.M., Mal'tseva, V.N., Maiorov, S.A. and Gaidin, S.G., 2025. The role of the endocannabinoid system in the pathogenesis and treatment of epilepsy. Reviews in the Neurosciences, 36(4), pp.351-370.

Kwong, K.L., Lam, D., Tsui, S., Ngan, M., Tsang, B., Lai, T.S. and Lam, S.M., 2016. Anxiety and depression in adolescents with epilepsy. Journal of child neurology, 31(2), pp.203-210.

Lima, E.M., Gois, J., Paiva, M.L., Vincentiis, S., Moschetta, S. and Valente, K.D.R., 2021. Anxiety symptoms are the strongest predictor of quality of life in temporal lobe epilepsy. Seizure, 88, pp.78-82.

Löscher, W., Potschka, H., Sisodiya, S.M. and Vezzani, A., 2020. Drug resistance in epilepsy: clinical impact, potential mechanisms, and new innovative treatment options. Pharmacological reviews, 72(3), pp.606-638.

Monteiro, Á.B., Alves, A.F., Portela, A.C.R., Pires, H.F.O., de Melo, M.P., Barbosa, N.M.M.V. and Felipe, C.F.B., 2024. Pentylenetetrazole: A review. Neurochemistry international, 180, p.105841.

Mula, M., Kanner, A.M., Jetté, N. and Sander, J.W., 2021. Psychiatric comorbidities in people with epilepsy. Neurology: Clinical Practice, 11(2), pp. e112-e120.

Naderi, R., Esmaeili-Mahani, S. and Abbasnejad, M., 2017. Phosphatidylinositol-3-kinase and protein kinase C are involved in the pro-cognitive and anti-anxiety effects of phytohormone abscisic acid in rats. Biomedicine & Pharmacotherapy, 96, pp.112-119.

Ngo, V. and Duennwald, M.L., 2022. Nrf2 and oxidative stress: a general overview of mechanisms and implications in human disease. Antioxidants, 11(12), p.2345.

Parajuli, K.R., Jung, Y. and Taichman, R.S., 2024. Abscisic acid signaling through LANCL2 and PPARγ induces activation of p38MAPK resulting in dormancy of prostate cancer metastatic cells. Oncology reports, 51(3), p.39.

Pearson-Smith, J.N. and Patel, M., 2017. Metabolic dysfunction and oxidative stress in epilepsy. International Journal of Molecular Sciences, 18(11), p.2365.

Peek, S.I., Twele, F., Meller, S., Packer, R.M. and Volk, H.A., 2024. Epilepsy is more than a simple seizure disorder: Causal relationships between epilepsy and its comorbidities. The Veterinary Journal, 303, p.106061.

Piazzini, A., Beghi, E., Turner, K. and Ferraroni, M., 2008. Health-related quality of life in epilepsy: findings obtained with a new Italian instrument. Epilepsy & Behavior, 13(1), pp.119-126.

Psarropoulou, C., Matsokis, N., Angelatou, F. and Kostopoulos, G., 1994. Pentylenetetrazol-induced seizures decrease γ-aminobutyric acid-mediated recurrent inhibition and enhance adenosine-mediated depression. Epilepsia, 35(1), pp.12-19.

Puttachary, S., Sharma, S., Stark, S. and Thippeswamy, T., 2015. Seizure-induced oxidative stress in temporal lobe epilepsy. BioMed research international, 2015(1), p.745613.

Sahu, M. and Jain, U., 2025. Activation, interaction and intimation of Nrf2 pathway and their mutational studies causing Nrf2 associated cancer. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1871(5), p.167764.

Shabani, M., Ranjbar, H., Soti, M. and Naderi, R., 2022. Central injection of abscisic acid attenuates mood disorders induced by subchronic stress in male mice. Brain and Behavior, 12(12), p.e2796.

Shahsavari, F., Abbasnejad, M., Raoof, M. and Esmaeili-Mahani, S., 2020. The rostral ventromedial medulla orexin 1 receptors and extracellular signal-regulated kinase in hippocampus are involved in modulation of anxiety behavior induced by dental pulp nociception in adult male rats. Archives of Oral Biology, 116, p.104778.

Soti, M., Abbasnejad, M., Kooshki, R. and Esmaeili-Mahani, S., 2019. Central microinjection of phytohormone abscisic acid changes feeding behavior, decreases body weight, and reduces brain oxidative stress in rats. Nutritional Neuroscience, 22(10), pp.678-687.

Spinelli, S., Begani, G., Guida, L., Magnone, M., Galante, D., D'Arrigo, C., Scotti, C., Iamele, L., De Jonge, H., Zocchi, E. and Sturla, L., 2021. LANCL1 binds abscisic acid and stimulates glucose transport and mitochondrial respiration in muscle cells via the AMPK/PGC-1α/Sirt1 pathway. Molecular metabolism, 53, p.101263.

Sturla, L., Fresia, C., Guida, L., Bruzzone, S., Scarfì, S., Usai, C., Fruscione, F., Magnone, M., Millo, E., Basile, G. and Grozio, A., 2009. LANCL2 is necessary for abscisic acid binding and signaling in human granulocytes and in rat insulinoma cells. Journal of Biological Chemistry, 284(41), pp.28045-28057.

Suleymanova, E.M., 2021. Behavioral comorbidities of epilepsy and neuroinflammation: Evidence from experimental and clinical studies. Epilepsy & Behavior, 117, p.107869.

Sumadewi, K.T., Harkitasari, S. and Tjandra, D.C., 2023. Biomolecular mechanisms of epileptic seizures and epilepsy: a review. Acta epileptologica, 5(1), p.28.

Sun, H., Li, X., Zhao, W., Zhang, W. and Meng, H., 2025. Mitochondria-Associated Endoplasmic Reticulum Membranes as Potential Therapeutic Targets in Epilepsy. CNS Neuroscience & Therapeutics, 31(12), p.e70726.

Tipton, A.E. and Russek, S.J., 2022. Regulation of inhibitory signaling at the receptor and cellular level; advances in our understanding of

GABAergic neurotransmission and the mechanisms by which it is disrupted in epilepsy. Frontiers in Synaptic Neuroscience, 14, p.914374.

Xu, L., Zhao, Z., Zhang, Y. and Ma, C., 2025. The interplay between metabolism and neuroinflammation in epilepsy: mechanisms and therapeutic perspectives. Journal of Neuroinflammation, 22(1), p.265.

Zamyad, Mahnaz, et al. "The anticonvulsant effects of Ducrosia anethifolia (Boiss) essential oil are produced by its main component alpha-pinene in rats." Arquivos de neuro-psiquiatria 77.02 (2019): 106-114.

Zhu, S., Noviello, C.M., Teng, J., Walsh Jr, R.M., Kim, J.J. and Hibbs, R.E., 2018. Structure of a human synaptic GABAA receptor. Nature, 559(7712), pp.67-72.