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Neuroprotective Effect of Brivaracetam and Perampanel Combination on Electrographic Seizures and Behavior Anomalies in Pentylenetetrazole-Kindled Mice

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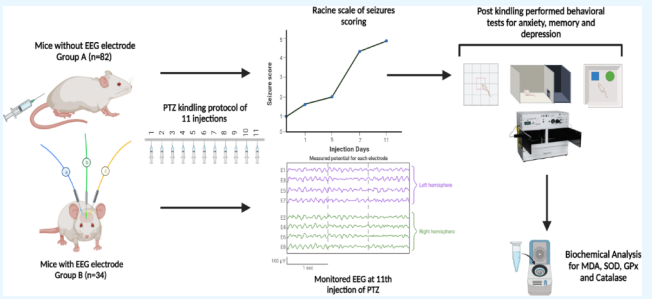
ABSTRACT: Pentylenetetrazole (PTZ)-induced kindling is a broadly used experimental model to study the anticonvulsive potential of new and existing chemical moieties with the aim of discovering drugs hindering seizure progression and associated neurological comorbidities. In the present study, the impact of brivaracetam (BRV) (10 and 20 mg/kg) as monotherapy as well as in combination with 0.25 mg/kg of perampanel (PRP) was investigated on seizure progression with simultaneous electroencephalographic changes in PTZ kindling mouse model. Subsequently, mice were experimentally analyzed for anxiety, cognition, and depression after which their brains were biochemically evaluated for oxidative stress. The outcomes demonstrated that BRV alone delayed the kindling process, but BRV + PRP combination significantly ($p < 0.0001$) protected the mice from seizures of higher severity and demonstrated an antikindling effect. The PTZ-kindled mice exhibited anxiety, memory impairment, and depression in behavioral tests, which were remarkably less ($p < 0.001$) in animals treated with drug combination (in a dose-dependent manner) as these mice explored central, illuminated, and exposed zones of open-field test, light/dark box, and elevated plus maze. Moreover, memory impairment was demonstrated by kindled mice, which was significantly ($p < 0.001$) protected by BRV + PRP as animal's spontaneous alteration, object discrimination, and step-through latencies were increased in various tests employed for the assessment of cognitive abilities. The brains of PTZ-kindled mice had increased malondialdehyde and reduced antioxidant enzymes while treatment with BRV + PRP combination prevented kindling-induced elevation in oxidative markers. The outcomes of this study demonstrate that combining the PRP at low dose augmented the antiseizure properties of BRV as both drugs when administered simultaneously hindered the process of kindling by reducing PTZ-induced excessive electrical activity and oxidative stress in the brain.



1. INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by abnormal and uncontrolled neuronal activity in the brain, resulting in spontaneous seizures as well as behavior comorbidities.[1] According to the WHO, around five million people are diagnosed every year with epilepsy and 50 million people are being affected by it worldwide.[2] Epileptic seizures refer to abnormal electrical activity in the brain leading to epileptogenesis, which is the conversion of a normal brain into an abnormal epileptic brain.[3] Various mechanisms have been proposed for epileptogenesis, including an imbalanced GABA and glutamate level, resulting in excess glutaminergic and diminished GABAergic neurotransmission as well as hyperexcitability of neurons and excitotoxic damage to the brain.[4] Excess glutamate levels in the brain also lead to a decline in cognitive and motor functions.[5]

In the recent past, many newer antiseizure drugs (ASDs) have received regulatory approval, and this number is growing. However, despite the emergence of newer agents with innovative mechanisms of action and modest side-effect profiles, one-third of epileptic patients still experienced partial seizures during treatment.[6] As a result, an appropriate combination of ASDs must be chosen.[7] The use of most of the ASDs from first and second generations has been

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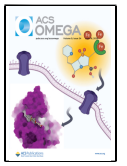

associated with adverse effects and drug resistance, while third-generation ASDs offer better safety and efficacy profiles.[8]

Brivaracetam is a newer third-generation antiseizure drug, and it is one of the recommended medications for the management of focal seizures. Brivaracetam, a chemical analog of levetiracetam, interacts with synaptic vesicular protein (SV2A) the same way as levetiracetam does but has a higher affinity for receptors, making it ten times more potent than levetiracetam.[9] Various preclinical studies report that BRV possesses the ability to reduce the epileptogenic activity in rat brain.[10] In animal models of cardiac stress-induced seizures, brivaracetam was found to have better antiseizure potential.[11]

Previous studies showed an increase in density as well as the hypersensitivity of AMPA receptors in the brains of epileptic patients.[12] Perampanel is an AMPA receptor antagonist that decreases the AMPA-mediated excitatory mechanisms in the brain.[13] Perampanel has been extensively studied in the preclinical phase and has been reported to be effective at lower doses as compared to traditional ASDs. The drug has also been tested in clinical trials, and findings demonstrate its effectiveness in patients with partial seizures.[14]

To recognize the effect of any drug or its combination on seizure development and progression, an appropriate animal model is required.[15] Various chemoconvulsants used for preclinical studies are pilocarpine, kainic acid, and pentylenetetrazole (PTZ).[16,17] In pilocarpine and kainic acid models, animals exhibit episodes of acute status epilepticus followed by recurrent seizures, while the PTZ model is used to study the effect of test drugs on seizure development and progression during the kindling process. The PTZ kindling model has the benefits of cost affordability and reduced risk of model-induced mortality in comparison to kainic acid and pilocarpine.[18] PTZ kindling is the process of injecting a subconvulsive dose of PTZ into animals, resulting in seizures of higher severity.[19] PTZ alters GABAergic neurotransmission by antagonizing GABA receptors, causing neurons to become hyperexcited. Additionally, PTZ also induces alterations in rodent behaviors, which indicates that this model also gives an insight view about behavioral changes that could occur in epilepsy.[20]

Brivaracetam is effective in focal seizures, and its effectivity in maximal electroshock, 6 Hz model, and audiogenic seizures has been previously established.[21] In a recent study, 20−40 mg/kg of brivaracetam in combination with 0.9 and 2.7 mg/kg of perampanel reduced DMCM-induced seizures in immature rats,[22] but the impact was not investigated for seizure-associated electrographic changes and neurobehavioral comorbid changes. So, in this current study, we evaluated the antiseizure potential of a low-dose combination of brivaracetam and perampanel on the seizure development and progression in PTZ kindling mouse model along with validation through real-time electroencephalographic (EEG) changes. Immediately after the kindling process, the animal's neurobehavioral changes were evaluated through behavior tests with an eventual biochemical analysis of isolated brains.

2. MATERIALS AND METHODS

2.1. Animals Used. In this study, 8- to 10-week-old male BALB/c mice with 30−35 g of body weight were used. These mice were obtained from the animal house located in the Faculty of Pharmacy, Bahauddin Zakariya University, Multan, and kept in polycarbonate cages placed in a clean and well-controlled environment at the animal house. The animals were provided with a 12-h light/dark cycle and access to free-flowing rodent food and water. Regarding ethical approval, all experiments and protocols were approved by the ethical committee of the Department of Pharmacology (03-PHL-S-21), Bahauddin Zakariya University, Multan.

2.2. Drugs and Chemical. Brivaracetam (BRV), perampanel (PRP), diazepam, and pentylenetetrazol (PTZ) were used in this study. The dose of each drug was adjusted according to previously published studies. Pentylenetetrazole (Sigma-Aldrich) was dissolved in normal saline to inject its dose of 40 mg/kg.[18] Brivaracetam (Lot no. NP17−13−2111003) was obtained from Zhejiang Eazy Pharmchem, dispensed in distilled water, and its 10 and 20 mg/kg doses were used.[23] Perampanel, available as a triturate tablet, named Fycompa (Eisai. Co. Ltd., Japan) was solubilized in 1% tween to use its dose of 0.25 mg/kg.[24] Diazepam, available as a commercial formulation named Valium, from Roche Pharma was diluted in distilled water, and its dose of 1 mg/kg was used.[25]

2.3. Animal Grouping. A total of 116 animals used in this study were divided into two subsets, i.e., subset A comprised 82 animals without EEG electrodes, while subset B comprised 34 animals with EEG electrodes. These animals were further divided into 8 subgroups given as

1. **Healthy control** received 0.9% normal saline intraperitoneally (i.p.), 1 mL/kg every other day for 21 days.
2. **Diazepam** received 1 mg/kg of diazepam i.p. + 40 mg/kg of PTZ on alternate days for 21 days.
3. **PTZ control** comprising kindled mice received 40 mg/kg of PTZ i.p. on alternate days for 21 days.
4. **BRV 10** received 10 mg/kg of brivaracetam i.p. daily + 40 mg/kg of PTZ on alternate days for 21 days.
5. **BRV 20** received 20 mg/kg of brivaracetam i.p. daily + 40 mg/kg of PTZ on alternate days for 21 days.
6. **PRP 0.25** received 0.25 mg/kg of perampanel i.p. + 40 mg/kg of PTZ on alternate days for 21 days.
7. **BRV 10 + PRP 0.25** received 10 mg/kg of brivaracetam and 0.25 mg/kg of perampanel i.p. daily + 40 mg/kg of PTZ on alternate days for 21 days.
8. **BRV 20 + PRP 0.25** received 20 mg/kg of brivaracetam and 0.25 mg/kg of perampanel i.p. daily + 40 mg/kg of PTZ on alternate days for 21 days.

All animals except healthy and PTZ control mice were treated with groupwise-designated treatments once daily on every alternate day (from day 1 to day 21), and after 40−45 min, a subconvulsive dose of PTZ (40 mg/kg; i.p.) was administered. The animals were observed for post-PTZ 30 min, and changes in their behavior were noted for seizure development and progression after every PTZ injection. Every animal was individually assigned a score, as described by the Racine scale, i.e., no response (stage 0), immobilization (stage 1), head nodding and facial twitches (stage 2), continuous myoclonic jerking (stage 3), rearing with colonic seizures (stage 4), full body tonic−clonic seizures with wild jumping (stage 5), and death (stage 6). The animals received 11 injections of PTZ, and the ones showing at least three consecutive stages 4 and 5 seizures were considered kindled.[26]

2.4. Stereotaxic Surgery. For surgical procedure to implant EEG electrodes, animals were administered the mixture of an anesthetic cocktail comprising ketamine and xylazine (87.5 mg/kg and 12.5 mg/kg, respectively).[27] This anesthetic mixture was given intraperitoneally, and the animal's consciousness was monitored by pinching its toe. Animals were

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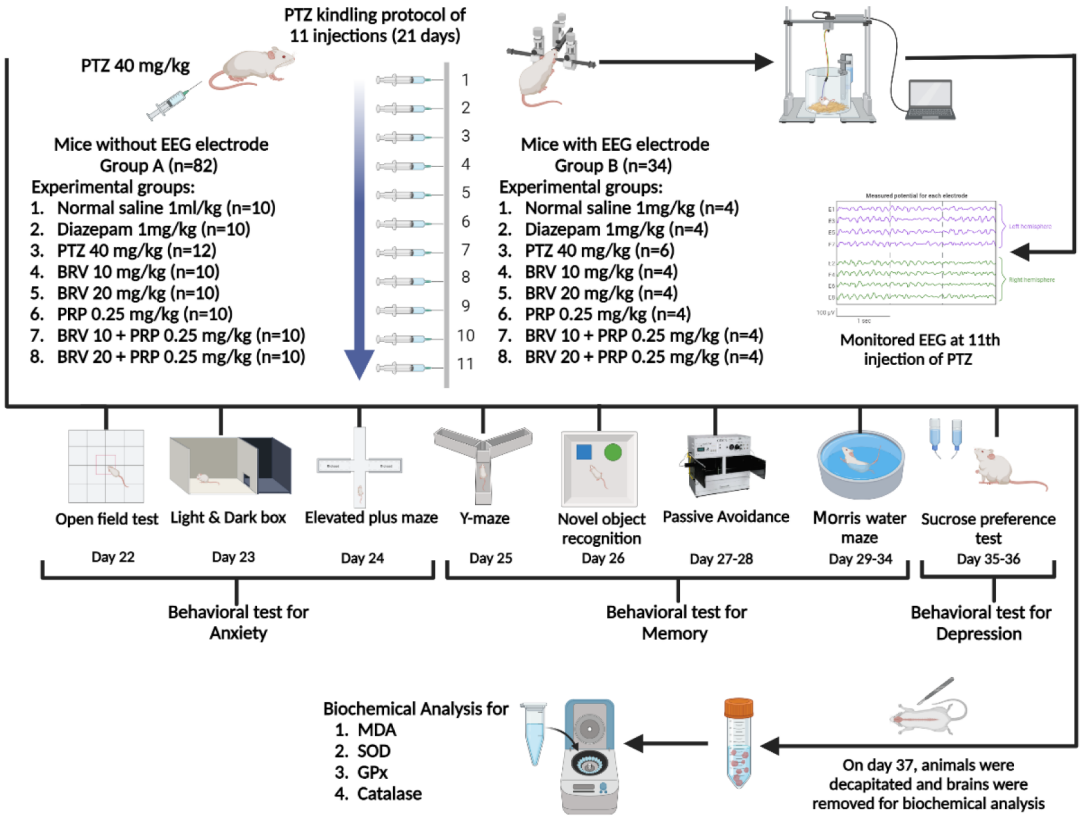

Figure 1. Pictorial illustration of the complete experimental layout. Subset A consisted of 82 animals without EEG electrodes. These animals, after PTZ kindling, passed through behavioral experiments for anxiety, memory, and depression. After the behavioral assessment, brains were removed and processed for biochemical analysis. Subset B consisted of 34 animals having EEG electrodes. These animals had stereotaxic surgery, and after the recovery period, EEG was recorded after the 11th injection of PTZ to assess the effect of test treatments on electrographic changes induced by kindling process. This layout figure has been designed using Biorender.com (SJ26947NQM: dated December 24, 2023).

considered ready for surgery when they did not show any response when pinching their toe. After successful anesthesia, animals were fixed onto the stereotaxic device (Stoelting, USA) supplied with a heating cushion for maintaining the constant physiological temperature. After shaving the hair from the required area on the skull, the skin was prepared for incision by cleaning with isopropyl alcohol. After cleaning, an incision of 2 cm was made by a sharp surgical blade, and soft tissue was removed so that the surface of the skull became prominent for proper visualization of bregma and lambda regions. An electric drill was used to drill holes into the skull. During drilling, it was ensured not to penetrate the meningeal membrane. After drilling, the cortical electrodes were implanted by placing two screws AP + 2 mm, LL ± 1.5 mm from the bregma, and for the reference electrode, one screw was implanted AP − 2 mm; L − 1.5 mm.[18] These electrodes were fixed onto their position with the aid of dental cement, and animals were given bolus injections of normal saline to prevent them from dehydration. After this, the animals were kept under observation and given 5−7 days for recovery from surgery before using them for the recording of EEG.

2.5. EEG Acquisition. After providing 5−7 days post surgery recovery period, the electrode-bearing animals of subset B were administered with respective treatments and PTZ. After the 11th injection given on the 21st day, the animal's post-PTZ behavioral changes were validated electrographically by connecting the animals with a Power lab data acquisition system joined with bio amplifiers. The EEGs were recorded using band-pass filter of 0.1 and 60 Hz and a sampling rate of 200 Hz, and data were acquired with Lab Chart data acquisition software (Pro version 8.1.19).[28]

Detailed animal grouping and experimental scheme are provided in Figure 1.

2.6. Behavioral Studies. On the next day after the 11th PTZ injection, animals of subset A were tested in a series of experiments to assess their anxiety, cognition, and depression-like behavior. Before starting behavior experiments, animals were permitted to acclimatize to the environment of the behavior room for at least 30 min before the commencement of experiments. All behavioral assessments were made by employing a sequence of tests from slightest to greatest aversive and conducted from 8:00 am to 6:00 pm. The behavioral experiments were handled by the experimenter kept unaware of mice grouping and designated treatments to eliminate the possibility of biasness in experimental outcomes. Furthermore, animal activity during the behavioral experiment was video-recorded by using Logitech camera, and these recorded videos were analyzed through trial version (7.1) of ANY-MAZE software (Stoelting Co., USA).

2.6.1. Open-Field Test (OFT). In this test, mice were permitted to discover the open arena for a specific period, and their response in terms of anxiety was evaluated by noticing their preference for a centrally exposed area. The apparatus consisted of an open arena of 50 cm in length and 50 cm in breadth surrounded by walls of 38 cm in height to avoid animal escape during testing. The box was made up of highly dense and nonporous plastic material. Each animal was tested once for 5 min, and between the testing of the two animals, the

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open-field chamber was cleaned with isopropyl alcohols.[29] The arena of the open-field box was separated into different zones like the central zone and peripheral zone. The number of entries and duration spent in the center of the maze were noted to assess the anxiety of animals, while the number of line crossings and distance traveled were noted to examine any possible CNS-suppressing impact of test treatments.

2.6.2. Light and Dark (L/D) Box. To evaluate the antianxiety potential of drugs, the L/D box has been widely used as it is a quite simple paradigm in which no animal training is required. The equipment comprised 21 × 21 × 25 cm illuminated and exposed compartment and 20 × 40 × 40 cm dark and closed compartment.[7] Innately, animals prefer to stay in dark and closed places and avoid lightened places as open places prove a source of anxiety for them. To evaluate the anxiety-like behavior of animals, each animal was tested for 5 min in light and dark boxes, and their anxiety was estimated by quantifying the amount of time spent by them in each zone.

2.6.3. Elevated Plus Maze (EPM). This experiment is based on the idea that mice naturally have a fear of high and open spaces but exhibit a natural desire to explore such places. The apparatus of EPM consisted of four arms, two open (25 × 5.5 cm), which were exposed, while two closed (25 × 5.5 cm) were surrounded by a wall. All four arms were arranged to constitute the plus-shaped apparatus, which was positioned 50 cm above the ground. Each animal was tested in EPM for 5 min and allowed to explore all arms.[30] To evaluate the anxiety in animals, the parameters observed were the % entries in open arms and % time spent there as literature reports that increased exploration of open arms is associated with reduced anxiety in rodents.

2.6.4. Y-Maze (Spontaneous Alteration). The apparatus of Y-maze has three arms (A, B, and C), each comprising the dimensions of 40 × 8 × 15 cm. Animals to be tested were individually introduced in the middle of the apparatus, and their tendency to discover all arms was evaluated for 5 min.[31] Due to the inherent curiosity of rodents toward novel surroundings, mice with remarkable cognitive abilities tend to investigate novel and previously undiscovered arms of the maze. By recalling previously visited arms, the sequence of entries of animals in arms is altered, which is expressed as spontaneous alternation (SAP) by calculating through the following formula:

% SAP = (number of alterations)/(total entries in three arms −2) × 100

2.6.5. Novel Object Recognition Test (NORT). This test evaluates the cognitive ability of rodents by noticing their capability to discriminate the novel object from familiarized object. The test was completed in two phases, designated as the acquisition phase and the testing phase. In the training phase, the animals were made familiar with two familiar objects for 5 min. Immediately after training, one previously familiarized object was removed, and a new object was introduced in the test arena. The animals were tested for another 5 min to note their interaction with the new object to calculate the discrimination index (DI), which if increased is considered a sign of good remembrance.[32]

$DI = T_1 - T_2 / T_{1+} T_2$

where T_1 is the time spent in exploring the novel object, and T_2 is the time spent in exploring the familiar object.

2.6.6. Passive Avoidance Test. In the passive avoidance test, the capability of the test animals to avoid the compartment in which shock or any stimulus was given is tested. Each animal was introduced to the Gemini Passive avoidance system divided into two chambers (each of 9.5 × 8 × 8 in), a light chamber and a dark chamber interconnected through a metallic door. The opening of this door and the shock stimulus were controlled by software. The whole protocol consists of 2 days comprising one training session and post-1-h and post-24-h test sessions.[33] During the training session, animals were introduced to the light chamber and allowed to acclimatize there for 30 s followed by opening the door to allow animals to enter the dark chamber. The entry of animals into the dark chamber was followed by the immediate closure of the door, and a foot shock was given having an intensity of 0.5 mA, 50 Hz for 2 s. In the testing session, the memory retention of animals was evaluated by placing them into the light chamber, and their latency to step into the dark zone was noted for 300 s after opening the door.

2.6.7. Morris Water Maze. The apparatus consisted of a 100 × 60 cm circular container virtually separated into four quadrants (NW, SW, NE, and SE). Inside the Morris water maze, various signs or cues of various geometric shapes and colors were placed to provide navigational assistance to animals to remember the location of the platform. Proximal cues were displayed on the inner wall of the maze above the water level, while distal cues were mounted on the stands positioned around the maze. The duration of the test comprised six consecutive days comprising 2 days for training, 3 days for testing, and 1 day for probe trial.[34] During acquisition days, the mice were trained to locate the visible platform placed in the NE quadrant of the maze by giving four trials in a day. The location of the rescue platform was maintained during the subsequent 3 test days, but the platform was disguised by adding a nontoxic murky substance (white nontoxic poster paint), which masked the previously exposed platform. In the probe trial, the platform was removed, animals were permitted to explore the entire maze for 2 min, and animal entries and duration of swimming in the NE quadrant were noted to evaluate their long-term spatial memory.

2.6.8. Sucrose Preference Test (SPT). In this test, animals' depression-like behavior was estimated by evaluating their preference for sweetened water over plain drinking water. This test was performed after the fasting period of approximately 24 h. After that, the animals were provided with two bottles comprising tap water and 1% sucrose. Both bottles were kept in the cage of animals for 24 h, and animals were allowed to consume any of the two provided solutions.[35] The % sucrose preference was calculated as follows:

% Sucrose preference = (volume (mL) of 1% sucrose consumed)/(volume (mL) of 1% sucrose consumed + volume (mL) of plain water consumed) x 100.

2.7. Biochemical Analysis. In this study, from each experimental group, animals (n = 6) were randomly chosen and decapitated by cervical dislocation for dissection of whole brains. After weighing the brains, they were homogenized in 0.1 M phosphate-buffered saline (PBS) at a 1:10 w/v ratio.[36] The homogenized mixture was centrifuged for 10 min at a low temperature of 4 °C at 12 000 rcf. After the centrifugation, the supernatant was collected, and aliquots were stored at −20 °C.

2.7.1. Malondialdehyde Assay. Malondialdehyde (MDA) is the marker of lipid peroxidation, and its elevated levels indicate cellular damage. It has also been reported that epileptic seizures induce the breakdown of phospholipids and subsequently glycerol levels. The MDA content was quantified by adding 100 μL of brain homogenate to the 1:1 mixture of

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trichloroacetic acid and thiobarbituric acid. Next, this whole mixture was mixed and then boiled, cooled, and then centrifuged at 3500 rpm for 10 min at 4 °C. The content of MDA in cells was quantified by a microplate reader (Spectramax 340 PC384 by Molecular Devices, CA, USA) with reference to the wavelength of 532 nm. The MDA content was expressed as nanomoles per milligram of brain tissue.[37]

2.7.2. Catalase Assay. To 10 μL of brain homogenate, 50 μL of PBS (Ph 7.4) and 40 μL of two molar hydrogen peroxide were added. After this step, this reaction mixture was incubated in the oven for 90 min at 37 °C. After the incubation, 100 μL of 5% potassium dichromate acetic acid was added. Further incubation was done at 100 °C for 10 min. The standard and blank were run simultaneously, absorbance was taken by a microplate reader at 570 nm, and results were expressed in μmol/min/mg of protein.[35]

2.7.3. Glutathione Peroxidase Assay. For the estimation of glutathione peroxidase levels, 20 μL of brain homogenate was mixed with 10 μL of hydrogen peroxide, 20 μL of glutathione, 10 μL of sodium azide, and 20 μL of PBS. Following mixing, the mixture was further processed for 15 min and 40 μL of TCA was added. After mixing all chemicals, centrifugation of the reaction mixture was carried out at 1500 rpm for 15 min, and 30 μL of dibasic sodium phosphate and 70 μL of DTNB were added. A microplate reader was used to read the mixture at a wavelength of 412 nm, and glutathione peroxidase content was noted.[18]

2.7.4. Superoxide Dismutase Assay. SOD levels were quantified by taking 50 μL of brain homogenate and mixed with sodium bicarbonate (50 μL), EDTA (20 μL), and NBT (40 μL). The reaction was initiated after the addition of HAC (40 μL). As a result of this reaction, the insoluble formazan dye was formed, indicated by the formation of a purple color. After mixing all these chemicals, sample absorbance was taken in a duplicate manner every 5 min up to 45 min by the microplate reader[38] at a wavelength of 570 nm. The content of SOD in the brain tissue was expressed in milliunits per milligram of protein.

2.8. Statistical Analysis. The GraphPad Prism version 8.0 software was used for statistical analysis of all experimental outcomes. The seizure score was expressed as median range and evaluated by repeated measure two-way ANOVA with the Fisher's LSD test. The behavioral data was tested for normality by Shapiro-Wilk and Kolmogorov−Smirnov tests, followed by parametric one-way ANOVA and the Dunnett's test. Two-way ANOVA with the Tukey's test was used to evaluate latencies in passive avoidance and Morris water maze tests. All behavioral data were presented as mean ± SEM for $n = 10$ in behavioral studies and $n = 6$ in biochemical analysis, and results were considered significant when $p < 0.05$.

3. RESULTS

3.1. Effect of Brivaracetam and Perampanel Alone and in Combination on Kindling Progression. The results revealed a prominent change in seizure progression in terms of seizure scores among differently treated animals [F (7,720) = 108.8; $p < 0.0001$]. In detail, most of the animals from the kindled control group showed seizures with head nodding and myoclonic jerking on the third PTZ injection ($p < 0.0001$), which progressed to convulsions with rearing on the ninth PTZ injection ($p < 0.0001$), as compared to healthy mice. The seizure score continued to increase in kindled control mice, and 80% of animals demonstrated the post-PTZ seizure progression to rearing and falling on consecutive 3−4 PTZ injections and were considered kindled (Figure 2A). The

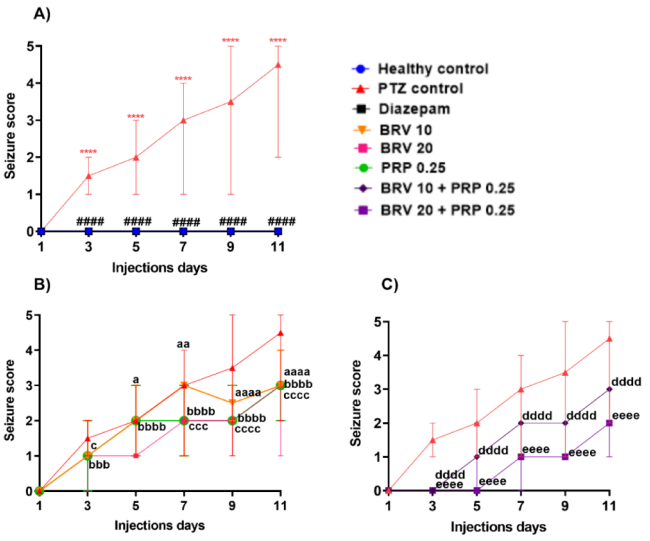

Figure 2. Demonstration of the impact of brivaracetam (BRV) alone and in combination with perampanel (PRP) on seizure severity during PTZ kindling. After treatment with respective treatments every other day and after 40−45 min, the animals were injected with a subconvulsive dose of PTZ for 11 injections to develop the kindling process in them. During post-PTZ 30 min, the animals were behaviorally noted for seizure score, and the outcomes were evaluated to express (**A**) comparison of healthy control and diazepam with kindled control, (**B**) comparison of BRV 10, BRV 20, and PRP 0.25 with kindled control, and (**C**) comparison of BRV 10 + PRP 0.25 and BRV 20 + PRP 0.25 with kindled control. The results were evaluated using repeated measure two-way ANOVA with the Fisher's LSD test. All data values in graphs have been expressed as median ± range (n = 10/group), and $p < 0.05$ was considered statistically significant. ****$p < 0.0001$ represents the significance between PTZ control and healthy control, $^{\#\#\#\#}p < 0.0001$ represents the significance between diazepam and PTZ control, $^{a}p < 0.05$, $^{aa}p < 0.01$, and $^{aaaa}p < 0.0001$ represent the significance between BRV 10 and PTZ control, $^{bbb}p < 0.001$, and $^{bbbb}p < 0.0001$ represent the significance between BRV 20 and PTZ control, $^{c}p < 0.05$, $^{ccc}p < 0.001$, and $^{cccc}p < 0.0001$ represent the significance between PRP 0.25 and PTZ control, $^{dddd}p < 0.0001$ represents the significance between BRV 10 + PRP 0.25 and PTZ control, and $^{eeee}p < 0.0001$ represents the significance between BRV 20 + PRP 0.25 and PTZ control.

diazepam at the dose of 1 mg/kg had significant effectivity in protecting the animals from seizure development as 100% of diazepam-treated animals remained seizure-free throughout the kindling process.

Treatment of animals with only BRV (10 and 20 mg/kg) provided dose-dependent protection from PTZ kindling as BRV 10 was effective in delaying the development of seizures of higher severity, and most of these animals demonstrated the full-body mycolic seizures only until the seventh PTZ injection. However, the results with BRV 20 were significant after the third PTZ injection with $p < 0.001$, and these animals were notably protected from seizure progression throughout the kindling process (Figure 2B). However, on combining BRV with PRP, the outcomes were significant at both doses, and 100% of combination-treated mice showed protection from generalized convulsions with rearing and falling (stages 4−5). Approximately 60% of BRV 10 + PRP 0.25 animals

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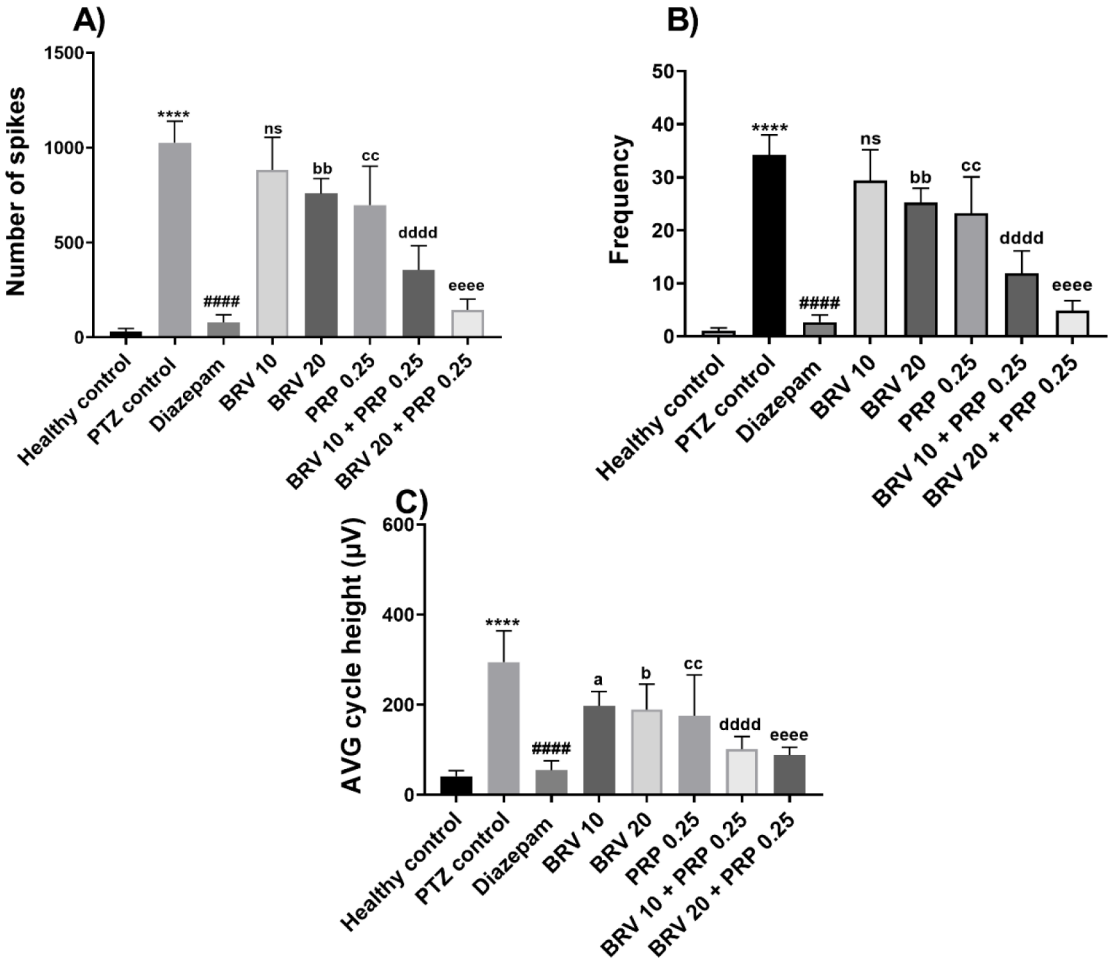

Figure 3. Quantification of post-PTZ EEG recorded for 30 min after the 11th PTZ injection to evaluate the (**A**) number of spikes, (**B**) frequency of spikes, and (**C**) amplitude of spikes expressed in average cyclic height. All data values in graphs have been expressed as mean ± SEM (n = 4). ****p < 0.0001 represents the significance between PTZ control and healthy control, $^{\#\#\#\#}p$ < 0.0001 represents the significance between diazepam and PTZ control, ^{a}p < 0.05 and ^{aa}p < 0.01 represent the significance between BRV 10 and PTZ control, ^{b}p < 0.05 and ^{bb}p < 0.01 represent the significance between BRV 20 and PTZ control, ^{cc}p < 0.01 represents the significance between PRP 0.25 and PTZ control^{dddd}p < 0.0001 represents the significance between BRV 10 + PRP 0.25 and PTZ control, and ^{eeee}p < 0.0001 represents the significance between BRV 20 + PRP 0.25 and PTZ control.

maximally progressed to full body myoclonus (stage 3), while 70% of BRV 20 + PRP 0.25 animals presented with the post-PTZ head nodding with partial myoclonus (stage 2) after 11 PTZ injections, as shown in Figure 2C.

Simultaneously, animals implanted with cortical electrodes were electrographically monitored to understand the effect of mono- and duotherapy on kindling-induced spiking activity in the brain. On the 21st day of the study after the administration of the 11th PTZ injection, the healthy animals had basal EEG activity, while treatment with diazepam prevented PTZ-induced epileptic spikes in the brains of animals (p < 0.0001), as presented in Figure 3A, B. The continuous administration of PTZ in control mice led to the development of kindling in the brains of the animals as they progressed into seizures of higher severity validated through electrographic changes as continuously appearing epileptic spikes progressed into spike discharges (p < 0.0001), and these spikes had significantly higher amplitude (p < 0.0001) as animals progressed to generalized tonic−clonic seizures, as shown in Figures 3C and 4. The animals treated with BRV 10 showed unaffected electrographic activity, which was nonsignificantly different in spike counts and frequency from kindled mice. These electrographic events were reduced (p < 0.01) in BRV 20 animals, revealing that there was an overlap between the behavioral manifestations and electrographic changes observed in animals. However, the BRV at both doses affected the amplitude of spikes as the average cyclic height of spikes was notably less (p < 0.05). The prolonged administration of PRP 0.25 also protected the animals from frequent spikes (p < 0.01), and animals presented with intermittently occurring spikes after a notable interspike interval. But the coadministration of BRV with PRP protected the animals from developing seizures of higher severity, as evidenced from recorded EEG where animals did not develop generalized convulsions, and the electrographic activity remained significantly (p < 0.0001) reduced epileptic spikes at both doses, in comparison to that in kindled mice.

3.2. OFT. After 24 h of completion of the kindling process, the mice were tested to examine the impact of prolonged administration of BRV and PRP on animal locomotion by evaluating their number of line crossings and distance traveled in a square-shaped open arena. A significant intergroup difference was present for a number of line crossings [F (7,72) = 2.68; p = 0.015] and distance traveled [F (7,72) =

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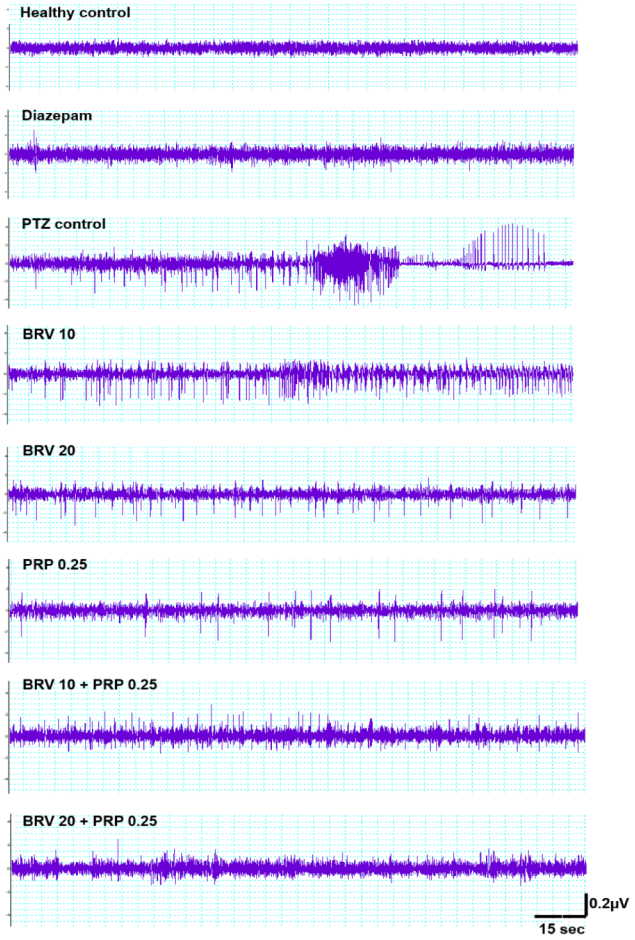

Figure 4. Representative tracings of EEG recorded in electrode-implanted animals to depict the impact of given treatments on kindling development after the 11th PTZ injection. The tracings demonstrate that no change was noted in healthy animals while kindled control animals were fully kindled, as validated by the post-PTZ appearance of epileptic spikes followed by a gust of spike discharges during generalized seizures of stages 4−5 with a postseizure reduction in electrographic activity with subsequent appearance of another discharge of epileptic spikes. In animals treated with BRV 10, there was frequent spiking activity while the interspike interval was increased when BRV dose was doubled to 20 mg/kg. PRP 0.25 was found effective in reducing spike frequency, but the amplitude of spikes remained apparently unaffected by drug alone. However, in animals administered with BRV + PRP, the effects were enhanced as only fewer spikes appeared on the electroencephalogram, and these spikes had very reduced amplitude in comparison to kindled brains, revealing the significance of this combination in protection from PTZ-induced kindling progression.

2.75; $p = 0.013$]. In detail, the animals treated with diazepam for a long duration had a significant reduction in line crossing ($p < 0.05$) and distance traveled ($p < 0.05$). However, BRV at 10 and 20 mg/kg did not affect the locomotor activity of animals, revealing that BRV in the dose range of 10−20 mg/kg has no CNS suppressant effects in mice. However, animals treated with PRP alone as well as with BRV + PRP combination showed a notable reduction in line crossing ($p < 0.05$) and distance traveled ($p < 0.01$), revealing the prolonged administration of PRP at the dose of 0.25 mg/kg might be exerting brain-suppressing effects in mice, as presented in Figure 5A,B.

Moreover, the kindling process and test treatments caused significant variations in central zone preference. The one-way ANOVA showed this variation for the number of central zone entries [$F(7,72) = 22.38$); $p < 0.0001$] and time in central zone [$F(7,72) = 35.94$; $p < 0.0001$]. The kindled animals showed anxiety-like behavior as compared to healthy controls as they preferred the central arena of the maze significantly less, and both observed parameters were statistically reduced in these mice ($p < 0.0001$). In comparison to diseased mice, treatment with BRV alone at both doses caused a significant reduction in anxiety-like behavior in mice ($p < 0.01$). However, when BRV was combined with PRP, the results were much improved ($p < 0.0001$) as these animals visited the central zone more frequently and spent more time in this zone (Figure 5C,D).

3.3. L/D Test. In the L/D box test, animals of differently treated groups had a notable variation in their duration spent in the exploration of the illuminated box [$F(7,72) = 72.65$; $p < 0.0001$] and dark box [$F(7,72) = 76.87$; $p < 0.0001$]. The PTZ-kindled animals showed marked anxiety-like behavior and spent significantly more time ($p < 0.0001$) in the safer and innately preferred zone of the apparatus, as compared to healthy mice. While animals treated with BRV 20 showed an increased preference for illuminated compartment, with $p < 0.01$ in comparison with kindled group. Similarly, PRP at a dose of 0.25 mg/kg worked effectively as the animal's preference for a lightened zone was significantly increased ($p < 0.05$). However, the outcomes were more pronounced when animals were treated with the combination of BRV and PRP as these animals showed remarkably reduced anxiety as they explored the illuminated zone for a significantly longer time in a dose-dependent manner, i.e., $p < 0.001$ was noted for BRV 10 + PRP 0.25 and $p < 0.0001$ was noted for BRV 20 + PRP 0.25, in comparison with kindled mice, as shown in Figure 6A,B.

3.4. EPM. In this test, statistical evaluation of outcomes showed a significant intergroup difference for % open-arm entries [$F(7,72) = 42.96$); $p < 0.0001$] and % time spent there [$F(7,72) = 44.88$); $p < 0.0001$]. In comparison to healthy animals, the kindled mice demonstrated a significantly reduced preference for the exposed zone of the maze as they visited the open arms less frequently and spent less duration there ($p < 0.0001$). However, the prolonged administration of BRV 20 caused the animals to show less anxiety for open arms than kindled mice, as deceptive from their amplified percentage of visits ($p < 0.01$) and duration of exploration ($p < 0.01$). Similarly, animals of the PRP 0.25 group had marked improvement in open-arm entries ($p < 0.001$) and demonstrated increased exploration of this anxiogenic zone for a longer time ($p < 0.05$). In animals receiving a combination of BRV and PRP, a remarkable rise in the % open-arm entries was observed ($p < 0.0001$) (Figure 7A). Moreover, BRV at both doses, when combined with PRP, caused improvement in % duration spent in open arms with $p < 0.0001$, in comparison to that in the kindled group (Figure 7B).

3.5. Y-Maze Test. A significant intergroup difference in SAP was noted [$F(7,72) = 19.57$; $p < 0.0001$]. The kindled control showed compromised remembrance capacity as they poorly remembered the just-visited arm of the Y-shaped apparatus and revisited already-explored arms significantly more often ($p < 0.0001$) as compared to healthy mice. The mice treated with BRV 20 and PRP 0.25 showed increased memory retention as their SAP was markedly increased ($p <$

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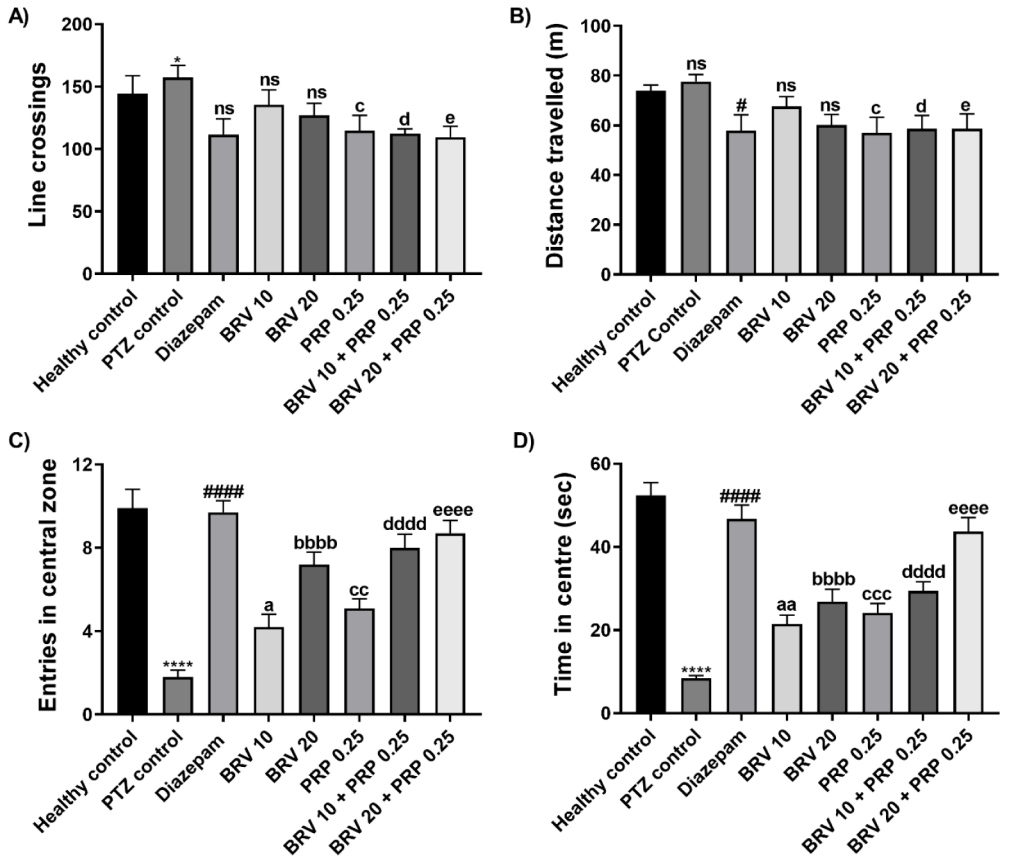

Figure 5. Impact of prolonged brivaracetam (BRV) and perampanel (PRP) administration on the general locomotor of mice was tested by monitoring (**A**) the number of line crossings and (**B**) distance traveled. Furthermore, assessment of the impact of BRV alone and in combination with PRP on animal's anxiety in open-field test was estimated by observing (**C**) entries in center and (**D**) time in center. All data in graphs have been presented as mean ± SEM (n = 10). *p < 0.05 and ****p < 0.0001 represent the significance between PTZ control and healthy control, $^{\#}p$ < 0.05 and $^{\#\#\#\#}p$ < 0.0001 represent the significance between diazepam and PTZ control, ^{a}p < 0.05 and ^{aa}p < 0.01 represent the significance between BRV 10 and PTZ control, ^{bbbb}p < 0.0001 represents the significance between BRV 20 and PTZ control, ^{c}p < 0.05, ^{cc}p < 0.01, and ^{ccc}p < 0.001 represent the significance between PRP 0.25 and PTZ control, ^{d}p < 0.05 and ^{dddd}p < 0.0001 represent the significance between BRV 10 + PRP 0.25 and PTZ control, and ^{e}p < 0.05 and ^{eeee}p < 0.0001 represent the significance between BRV 20 + PRP 0.25 and PTZ control.

0.05) than those of kindled mice. A remarkable improvement in animal memory was noted in animals receiving BRV and PRP combination. The animals treated with BRV 10 + PRP 0.25 had increased SAP with p < 0.01, and these results were further upgraded in the group chronically administered with BRV 20 + PRP 0.25 (p < 0.0001), as shown in Figure 8.

3.6. NORT. The animal's intellect to recall and recognize the novelty was evaluated in the test phase of NORT, and a notable difference in the discrimination index was noted among all groups [F (7,72) = 11.95; p < 0.0001]. When outcomes were compared among healthy and kindled mice, the kindled control group had reduced capability (p < 0.0001) to retain the identity of familiarized object and spent time with both objects undistinguishably. The treatment with BRV 20 and PRP 0.25 alone caused a significant increase in discrimination with p < 0.05 and p < 0.01, respectively. But this improvement in the animal's capability to differentiate the novel object from the familiarized one was conspicuously improved by treating the mice with BRV and PRP combination. The rise in the discrimination index was noted as animals treated with BRV 10 + PRP 0.25 showed improvement with p < 0.01, while BRV 20 + PRP 0.25 caused more significant outcomes with p < 0.0001, as depicted in Figure 9.

3.7. Passive Avoidance Test. The statistical evaluation demonstrated a significant variation in step-through latencies (Figure 10) among differently treated mice [F (7,144) = 147.9; p < 0.0001]. In detail, mice receiving different treatments did not vary for step-through latencies during the training phase. In post 1-h test, the PTZ-kindled animals showed poorer remembrance of the aversive stimuli zone as they stepped into dark chamber notably earlier than healthy animals (p < 0.0001), revealing their compromised capability to remember the aversive stimuli delivered an hour ago. The step-through latencies noted in animals treated with BRV 20 showed that these animals remembered the aversive stimuli zone as they avoided the zone for a long time, which resulted in their longer latencies to step into the dark compartment (p < 0.05). However, treatment of mice with BRV 10 and PRP 0.25 alone did not cause notable differences in step-through latencies in comparison to those in kindled mice. But, when the BRV and PRP were simultaneously administered, the outcomes were significantly improved. When PRP was combined, BRV at both doses (10 and 20 mg/kg) showed better memory retention as the animals revealed longer step-through latencies in a dose-dependent manner, i.e., p < 0.001 was noted with BRV 10 + PRP 0.25, and p < 0.0001 was noted with BRV 20 + PRP 0.25.

Similarly, testing was done after 24 h of the training phase to estimate the effect of test drugs on long-term remembrance

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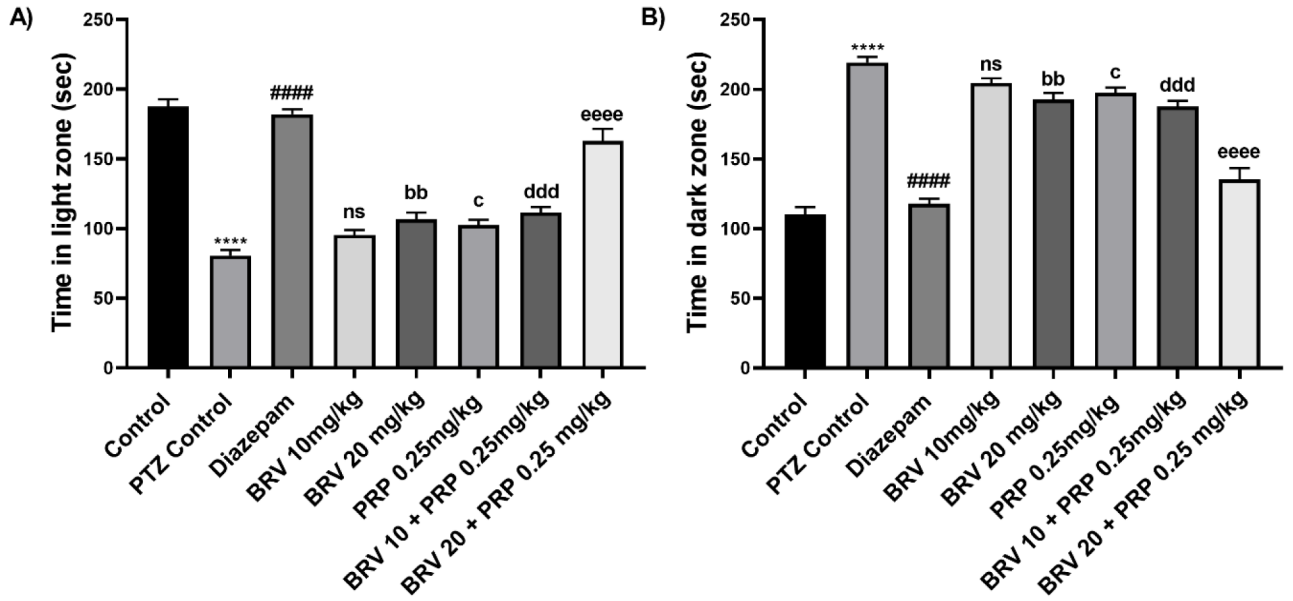

Figure 6. Assessment of anxiolytic action of brivaracetam (BRV) alone and in combination with perampanel (PRP) on PTZ-kindled mice in the light and dark test. The parameters observed were (**A**) time spent in the light zone and (**B**) time spent in the dark zone. All data in graphs have been presented as mean ± SEM (n = 10). ****p < 0.0001 represents the significance between PTZ control and healthy control, $^{\#\#\#\#}p$ < 0.0001 represents the significance between diazepam and PTZ control, ^{bb}p < 0.01 represents the significance between BRV 20 and PTZ control, ^{c}p < 0.05 represents the significance between PRP 0.25 and PTZ control, ^{ddd}p < 0.001 represents the significance between BRV 10 + PRP 0.25 and PTZ control, and ^{e}p < 0.05 and ^{eeee}p < 0.0001 represent the significance between BRV 20 + PRP 0.25 and PTZ control.

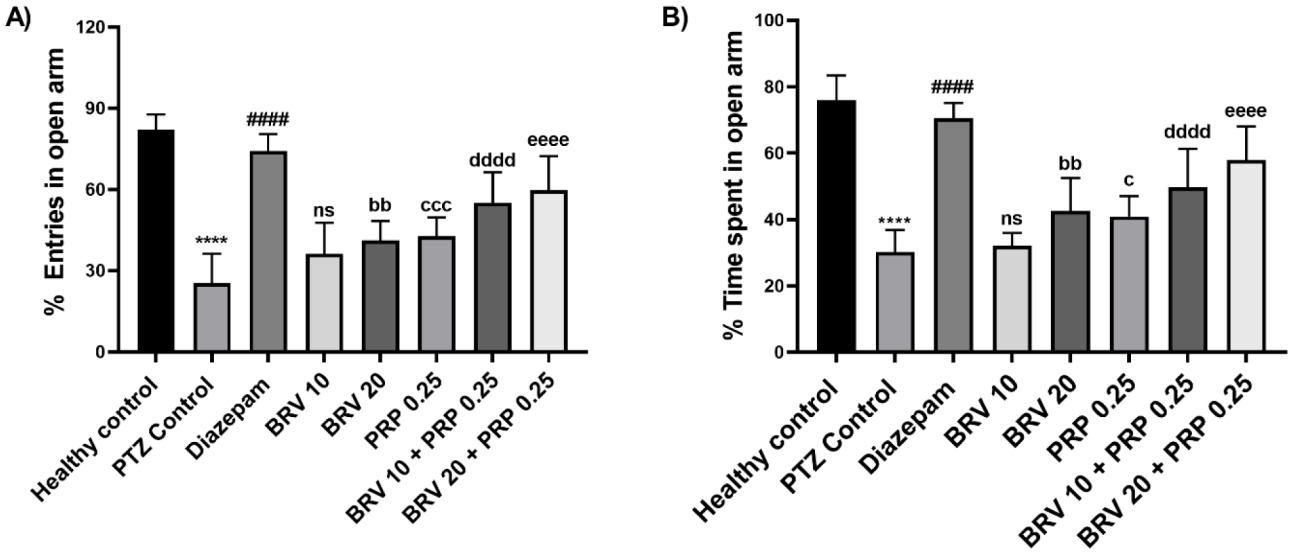

Figure 7. Assessment of the anxiolytic action of brivaracetam (BRV) alone and in combination with perampanel (PRP) on PTZ-kindled mice in an elevated plus maze test. The parameters observed were the (**A**) total number of entries in open arms and (**B**) % time spent by animals in open arms. All data in the graphs have been presented as mean ± SEM (n = 10). ****p < 0.0001 represents the significance between PTZ control and healthy control, $^{\#\#\#\#}p$ < 0.0001 represents the significance between diazepam and PTZ control, ^{bb}p < 0.01 represents the significance between BRV 20 and PTZ control, ^{c}p < 0.05 and ^{ccc}p < 0.001 represent the significance between PRP 0.25 and PTZ control, ^{dddd}p < 0.0001 represents the significance between BRV 10 + PRP 0.25 and PTZ control, and ^{eeee}p < 0.0001 represents the significance between BRV 20 + PRP 0.25 and PTZ control.

capability. As noted in the post 1-h test session, the kindled mice displayed shorter step-through latencies (p < 0.0001), showing that they did not remember the aversive stimuli, in comparison to healthy mice. The BRV 20 and PRP 0.25 worked effectively in preventing the kindling-associated memory deficit as these animals had longer step-through latencies with p < 0.01 and p < 0.05, respectively. Moreover, the mice treated with BRV and PRP combination had a remarkable increase in step-through latencies (p < 0.0001), revealing that this polypharmacy approach had possibly protected them from seizure-induced neuronal injury and postkindling cognitive impairment.

3.8. MWM. Significant intergroup differences were noted for escape latencies in the first 5 days [F (7,288) = 29.30; p < 0.0001]. During the acquisition phase, the mice had training to trace the position of the platform in the NE quadrant, and

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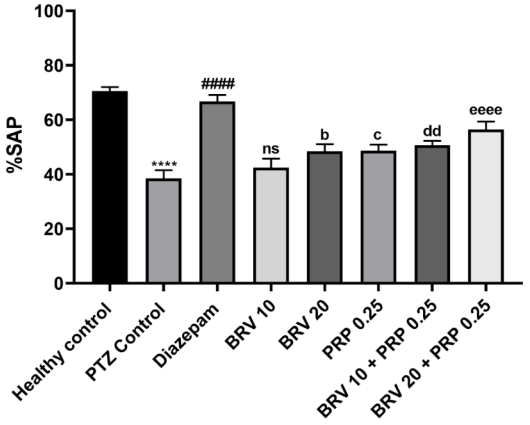

Figure 8. Impact of the combination of brivaracetam (BRV) alone and in combination with perampanel (PRP) on animal learning and memory in terms of SAP noted in Y-maze test. All data in the graph have been presented as mean ± SEM (n = 10). ****p < 0.0001 represents the significance between PTZ control and healthy control, $^{\#\#\#\#}p$ < 0.0001 represents the significance between diazepam and PTZ control, ^{b}p < 0.05 represents the significance between BRV 20 and PTZ control, ^{c}p < 0.05 represents the significance between PRP 0.25 and PTZ control, ^{dd}p < 0.01 represents the significance between BRV 10 + PRP 0.25 and PTZ control, and ^{eeee}p < 0.0001 represents the significance between BRV 20 + PRP 0.25 and PTZ control.

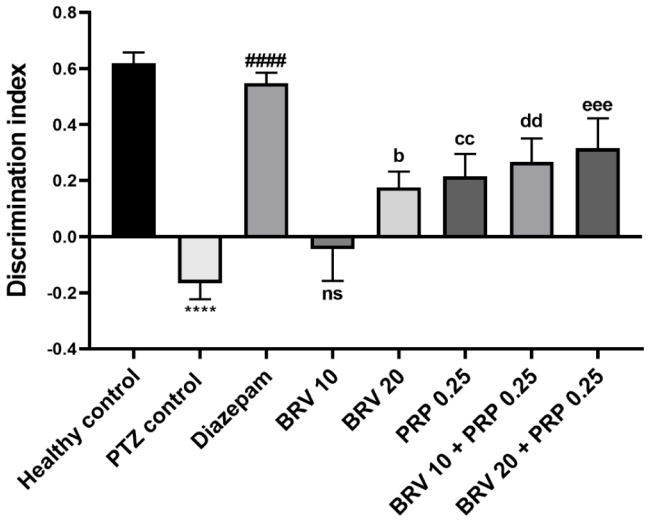

Figure 9. Impact of combination of brivaracetam (BRV) alone and in combination with perampanel (PRP) on animal's learning and memory in terms of the discrimination index noted in novel object recognition test. All data in the graph have been presented as mean ± SEM (n = 10). ****p < 0.0001 represents the significance between PTZ control and healthy control, $^{\#\#\#\#}p$ < 0.0001 represents the significance between diazepam and PTZ control, ^{b}p < 0.05 represents the significance between BRV 20 and PTZ control, ^{cc}p < 0.01 represents the significance between PRP 0.25 and PTZ control, ^{dd}p < 0.01 represents the significance between BRV 10 + PRP 0.25 and PTZ control, and ^{eee}p < 0.001 represents the significance between BRV 20 + PRP 0.25 and PTZ control.

different cues mounted around the perimeter of the maze helped the mice to navigate toward the platform. After the acquisition phase of 2 days comprising four trials per day, the PTZ-kindled animals were incapable of remembering the location of the platform on test days, which was evident from their longer escape latencies, in comparison to the healthy

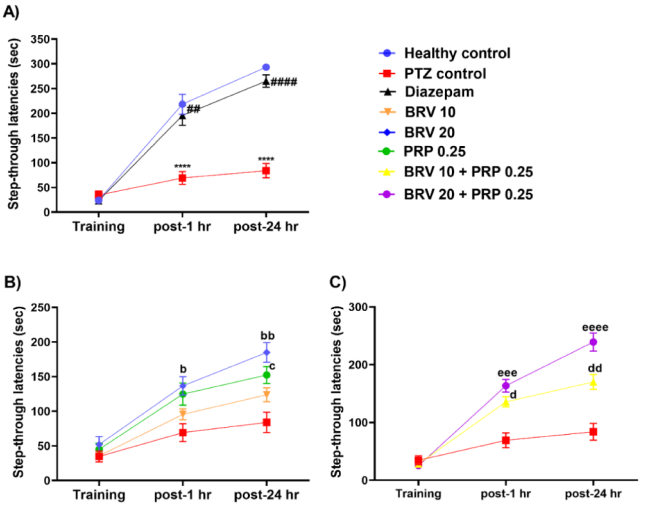

Figure 10. Impact of combination of brivaracetam (BRV) alone and in combination with perampanel (PRP) on step-through latencies in the passive avoidance test. (**A**) Comparison of healthy control and diazepam with kindled control, (**B**) comparison of BRV 10, BRV 20, and PRP 0.25 with kindled control, and (**C**) comparison of BRV 10 + PRP 0.25 and BRV 20 + PRP 0.25 with kindled control. All data in the graphs have been presented as mean ± SEM (n = 10). ****p < 0.0001 represents the significance between PTZ control and healthy control, $^{\#\#}p$ < 0.01 and $^{\#\#\#\#}p$ < 0.0001 represent the significance between diazepam and PTZ control, ^{b}p < 0.05 and ^{bb}p < 0.01 represent the significance between BRV 20 and PTZ control, ^{c}p < 0.05 represents the significance between PRP 0.25 and PTZ control, ^{d}p < 0.05 and ^{dd}p < 0.01 represent the significance between BRV 10 + PRP 0.25 and PTZ control, and ^{eee}p < 0.001 and ^{eeee}p < 0.0001 represent the significance between BRV 20 + PRP 0.25 and PTZ control.

control group [F (1,36) = 217.1; p < 0.0001], as presented in Figure 11A. However, significantly reduced escape latencies were noted in the animals administered with BRV 20 and PRP 0.25 mg/kg, which shows that both drugs worked when administered alone effectively in protecting the animals from PTZ-induced brain damage and cognitive insufficiency. The BRV 20 animals had longer escape latencies on day 3 (p < 0.05), and the difference was more evident on day 5 (p < 0.001). However, BRV at the dose of 10 mg/kg did not produce any significant outcomes, revealing that BRV alone at this dose was not potent enough to protect the mice from kindling-associated cognitive impairment (Figure 11B). However, when combined with PRP 0.25, BRV at both doses caused a remarkable reduction in escape latencies. These animals were comparatively quicker in locating the hidden platform during test days, which was clearly portrayed by their shorter escape latencies noted. On day 5, BRV 10 + PRP 0.25 group had significantly shorter escape latencies (p < 0.001), and a dose-dependent improvement in outcomes was noted in animals receiving BRV 20 + PRP 0.25 (p < 0.0001), as shown in Figure 11C.

On the probe day, the animal's ability to recall the location of the rescue platform was estimated by allowing the animals to swim the entire water maze, and their entries and time spent in the target quadrant zone were estimated. The results of one-way ANOVA elaborated significant differences among groups for the number of entries in the target quadrant [F (7,72) = 33.00, p < 0.0001] and time spent there [F (7,72) = 27.28; p < 0.0001]. In detail, when compared with healthy mice, the

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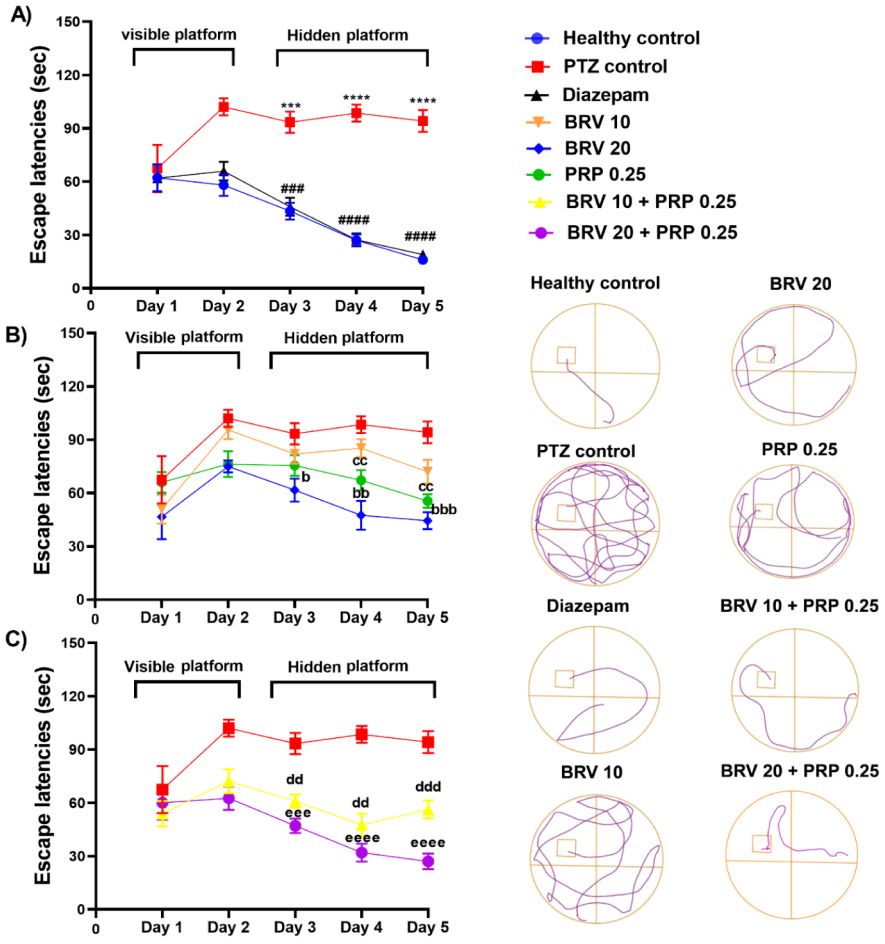

Figure 11. Impact of the combination of brivaracetam (BRV) alone and in combination with perampanel (PRP) on escape latencies along with representative track plots in the Morris water maze test. (**A**) Comparison of healthy control and diazepam with kindled control, (**B**) comparison of BRV 10, BRV 20, and PRP 0.25 with kindled control, and (**C**) comparison of BRV 10 + PRP 0.25 and BRV 20 + PRP 0.25 with kindled control. All data in the graphs have been presented as mean ± SEM (n = 10). ***p < 0.001 and ****p < 0.0001 represent the significance between PTZ control and healthy control, $^{###}p$ < 0.001 and $^{####}p$ < 0.0001 represent the significance between diazepam and PTZ control, ^{b}p < 0.05, ^{bb}p < 0.01, and ^{bbb}p < 0.001 represent the significance between BRV 20 and PTZ control, ^{cc}p < 0.01 represents the significance between PRP 0.25 and PTZ control, ^{dd}p < 0.01 and ^{ddd}p < 0.001 represent the significance between BRV 10 + PRP 0.25 and PTZ control, and ^{eee}p < 0.001 and ^{eeee}p < 0.0001 represent the significance between BRV 20 + PRP 0.25 and PTZ control.

kindled mice remained thigmotaxic in the whole water maze and entered the NE quadrant less frequently (p < 0.0001) (Figure 12A) and swam in the target quadrant for a shorter duration (p < 0.0001) (Figure 12B). However, BRV 20 and PRP 0.25 animals showed improved memory retention, which was depicted from their frequent visits of the target zone (p < 0.05) and more duration of swimming there (p < 0.01), as compared to the kindled group. These outcomes were further augmented in animals treated with the combination of BRV and PRP. The animals treated with the combination of BRV 10 + PRP 0.25 had significantly improved remembrance of the NE zone, which was depicted by their frequent visits and swimming duration (p < 0.01). Similarly, the animal's treatment with BRV 20 + PRP 0.25 caused further improvement in memory retention as the swimming duration of these animals in the NE zone was significantly increased (p < 0.001).

3.9. SPT. This test was performed to evaluate the depression-like behavior in mice subject to PTZ-induced epilepsy. Data were statistically analyzed by one-way ANOVA, and a significant difference in sucrose preference was noted in animals of all groups [F (7,72) = 41.00; p < 0.0001]. The animals of the kindled group showed marked depression-like behavior in comparison with the healthy control group (p < 0.0001). BRV and PRP alone were found significantly effective in reducing depressive behavior as the animals of these groups significantly consumed more sucrose with p < 0.001 and p < 0.05, respectively, in comparison to kindled control. However, animals treated with the combination of both drugs had a remarkable increase in preference for sucrose solution over water (p < 0.0001), as shown in Figure 13.

3.10. Biochemical Assays. A significant difference in MDA levels among kindled and treated groups was noted [F (7,40) = 31.3; p < 0.0001]. The intermittently administered subconvulsive doses of PTZ caused increased MDA levels in the brains of kindled mice (p < 0.0001). The MDA levels were significantly reduced in BRV 20 and PRP 0.25 animals (p < 0.05). However, the PTZ-induced lipid peroxidation was remarkably prevented in animals treated with BRV and PRP combination, and the MDA levels were dose dependently affected as BRV 10 + PRP 0.25 animals had a significance of p < 0.01, which was further increased to p < 0.0001 in mice of the BRV 20 + PRP 0.25 group (Figure 14A).

The outcomes of one-way ANOVA showed a significant intergroup difference for catalase [F (7,40) = 215.2; p <

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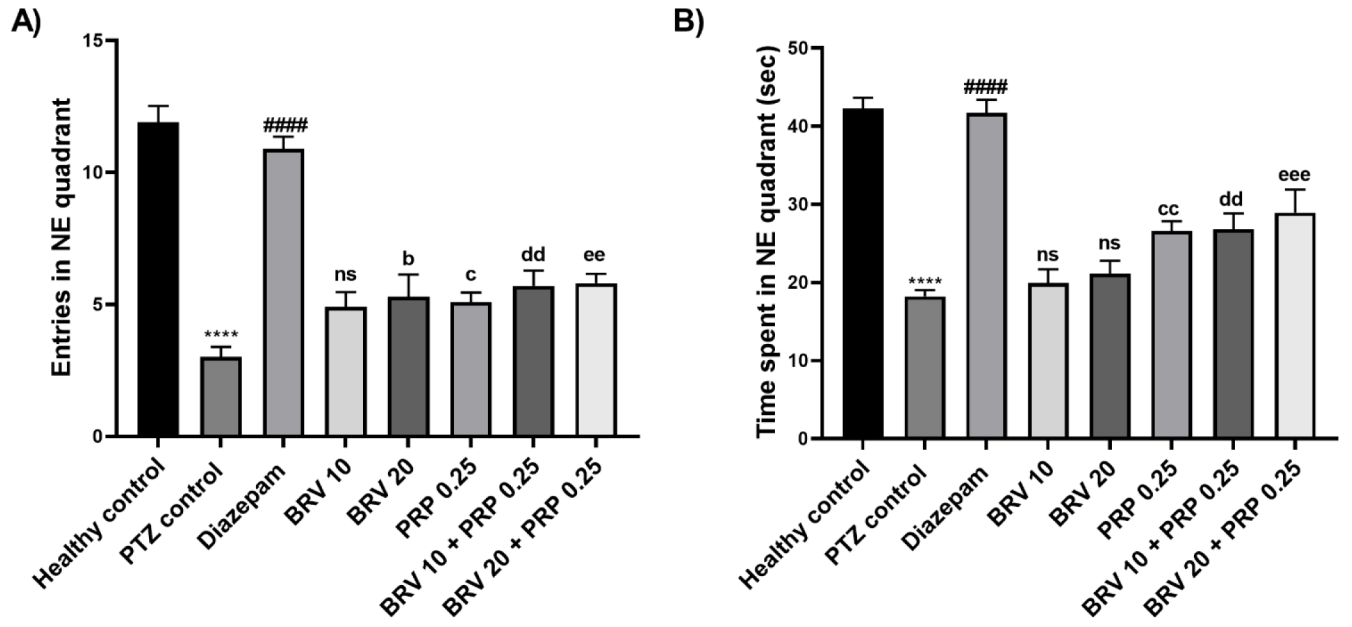

Figure 12. Impact of the combination of brivaracetam (BRV) alone and in combination with perampanel (PRP) on (**A**) entries in the NE quadrant and (**B**) time spent in the NE quadrant on probe day in the Morris water maze test. All data in the graphs have been presented as mean ± SEM (n = 10). ****p < 0.0001 represents the significance between PTZ control and healthy control, $^{\#\#\#\#}p$ < 0.0001 represents the significance between diazepam and PTZ control, ^{b}p < 0.05 represents the significance between BRV 20 and PTZ control, ^{c}p < 0.05 and ^{cc}p < 0.01 represent the significance between PRP 0.25 and PTZ control, ^{dd}p < 0.01 represents the significance between BRV 10 + PRP 0.25 and PTZ control, ^{ee}p < 0.01 and ^{eee}p < 0.001 represent the significance between BRV 20 + PRP 0.25 and PTZ control.

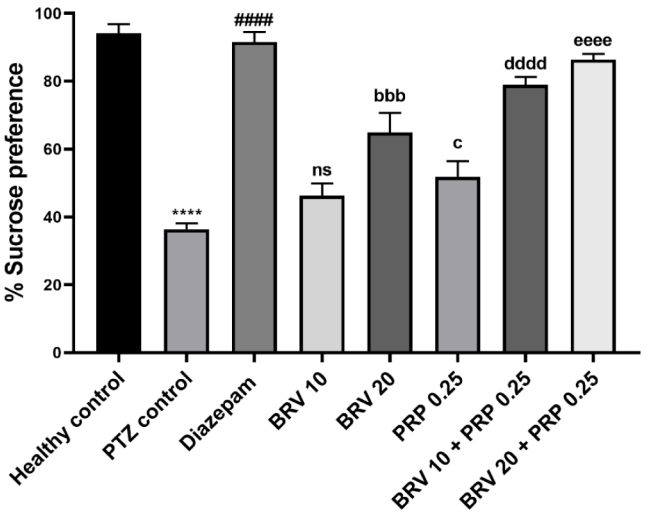

Figure 13. Impact of the combination of brivaracetam (BRV) alone and in combination with perampanel (PRP) on % sucrose preference in the sucrose preference test. All data in the graph have been presented as mean ± SEM (n = 10). ****p < 0.0001 represents the significance between PTZ control and healthy control, $^{\#\#\#\#}p$ < 0.0001 represents the significance between diazepam and PTZ control, ^{bbb}p < 0.001 represents the significance between BRV 20 and PTZ control, ^{c}p < 0.05 represents the significance between PRP 0.25 and PTZ control, ^{dddd}p < 0.0001 represents the significance between BRV 10 + PRP 0.25 and PTZ control, and ^{eeee}p < 0.0001 represents the significance between BRV 20 + PRP 0.25 and PTZ control.

0.0001], SOD [F (7,40) = 26.11; p < 0.0001], and GPx [F (7,40) = 72.38; p < 0.0001]. In comparison to healthy control, the levels of these antioxidant enzymes were significantly reduced in brains of kindled mice (p < 0.0001). BRV alone at a dose of 10 mg/kg remained ineffective in kindling-induced reduction in antioxidant enzyme levels. However, catalase and GPx levels were significantly increased in animals receiving 20 mg/kg of BRV (p < 0.01) and 0.25 mg/kg of PRP (p < 0.05), as shown in Figure 14B,C. Similarly, SOD levels were increased in BRV 20 animals (p < 0.05) but PRP 0.25 did not work significantly in protecting the brains of animals from these kindling-associated deteriorative changes (Figure 14D). When simultaneously administered, BRV and PRP combination yielded additive benefits, and dose-dependent protection from oxidative stress was noted. The BRV 10 + PRP 0.25 treatment caused increased catalase (p < 0.001), GPx (p < 0.001), and SOD (p < 0.01) levels in comparison to the kindled group. These outcomes were further improved in animals receiving BRV 20 + PRP 0.25 for all evaluated enzymes and catalase, GPx levels were notably higher (p < 0.0001), and SOD was also dose dependently elevated (p < 0.001).

4. DISCUSSION

Approximately 20−40% of epileptic patients become resistant to monotherapy and continue to endure seizures even after an initial improvement in the symptoms.[39] The management of refractory epilepsy becomes a challenge and neurologists tend to prefer a polytherapy approach to overcome the drug resistance.[40] Brivaracetam is a rationally designed chemical analog of levetiracetam with higher blood−brain barrier permeability and affinity for SV2A protein, thus regulating the neurotransmitter release.[41] The current study evaluates the impact of combining brivaracetam with a novel AMPA-antagonizing antiseizure drug, perampanel, on seizure progression in the PTZ-induced kindling model. PTZ is a chemoconvulsant that interrupts GABAergic neurotransmission, and its repetitive administration causes increased seizure susceptibility in laboratory animals.[42] In the present study, it

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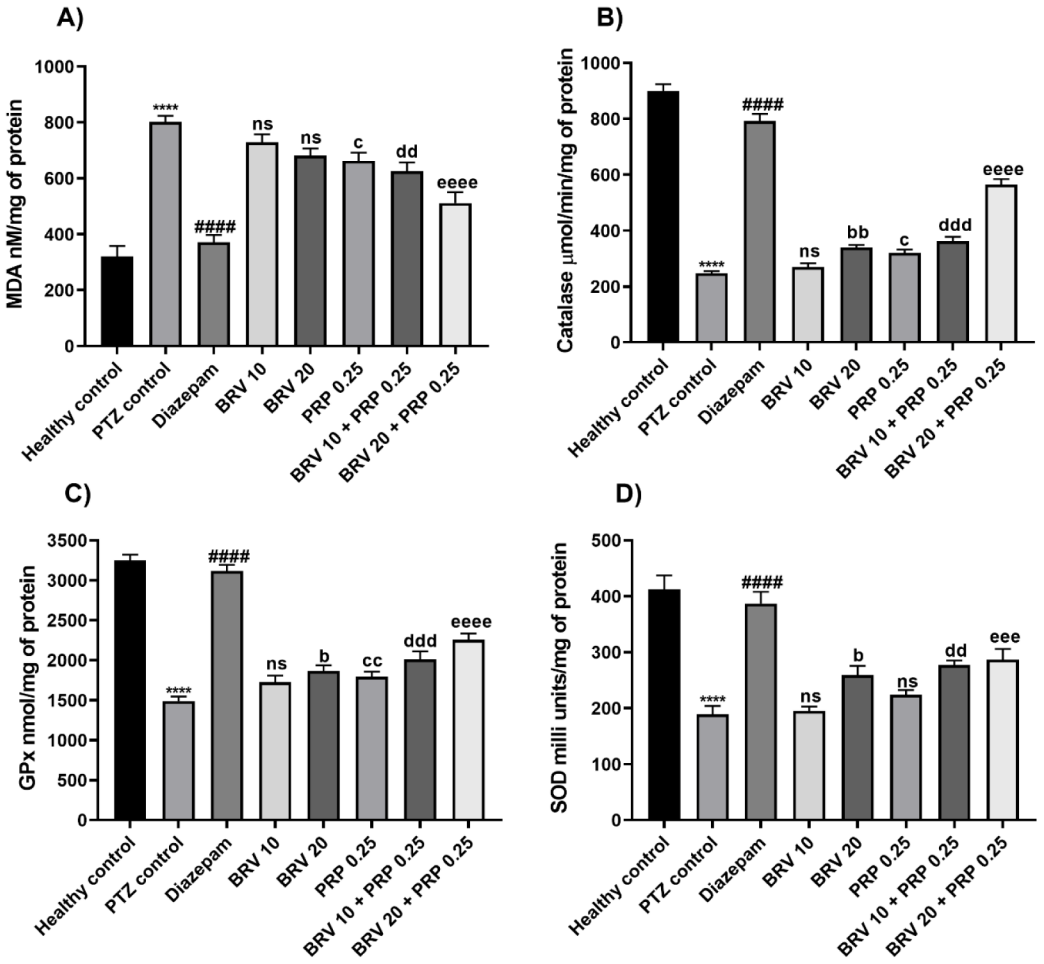

Figure 14. Impact of brivaracetam alone and in combination with perampanel on neurochemical analysis on isolated brain samples. (**A**) malondialdehyde, (**B**) catalase, (**C**) superoxide dismutase, and (**D**) glutathione peroxidase. All data in the graphs have been presented as mean ± SEM (n = 6). ****p < 0.0001 represents the significance between PTZ control and healthy control, $^{\#\#\#\#}p$ < 0.0001 represents the significance between diazepam and PTZ control, ^{b}p < 0.05 and ^{bb}p < 0.01 represent the significance between BRV 20 and PTZ control, ^{c}p < 0.05 and ^{cc}p < 0.01 represent the significance between PRP 0.25 and PTZ control, ^{dd}p < 0.01 and ^{ddd}p < 0.001 represent the significance between BRV 10 + PRP 0.25 and PTZ control, and ^{eee}p < 0.001 and ^{eeee}p < 0.0001 represent the significance between BRV 20 + PRP 0.25 and PTZ control.

was noted that animals pretreated with diazepam remained free from behavioral progression to seizures of higher severity and associated electrographic alterations. Diazepam belongs to benzodiazepines and is known to reduce neuronal excitation by increasing the binding of GABA with the GABA-A receptor, leading to prolonged opening of associated chloride channels.[43] The present study evaluated brivaracetam and perampanel as an alone and combination therapy against PTZ-induced kindling. Brivaracetam works as SV2A ligand in the brain, and SV2A glycoprotein plays a crucial role in the regulation of neuronal transmission by regulating vesicle fusion,[44] while perampanel works as an AMPA antagonist. The drugs when used alone worked less effectively in preventing the seizures as compared to diazepam, and it might be due to the mechanism of action of diazepam as it directly prevents the GABA-antagonizing effects of PTZ. A previous study by Faizi et al. reported the high effectivity of diazepam against PTZ as the ED_{50} of diazepam was very low, i.e., 0.96 mg/kg in mice exposed to a lethal dose of PTZ (100 mg/kg).[45]

The pretreatment of mice with brivaracetam alone at 10 mg/kg caused a delay in initiation and progression to PTZ-induced seizures to convulsions of higher severity, and the difference was noteworthy after the ninth PTZ injection.[46] However, when the dose of brivaracetam was doubled to 20 mg/kg, earlier protection from the kindling process was notable after the fifth PTZ injection, but the EEGs recorded in electrode-bearing mice demonstrated the frequently occurring spikes of higher amplitude. These findings bridge with previous studies reporting that brivaracetam at the dose of 20−40 mg/kg and perampanel at the dose of 0.9−2.4 mg/kg suppressed DMCM-induced postnatal seizures in developing rats.[22] In another study, animals pretreated with brivaracetam at the dose of 21.2 mg/kg resulted in significant protection from generalized convulsions in the amygdala-kindling process.[10] The literature reports the significance of SV2A protein in seizure modulation as SV2A knockout animals were noted to develop seizures of higher severity, resulting in their death within 3 weeks. The SV2A-GABA system has been associated with kindling progression as Tokudome et al. reported that as SV2A protein modulates GABA release[47] and its dysfunction leads to impaired release of synaptic GABA in the hippocampus, leading to elevated susceptibility of rodents to PTZ kindling.[48]

Perampanel is a drug with a novel mechanism of action and seems attractive for rational polypharmacy designs. In our study, when brivaracetam was combined with perampanel, the

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mice remained completely protected from kindling-induced full-bloom generalized seizures, and their simultaneous EEGs validated that epileptic spikes were significantly less severe in terms of both frequency and amplitude. The findings reveal that this multitarget drug combination might be contributing synergistically to modify the seizure progression during kindling process through their diverse mechanism of action targeting SV2A protein and AMPA receptor to modify neurotransmitter release, neuronal excitation, and seizure severity.

Moreover, the locomotor parameters noted in OFT depicted that brivaracetam at the dose of 10−20 mg/kg has no behavioral adverse impact as no sedative effects were noted in animals. A review on the behavioral impact of brivaracetam treatment has reported that its use has less association with adverse behavioral changes in rodents.[49] Moreover, the outcomes of randomized blinded trials also showed that the risk of adverse psychiatric events was lower than 8% after brivaracetam treatment.[44]

Epilepsy has been associated with comorbid neurological conditions including anxiety and depression during the course of the disease.[50] In this study, the impact of brivaracetam in combination with perampanel showed promising protection from kindling-associated anxiety and depression-like behaviors as these animals were more exploratory and fearless toward open, bright, and exposed areas of experimental mazes and preferred sucrose over tap water. Among various behavioral discrepancies following the kindling process, cognitive impairment has been frequently reported.[51] It is extensively believed that epileptiform activity occurring during the kindling process causes hippocampal damage, which is accompanied by memory impairment.[52] The currently used polytherapy concept protected the mice from kindling and protected their brains from pathophysiological changes associated with reminiscence impairment. The simultaneous and prolonged administration of brivaracetam and perampanel might protect from memory dysfunction by reducing PTZ-mediated epileptic discharges, neuronal excitotoxicity, and oxidative stress. These findings are supported by Nygaard et al., who revealed that chronically administered brivaracetam reversed spatial memory impairment in transgenic mouse model of AD.[53] The exact mechanism behind how SV2A modulators regulate cognition is unclear, but a study identifies that SV2A proteins are capable of transporting galactose into the cell, which plays crucially in regulating neuronal physiology and synaptic plasticity, which are important for learning and memory.[54]

The neuronal hyperexcitability during PTZ kindling results in disturbed excitatory−inhibitory pathways that contribute toward elevated brain oxidative stress.[55] In our study, the kindling process was associated with increased oxidative stress, which was evident from reduced levels of endogenously present antioxidant enzymes in the brain. These findings are strengthened by previous studies associating kindling-mediated neuronal excitation with elevated oxidative stress.[35,56] The brivaracetam and perampanel combination exerted neuroprotective effects as the kindling-induced deteriorative changes in antioxidant enzymes were ameliorated in a dose-dependent manner.

Brivaracetam, being the novel SV2A modulator, has very limited literature available regarding preclinical studies on epilepsy-associated neurobehavioral impairments. However, the outcomes of this study provide the beneficial dose-dependent antikindling potential of brivaracetam, which was further improved when it was combined with perampanel. Though the data presented in this study provide a rationale for the evaluation of brivaracetam and perampanel combination in the future in other experimental epilepsy models, it is difficult to obtain any clinical conclusion from this preclinical study unless similarly designed studies are carried out in the future.

5. CONCLUSION

The outcomes of the present study support that brivaracetam in combination with perampanel provided extraordinary protection against experimentally induced kindled brains and associated neurobehavioral impairments. The outcomes might be attributed to the synergistic contribution of distinctly working drugs in combating seizure onset and progression. The combination also protected the mice from kindling-associated neurobehavioral impairments including anxiety, cognitive deficit, and depression, which might be attributed to potential of both drugs to reduce the kindling-induced neuronal damage by mitigating the brain oxidative stress.

■ ASSOCIATED CONTENT

Data Availability Statement

All underlying data are available in the article itself.

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Author Contributions

[▽]T.F. and S.J. contributed equally. T.F. performed the experiments, analyzed the data, and wrote the paper. S.J. performed the experiments, interpreted the data, and wrote the paper. W.A. designed the experiments and analyzed and interpreted the data. M.F.R. conceptualized the experiment and interpreted the data. S.M.M.A. designed the experiments and contributed analysis tools. A.S. performed the experiments, analyzed the data, and wrote the paper. T.A and S.A.A reviewed the paper. F.A. conceptualized the experiment, contributed materials, and proofread the manuscript. I.I. conceived and conceptualized the experiment, contributed chemicals and materials, and analyzed and interpreted the data.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Sirven, J. I. Epilepsy: A Spectrum Disorder. *Cold Spring Harb. Perspect. Med.* **2015**, *5* (9), a022848.
(2) Beghi, E. The Epidemiology of Epilepsy. *Neuroepidemiology* **2020**, *54* (2), 185−191.
(3) Goldberg, E. M.; Coulter, D. A. Mechanisms of Epileptogenesis: A Convergence on Neural Circuit Dysfunction. *Nat. Rev. Neurosci.* **2013**, *14* (5), 337−349.
(4) Staley, K. Molecular Mechanisms of Epilepsy. *Nat. Neurosci.* **2015**, *18* (3), 367−372.
(5) 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.; et al. Coadministration of Ketamine and Perampanel Improves Behavioral Function and Reduces Inflammation in Acute Traumatic Brain Injury Mouse Model. *BioMed. Res. Int.* **2020**, *2020*, 3193725.
(6) Stafstrom, C. E.; Carmant, L. Seizures and Epilepsy: An Overview for Neuroscientists. *Seizures and Epilepsy: An Overview for Neuroscientists* **2015**, *5*, 1−19.
(7) Shakeel, W.; Javaid, S.; Anjum, S. M. M.; Rasool, M. F.; Samad, N.; Alasmari, F.; Alasmari, A. F.; Alaqil, F. A.; Alqarni, S. A.; Alotaibi, F. M.; et al. Time Course Evaluation of Lacosamide Alone and in Polypharmacy on Behavioral Manifestations and Oxidative Stress in Lithium-Pilocarpine-Induced Model. *J. Physiol. Pharmacol.* **2020**, *71* (4), 547−564.
(8) Zhuo, C.; Jiang, R.; Li, G.; Shao, M.; Chen, C.; Chen, G.; Tian, H.; Li, J.; Xue, R.; Jiang, D. Efficacy and Tolerability of Second and Third Generation Anti-Epileptic Drugs in Refractory Epilepsy: A Network Meta-Analysis. *Sci. Rep.* **2017**, *7* (1), 2535.
(9) Gayke, M.; Narode, H.; Eppa, G.; Bhosale, R. S.; Yadav, J. S. Synthetic Approaches toward the Synthesis of Brivaracetam: An Antiepileptic Drug. *ACS Omega* **2022**, *7* (3), 2486−2503.
(10) Matagne, A.; Margineanu, D. G.; Kenda, B.; Michel, P.; Klitgaard, H. Anti-Convulsive and Anti-Epileptic Properties of Brivaracetam (Ucb 34714), a High-Affinity Ligand for the Synaptic Vesicle Protein, SV2A. *Br. J. Pharmacol.* **2008**, *154* (8), 1662−1671.
(11) Tai, K. K.; Truong, D. D. Brivaracetam Is Superior to Levetiracetam in a Rat Model of Post-Hypoxic Myoclonus. *J. Neural Transm.* **2007**, *114* (12), 1547−1551.
(12) Hanada, T.; Hanada, T. The AMPA Receptor as a Therapeutic Target in Epilepsy: Preclinical and Clinical Evidence. *J. Recept., Ligand Channel Res.* **2014**, *7*, 39−50.
(13) Taniguchi, S.; Stolz, J. R.; Swanson, G. T. The Antiseizure Drug Perampanel Is a Subunit-Selective Negative Allosteric Modulator of Kainate Receptors. *J. Neurosci.* **2022**, *42* (28), 5499−5509.
(14) Tsai, J. J.; Wu, T.; Leung, H.; Desudchit, T.; Tiamkao, S.; Lim, K. S.; Dash, A. Perampanel, an AMPA Receptor Antagonist: From Clinical Research to Practice in Clinical Settings. *Acta Neurol. Scand.* **2018**, *137* (4), 378−391.
(15) Imran, I.; Hillert, M. H.; Klein, J. Early Metabolic Responses to Lithium/Pilocarpine-Induced Status Epilepticus in Rat Brain. *J. Neurochem.* **2015**, *135* (5), 1007−1018.
(16) Löscher, W.; Brandt, C. Prevention or Modification of Epileptogenesis after Brain Insults: Experimental Approaches and Translational Research. *Pharmacol. Rev.* **2010**, *62* (4), 668−700.
(17) Hillert, M. H.; Imran, I.; Zimmermann, M.; Lau, H.; Weinfurter, S.; Klein, J. Dynamics of Hippocampal Acetylcholine Release during Lithium-Pilocarpine-Induced Status Epilepticus in Rats. *J. Neurochem.* **2014**, *131* (1), 42−52.
(18) 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. Tiagabine Suppresses Pentylenetetrazole-Induced Seizures in Mice and Improves Behavioral and Cognitive Parameters by Modulating BDNF/TrkB Expression and Neuroinflammatory Markers. *Biomed. Pharmacother.* **2023**, *160*, 114406.
(19) Shimada, T.; Yamagata, K. Pentylenetetrazole-Induced Kindling Mouse Model. *J. Vis. Exp.* **2018**, *136*, No. e56573.
(20) Kaur, D.; Pahwa, P.; Goel, R. K. Protective Effect of Nerolidol Against Pentylenetetrazol-Induced Kindling, Oxidative Stress and Associated Behavioral Comorbidities in Mice. *Neurochem. Res.* **2016**, *41* (11), 2859−2867.
(21) Stephen, L. J.; Brodie, M. J. Brivaracetam: A Novel Antiepileptic Drug for Focal-Onset Seizures. *Ther. Adv. Neurol. Disord.* **2018**, *11*, 1756285617742081.
(22) Culjat, M.; Huizenga, M. N.; Forcelli, P. A. Age-Dependent Anticonvulsant Actions of Perampanel and Brivaracetam in the Methyl-6,7-Dimethoxy-4-Ethyl-Beta-Carboline-3-Carboxylate (DMCM) Model of Seizures in Developing Rats. *Pharmacol. Rep.* **2021**, *73* (1), 296−302.
(23) Klein, P.; Diaz, A.; Gasalla, T.; Whitesides, J. A Review of the Pharmacology and Clinical Efficacy of Brivaracetam. *Clin. Pharmacol. Adv. Appl.* **2018**, *10*, 1−22.
(24) Zolkowska, D.; Dhir, A.; Rogawski, M. A. Perampanel, a Potent AMPA Receptor Antagonist, Protects against Tetramethylenedisulfotetramine-Induced Seizures and Lethality in Mice: Comparison with Diazepam. *Arch. Toxicol.* **2021**, *95* (7), 2459−2468.
(25) Abdulsahib, W. K.; Kathem, S. H.; Al-Radeef, M. Y.; Jasim, L. S. Mentha Piperita Oil Exerts an Antiepileptic Effect in Pilocarpine and Pentylenetetrazol-Induced Seizures in Mice. *Vet Med. Int.* **2022**, *2022*, 4431317.
(26) Nieoczym, D.; Socała, K.; Zelek-Molik, A.; Pieróg, M.; Przejczowska-Pomierny, K.; Szafarz, M.; Wyska, E.; Nalepa, I.; Wlaź, P. 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* **2021**, *238* (11), 3167−3181.
(27) Bielefeld, P.; Sierra, A.; Encinas, J. M.; Maletic-Savatic, M.; Anderson, A.; Fitzsimons, C. P. A Standardized Protocol for Stereotaxic Intrahippocampal Administration of Kainic Acid Combined with Electroencephalographic Seizure Monitoring in Mice. *Front. Neurosci.* **2017**, *11*, 160.
(28) Anjum, S. M. M.; Käufer, C.; Hopfengärtner, R.; Waltl, I.; Bröer, S.; Löscher, W. 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.* **2018**, *88*, 189−204.
(29) Sajjad Haider, M.; Ashraf, W.; Javaid, S.; Fawad Rasool, M.; Muhammad Abdur Rahman, H.; Saleem, H.; Muhammad Muneeb Anjum, S.; Siddique, F.; Morales-Bayuelo, A.; Kaya, S.; Alqahtani, F.; Alasmari, F.; Imran, I. Chemical Characterization and Evaluation of the Neuroprotective Potential of Indigofera Sessiliflora through In-Silico Studies and Behavioral Tests in Scopolamine-Induced Memory Compromised Rats. *Saudi J. Biol. Sci.* **2021**, *28*, 4384−4398.
(30) Stanely Mainzen Prince, P.; Kamalakkannan, N.; Menon, V. P. Syzigium Cumini Seed Extracts Reduce Tissue Damage in Diabetic Rat Brain. *J. Ethnopharmacol.* **2003**, *84* (2−3), 205−209.
(31) Tariq, M.; Javaid, S.; Ashraf, W.; Anjum, S. M. M.; Rasool, M. F.; Siddique, F.; Ahmad, T.; Alsanea, S.; Alasmari, F.; Alqahtani, F.; et al. Unveiling the Potential of Perampanel and Pregabalin in Addressing Pentylenetetrazole-Induced Electrographic Alterations and Neurobehavioral Anomalies. *Biomed. Pharmacother.* **2024**, *170*, 115935.
(32) Kersten, M.; Rabbe, T.; Blome, R.; Porath, K.; Sellmann, T.; Bien, C. G.; Köhling, R.; Kirschstein, T. Novel Object Recognition in Rats with NMDAR Dysfunction in CA1 after Stereotactic Injection of Anti-NMDAR Encephalitis Cerebrospinal Fluid. *Front. Neurol.* **2019**, *10*, 586.
(33) Malik, H.; Javaid, S.; Fawad Rasool, M.; Samad, N.; Rizwan Ahamad, S.; Alqahtani, F.; Imran, I. Amelioration of Scopolamine-Induced Amnesic, Anxiolytic and Antidepressant Effects of Ficus Benghalensis in Behavioral Experimental Models. *Medicina* **2020**, *56* (3), 144.
(34) Rahman, H. M. A.; Javaid, S.; Ashraf, W.; Rasool, M. F.; Anjum, S. M. M.; Saleem, H.; Siddique, F.; Chtita, S.; Sivandzade, F.; Alqahtani, F.; Alotaibi, M. R.; Imran, I. Neuropharmacological Investigation, Ultra-High Performance Liquid Chromatography Analysis, and in Silico Studies of Phyla Nodiflora. *J. Physiol. Pharmacol.* **2021**, *72* (4), 637−654.
(35) Rehman, Z.; Farooq, T.; Javaid, S.; Ashraf, W.; Fawad Rasool, M.; Samad, N.; Tariq, M.; Muhammad Muneeb Anjum, S.; Sivandzade, F.; Alotaibi, F.; et al. Combination of Levetiracetam with Sodium Selenite Prevents Pentylenetetrazole-Induced Kindling and Behavioral Comorbidities in Rats. *Saudi Pharm. J.* **2022**, *30*, 494−507.
(36) Malik, N.; Javaid, S.; Ashraf, W.; Siddique, F.; Rasool, M. F.; Alqahtani, F.; Ahmad, T.; Abrar, M. A.; Imran, I. 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* **2023**, *15* (3), 666.
(37) Imran, I.; Javaid, S.; Waheed, A.; Rasool, M. F.; Majeed, A.; Samad, N.; Saeed, H.; Alqahtani, F.; Ahmed, M. M.; Alaqil, F. A. Grewia Asiatica Berry Juice Diminishes Anxiety, Depression, and Scopolamine-Induced Learning and Memory Impairment in Behavioral Experimental Animal Models. *Front. Nutr.* **2021**, *7*, 587367.
(38) Perveen, N.; Alqahtani, F.; Ashraf, W.; Fawad Rasool, M.; Muhammad Muneeb Anjum, S.; Kaukab, I.; Ahmad, T.; Alqarni, S. A.; Imran, I. Perampanel Increases Seizure Threshold in Pentylenetetrazole-Kindled Mice and Improves Behavioral Dysfunctions by Modifying MRNA Expression Levels of BDNF/TrkB and Inflammatory Markers. *Saudi Pharm. J.* **2024**, *32* (1), 101930.
(39) Schmidt, D.; Löscher, W. Drug Resistance in Epilepsy: Putative Neurobiologic and Clinical Mechanisms. *Epilepsia* **2005**, *46* (6), 858−877.
(40) Lee, J. W.; Dworetzky, B. Rational Polytherapy with Antiepileptic Drugs. *Pharmaceuticals* **2010**, *3* (8), 2362−2379.
(41) Nicolas, J. M.; Hannestad, J.; Holden, D.; Kervyn, S.; Nabulsi, N.; Tytgat, D.; Huang, Y.; Chanteux, H.; Staelens, L.; Matagne, A.; et al. Brivaracetam, a Selective High-Affinity Synaptic Vesicle Protein 2A (SV2A) Ligand with Preclinical Evidence of High Brain Permeability and Fast Onset of Action. *Epilepsia* **2016**, *57* (2), 201−209.
(42) Rasool, N.; Razzaq, Z.; Gul Khan, S.; Javaid, S.; Akhtar, N.; Mahmood, S.; Christensen, J. B.; Ali Altaf, A.; Muhammad Muneeb Anjum, S.; Alqahtani, F.; et al. A Facile Synthesis of 1,3,4-Oxadiazole-Based Carbamothioate Molecules: Antiseizure Potential, EEG Evaluation and in-Silico Docking Studies. *Arab. J. Chem.* **2023**, *16* (4), 104610.
(43) Griffin, C. E.; Kaye, A. M.; Rivera Bueno, F.; Kaye, A. D. Benzodiazepine Pharmacology and Central Nervous System−Mediated Effects. *Ochsner J.* **2013**, *13* (2), 214−223.
(44) Steinhoff, B. J.; Staack, A. M. Levetiracetam and Brivaracetam: A Review of Evidence from Clinical Trials and Clinical Experience. *Ther. Adv. Neurol. Disord* **2019**, *12*, 1756286419873518.
(45) Faizi, M.; Jahani, R.; Ebadi, S. A.; Tabatabai, S. A.; Rezaee, E.; Lotfaliei, M.; Amini, M.; Almasirad, A. Novel 4-Thiazolidinone Derivatives as Agonists of Benzodiazepine Receptors: Design, Synthesis and Pharmacological Evaluation. *Excli J.* **2017**, *16*, 52−62.
(46) Crowder, K. M.; Gunther, J. M.; Jones, T. A.; Hale, B. D.; Zhang, H. Z.; Peterson, M. R.; Scheller, R. H.; Chavkin, C.; Bajjalieh, S. M. Abnormal Neurotransmission in Mice Lacking Synaptic Vesicle Protein 2A (SV2A). *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96* (26), 15268−15273.
(47) Tokudome, K.; Okumura, T.; Shimizu, S.; Mashimo, T.; Takizawa, A.; Serikawa, T.; Terada, R.; Ishihara, S.; Kunisawa, N.; Sasa, M.; et al. Synaptic Vesicle Glycoprotein 2A (SV2A) Regulates Kindling Epileptogenesis via GABAergic Neurotransmission. *Sci. Rep.* **2016**, *6*, 27420.
(48) Li, K. Y.; Hsu, C. Y.; Yang, Y. H. A Review of Cognitive and Behavioral Outcomes of Brivaracetam. *Kaohsiung J. Med. Sci.* **2023**, *39* (2), 104−114.
(49) Steinhoff, B. J.; Staack, A. M. Levetiracetam and Brivaracetam: A Review of Evidence from Clinical Trials and Clinical Experience. *Ther Adv. Neurol Disord.* **2019**, *12*, 1756286419873518.
(50) Rodríguez, C. A.; Kubis, M. M.; Arteaga, C. B. T.; Fustes, O. J. H. Psychiatric Comorbidities in Epilepsy. *J. Epilepsy Res.* **2022**, *12* (1), 21−26.
(51) Bertoncello, K. T.; Zanandrea, R.; Bonan, C. D. Pentylenetetrazole-Induced Seizures Cause Impairment of Memory Acquisition and Consolidation in Zebrafish (Danio Rerio). *Behav Brain Res.* **2022**, *432*, 113974.
(52) Das, R.; Luczak, A. Epileptic Seizures and Link to Memory Processes. *AIMS Neurosci.* **2022**, *9* (1), 114−127.
(53) Nygaard, H. B.; Kaufman, A. C.; Sekine-Konno, T.; Huh, L. L.; Going, H.; Feldman, S. J.; Kostylev, M. A.; Strittmatter, S. M. Brivaracetam, but Not Ethosuximide, Reverses Memory Impairments in an Alzheimer's Disease Mouse Model. *Alzheimer's Res. Ther.* **2015**, *7* (1), 25.
(54) Madeo, M.; Kovács, A. D.; Pearce, D. A. The Human Synaptic Vesicle Protein, SV2A, Functions as a Galactose Transporter in Saccharomyces Cerevisiae. *J. Biol. Chem.* **2014**, *289* (48), 33066−33071.
(55) Aguiar, C. C. T.; Almeida, A. B.; Araujo, P. V. P.; de Abreu, R. N. D. C.; Chaves, E. M. C.; Vale, O. C. D.; Macedo, D. S.; Woods, D. J.; Fonteles, M. M. D. F.; Vasconcelos, S. M. M. Oxidative Stress and Epilepsy: Literature Review. *Oxid. Med. Cell. Longev.* **2012**, *2012*, 795259.
(56) Eraković, V.; Župan, G.; Varljen, J.; Simonić, A. Pentylenetetrazol-Induced Seizures and Kindling: Changes in Free Fatty Acids, Superoxide Dismutase, and Glutathione Peroxidase Activity. *Neurochem. Int.* **2003**, *42* (2), 173−178.

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