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Perampanel increases seizure threshold in pentylenetetrazole-kindled mice and improves behavioral dysfunctions by modifying mRNA expression levels of BDNF/TrkB and inflammatory markers

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ABSTRACT

Perampanel (PER), a novel 3rd-generation antiseizure drug that modulates altered post-synaptic glutamatergic storming by selectively inhibiting AMPA receptors, is recently approved to treat intractable forms of seizures. However, to date, presumably consequences of long-term PER therapy on the comorbid deleterious psychiatric disturbances and its correlation with neuroinflammatory parameters are not fully investigated in chronic models of epilepsy. Therefore, we investigated the real-time effect of PER on brain electroencephalographic (EEG) activity, behavioral alterations, redox balance, and relative mRNA expression in pentylenetetrazole (PTZ) induced kindling. Male BALB/c mice were pretreated with PER (0.125, 0.25, and 0.5 mg/kg) for 3 weeks and challenged with 11 injections of PTZ at the sub-threshold dose of 40 mg/kg every other day. vEEG from implanted cortical electrodes was monitored to elucidate seizure propagation and behavioral manifestations. Recorded EEG signals exhibited that PER 0.5 mg/kg pretreatment exceptionally impeded the onset of sharp epileptic spike-wave discharges and associated motor symptoms. Additionally, qEEG analysis showed that PER prevented alterations in absolute mean spectral power and reduced RMS amplitude of epileptogenic spikes vs PTZ control. Furthermore, our outcomes illustrated that PER dose-dependently attenuated PTZ-evoked anxiety-like behavior, memory deficits, and depressive-like behavior that was validated by a series of behavioral experiments. Moreover PER, significantly reduced lipid peroxidation, AChE, and increased levels of SOD and total thiol in the mice brain via AMPAR antagonism. Post-PTZ kindling provoked overstimulation of BDNF/TrkB signaling and increased release of pro-inflammatory cytokines that were reversed by PER with suppression of iNOS in brain immune cells. In conclusion, our findings highlight that PER might play an auspicious preventive role in the proepileptic transformation of brain circuits via suppression of BDNF/TrkB signaling and reduced transcriptional levels of neuroinflammatory markers leading to improvised epilepsy-induced neurobehavioral and neurochemical effects.

1. Introduction

Epilepsy is a progressive brain disorder that strikes nearly 1% of the world's population (Stafstrom and Carmant, 2015). It is characterized by exaggerated neuronal firing in the central nervous system resulting in recurrent episodic seizures with associated neurobehavioral comorbidities (Mormann and Jefferys, 2013). It is generally accepted that in the precipitation of a seizure attack, the Glutamatergic system becomes hyper-excited, and GABAergic signaling declines, nevertheless the involvement of other systems such as cholinergic, neuropeptides, metabolic alterations, and many other neuromodulators are integrated part of epileptogenesis and active epilepsy (Barker-Haliski and Steve

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White, 2015; Imran et al., 2015). Hitherto, many mechanistic insights of typical antiseizure drugs (ASDs) are yet to be unraveled particularly their putative neuroprotective role in the development of epileptogenesis.

In the pentylenetetrazole (PTZ) kindled rodent model a repetitive time course administration of chemoconvulsant at a subconvulsive dose initiates a cascade of events that results in full-bloom established seizures (Shimada and Yamagata, 2018). Subsequent to PTZ, the down-regulation of inhibitory balance, the exaggerated influx of calcium ions, activation of the enzymes that generate reactive oxygen species (ROS), and many other simultaneous unexplored events contribute to the process of epileptogenesis (Łukawski and Czuczwar, 2023). In the full-established PTZ-kindled seizure models involvement of other co-morbidities such as learning/memory impairment, anxiety/depression-like behavior and many other psychological disorders are common (Hoeller et al., 2017*)*. This model mimics the human model and is substantially helpful for the investigation of neurophysiological, behavioral, and morphological damage during the time-course of seizure development and simultaneously for the provision of efficacy of existing and development of new ASDs (Kandratavicius et al., 2014).

α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, the key receptors responsible for conducting glutamatergic-driven fast excitatory neurotransmission postsynaptically, play an eminent role in the proepileptic transformation of brain circuits and thus AMPAR receptors have long emerged as a promising molecular target for antiseizure drug development (Adotevi et al., 2020). Several discrete competitive and non-competitive AMPAR antagonists have been investigated for their broad spectrum anti-convulsant activity in preclinical models. AMPAR antagonists have been reported to prevent the spread of epileptic activity and epileptogenesis-associated neurodegeneration.

Perampanel (PER) is a novel and selective non-competitive high-affinity AMPA receptor antagonist approved by the FDA in 2012 with some serious black box warnings as an adjunctive therapy to treat focal seizures and primarily generalized tonic-clonic convulsions (Rektor, 2013). PER has been reported to exhibit seizure modifying effects in different preclinical models of epilepsy i.e. maximal electroshock seizures (MES), 6-Hz model of partial seizures, PTZ provoked seizures, lithium pilocarpine-induced status epilepticus, and audiogenic seizures in DBA/2 mice (Hanada, 2014). However, presumably to date no data have been published on its dose-dependent impact in post-PTZ induced electrographic (EEG) changes in the cortex of mice brains, and its correlation with the amalgamated complex of psychiatric comorbidities such as cognitive deficits, anxiety, and depressive-like behavior. Therefore, the current study intended to understand the neuroprotective prospects of PER and PTZ evoked behavioral ramifications in PTZ kindled animals. Moreover, the concentration dependent impact of PER in protection against epileptogenesis via modulation of the BDNF/TrkB signaling pathway and attenuating seizure-mediated neuro-inflammation has also been probed in this study.

2. Material and methods

2.1. Animals

At the start of the experiment, male BALB/c mice weighing 25–35 g were procured from the animal house of the Faculty of Pharmacy, Bahauddin Zakariya University Multan. A total of 84 adult mice were used in this study. The mice were accommodated in standard polycarbonate cages with strictly controlled environmental conditions i.e. 12 h light–dark cycle, consistent temperature of 23 ± 2 °C, sterile bedding, rodent food, and water ad libitum. To prevent overcrowding and cross-contamination only 4 mice were housed per cage. All cages were situated in the conventional noiseless animal house facility to prevent external stress. Animals were checked daily in the cage to observe seizure-related complications or distress. All in-vivo experimental tests were conducted during the light cycle i.e. 8:00 a.m.–6:00 p.m. The stringent experimental and housing protocol complied with standard ethical guidelines and was approved by the Departmental Ethics Committee (07-PHDL S18, Dated 08-February 2021) of the Department of Pharmacology, B.Z. University, Multan. Strict care was provided to animals and housing conditions were maintained throughout the PTZ kindling process to ensure animal well-being and coherence of our experimental results.

2.2. Drugs and chemicals

PER 0.125–0.5 mg/kg (FYCOMPA® 8 mg by Eisai Co., Ltd., Japan) was suspended in a solution of 1% tween 80, and mice were pretreated 30 min before PTZ administration (Mareš and Kubová, 2021). The chemo-convulsant PTZ (Sigma-Aldrich) was dissolved in 0.9% NS (normal saline) to administer at an appropriate sub-threshold dose of 40 mg/kg for kindling (Kamiński et al., 2020). A commercially available formulation of diazepam (Valium 10 mg/2 ml) was procured from Roche Pharma, Pakistan, and used as a positive control *(*Georgiev et al., 1991). All drug concoctions were freshly formulated to ensure consistency in drug potency and dispensed via the intraperitoneal (i.p.) route. Drug dosages (PER 0.125, 0.25, and 0.5 mg/kg), route of administration, and treatment therapies were meticulously designated and adhered to during the entire duration of this study.

2.3. Stereotaxic surgery

The cortical electrode implantation was performed according to our previously published method (Javaid et al., 2023). Briefly, Balb/c mice were anesthetized with chloral hydrate (400 mg/kg i.p.), and degree of consciousness was observed through crossed-extension reflex (foot withdrawal reflex) and then the mice head was fixed on the stereotaxic frame (Stoelting, USA). The fur from the animal's head was shaved and with a sterile surgical blade, a midline incision (2 cm) was made over the skull, and the periosteum was scrubbed to expose the skull surface. For surface EEG recording, 4 small holes were carefully drilled to minimize brain damage and meningeal penetration. The cortical tripolar electrodes were screwed at AP + 3 mm; LL ± 1.5 mm from bregma taking one screw implanted at AP − 2 mm; L − 1.5 mm as reference electrode. The screwed electrode was fixed with the help of dental acrylic and immediately after completion of surgery animals received 1 ml of ringer lactate i.p. to avert dehydration and were observed for post-anesthesia recovery. Throughout the surgical procedure, the body temperature of the mice was strictly maintained at 37 °C with the application of a heating pad. After surgery, animals were given a recovery period of 7 days before using them for PTZ-induced kindling.

2.4. Experimental design

A total of 84 adult Balb/c mice were bifurcated into two major groups and the PTZ kindling was prompted simultaneously in mice devoid of cortical EEG (electroencephalography) electrode implants (n = 48); for behavioral, neurochemical, and real-time polymerase chain reaction experiments and mice with implanted EEG electrodes (n = 36; for surface EEG recording).

The 48 mice without EEG electrodes were randomly sorted into six groups (n = 8) labeled as **Tween 80:** vehicle control (1 ml/kg, i.p.), **PTZ control:** PTZ (40 mg/kg, i.p.), **Dia 1:** Diazepam (1 mg/kg, i.p.). The mice of **PER 0.125**, **PER 0.25,** and **PER 0.5** were pretreated with perampanel 0.125, 0.25, and 0.5 mg/kg on every alternate day before PTZ administration for 3 weeks. The animals in Dia 1 and tween 80 groups received a single dose of pretreatment with their respective aforementioned treatments on alternate days for 21 days. To induce kindling, repetitive exposure at the sub-convulsive dose of 40 mg/kg was done every other day in all groups except for the vehicle control and animals were observed for 30 min to quantify seizure severity as per the Racine scale. According to the modified Racine scale, the following stages were

considered subsequent to PTZ injection; **Stage 1**: no response, **stage 2:** Straub tail, head nodding, **Stage 3:** myoclonic jerks, whole body clonus **Stage 4** Forelimb clonus with rearing and falling, **stage 5:** wild jumping, GTCS; generalized tonic-clonic seizure, **Stage 6:** death. The animals exhibiting continual high-grade convulsions of stage 4–5 on three successive PTZ injections were designated as fully kindled (Rehman et al., 2022). By the end of the 11th PTZ injection, all animals without electrode implants were tested for post-kindling associated psychiatric comorbidities in a series of least to most repugnant neurobehavioral experiments. Mice were acquainted in the investigational facility for at least 30 min prior to the commencement of any behavioral test.

2.5. EEG acquisition

For EEG recordings, the 36 animals with cortical electrode implants were sorted into six groups (n = 6) and were treated with respective treatment regimens on every alternate day and challenged PTZ (40 mg/kg) every other day for 3 weeks. Animals after PER pretreatment (0.125–0.5 mg/kg) were connected to the 8-channel bioamplifiers (ADInstruments Ltd., Sydney, Australia) and an analog–digital converter

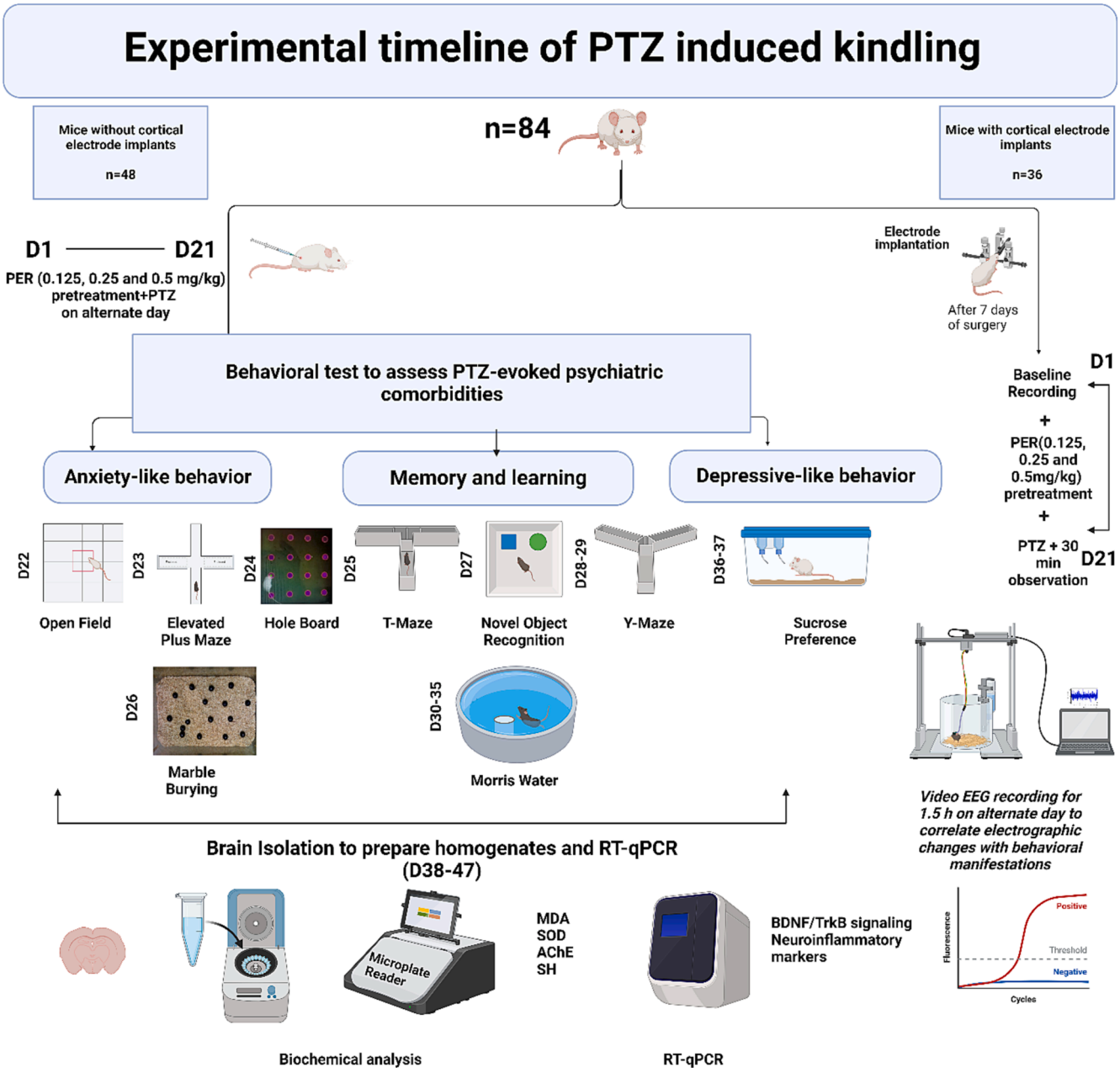

Fig. 1. Experimental layout representing the process of PTZ-induced kindling. A total of 84 Balb-c mice were randomly sorted into 2 groups, mice with EEG electrode implants (n = 36) and mice without EEG electrode implants (n = 48). All mice received PER (0.125, 0.25, and 0.5 mg/kg) pretreatment with subsequent PTZ challenge (40 mg/kg) on every other day for 3 weeks. vEEG was recorded to correlate EEG manifestations with behavioral severity. By the 11th injection, mice without EEG electrode implants were passed through a course of neurobehavioral tests to assess anxiety-like behavior (Open field test; OFT, Elevated plus maze; EPM, Hole board test; HBT, Marble burry test; MBT), short-term memory (T-maze; spontaneous alternation, Novel object recognition; NOR), long-term spatial memory (Y-maze; Novel arm recognition, Morris water maze; MWM), and depressive-like behavior (Sucrose preference test; SPT). At the end of behavioral experiments, brains were isolated for neurochemical analysis (Malondialdehyde; MDA, Super oxide dismutase; SOD, Acetylcholinesterase; AChE, and Thiol content; SH) and to check transcriptional levels of BDNF (brain-derived neurotrophic factor), TrkB (Tropomyosin receptor kinase B), and neuroinflammatory markers (Tumor necrosis factor; TNF-α, Interleukin 1 beta; IL-1β, Inducible nitric oxide synthase; iNOS via RT-qPCR.

(Power Lab 8/35, ADInstruments). After plugging the electrodes with the EEG acquisition system leads, the mice were acclimatized inside the Plexiglas EEG cage, and stable baseline EEG was recorded for 30 min prior to the administration of any drug. Subsequent to drug pretreatment after 60 min except in tween 80, brain EEG activity was observed to quantify epileptiform activity post-PTZ for 30 min using a signal sampling rate of 200 Hz and band pass filtered between 0.1 and 60 Hz. Concurrently, the video was recorded to correlate EEG findings with behavioral changes. Electrographic epileptic spikes were identified as events with peak amplitude 2 × baseline. EEG quantification was done with the previously reported algorithm by Anjum et al. (2018). Artifact-free EEG signals were analyzed with LabChart Pro version 8.1.19 for absolute spike power and RMS amplitude of epileptic spikes for 30 min following PTZ administration (see Fig. 1).

2.6. Open field test (OFT)

OFT is a well-known paradigm for anxiolytic profiling of therapeutic drugs and evaluating locomotive activity (Nesci et al., 2020; Parlar et al., 2020, 2022). For the assessment of anxiety-like behavior, a square arena (45 × 45 cm × 20 cm) was segregated between two zones i.e. peripheral and central. Acclimated mice were introduced at the center of a square chamber. Animals were allowed to spontaneously investigate the novel square chamber for 5 min and tested once only. Prior to the trial commencement with the next mice, 70% isopropyl alcohol was deployed in the open chamber to eliminate scent clues from the previously tested animal. The test session was recorded for each mouse separately using Logitech software and the rodent's tendency/avoidance towards the central zone was evaluated by the ANY-maze video tracking system (trial version 7.15). The number of visits and duration of stay in the central area of the open maze were observed to contemplate differences in levels of anxiety between kindled and non-kindled animals.

2.7. Elevated plus maze (EPM)

Mice challenged with PTZ were further evaluated in a plus-shaped maze with two bare and two walled arms (15 × 5.5 cm) raised 45 cm above the floor. Innately, rodents tend to stay in covered dark spaces but also depict curiosity-driven exploration of novel open surroundings (Nieoczym et al., 2021). All mice were allowed to freely roam both open and closed arms of the maze for the duration of 5 min. The number of visits and time spent in the exploration of open arms were monitored to elucidate intergroup variability in anxiogenic behavior.

2.8. Hole board test (HBT)

HBT is the commonly used ethologically based tool for quantification of the potential anxiolytic character of test drugs and associated rodent emotionality in behavioral pharmacology (Casarrubea et al., 2021). The HB comprises of closed square arena (40 × 40 cm) with sixteen equidistant anxiogenic holes (2.5 cm in dia) through which mice can snout. The increase in head poking (head dipping) behavior is inversely correlated with anxiety levels. In particular, a decrease in the frequency of head poking is transcribed as an altered exploratory behavior indicating a pronounced feeling of apprehension or disturbance in the animal's emotional assets. To test the impact of pharmacological manipulation on central anxiety levels animals were permitted to investigate the test apparatus for a total duration of 5 min and the number of head dipping was noted.

2.9. Marble burying test (MBT)

The MBT is a useful tool to screen anxiety-like behavior in preclinical studies. For the test, mice were placed in a plastic cage (28 × 45 × 14 cm) filled with sawdust litter and a total of 15 little balls arranged in regularly spaced five rows, with 3 marbles per row. Marble burying is directly associated with anxiousness in mice, as animals with the help of forepaws can dig the novel intimidating unknown objects into the sawdust (Thomas et al., 2009). All mice were tested separately and conceded to traverse the marble balls for 30 min, and the total count of buried and unburied marbles was noted. A marble ball is considered buried if 2/3 was camouflaged by sawdust litter.

2.10. T-maze test (spontaneous alternation)

Episodic spatial working memory in rodents can be assessed with the help of less aversive T-maze tests. The test equipment is the T-shaped apparatus comprising a stem arm or start arm (30 × 10 × 20 cm) and two lateral goal arms (left and right) at 90°. This task relies on the natural willingness of mice to delve into novel arms rather than the familiar ones which necessitates the alternation in the choice of the lateral goal arms across the repeated trials (d'Isa et al., 2021). During a trial of 5 min, mice without memory deficits usually show good reminiscence of the previously investigated arm and tend to enter new unexplored arm of the T-maze. The pattern of entries to each arm was observed and the percentage spontaneous alternation behavior was computed (% SAB) according to the formula:

% SAB = number of alternations/total number of entries-2 × 100.

2.11. Novel object recognition test (NOR)

The working memory can be assessed by observing the rodent's adroitness to distinguish between two unidentical objects. Due to their innate propensity, mice tend to traverse unknown novel environments, as the duration of sniffing with the novel object will be longer in animals with intact memory (Malik et al., 2023). For this purpose, two sessions were commenced s in the square arena (40 × 40 cm) with high walls to prevent the escape of animals (38 cm). In the training session, rodents were conceded to traverse two indistinguishable objects of the same shape, size, and height for 10 min followed by the substitution of one previously familiar object by an unknown different object. Mice interactions in the test session with both previously familiar and novel objects were observed to quantify time spent in the investigation of both objects. Object recognition score or discrimination index was computed which if raised, suggests that mice had good learning and recall capabilities. The object recognition score was computed by using the formula:

DI = Time with novel object-time with familiar/time with novel + time with familiar.

2.12. Y-maze (Novel arm) preference test

Long-term retention of spatial reference memory was estimated with the help of Y-maze. The test was conducted in two 5 min sessions over two days and the y-maze arms were divided as the start arm (SA), the other arm (OA), and the novel arm (NA). During training mice weren't allowed to explore the novel arm of the trio maze by blocking it with the help of a black baffle and animals only investigated the SA and OA arms of the maze (Conrad et al., 1997). After 24 h, NA was unblocked and mice were conceded to freely traverse three arms of the Y-shaped maze for a duration of 5 min. To elucidate intergroup differences in PTZ-induced memory impairment entries and investigation time in NA was noted.

2.13. Morris water maze test (MWM)

In MWM rodents were primed to track down hidden platform to evaluate long-term hippocampal-dependent learning and memory in rodents (Vorhees and Williams, 2006). A circular pool (100 × 60 cm) filled with water and opacified with some non-toxic dye i.e. milk or tempera paint supplemented with extra-maze distal and proximal colored geometric cues was used. The water tank was sectioned into four

quadrants (SW, NW, SE, and NE) and the rescue platform (12 × 12 cm) was positioned in the NW quadrant. The test was conducted in three phases: cued associated learning, and spatial acquisition followed by retention/probe trials. Prior to the experimental phase, mice were trained for 2 days, 3 sessions per day to locate visible platform. Each animal was gently lowered into the water tank and then allowed to navigate to find a rescue platform. Once found, mice were left on the platform for 10 s. Moreover, animals that didn't detect the rectangular rescue podium within 120 s were amiably pushed toward the rescue platform. After training, the platform was immersed approximately 1 in. below the surface of the water, and the time taken to reach the safe platform (escape latency) was noted for the next three days by testing. On the 6th day, a probe trial was commenced, the rescue platform was extirpated and animals were then allowed to navigate in the water for 2 min and their visits and duration of stay in the target quadrant were observed to estimate post-kindling memory dysfunction.

2.14. Sucrose preference test (SPT)

SPT estimates the animal's proclivity for sweet-tasting 1% sucrose solution over unsweetened simple water. A decrease in the percentage of sucrose preference is directly correlated with anhedonia, a hallmark of depressive-like behavior in rodents (Fonseca-Rodrigues et al., 2022). After 12–14 h of fasting, mice accommodated in their home cage were presented with two water bottles, one filled with sweet-tasting 1 % sucrose solution and the other with normal unsweetened tap water. After 24 h, the consumed volume for both sucrose solution and simple water was noted to compute the percentage sucrose preference.

% SPT = sucrose solution intake (ml)/sucrose solution intake + water intake (ml) × 100.

2.15. Brain homogenization for neurochemical analysis

Immediately after the end of behavioral experiments, mice were sacrificed (n = 6), and brains were isolated and individually homogenized (10 w/v) with PBS of pH 7.4 (Solarbio, Life Sciences). Homogenized brain samples were centrifuged at 12,000 rpm for 10 min at the low temperature of 4 ℃. The pellet was discarded and clear supernatants were carefully stored at −40 ℃ to assess markers of oxidative stress in kindled and non-kindled animals (Chaudhary and Parvez, 2012). Protein quantification was done for each brain sample according to the Lowry method (Waterborg, 2009).

2.15.1. Malondialdehyde (MDA)

To estimate PTZ-induced lipid peroxidation, levels of MDA were analyzed colorimetrically. The reaction of MDA with 0.375% thiobarbituric acid (TBA) generates pink colored TBA- MDA adduct, while 15% trichloroacetic acid increases its rate of condensation. Briefly, 100 µl of brain supernatant was added to TBA: TCA in an equal ratio of 1:1. The reaction mixture was boiled in a water bath maintained at 100 ℃ and then cooled followed by centrifugation at 10,000*g* for 10 min at 4 ℃. Lastly, the reading in duplicates was taken at 532 nm with the microplate reader (Spectramax 340 PC384 by Molecular Devices, CA, USA) at 532 nm and MDA levels were individually normalized with protein content for all mice brains (Chow and Tappel, 1972; Haider et al., 2015).

2.15.2. Superoxide dismutase (SOD)

To assess SOD activity, 50 µl of 50 mm Na_2CO_3 (Sodium carbonate, Sigma, Aldrich), 20 µl of 0.1 mm EDTA (ethylene diamine tetraacetic acid, Sigma, Aldrich), and 40 µl of 0.56 mM of NBT (nitro blue tetrazolium, Molekula, England) was added to 50 µl of brain supernatant. Then 40 µl of 0.1 mM HAC (hydroxyl amine chloride) was added to the reaction mixture and sample readings in duplicate were observed for up to 45 min with an interim of 5 min (Chidambara Murthy et al., 2002; Naskar et al., 2010).

2.15.3. Acetylcholinesterase (AChE)

To measure AChE activity, 40 µl of brain homogenate was blended with 138 ul of phosphate buffer and 20 µl of 0.01 M 5,5′-dithiobis (2-nitrobenzoic acid); (DTNB) to note basal reading at 412 nm followed by the addition of 2 µl acetylthiocholine iodide (Sigma, Aldrich). Reading was taken at 412 nm for up to 20 min with a time interval of 2 min (Tor et al., 1994).

2.15.4. Estimation of thiol contents

Thiol content was measured with Ellman's reagent. Briefly, 15 µl of supernatants were incubated with 175 µl of tris-EDTA buffer (pH = 8.6) and absorbance was taken at 412 nm against the buffer alone. Then 10 µl of DTNB (10 mm in absolute methanol) was mixed and readings were taken again after 15 min at 412 nm (Mansouri et al., 2021).

2.16. Quantitative Real-Time polymerase chain reaction (qRT-PCR)

To compute relative mRNA transcription levels, the brains of mice challenged with PTZ were removed and TRIzol reagent (Biobasic, Lot: BS410A-MA18DR0J) was utilized to extract total RNA. The purity and relative concentration of extracted RNA were determined on a Nano-Drop Lite spectrophotometer (Thermo Fischer Scientific, USA). Immediately after isolation, cDNA from 2000 ng RNA was synthesized using a commercial kit (Thermo scientific RevertAid kit; K1622. In detail, 0.5 µl of cDNA in a total 10 µl volume of the reaction mixture with SYBR Green reagents (PowerUp SYBR Green Master Mix; A25742) on Step One Plus Real-time PCR system (Applied Biosystems, USA) was used (Ashraf et al., 2013). The RT-PCR conditions included initial denaturation at 95 °C for 5 min, 40 cycles at 95 °C for 20 s, 60 °C for 20 s and 72 °C for 45 s. The expression of each gene was normalized against HPRT as an internal control and the $2-\Delta\Delta Ct$ method was used to analyze the relative fold changes. The primers for BDNF, TrkB, TNF-α, IL-1β, iNOS, and HPRT were designed using Primer-Blast. 22 and are presented in supplementary Table S1.

2.17. Statistical analysis

To illustrate intergroup differences between kindled and PER-treated mice the one-way ANOVA followed by Dunnett's multiple comparison tests was utilized to analyze all parameters of neurobehavioral, neurochemical, and RT-PCR studies except seizure score and escape latencies in Morris water maze test were evaluated by two-way ANOVA, and for EEG quantification, non-parametric Kruskal-Wallis test was applied by using GraphPad prism v. 8 for Windows (GraphPad Software, San Diego, CA, USA). Data is reported as means ± S.E.M and a probability value (P) of < 0.05 was considered statistically significant.

3. Results

3.1. Impact of PER on the progression of PTZ-prompted kindling process

To assess the dose-dependent impact of PER on the propagation of PTZ-spawned convulsive intensity, animals in control and treated groups were visually monitored meticulously after each PTZ injection at the subconvulsive dose of 40 mg/kg for the duration of 30 min. Two-way ANOVA depicted significant differences for both diseased and treated groups ($F(5,330) = 18.75$, ($P < 0.0001$)) (Fig. 2). The intermittent PTZ exposure caused a gradual escalation in seizure severity from no response (stage 1) to myoclonus jerking (stage 3) which then culminates in high-grade convulsive GTCS (generalized tonic-clonic convulsions) of stage 5. By the 5th PTZ injection, animals in disease control presented generalized clonus and rearing. Repetitive PTZ exposure in an alternate manner for 21 days provoked repetitive seizures of higher severity of stage 4–5 as per the Racine scale and the mice were fully kindled by the 11th injection. Dia 1 as a positive treatment control, significantly halted the progression of PTZ-induced kindling. Like wisely, PER treatment

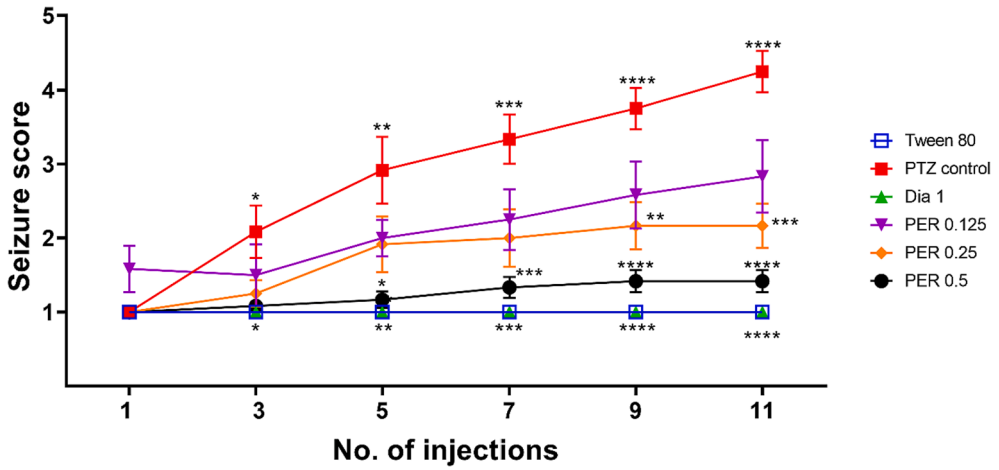

Fig. 2. Effect of PER (0.125, 0.25, and 0.5 mg/kg) pretreatment on average seizure score in animals challenged with PTZ. Mice were visually monitored meticulously after each PTZ injection at the sub-convulsive dose of 40 mg/kg for the duration of 30 min for seizure as per the Racine scale. All the values are expressed as mean ± SEM (n = 12), while *P < 0.05, ***P < 0.001, and ****P < 0.0001 represents the comparison between kindled and non-kindled mice.

arrested kindling propagation in a dose-dependent manner. Chronic treatment with PER 0.5 showed exceptional latency and remarkably attenuated kindling progression as 100 percent of the PTZ-challenged mice remained free from motor seizures and these outcomes were comparable with the mice receiving Dia 1 therapy. Similarly, the treatment with PER 0.25 interrupted PTZ-induced epileptogenesis as only 10 percent of the mice showed stage 4 seizures while others depicted full body clonus (stage 3) and mild seizures of stage 2

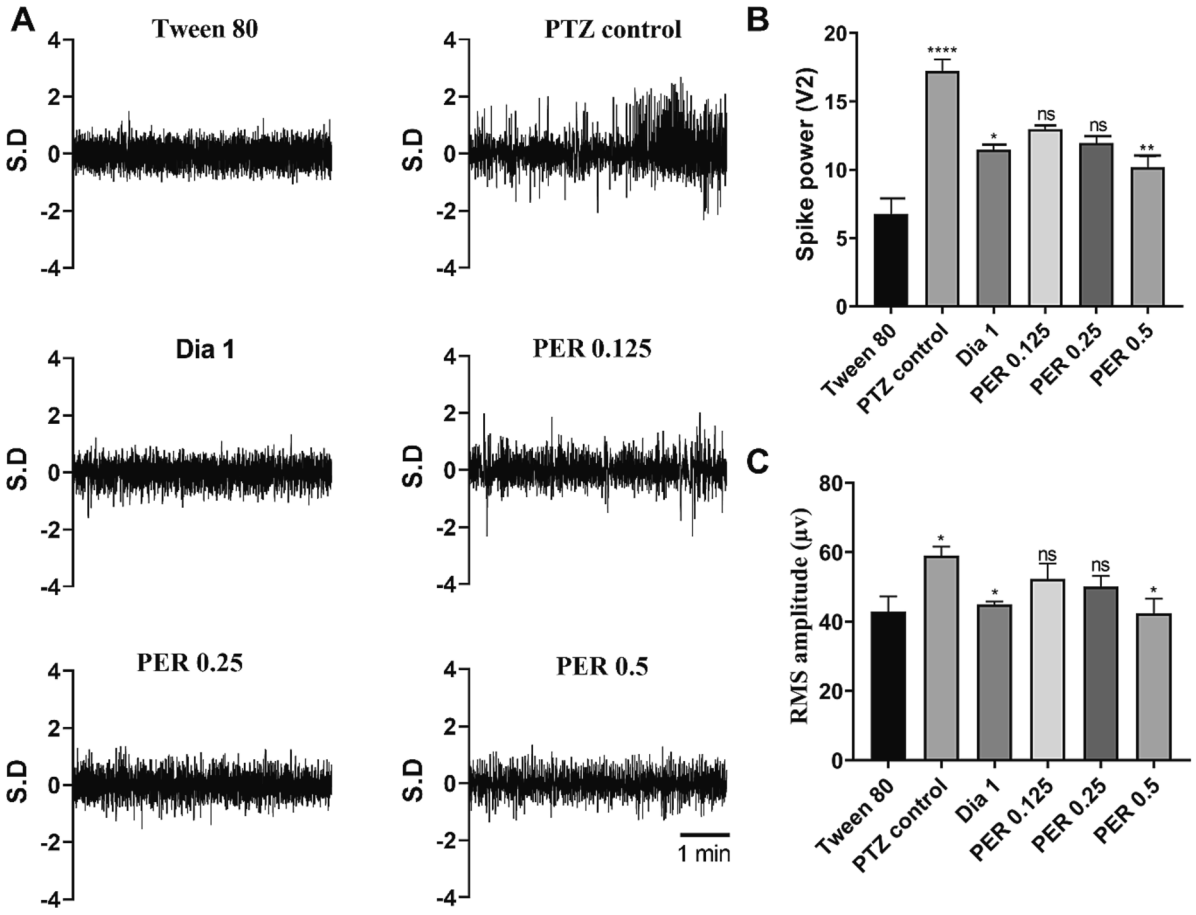

Fig. 3. (A) EEG tracings illustrating electrographic changes in kindled vs. non-kindled animals following PTZ treatment for 21 days. Administration of PTZ induced high amplitude rhythmic spike-wave discharges with behavioral GTCS of stage 5 in comparison with Tween80. Dia 1, PER 0.25, and PER 0.5 showed protection against unabated PTZ provoked EEG changes whereas low dose PER 0.125 depicted recurrent epileptic spikes of high amplitude. Data is represented in normalized SD values. **(B)** Absolute EEG power (V^2). **(C)** RMS amplitude which is computed by taking the root mean square of peak amplitude. Non-parametric Kruskal-Wallis test was used to quantify EEG changes and data are expressed as mean ± S.E.M (n = 6). *P < 0.05, **P < 0.01 and ****P < 0.0001 represents comparison between kindled and non-kindled mice.

continually in successive injections and therefore categorized as non-kindled. The PER 0.125 remained ineffective in delaying the PTZ instigated seizure severity and behavioral manifestations as 41 percent of the animals were fully kindled by the 11th injection similar to animals in the PTZ disease group.

3.2. EEG analysis of spike power and RMS amplitude

To correlate post-PTZ electrographic changes and behavioral manifestations, video-EEG for 30 min following PTZ administration was recorded in animals with implanted cortical electrodes (Fig. 3A). Quantification of spike power and RMS amplitude was done during the kindling process to compute the impact of chronic PER treatment for 21 days on PTZ-induced aberrant brain EEG activity. Spikes Power (V^2) was analyzed in the frequency domain (1–50 Hz) with FFT analysis at 1 K (1024). Kruskal-Wallis test revealed notable differences in the trend of mean spectral power in PTZ control and treated group ($F(5,30) = 28.13$, ($P < 0.0001$)) with $P < 0.0001$. Mice in the PTZ control group showed recurrent epileptic spikes with increased absolute power (17.21 ± 0.88) as compared to the tween-treated healthy control (6.78 ± 1.14) with $P < 0.0001$. Treatment with diazepam significantly lowered power intensity of electrographic spikes (11.45 ± 0.37) as compared to PTZ control with $P = 0.0259$ (Fig. 3B). However in comparison with the PTZ control, animals treated with PER 0.125 (12.98 ± 0.25) and PER 0.25 (11.96 ± 0.50) showed non-significant decrease in absolute mean EEG power post PTZ 30 min with $P = 0.8532$, and $P = 0.1232$, respectively. Conversely, treatment with PER 0.5 significantly reserved normal EEG activity as the mean absolute power following PTZ administration was significantly lowered (10.16 ± 0.82) as compared to diseased animals with $P = 0.0015$.

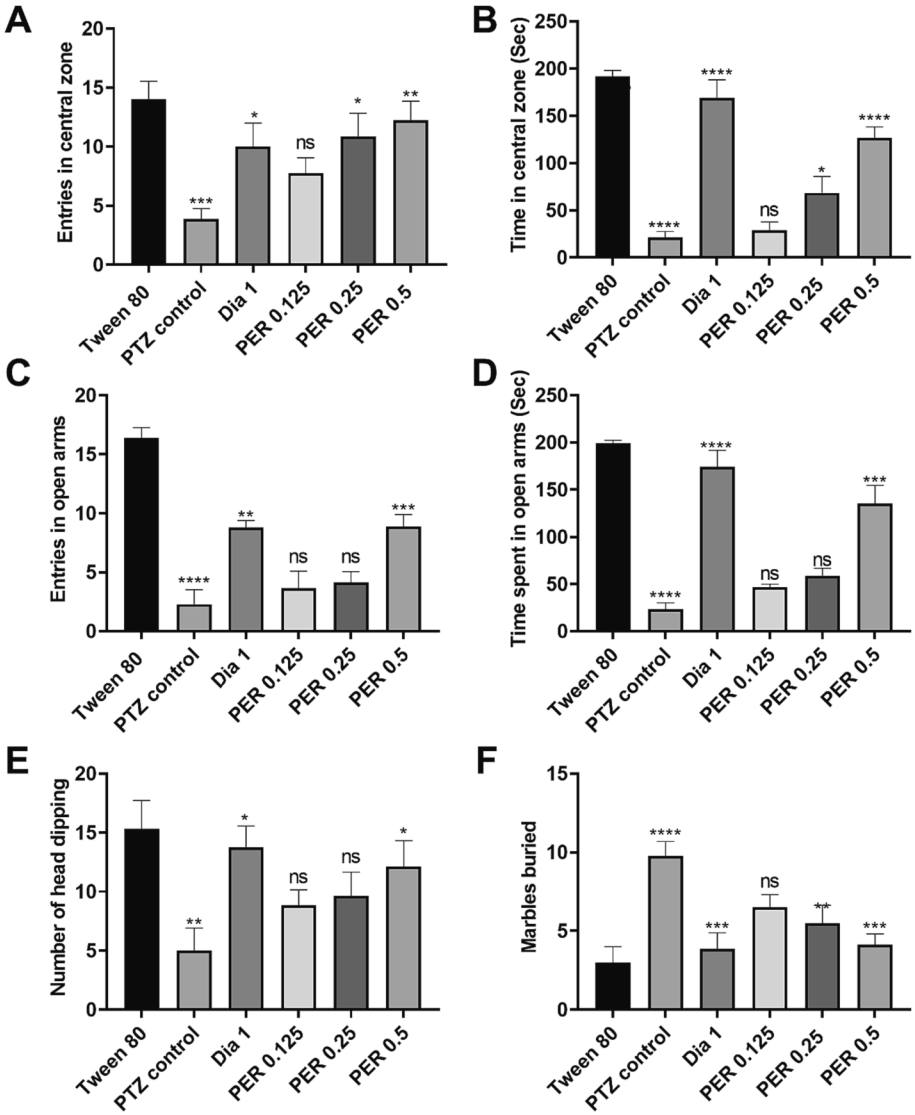

Fig. 4. Assessment of anxiolytic impact in mice chronically administered with PER for 21 days (0.125, 0.25, and 0.5 mg/kg) via a battery of neurobehavioral tests for anxiety such as open field, elevated plus maze, hole board, and marble bury tests. On the 22nd day, the animals were conceded to traverse the open arena for 5 min and their anxiety was evaluated by monitoring their **(A)** entries in the central zone and **(B)** time spent in the central zone. Mice were further tested by EPM on the 23rd day for the duration of 5 min **(C)** number of visits in open aversive arms, **and (D)** Duration of stay in open arms were observed to quantify post-kindling anxiogenic behavior. **(E)** On the 24th day, a hole board test was carried out to elucidate the dose-dependent impact of chronic treatment with PER on the exploratory tendency of anxiogenic holes by noting their head dipping behavior as hole poking is inversely correlated with degree of anxiety. **(F)** Moreover, animals were further tested to estimate the anxiolytic profile of PER at a low and high dose for 30 min on the 25th day in a rectangular cage with 15 equally spaced marbles, and the number of buried marbles was observed. Data are presented as mean ± S.E.M (n = 8) while $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{****}P < 0.0001$ represents comparison between kindled and non-kindled mice.

Furthermore, RMS amplitude was computed to discriminate seizure vs. non-seizure EEG activity after PTZ treatment (Kruskal-Wallis test: ($F(5,30) = 13.43$, ($P < 0.0197$)). PTZ administration provokes ictal electrographic events of high amplitude (59.06 ± 2.56) as compared to vehicle control with a mean of 42.93 ± 4.34 μv and $P = 0.0241$ (Fig. 3C). Treatment with PER 0.125 (52.36 ± 4.41 μv) and 0.25 (50.12 ± 3.043 μv) depicted non-significant decrease in EEG amplitude with $P = 0.9999$ and $P = 0.3347$, respectively. In comparison with the PTZ control, mice receiving PER 0.5, showed a normal EEG pattern with amplitude (42.43 ± 4.16 μv) similar to healthy tween-treated animals and $P = 0.0372$.

3.3. Dose-dependent anxiolytic effects of PER in OFT, EPM, hole board, and marble burying test

To elucidate the effect of PER on kindling-associated anxiety-like behavior, the animal's exploratory activity in the open maze was examined by their tendency towards open arena by observing animals number of visits in the central zone ($F(5,42) = 3.507$, ($P = 0.0097$)) and total time spent ($F(5,42) = 32.83$, ($P < 0.0001$)) in that particular zone. The vehicle-treated mice exhibited reduced apprehension for the unconditioned open arena as evidenced by their increased number of entries 14.00 ± 1.53 and duration of stay 191.88 ± 6.16 s (Fig. 4A and B). Reciprocally, the PTZ-treated animals depicted a marked increase in anxiety-like behavior with the reduced number of entries 3.87 ± 0.87 ($P = 0.0003$) and stay 21.19 ± 6.35 s in the central zone ($P < 0.0001$). This kindling-prompted anxiety was significantly alleviated by PER in a dose-dependent manner. In comparison with the PTZ control, the mice receiving PER 0.25 entered the central arena 10.87 ± 1.95 times ($P < 0.0146$) and loitered there for 68.38 ± 17.47 s with $P = 0.0467$. PER 0.5 showed the highest anxiolytic effect as animals presented further increase in total number of entries 12.25 ± 1.60 ($P = 0.0027$) with prolonged duration of stay 126.66 ± 11.51 ($P < 0.0001$). However, animals receiving PER at the low dose of 0.125 mg/kg showed a non-significant decrease in PTZ-kindling provoked anxiogenic behavior with a slight increase in the number of entries 7.75 ± 1.30 ($P = 0.3063$) and duration of stay 28.82 ± 8.77 ($P = 0.9916$) as compared to PTZ control.

The results of the elevated plus maze test demonstrated that a notable inter-group difference was there for the number of entries in open arms ($F(5,42) = 20.65$, ($P < 0.0001$)) and duration spent in exploration of open arms ($F(5,42) = 26.13$, ($P < 0.00010$)). The PTZ-kindled animals showed reduced risk-taking behavior with a lesser number of visits 2.28 ± 1.24 and a decrease in duration of stay 23.43 ± 6.70 s in aversive open arms of the plus maze as compared to non-kindled healthy animals with 16.40 ± 0.87 and 199.14 ± 3.08 s, respectively with $P < 0.0001$. The repeated administration of PER on alternate days for 3 weeks depicted dose-dependent protection from kindling-induced anxiety-like behavior and resulted in increased preference towards open arms of the maze. Fig. 4 C and D clearly illustrate that mice in the PER 0.5 group showed increased curiosity towards open arms of the equipment with more frequent visits 8.85 ± 1.03 ($P = 0.0004$) and prolonged stay for 135.13 ± 19.39 s ($P = 0.0001$), thus, exhibiting marked fearlessness and improved anxiolytic effect of PER at the dose of 0.5 mg/kg in comparison to PTZ challenged animals. However, the outcomes with PER 0.125 and PER 0.5 treatment remained statistically non-significant for both the number of entries and time spent in open arms of the maze in contrast with the PTZ-treated mice.

In the HBT, the animal's anxious response to an unfamiliar environment and exploratory tendency was assessed by the frequency of spontaneously elicited hole-poking behavior in all groups ($F(5,42) = 3.48$, ($P = 0.0108$)) for a duration of 5 min. Healthy mice showed a prominent exploratory tendency towards anxiogenic holes with an increase in head-dipping behavior (15.33 ± 2.39). In contrast, PTZ-treated mice showed a pronounced withdrawal tendency towards novel environments and increased levels of anxiety as evidenced by their reduced number of hole poking (5.00 ± 1.91) with $P = 0.0049$. Furthermore, PER 0.5 animals demonstrated a marked reduction in post-kindling anxiety-like behavior with increased head dipping 12.12 ± 2.18 ($P = 0.0498$) as compared to kindled mice (Fig. 4 E). However, in the hole board test, outcomes remained non-significantly different from PTZ epileptic animals in PER 0.125 (8.83 ± 1.32) and PER 0.5 (9.62 ± 2.02) groups with $P = 0.5679$ and $P = 0.3195$, respectively.

Moreover, the anxiolytic effects of PER were further validated with the marble burry test as the number of buried marbles is directly correlated with the anxiety-like behavior in rodents ($F(5,42) = 4.26$, ($P = 0.002$)). In line with the previous results, PTZ-treated animals exhibited an increase in digging behavior and covered more marbles (9.75 ± 0.94) by the bedding in the cage as compared to vehicle-treated animals (3.00 ± 1.00) with $P < 0.0001$. PER 0.25 and PER 0.5 buried 5.50 ± 1.13 ($P = 0.0116$) and 4.12 ± 0.69 ($P = 0.0006$) respectively which is significantly less in comparison with the PTZ control group and thus hints at a dose-dependent attenuation of kindling induced comorbid anxiety-like behavior in rodents (Fig. 4 F). Treatment with PER 0.125 remained statistically non-significant for buried marbles (6.50 ± 0.80) as compared to PTZ control group with $P = 0.0739$.

3.4. Assessment of short-term spatial recognition memory in T-maze and NOR

The one-way ANOVA showed notable intergroup differences in cognitive and learning function as assessed by estimating % spontaneous alternation behavior (SAB) ($F(5,42) = 7.694$, ($P < 0.0001$)). The epileptic mice showed poor cognitive abilities as evidenced by their reduced reminiscence of the previously explored arm resulting in a marked decrease in % SAB (6.25 ± 1.09) as compared to vehicle-treated mice (14.87 ± 1.69) with $P < 0.0001$. Animals receiving PER 0.25 and PER 0.5 showed a reduction in post-kindling-induced amnesia as these mice didn't show an inclination to re-enter and explore the previously visited arm of the T-shaped maze and exhibited a significant increase in alternation behavior with mean of 10.65 ± 0.73 ($P = 0.0255$) and 12.87 ± 1.06 ($P = 0.0004$), respectively (Fig. 5A). Animals in PER 0.125 group represented amnesia as evident by their reduced remembrance of recently explored arm similar to PTZ-control group with mean % SAB of 9.12 ± 0.39 ($P = 0.2281$).

The NOR test comprised of acquisition and trial session and the object recognition score was estimated for the trial session. In the acquisition phase of the NOR test, where animals investigated two indistinguishable objects of the same shape, height, and color, treated groups explored two novel objects without any preference for side or other relevant factors, while diseased mice mostly showed no exceptional interest in investigation of unknown novel articles. In the trial session of the NOR test, PER-treated animals dose-dependently showed object recognition memory with respect to the discrimination index (object recognition score), calculated as total time spent in proximity to the known object vs. an unknown novel object ($F(5,42) = 6.790$, ($P = 0.0001$)). Between-group comparison illustrated that healthy non-diseased animals spent more time in the investigation of the unfamiliarized object with a mean score of 0.43 ± 0.08 as compared to the PTZ control group which showed a recognition score of −0.25 ± 0.11 ($P < 0.0001$). In contrast, animals receiving PER 0.25 and 0.5 depicted improved recognition memory with mean object recognition scores of 0.17 ± 0.07 ($P = 0.0138$) and 0.23 ± 0.06 ($P = 0.0037$), respectively as most of the animals efficiently discriminate novel object from the previously known object. However, in comparison with the PTZ control outcomes with PER 0.5 remained statistically non-significant as evident by their reduced object recognition score (0.08 ± 0.12) with $P = 0.0714$ (Fig. 5B).

3.5. Evaluation of long-term working memory in y-maze and Morris water maze tests

In the novel arm recognition test (Y-maze), the mice were permitted to enter the forbidden arm which was unbridled during the test session,

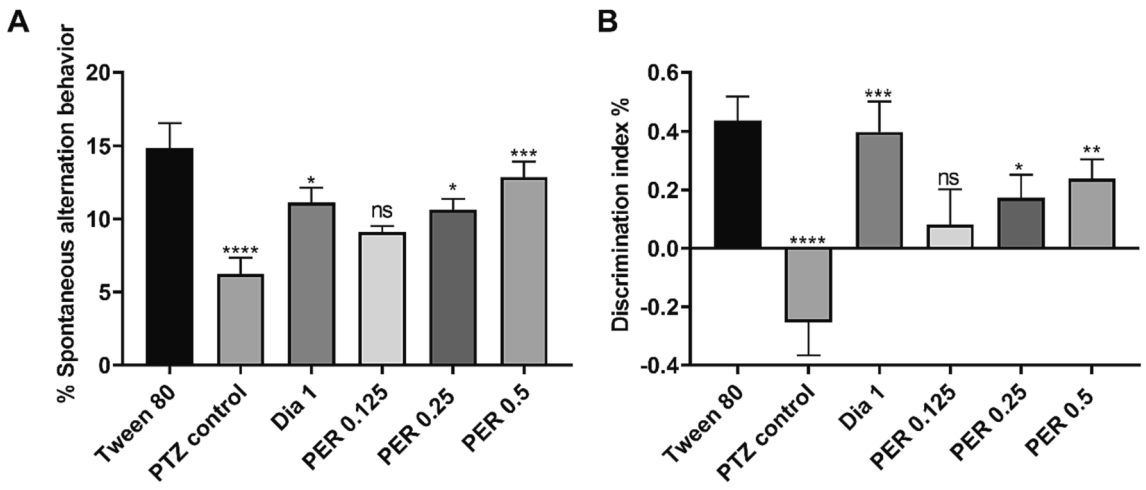

Fig. 5. Evaluation of chronic treatment of PER (0.125, 0.25, and 0.5 mg/kg) on post-kindling induced memory deficits in PTZ-challenged animals by observing their behavioral response in T-maze and object recognition test. On the 26th day, the animals were conceded to spontaneously traverse three arms of the T-maze, and cognitive dysfunction was analyzed by monitoring the pattern of arm visits for 5 min to estimate **(A)** Percentage spontaneous alternation behavior**.** On the 27th day, the animals in all groups were familiarized with two identical objects in the acquisition phase for 10 min accompanied by the subsequent substitution of one familiar object with a new unidentical one to allow another investigation phase for the duration 5 min to observe the **(B)** Discrimination index (Object recognition score). Data are expressed as mean ± S.E.M (n = 8) while *P < 0.05, **P < 0.01 ***P < 0.001 and ****P < 0.0001 represents comparison between kindled and non-kindled mice.

and the number of visits (F (5,42) = 6.776, (P = 0.0001)) in the novel arm and investigation time (F (5,42) = 4.129, (P < 0.0039)) there was noted to assess post-kindling associated memory impairments. The innate preference towards novelty was more pronounced in healthy animals as evidenced by their increased number of visits 11.75 ± 1.31 and exploration time 135.60 ± 19.20 s in the unfamiliar novel arm of the equipment (Fig. 6A and B). The PTZ control diseased group showed poor memory recall of previously investigated arenas of the maze and thus, results in reduced entries 4.50 ± 0.37 (P < 0.0001) and investigation time 60.37 ± 10.69 s (P = 0.0010). The animals receiving PER 0.25 and PER 0.5 showed dose-dependent amelioration of PTZ-provoked memory deficits as they presented frequent visits with a mean of 8.75 ± 0.96 (P = 0.0300) and 9.00 ± 1.26 (P = 0.0197), respectively in the novel zone. Moreover, increased exploration time further validated the dose-dependent memory restorative effects of PER as PER 0.25 and PER 0.5 treated mice stayed there for 109.43 ± 3.59 (P = 0.0461) and 116.01 ± 16.53 s (P = 0.0191), respectively as compared to PTZ control group. However, in comparison to the kindled mice, PER 0.125 failed to show any remarkable improvement in post-kindling memory decline as animals depicted a non-significant increase in number of entries 5.25 ± 0.81 (P = 0.9826) and investigation time 94.80 ± 9.30 s (P = 0.2410) in the novel zone of the y-maze.

Furthermore, the outcomes of the MWM analyzed by two-way ANOVA showed marked intergroup differences in escape latencies (F (5,84) = 52.92, (P < 0.0001)) validating that kindling might affect hippocampal-dependent learning in mice (Fig. 7A). Most of the kindled mice showed thigmotaxic behavior during the experimental phase instead of the targeted search of the platform zone submerged in NW quadrant of the water maze. The kindled epileptic mice showed pronounced impairments in cue-associated learning evident from their

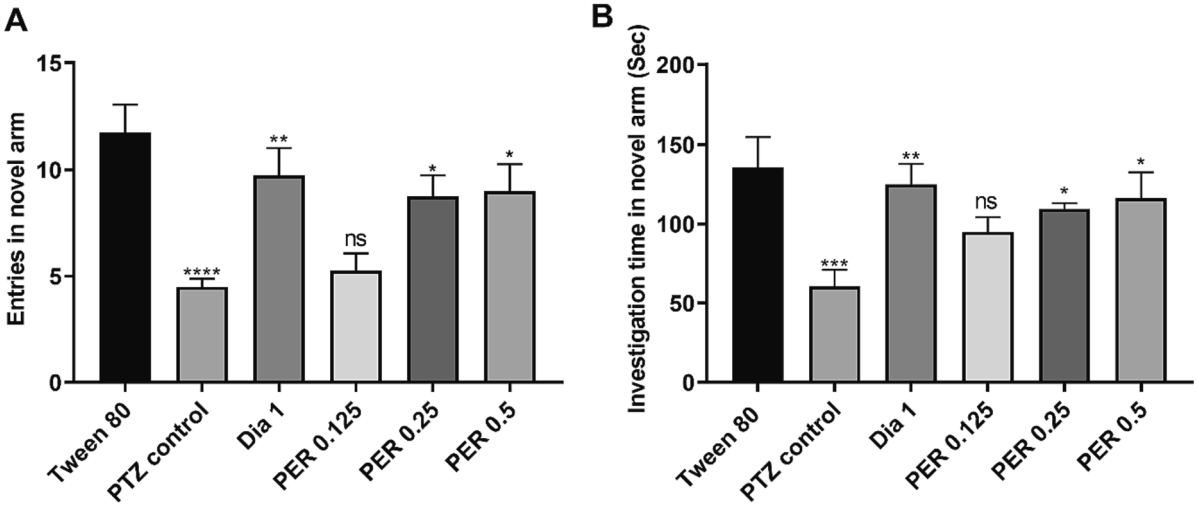

Fig. 6. Estimation of neuroprotective impact of long-term therapy with PER (0.125, 0.25, and 0.5 mg/kg) on PTZ provoked memory deficits in the Y-maze test. On the 28th day, the animals were allowed to traverse spontaneously two arms of the Y-maze while one arm was kept blocked. On the 29th day, the mice in all groups acquiesced to investigate the trio-arm maze for a duration of 5 min, and their working memory was analyzed by observing **(A)** the number of visits in the novel arm. **(B)** Investigation time in the novel arm. Data are expressed as mean ± S.E.M (n = 8) while *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 represents comparison between kindled and non-kindled mice.

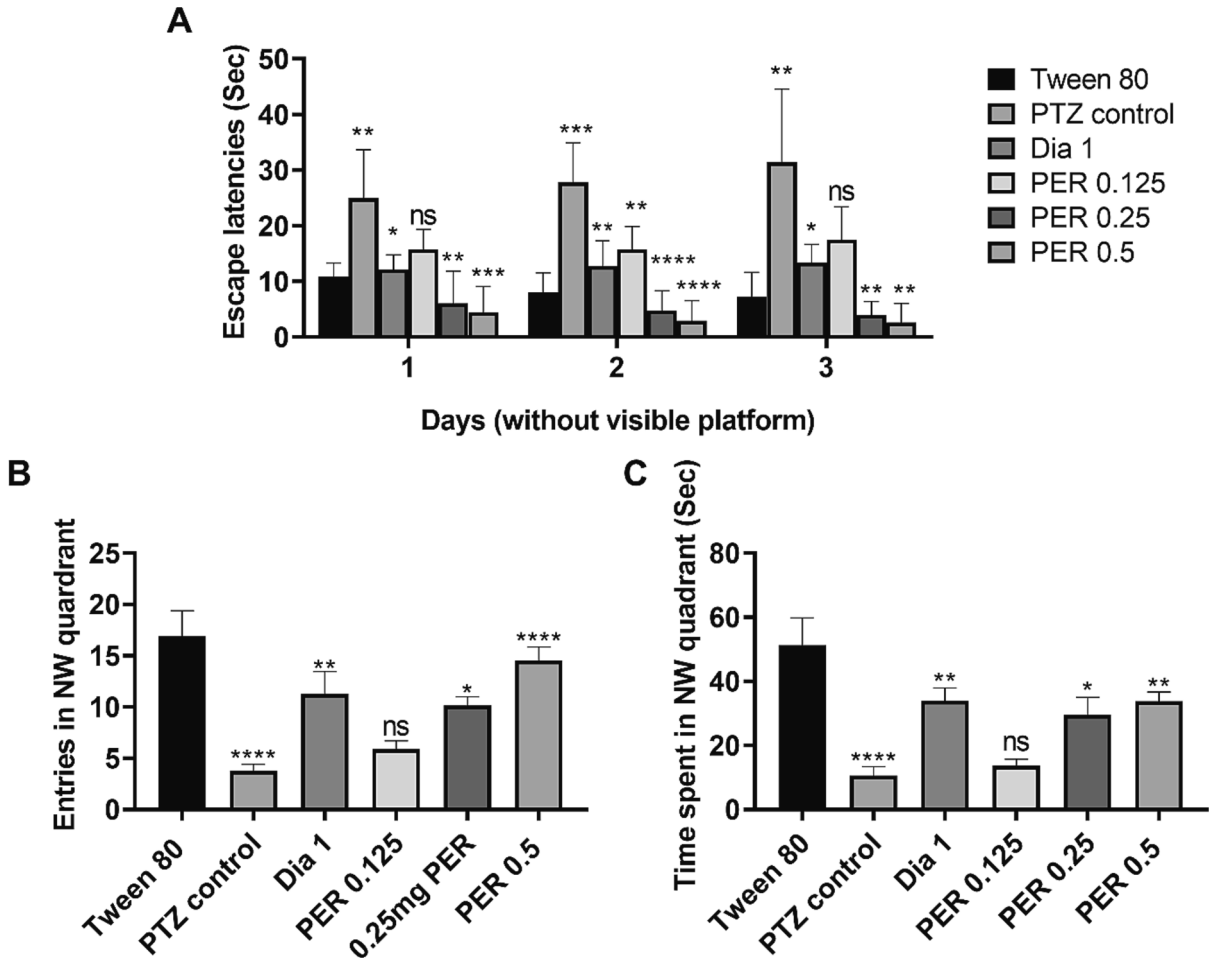

Fig. 7. Evaluation of hippocampal-dependent memory impairments in animals administered with PER (0.125, 0.25, and 0.5 mg/kg) by Morris water maze test. During the acquisition phase from the 30th-31st day, animals were given three sessions per day to locate visible platform and to build memory. During the experimental trial from the 32nd-34th day, animals were tested in a water maze to assess long-term working memory by observing their (A) escape latencies (time taken to reach platform). On the 35th day, a probe trial for 120 sec was conducted to monitor (B) entries in the target quadrant and (D) Duration spent in the NW quadrant. Data are expressed as mean ± S.E.M (n = 8) while $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{****}P < 0.0001$ represents comparison between kindled and non-kindled mice.

prolonged time navigating the submerged platform. On 3rd day, the escape latencies in vehicle-treated animals mice were 10.90 ± 0.84 s which was significantly <24.93 ± 3.08 s observed in a diseased animal (P = 0.0087). Likewise, the vehicle control showed good learning capability and escaped from water in a much shorter duration 7.19 ± 1.56 s as compared to kindled animals 31.45 ± 4.64 s by the 5th day with P = 0.0036. Administration of PER dose-dependently reduced escape latencies in consecutive three experimental days with a hidden platform. Likewise, on the 5th day PER 0.25 and PER 0.5 mice found the platform in 3.97 ± 0.85 s (P = 0.0020) and 2.58 ± 1.21 s (P = 0.0013), respectively. In comparison with PTZ control, the outcomes of treatment with PER 0.125 (17.54 ± 2.07 s) remained non-significant by the end of the experimental trial with P = 0.0771.

On probe day, pronounced intergroup variations were noted for the number of entries into the NW rescue zone ($F_{(5,42)} = 10.16$, ($P < 0.0001$)) and duration of stay there ($F_{(5,42)} = 9.809$, ($P < 0.001$)). The diseased mice showed altered remembrance of the rescue zone with a reduced number of visits 3.75 ± 0.64 and time spent there 10.64 ± 2.75 s ($P < 0.0001$) and they kept spanning in the water maze to navigate the target platform as collated to the vehicle control group in which 16.87 ± 2.48 number entries and time spun 51.31 ± 8.43 s there was noted (Fig. 7B). Treatment with PER 0.25 and 0.5 significantly reverted kindling induced progression of memory dysfunction in a dose-dependent manner. In comparison to PTZ control, mice in PER 0.25 and 0.5 showed an increased number of entries 10.12 ± 0.85 (P = 0.0260) and 14.50 ± 1.35 ($P < 0.0001$), respectively into the target quadrant in which the platform was previously situated. Moreover, increased swimming time in the NW zone of the water maze by both animals of PER 0.25 (29.54 ± 5.44) and PER 0.5 (33.75 ± 2.85) further strengthened the memory-preserving impact of PER with P = 0.0316 and P = 0.0061, respectively. However, outcomes with PER 0.125 remained non-significant and similar to PTZ kindled mice in terms of both entries in target quadrant 5.87 ± 0.83 and time spent swimming 13.73 ± 1.94 indicating their poor memory recall capabilities.

3.6. Assessment of post-kindling depression-like behavior in a sucrose preference test

Post-kindling associated depression-like behavior was assessed by using a sucrose preference test, a two-bottle paradigm, and the taste preference towards sweetened 1% sucrose solution was noted ($F_{(5,12)} = 16.96$, ($P < 0.0001$)). PTZ kindled group depicted diminished preference towards the 1% sucrose water (38.33 ± 4.41 %) indicating marked anhedonia which is a hallmark of depressive-like behavior as compared to healthy tween-treated mice that showed a percentage sucrose preference of 91.00 ± 0.57 with $P < 0.0001$. Our data further showed that treatment with PER 0.25 and PER 0.5 markedly prevented the development of kindling-induced comorbid depression evident from

their increased preference towards sweetened water, 66.66 ± 3.75% (P = 0.0046) and 74.66 ± 2.02% (P = 0.0006), respectively. However, treatment with a low dose PER 0.125 showed a non-significant decrease in hedonic behavior (44.66 ± 5.17%) as compared to depressive mice in the PTZ control group with P = 0.8037 (see Fig. 8).

3.7. Biochemical analysis

Biochemical data revealed high intergroup variability for MDA levels, a marker to assess lipid peroxidation (F (5,18) = 5.544, (P = 0.0029)), and MDA levels were increased in the epileptic brains of kindled mice (1102.15 ± 184.52 nmol/mg) as compared to vehicle-treated control (483.73 ± 33.97 nmol/mg) with P = 0.0025 (Fig. 9A). However, PER dose-dependently reduced aberrant production of MDA in mice challenged with PTZ for 21 days. PER 0.25 and 0.5 showed low levels of MDA 566.98 ± 113.17 nmol/mg (P = 0.0084) and 422.48 ± 42.33 nmol/mg (P = 0.0010), respectively as compared to epileptic brains of PTZ control group. In contrast, outcomes with PER 0.125 remained non-significant (705.88 ± 20.23 nmol/mg) with P = 0.0594.

Furthermore, the assessment of the endogenous antioxidant enzyme SOD showed different activity in treated and kindled animals (F (5,18) = 7.918, (P = 0.0004)). In kindled brains, SOD activity significantly weakened (170.63 ± 25.16 milliunits/mg) as compared to healthy non-epileptic brains (915.81 ± 138.38 milliunits/mg) with P = 0.0005. Treatment with PER 0.25 and 0.5 remarkably restored redox balance evident from amplified SOD activity of 799.64 ± 167.17 milliunits/mg (P = 0.0025) and 843.71 ± 44.83 milliunits/mg (P = 0.0013), respectively. However, PER at the low dose of 0.125 mg/kg didn't yield a prominent antioxidant effect (460.94 ± 112.94 milliunits/mg) as collated to PTZ control with P = 0.2344 (Fig. 9B).

Additionally, one-way ANOVA showed marked intergroup differences for AChE activity (F (5,18) = 4.104, (P = 0.0116)). AChE activity was significantly higher in diseased brains (2.05 ± 0.43 μm/min/mg of protein) as compared to healthy brains of tween control group (0.57 ± 0.23 μm/min/mg) with P = 0.0156 (Fig. 9C). Mice receiving PER 0.25 and PER 0.5 showed downregulation of AChE activity 0.59 ± 0.28 μm/min/mg (P = 0.0172) and 0.35 ± 0.03 μm/min/mg (P = 0.0052), respectively as compared to PTZ control. Conversely, in comparison to the kindled brains, PER 0.125 chronic administration didn't show any beneficial effects on AChE activity (1.34 ± 0.44 μm/min/mg with P = 0.3892.

Likewise, our data showed statistical differences in the content of total thiol of kindled vs. non-kindled mice (F (5,18) = 4.733, (P = 0.0062)). PTZ kindled brains showed decreased content of total thiol (1.56 ± 0.53 μmol/mg) as compared to healthy brains (6.32 ± 1.26 μmol/mg) with P = 0.0052 (Fig. 9D). Brains of animals receiving PER 0.5 showed replenished total thiol content of 5.75 ± 0.47 μmol/mg (P = 0.0140) as compared to PTZ epileptic brains. In comparison to the kindled brains, outcomes of PER 0.125 and PER 0.25 showed a non-significant slight increase in total thiol content with mean levels of 2.42 ± 0.36 μmol/mg (P = 0.9324) and 4.89 ± 1.40 μmol/mg (P = 0.0588), respectively.

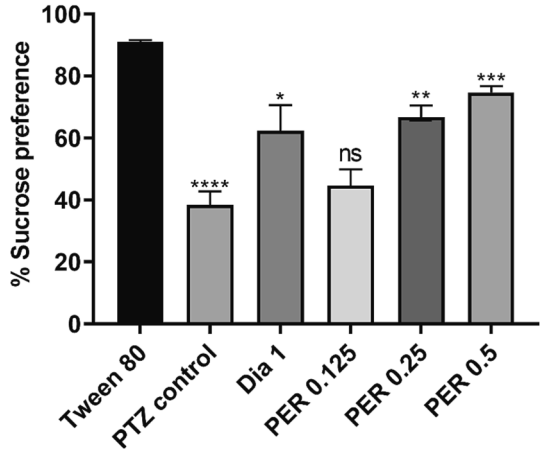

Fig. 8. Assessment of kindling-induced depression-like behavior in mice treated with PER (0.125, 0.25, and 0.5 mg/kg) by testing them through the behavioral paradigm of taste preference towards tap and sweetened water. On the 36th day, mice were kept in the state of fasting for 12–14 h and then given free access to two bottles, one containing simple tap water and the other with 1% sucrose solution. After 24 h on the 37th day, the volume consumed by animals in both bottles was noted to calculate % sucrose preference. Data are expressed as mean ± S.E.M (n = 3) while *P < 0.05, **P < 0.01, *** P < 0.001 and ****P < 0.0001 represents comparison between kindled and non-kindled mice.

3.8. PER dose-dependently downregulated BDNF/TrkB signaling in PTZ-challenged mice

One-way ANOVA revealed high variability in relative mRNA expression of BDNF and TrkB with (F (5,18) = 8.43, (P = 0.0003)) and (F (5,18) = 7.139, (P = 0.0008), respectively. In comparison with the tween 80, PTZ administration propagates epileptogenesis resulting in progressive change in BDNF and TrkB signaling due to neuronal epileptiform activity with increased mRNA expression levels of 4.06 ± 0.48 (P = 0.0007) and 2.95 ± 0.53 folds (P = 0.0181), respectively. The chronic pretreatment with PER showed dose-dependent downregulation of BDNF and TrkB. PER 0.125 administration slightly reduced BDNF relative expression to 1.74 ± 0.412 (P < 0.0031) and TrkB to 0.95 ± 0.07 (P < 0.0016) folds. PER 0.25 showed further reduction in mRNA expression levels of both BDNF and TrkB as levels were downregulated to 1.09 ± 0.51 folds (P = 0.0003) and 0.77 ± 0.22 folds (P = 0.0007), respectively. In contrast, PER 0.5 depicted a marked reduction in levels of BDNF to 0.97 ± 0.15 folds (P = 0.0002) and TrkB to 0.58 ± 0.08 folds (P = 0.0003) as compared to PTZ kindled animals (Fig. 10A and B).

3.9. PER dose-dependently reverted post-kindling alterations in mRNA expression of neuroinflammatory markers

Our outcomes revealed marked dynamic changes in mRNA expression levels of neuroinflammatory markers i.e. IL-1β (F (5,18) = 8.624, (P = 0.003)), TNF-α (F (5,18) = 4.932, (P = 0.0051)) and iNOS (F (5,18) = 11.45, (P < 0.0001)). The relative expression of IL-1β in the cortex of healthy brains was 12.82 ± 2.16 fold which was raised to 26.80 ± 5.09 folds in epileptic brains with P = 0.0046. Animals pretreated with PER 0.125, PER 0.25, and PER 0.5 showed dose-dependent downregulation of IL-1β expression to 16.07 ± 2.23 (P = 0.0316), 8.58 ± 0.85 (P = 0.0004) and 7.04 ± 1.42 (P = 0.0002) fold, respectively as shown in Fig. 11A.

Moreover, chronic exposure to PTZ significantly upregulated mRNA transcription of TNF-α and iNOS. The relative expression of TNF-α in healthy brains was 3.66 ± 0.39 which was upregulated by repeated PTZ administration to 17.59 ± 5.92 with P = 0.0056. As collocated with the kindled brains, mice receiving PER 0.125, PER 0.25, and PER 0.5 showed significant downregulation of TNF-α to 6.45 ± 1.51 (P = 0.0284), 2.90 ± 0.59 (P = 0.0036) and 1.85 ± 0.82 (P = 0.0019) as depicted in Fig. 11B.

Furthermore, in tween 80 treated healthy brains the iNOS levels were 1.46 ± 0.47 but PTZ kindling significantly upregulated its expression level to 7.17 ± 1.08 with P < 0.0001. Mice treated with PER 0.125, PER 0.25, and PER 0.5 dose-dependently reduced mRNA expression levels to 3.22 ± 0.89 (P = 0.0023), 2.29 ± 0.35 (P = 0.0003) and 1.28 ± 0.50 folds (P < 0.0001), respectively as presented in Fig. 11C.

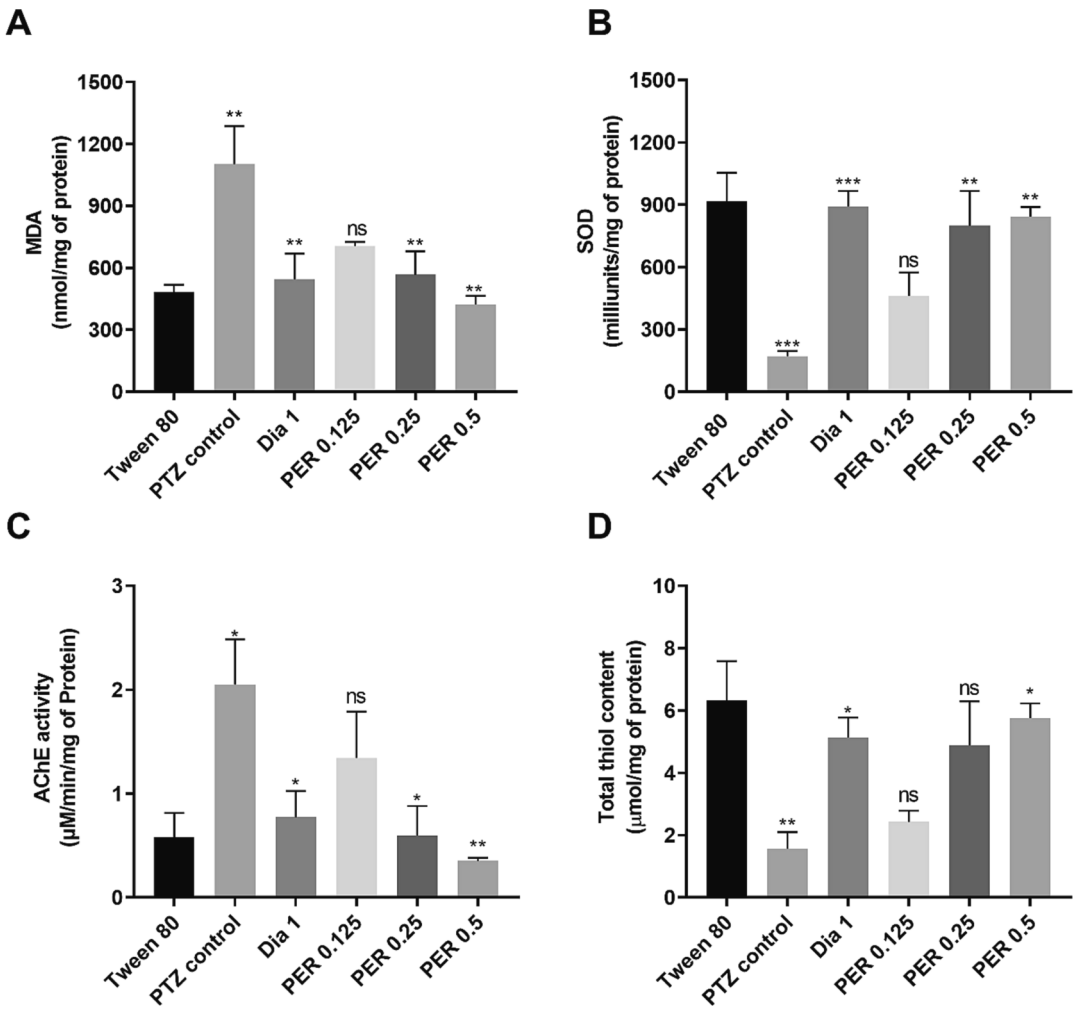

Fig. 9. Assessment of PTZ-induced redox impairments in mice brains to elucidate MDA, SOD, AChE, and total thiol levels. On the 36th day, after completion of the water maze, the kindled and non-kindled whole brains were carefully isolated and homogenized for neurochemical analysis. Data is reported as mean ± S.E.M (n = 4) while $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ represents the comparison between kindled and non-kindled mice.

4. Discussion

In the current study, PTZ was administered chronically at a sub-convulsive dose of 40 mg/kg to elucidate the potential neuroprotective effects of PER in the propagation of PTZ-induced kindling with behavioral manifestation of stage 4–5 seizures. PER pretreatment dose-dependently impacted PTZ provoked epileptogenesis and significantly prevented kindling-associated brain insults at the dose of 0.25–0.5 mg/kg. The group receiving PER 0.125 showed protection from seizures from day 1st-11th but later repetitive PTZ exposure instigated convulsions of higher severity and by the end of the 3rd week, 40% of the animals were kindled. In concert, vEEG illustrated that kindling incited high polyspikes, and sharp spike-wave discharges were notably precluded in animals pretreated with perampanel. On EEG, mice pretreated with PER 0.125 portrayed occasional motor seizures (stage 4–5 i.e. rearing and GTCS), while administration of PER at the dose of 0.25 and 0.5 mg/kg dose-dependently mitigates symptom severity as they just presented non-convulsive motor seizures (stage 1–2) with significant protection from PTZ evoked ictal epileptic spikes by the end of 11th injection. However, the lowest dose of PER 0.125 mg didn't yield any beneficial effects in the prevention of PTZ prompted high amplitude ictal events, and showed an increased frequency of epileptiform discharges similar to PTZ kindled mice and thus these outcomes insinuate the dose-dependent anti-epileptogenic impact of PER via modulation of glutamatergic storming through AMPA receptors antagonism.

Epilepsy is a network disorder necessitating chronic administration of drugs at a high dose which might trigger interictal psychiatric comorbidities and movement disorder as they share overlapping phenomenology (Kanner, 2017). The findings of OFT confirmed that long-term therapy with PER at doses of 0.125–0.5 mg/kg didn't exert any detrimental effect on animal's spontaneous exploratory activity as AMPA antagonists are perilously notorious for declined locomotor activity and connately have some adverse effects such as ataxia and sedation particularly at higher doses (Tsai et al., 2018). Sedation and ataxia are related to the upregulation of GABAergic transmission or recession in glutamatergic transmission in the CNS (Lee and Jeong, 2009). Thus, No CNS depressant effects were observed with PER up to 0.5 mg/kg. However, notable apprehension was observed in PTZ-kindled animals but ameliorated by PER dose-dependently in all neurobehavioral tests used in this current study to assess anxiety-like behavior. PTZ kindled mice depicted reduced preference towards the central arena of the open field, open arms of EPM, reduced number of head dipping in HBT, and increased number of buried marbles in MBT. PER at the higher dose of 0.5 mg/kg markedly halted the development of PTZ-evoked comorbid anxiety-like behavior as mice fearlessly explored novel central arena and open arms of EPM. Moreover, PER-treated mice showed increased exploration of anxiogenic holes in HBT and reduced number of buried marbles validating its anxiolytic potential. Mechanistic studies have reported that stimulation of GABAA/BZ, α2-adrenergic receptors as well as serotonin 5-HT1A, endowed to the anxiolytic

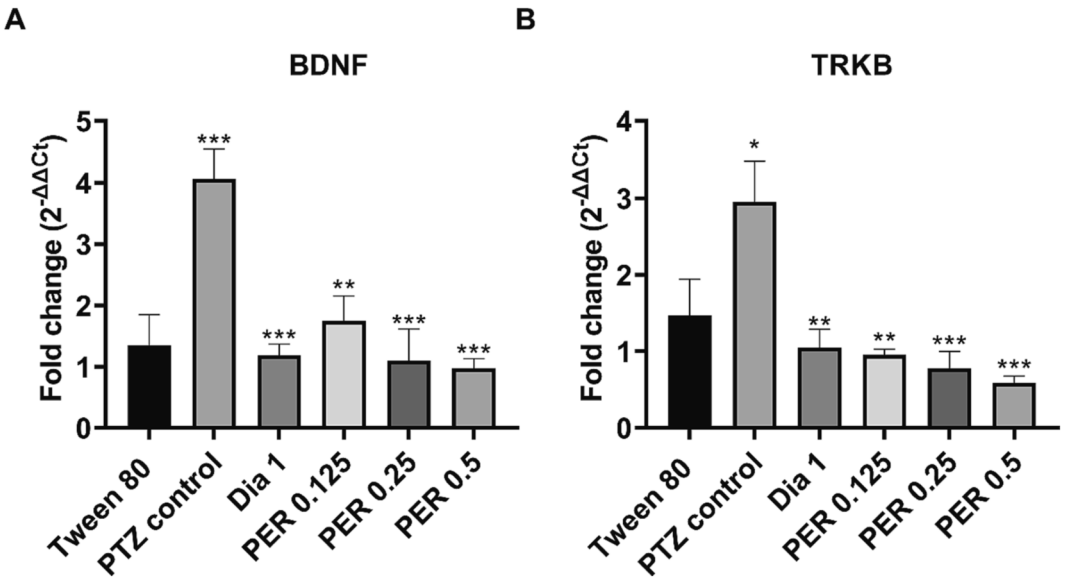

Fig. 10. Evaluation of PTZ-kindling induced alterations in relative mRNA expression in the cerebral cortex of mice brain. The mRNA expression of **(A)** BDNF and **(B)** TrkB was assessed by qRT-PCR using the relative Ct ($^{\Delta\Delta}$Ct) method and normalized against HPRT gene $^{\Delta}$Ct as an internal control and portrayed as fold changes in comparison to the PTZ kindled brains. Data are expressed as mean ± S.E.M (n = 4) while $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ represents comparison between kindled and non-kindled mice.

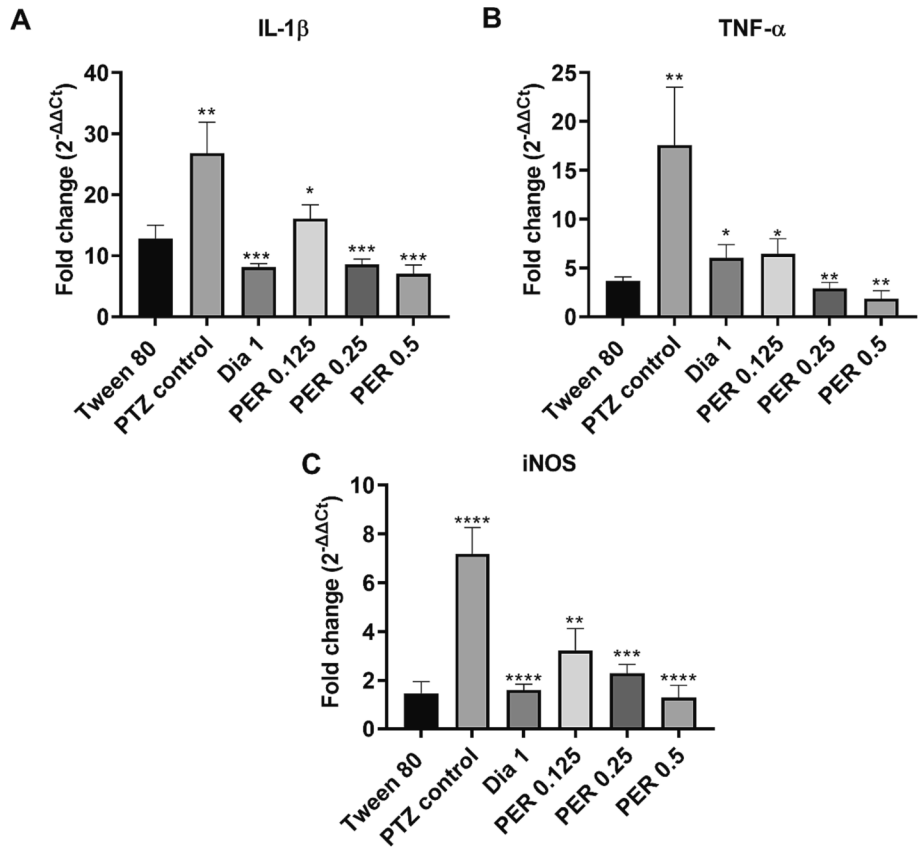

Fig. 11. Relative mRNA expression of neuroinflammatory markers. **(A)** IL-1β, **(B)** TNF-α, **(C)** iNOS. Relative mRNA expression of neuroinflammatory markers was quantified via qRT/PCR using the relative Ct ($^{\Delta\Delta}$Ct) method and normalized against HPRT gene $^{\Delta}$Ct as an endogenous control. Data is portrayed as fold changes to depict inter-group differences (n = 4). Data are expressed as mean ± S.E.M (n = 4) while $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{****}P < 0.0001$ represents comparison between kindled and non-kindled mice.

impact of PER. PER has been proclaimed to modulate GABAergic tonus in the brain areas related to anxiety i.e. the amygdala as synaptic stimulation of AMPA receptors impedes the GABA emancipation/ acquittal from cerebellar interneurons in brain regions responsible for anxious response (Nuss, 2015; Satake et al., 2000). These results were in line with Bektas and Arslan who published that PER 0.25–1 mg/kg

treatment in mice led them to brazenly explore innately aversive open arena in OFT and open arms of heightened elevated plus maze. Moreover, they reported that administration of flumazenil significantly antagonized the anxiolytic activity of PER in the EPM and on the "duration of stay in the central zone" parameter in the OFT (Bektas et al., 2020). Furthermore, obtaining exceptional anxiolytic effect from PER therapy at the dose of 0.5 mg/kg but not at the doses of 0.125 and 0.5 mg/kg in mice challenged with PTZ leads to an epilogue that definite magnitude of glutamatergic innervation must be conserved conducive to attain anxiolytic impact with AMPA antagonists.

Changes in cerebral structure and function impart increased vulnerability towards cognitive dysfunction in patients with epilepsy (Novak et al., 2022). Two retrospective cohort studies have published no negative influence of PER on attention and executive function as serious psychiatric and behavioral side effects are cited in the warning box of the PER label (Ahn et al., 2021; Meschede et al., 2018). In the present study kindled mice showed deteriorated perception and decline in both episodic and long-term spatial memory which were markedly improvised by PER in a concentration-dependent manner. PTZ kindled animals illustrated prominent amnesia and short-term memory dysfunction as assessed by the reduced number of alternations in T-maze and object recognition score in the NOR test while PER 0.5 treated mice illustrated good performance. Moreover, dose-dependent improved outcomes were observed with PER therapy in the MWM test as animals took less time to discover submerged platform. These findings are consistent with Wang *et al* who reported that PER at the dose of 3 mg/kg prevented cognitive impairments in young TLE mice via inhibition of GluR1 and AP-1 expression (Wang et al., 2022). Alqahtani *et al* also reported that coadministration of PER at the dose of 4 Pamg/kg along with ketamine prevented long-term cognitive impairments in mice challenged with TBI (Alqahtani et al., 2020).

Previously published short-term prospective studies have reported that PER dose-dependently may attenuate or aggravate aggression and depressive-like behavior in epileptic patients and in certain cases slight mood changes were well tolerated (Goji and Kanemoto, 2019). Moreover, its co-administration with other anti-epileptic drugs didn't provoke depression in patients with epilepsy. In our study, PTZ kindled mice showed anhedonia indicating depression phenotype whereas PER attenuated these comorbid behavioral alterations as animals presented increased preference towards sweetened sucrose solution vs normal tap water in SPT. Citraro *et al* have also reported that chronic treatment with PER at the dose range of (0.25–3 mg/kg) for 119 days in the WAG/Rij rat model of absence epilepsy remarkably reduced comorbid depressive-like behavior characterized by the force swim test (Goji and Kanemoto, 2019).

Epileptogenesis prompts a cascade of events at the cellular level i.e. altered neurogenesis, upregulation of cytokine expression, and increased oxidative stress that directly stimulates apoptotic and inflammatory signaling pathways responsible for neuronal damage as well as cognitive decline (Borowicz-Reutt and Czuczwar, 2020). To compare post-kindling behavioral manifestations with excitatory neurobiochemical changes, we assessed the direct correlation between oxidative markers and increased seizure severity. The findings were supported by Aguiar *et al.* who reported that the PTZ-evoked recurrent epileptic model results in excitotoxic oxidative damage with subsequent propagation of the process of epileptogenesis (Aguiar et al., 2012). Our outcomes showed increased lipid peroxidation evident from enhanced MDA levels, weakened activity of SOD antioxidant enzyme, and upregulation of AChE activity as well as reduced thiol content in whole epileptic brains of the PTZ control group. However, the long-term treatment for 21 days with PER ameliorated altered redox balance.

Overexpression of BDNF aggravates neuronal hypersynchrony and thus propagates susceptibility towards epilepsy via TrkB receptor signaling pathways (Wang et al., 2021). PTZ-induced epileptiform discharges induce striking upregulation of BDNF and its binding receptor TrkB in the mossy fiber pathway resulting in proepileptic transformation of neuronal circuits and neuroinflammation (Iughetti et al., 2018). Our outcomes portrayed a direct relation between BDNF/TrKB signaling and the degree of seizure severity following PTZ-induced kindling. In this current study, PER dose-dependently reversed altered BDNF/TrkB signaling elucidating that PER-promoted attenuation of seizure severity might be due to the downregulation of the BDNF/TrkB signaling pathway.

A growing body of literature provides evidence that proinflammatory cytokines directly exaggerate spontaneous seizure burden (Semple et al., 2020). Furthermore, PTZ-induced excitotoxic brain insults can upregulate iNOS in brain immune cells leading to activation of inflammatory signaling pathways and neuronal apoptosis (Murashima et al., 2000). In the present study, PER significantly reduced levels of pro-inflammatory cytokines and iNOS expression subsequent to PTZ administration for 21 days, and outcomes are validated by the previous studies in which AMPAR antagonist PER pre-treatment suppressed upregulation of proinflammatory cytokines and enhanced production of anti-inflammatory cytokines at the dose of 5 mg/kg in rats challenged with TBI (Chen et al., 2017).

5. Conclusion

In view of current experimental findings, we can conclude that PER, a novel AMPAR antagonist has an auspicious role in hampering post-PTZ development of epilepsy and associated psychiatric comorbidities. PER 0.5 mg/kg interrupted unabated high amplitude sharp spike-wave discharges in mice challenged with PTZ as compared to the low dose of 0.125 mg/kg. Long-term therapy with PER dose-dependently prompted protection against PTZ-induced excitotoxic oxidative damage. Additionally, the downregulation of BDNF/TrkB signaling pathway and proinflammatory markers might be attributed to its neuroprotective role in PTZ-induced neuronal excitability and associated plethora of behavioral alterations.

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Ethics Statement

The stringent experimental and housing protocol complied with standard ethical guidelines and was approved by the Departmental Ethics Committee (07-PHDL S18, Dated 08-February 2021) of the Department of Pharmacology, B.Z. University, Multan. Strict care was provided to animals and housing conditions were maintained throughout the PTZ kindling process to ensure animal well-being and coherence of our experimental results.

CRediT authorship contribution statement

Nadia Perveen: Methodology, Software, Investigation, Writing – original draft, Writing – review & editing. **Faleh Alqahtani:** Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition. **Waseem Ashraf:** Methodology, Validation, Writing – original draft, Writing – review & editing. **Muhammad Fawad Rasool:** Conceptualization. **Syed Muhammad Muneeb Anjum:** Methodology, Software. **Iram Kaukab:** Methodology, Writing – original draft, Writing – review & editing. **Tanveer Ahmad:** Writing – original draft, Writing – review & editing. **Saleh A. Alqarni:** Writing – original draft, Writing – review & editing. **Imran Imran:** Conceptualization, Resources, Writing – original draft, Writing – review & editing, Supervision, Visualization, Funding acquisition.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2023.101930.

References

Adotevi, N., Lewczuk, E., Sun, H., Joshi, S., Dabrowska, N., Shan, S., Williamson, J., Kapur, J., 2020. α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor plasticity sustains severe, fatal status epilepticus. Ann. Neurol. 87 (1), 84–96. https://doi.org/10.1002/ANA.25635.

Aguiar, C.C.T., Almeida, A.B., Arajo, P.V.P., De Abreu, R.N.D.C., Chaves, E.M.C., Vale, O. C., Do, MacÊdo, D. S., Woods, D. J., Fonteles, M. M. D. F., & Vasconcelos, S. M. M., 2012. Oxidative stress and epilepsy: Literature review. Oxid. Med. Cell. Longev. 1–12 https://doi.org/10.1155/2012/795259.

Ahn, S.J., Kim, T.J., Cha, K.S., Jun, J.S., Byun, J.I., Shin, Y.W., Sunwoo, J.S., Lee, S.H., Yu, K.S., Jang, I.J., Chu, K., Lee, S.K., Jung, K.Y., 2021. Effects of perampanel on cognition and quantitative electroencephalography in patients with epilepsy. Epilepsy Behav. 115 https://doi.org/10.1016/J.YEBEH.2020.107514.

Alqahtani, F., Assiri, M. A., Mohany, M., Imran, I., Javaid, S., Rasool, M. F., Shakeel, W., Sivandzade, F., Alanazi, A. Z., Al-Rejaie, S. S., Alshammari, M. A., Alasmari, F., Alanazi, M. M., Alamri, F. F., 2020. Coadministration of ketamine and perampanel improves behavioral function and reduces inflammation in acute traumatic brain injury mouse model. Biomed Res. Int.. https://doi.org/10.1155/2020/3193725.

Anjum, S.M.M., Käufer, C., Hopfengärtner, R., Waltl, I., Bröer, S., Löscher, W., 2018. Automated quantification of EEG spikes and spike clusters as a new read out in Theiler's virus mouse model of encephalitis-induced epilepsy. Epilepsy Behav. 88, 189–204. https://doi.org/10.1016/J.YEBEH.2018.09.016.

Ashraf, W., Manzoor, S., Ashraf, J., Ahmed, Q.L., Khalid, M., Tariq, M., Imran, M., Aziz, H., 2013. Transcript analysis of P2X receptors in PBMCs of chronic HCV patients: an insight into antiviral treatment response and HCV-induced pathogenesis. Viral Immunol. 26 (5), 343–350. https://doi.org/10.1089/VIM.2013.0044.

Barker-Haliski, M., Steve White, H., 2015. Glutamatergic mechanisms associated with seizures and epilepsy. Cold Spring Harb. Perspect. Med. 5 (8), 1–15. https://doi.org/10.1101/CSHPERSPECT.A022863.

Bektas, N., Arslan, R., Alyu, F., 2020. The anxiolytic effect of perampanel and possible mechanisms mediating its anxiolytic effect in mice. Life Sci. 261 https://doi.org/10.1016/J.LFS.2020.118359.

Borowicz-Reutt, K.K., Czuczwar, S.J., 2020. Role of oxidative stress in epileptogenesis and potential implications for therapy. Pharmacol. Reports 72 (5), 1218–1226. https://doi.org/10.1007/S43440-020-00143-W.

Casarrubea, M., Di Giovanni, G., Crescimanno, G., 2021. Effects of different anxiety levels on the behavioral patternings investigated through T-pattern analysis in wistar rats tested in the hole-board apparatus. Brain Sci. 11 (6), 714. https://doi.org/10.3390/BRAINSCI11060714.

Chaudhary, S., Parvez, S., 2012. An in vitro approach to assess the neurotoxicity of valproic acid-induced oxidative stress in cerebellum and cerebral cortex of young rats. Neuroscience 225, 258–268. https://doi.org/10.1016/J.NEUROSCIENCE.2012.08.060.

Chen, T., Dai, S.H., Jiang, Z.Q., Luo, P., Jiang, X.F., Fei, Z., Gui, S.B., Qi, Y.L., 2017. The AMPAR antagonist perampanel attenuates traumatic brain injury through anti-oxidative and anti-inflammatory activity. Cell. Mol. Neurobiol. 37 (1), 43–52. https://doi.org/10.1007/S10571-016-0341-8.

Chidambara Murthy, K.N., Jayaprakasha, G.K., Singh, R.P., 2002. Studies on antioxidant activity of pomegranate (Punica granatum) peel extract using in vivo models. J. Agric. Food Chem. 50 (17), 4791–4795. https://doi.org/10.1021/JF0255735.

Chow, C.K., Tappel, A.L., 1972. An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone-exposed rats. Lipids 7 (8), 518–524. https://doi.org/10.1007/BF02533017.

Conrad, C.D., Lupien, S.J., Thanasoulis, L.C., McEwen, B.S., 1997. The effects of type I and type II corticosteroid receptor agonists on exploratory behavior and spatial memory in the Y-maze. Brain Res. 759 (1), 76–83. https://doi.org/10.1016/S0006-8993(97)00236-9.

d'Isa, R., Comi, G., Leocani, L., 2021. Apparatus design and behavioural testing protocol for the evaluation of spatial working memory in mice through the spontaneous alternation T-maze. Sci. Reports 11 (1), 1–13. https://doi.org/10.1038/s41598-021-00402-7.

Fonseca-Rodrigues, D., Gonçalves, J., Laranjeira, I., Almeida, A., Pinto-Ribeiro, F., 2022. Sucrose intake and preference by Wistar Han rats are not influenced by sex or food/water deprivation. Pharmacol. Biochem. Behav. 216, 173387 https://doi.org/10.1016/J.PBB.2022.173387.

Georgiev, V.P., Lazarova, M.B., Kambourova, T.S., 1991. Interactions between angiotensin II, diazepam, clonazepam and di-n-propylacetate in pentylenetetrazol kindling seizures in mice. Neuropeptides 18 (4), 187–191. https://doi.org/10.1016/0143-4179(91)90146-A.

Goji, H., Kanemoto, K., 2019. The effect of perampanel on aggression and depression in patients with epilepsy: a short-term prospective study. Seizure 67, 1–4. https://doi.org/10.1016/J.SEIZURE.2019.02.009.

Haider, S., Naqvi, F., Batool, Z., Tabassum, S., Sadir, S., Liaquat, L., Naqvi, F., Zuberi, N. A., Shakeel, H., Perveen, T., 2015. Pretreatment with curcumin attenuates anxiety while strengthens memory performance after one short stress experience in male rats. Brain Res. Bull. 115, 1–8. https://doi.org/10.1016/J.BRAINRESBULL.2015.04.001.

Hanada, T., 2014. The discovery and development of perampanel for the treatment of epilepsy. Expert Opin. Drug Discov. 9 (4), 449–458. https://doi.org/10.1517/17460441.2014.891580.

Hoeller, A.A., de Carvalho, C.R., Franco, P.L.C., Formolo, D.A., Imthon, A.K., dos Santos, H.R., Eidt, I., Souza, G.R., Constantino, L.C., Ferreira, C.L., Prediger, R.D., Leal, R.B., Walz, R., 2017. Behavioral and neurochemical consequences of pentylenetetrazol-induced kindling in young and middle-aged rats. Pharmaceuticals 10 (3), 75. https://doi.org/10.3390/PH10030075.

Imran, I., Hillert, M.H., Klein, J., 2015. Early metabolic responses to lithium/pilocarpine-induced status epilepticus in rat brain. J. Neurochem. 135 (5), 1007–1018. https://doi.org/10.1111/JNC.13360.

Iughetti, L., Lucaccioni, L., Fugetto, F., Predieri, B., Berardi, A., Ferrari, F., 2018. Brain-derived neurotrophic factor and epilepsy: a systematic review. Neuropeptides 72, 23–29. https://doi.org/10.1016/J.NPEP.2018.09.005.

Javaid, S., Alqahtani, F., Ashraf, W., Anjum, S.M.M., Rasool, M.F., Ahmad, T., Alasmari, F., Alasmari, A.F., Alqarni, S.A., Imran, I., 2023. Tiagabine suppresses pentylenetetrazole-induced seizures in mice and improves behavioral and cognitive parameters by modulating BDNF/TrkB expression and neuroinflammatory markers. Biomed. Pharmacother. 160, 114406 https://doi.org/10.1016/J.BIOPHA.2023.114406.

Kamiński, K., Socała, K., Zagaja, M., Andres-Mach, M., Abram, M., Jakubiec, M., Pieróg, M., Nieoczym, D., Rapacz, A., Gawel, K., Esguerra, C.V., Latacz, G., Lubelska, A., Szulczyk, B., Szewczyk, A., Łuszczki, J.J., Wlaź, P., 2020. N-benzyl-(2,5-dioxopyrrolidin-1-yl)propanamide (AS-1) with hybrid structure as a candidate for a broad-spectrum antiepileptic drug. Neurotherapeutics 17 (1), 309–328. https://doi.org/10.1007/S13311-019-00773-W/FIGURES/11.

Kandratavicius, L., Alves Balista, P., Lopes-Aguiar, C., Ruggiero, R.N., Umeoka, E.H., Garcia-Cairasco, N., Bueno-Junior, L.S., Leite, J.P., 2014. Animal models of epilepsy: use and limitations. Neuropsychiatr. Dis. Treat. 10, 1693–1705. https://doi.org/10.2147/NDT.S50371.

Kanner, A.M., 2017. Psychiatric comorbidities in new onset epilepsy: should they be always investigated? Seizure 49, 79–82. https://doi.org/10.1016/J.SEIZURE.2017.04.007.

Lee, N.S., Jeong, Y.G., 2009. Pogo: a novel spontaneous ataxic mutant mouse. Cerebellum 8 (3), 155–162. https://doi.org/10.1007/s12311-009-0096-6.

Łukawski, K., Czuczwar, S.J., 2023. Oxidative stress and neurodegeneration in animal models of seizures and epilepsy. Antioxidants 12 (5), 1049. https://doi.org/10.3390/ANTIOX12051049.

Malik, N., Javaid, S., Ashraf, W., Siddique, F., Rasool, M.F., Alqahtani, F., Ahmad, T., Abrar, M.A., Imran, I., 2023. Long-term supplementation of Syzygium cumini (l.) skeels concentrate alleviates age-related cognitive deficit and oxidative damage: a comparative study of young vs. old mice. Nutrients 15 (3), 666. https://doi.org/10.3390/NU15030666/S1.

Mansouri, S., Hosseini, M., Beheshti, F., Sobhanifar, M.A., Rakhshandeh, H., Anaeigoudari, A., 2021. Neuroprotective effects of Pinus eldarica in a mouse model of pentylenetetrazole-induced seizures. Avicenna J. Phytomed. 11 (6), 610–621. https://doi.org/10.22038/AJP.2021.18562.

Mareš, P., Kubová, H., 2021. Perampanel exhibits anticonvulsant action against pentylentetrazol-induced seizures in immature rats. Epilepsy Res. 169, 106523 https://doi.org/10.1016/J.EPLEPSYRES.2020.106523.

Meschede, C., Witt, J.A., Rademacher, M., von Wrede, R.D., Elger, C.E., Helmstaedter, C., 2018. Evaluating the longer-term cognitive effects of adjunctive perampanel compared to lacosamide in a naturalistic outpatient setting. Seizure 58, 141–146. https://doi.org/10.1016/J.SEIZURE.2018.04.015.

Mormann, F., Jefferys, J.G.R., 2013. Neuronal firing in human epileptic cortex: the ins and outs of synchrony during seizures. Epilepsy Curr. 13 (2), 100. https://doi.org/10.5698/1535-7597-13.2.100.

Murashima, Y.L., Yoshii, M., Suzuki, J., 2000. Role of nitric oxide in the epileptogenesis of EL mice. Epilepsia 41 (Suppl 6). https://doi.org/10.1111/J.1528-1157.2000.TB01581.X.

Naskar, S., Islam, A., Mazumder, U.K., Saha, P., Haldar, P.K., Gupta, M., 2010. In vitro and in vivo antioxidant potential of hydromethanolic extract of phoenix dactylifera fruits. J. Sci. Res. 2 (1), 144–157. https://doi.org/10.3329/JSR.V2I1.2643.

Nesci, V., Russo, E., Arcidiacono, B., Citraro, R., Tallarico, M., Constanti, A., Brunetti, A., De Sarro, G., Leo, A., 2020. Metabolic alterations predispose to seizure development in high-fat diet-treated mice: the role of metformin. Mol. Neurobiol. 57 (11), 4778–4789. https://doi.org/10.1007/S12035-020-02062-6.

Nieoczym, D., Socała, K., Zelek-Molik, A., Pieróg, M., Przejczowska-Pomierny, K., Szafarz, M., Wyska, E., Nalepa, I., Wlaź, P., 2021. Anticonvulsant effect of pterostilbene and its influence on the anxiety- and depression-like behavior in the pentetrazol-kindled mice: behavioral, biochemical, and molecular studies.

Psychopharmacology (Berl). 238 (11), 3167–3181. https://doi.org/10.1007/S00213-021-05933-5.
Novak, A., Vizjak, K., Rakusa, M., 2022. Cognitive impairment in people with epilepsy. J. Clin. Med. 11 (1), 267. https://doi.org/10.3390/JCM11010267.
Nuss, P., 2015. Anxiety disorders and GABA neurotransmission: a disturbance of modulation. J. Clin. Med. 11, 165–175. https://doi.org/10.2147/NDT.S58841.
Parlar, A., Arslan, S.O., Çam, S.A., 2020. Glabridin alleviates inflammation and nociception in rodents by activating BKCa channels and reducing NO levels. Biol. Pharm. Bull. 43, 884–897. https://doi.org/10.1248/BPB.B20-00038.
Parlar, A., Parlar Köprülü, R.E., Arslan, S.O., Çam, S.A., Özdoğan, F.P., Yumrutaş, Ö., Üçkardeş, F., Ríos, M., Martinez, J.L., Rasekhian, M., n.d., 2022. Extract of Glycyrrhiza glabra root attenuates nociception in experimental pain models: the role of BKCa channels. Bol Latinoam Caribe Plant Med Aromat. 21 (4), 464–484. https://doi.org/10.37360/blacpma.22.21.4.29.
Rehman, Z., Farooq, T., Javaid, S., Ashraf, W., Fawad Rasool, M., Samad, N., Tariq, M., Muhammad Muneeb Anjum, S., Sivandzade, F., Alotaibi, F., Alqahtani, F., Imran, I., 2022. Combination of levetiracetam with sodium selenite prevents pentylenetetrazole-induced kindling and behavioral comorbidities in rats. Saudi Pharm. J. 30 (5), 494–507. https://doi.org/10.1016/J.JSPS.2022.03.003.
Rektor, I., 2013. Perampanel, a novel, non-competitive, selective AMPA receptor antagonist as adjunctive therapy for treatment-resistant partial-onset seizures. Expert Opin. Pharmacother. 14 (2), 225–235. https://doi.org/10.1517/14656566.2013.754883.
Satake, S., Saitow, F., Yamada, J., Konishi, S., 2000. Synaptic activation of AMPA receptors inhibits GABA release from cerebellar interneurons. Nat. Neurosci. 3 (6), 551–558. https://doi.org/10.1038/75718.
Semple, B.D., Dill, L.K., O'Brien, T.J., 2020. Immune challenges and seizures: how do early life insults influence epileptogenesis? Front. Pharmacol. 11, 2. https://doi.org/10.3389/FPHAR.2020.00002.
Shimada, T., Yamagata, K., 2018. Pentylenetetrazole-induced kindling mouse model. J. Vis. Exp. 136, 56573. https://doi.org/10.3791/56573.
Stafstrom, C.E., Carmant, L., 2015. Seizures and epilepsy: an overview for neuroscientists. Cold Spring Harb. Perspect. Med. 5 (6), 1–19. https://doi.org/10.1101/CSHPERSPECT.A022426.
Thomas, A., Burant, A., Bui, N., Graham, D., Yuva-Paylor, L.A., Paylor, R., 2009. Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. Psychopharmacology (Berl). 204 (2), 361–373. https://doi.org/10.1007/S00213-009-1466-Y.
Tor, E.R., Holstege, D.M., Galey, F.D., 1994. Determination of cholinesterase activity in brain and blood samples using a plate reader. J. AOAC Int. 77 (5), 1308–1313. https://doi.org/10.1093/JAOAC/77.5.1308.
Tsai, J.J., Wu, T., Leung, H., Desudchit, T., Tiamkao, S., Lim, K.S., Dash, A., 2018. Perampanel, an AMPA receptor antagonist: from clinical research to practice in clinical settings. Acta Neurol. Scand. 137 (4), 378–391. https://doi.org/10.1111/ANE.12879.
Vorhees, C.V., Williams, M.T., 2006. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat. Protoc. 1(2), 848–858, doi:10.1038/nprot.2006.116.
Wang, X., Hu, Z., Zhong, K., 2021. The role of brain-derived neurotrophic factor in epileptogenesis: an update. Front. Pharmacol. 12, 758232 https://doi.org/10.3389/FPHAR.2021.758232.
Wang, T., Wang, L., Li, L., Ma, L., Liu, X., 2022. Effects of perampanel on cognitive behavior and GluR1 expression in immature mice of temporal lobe epilepsy. Biochem. Biophys. Res. Commun. 588, 68–74. https://doi.org/10.1016/J.BBRC.2021.12.038.
Waterborg, J.H., 2009. The lowry method for protein quantitation. The Protein Protocols Handbook 7–10. https://doi.org/10.1007/978-1-59745-198-7_2.