

Neuroprotective Effects of *Bryophyllum pinnatum* on Lithium-Pilocarpine-Induced Prefrontal Cortex Alterations in a Wistar Rat Model of Seizure Disorder

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ABSTRACT

This study evaluated the neuroprotective effects of *Bryophyllum pinnatum* (BP) against Lithium Pilocarpine-induced prefrontal cortex (PFC) alterations in a Wistar rat model of seizure disorder. Forty male Wistar rats (120-150g each) were divided into five groups (n=8). Group A (control) received distilled water, while Group B (LiCl-P only) were administered LiCl-P only intraperitoneally {lithium chloride (127 mg/mL) first, followed by pilocarpine (30 mg/mL) after 24 hours} Group C received LiCl-P with BP (250mg/kg bw), Group D received LiCl-P with BP (500mg/kg.bw) and Group E received LiCl-P with Sodium valproate (200mg/kg bw). Weekly weight changes were recorded. At the end of the experiment, animals underwent neurobehavioral assessments (open field and Y-maze) and were then euthanised; and the prefrontal cortex tissues were either homogenised for biochemical analysis of oxidative stress markers, inflammatory markers, and antioxidant enzyme activities, or processed for histology and immunohistochemical analysis. Results showed that exposure to LiCl-P significantly decreases rearing, freezing and centre squares entry frequencies. Results also showed significant increase in glutamate, acetylcholine, MDA, TNF- α ; and decrease in GABA and catalase. Histological analysis showed neuronal loss in the LiCl-P groups; and for the BP treated groups, ameliorating effects were observed at high doses. *Bryophyllum pinnatum* treatment protects against LiCl-P induced seizures through reduction of oxidative stress, inflammation and neuronal damage; highlighting its potentials as a neuroprotective agent.

KEYWORDS: *Bryophyllum pinnatum*; Neuroprotection; Lithium–pilocarpine model; Seizure disorder, Oxidative Stress

1. Introduction

Seizure disorders (including epilepsy) are neurological conditions characterised by recurrent and abnormal electrical activities in the brain [1]. This abnormal electrical activity occur when there is sudden imbalance between excitatory and inhibitory neuronal activity, leading to hyper-synchronous firing of a group of neurons [2]. This imbalance can occur as a result of excess glutamate release, reduced GABA function, altered ion channel function, trauma or tumor [3]. Epilepsy manifests in various forms, including generalised epilepsy, where seizures affect both hemispheres of the brain simultaneously, and focal (partial) epilepsy, where seizures originate in a specific area of the brain [4]. One common form of focal epilepsy is temporal lobe epilepsy (TLE), which arises

from abnormal activity in the temporal lobe [5]. Temporal lobe epilepsy is distinctive for its potential resistance to standard antiepileptic drugs, requiring surgical intervention in drug-refractory cases [5].

The lithium-pilocarpine model is a well-established experimental paradigm for inducing status epilepticus (SE) in rodents, closely mimicking the full progression of temporal lobe epilepsy, including the acute, latent and chronic phases [6]. Pilocarpine causes massive neuronal depolarisation thereby leading to increase in intracellular calcium and glutamate release [7]. This model induces SE by first sensitising the animal with lithium chloride, which enhances the brain's sensitivity to pilocarpine, a muscarinic cholinergic agonist that triggers sustained seizure activity [8]. With this approach, mortality is reduced in rodents unlike high mortality rate in

other models (such as kainic acid), thereby providing a suitable platform for evaluating antiepileptic and neuroprotective therapies [9]. This model also leads to significant neurodegenerative changes, neuro-inflammation, altered neurogenesis, and increased oxidative stress; especially in brain regions such as the hippocampus and the prefrontal cortex, which play crucial roles in cognition, emotional regulation, and executive functions [8]. The prefrontal cortex (PFC) is highly vulnerable to seizure-induced oxidative stress, excitotoxicity, inflammation, and neuronal loss [10]. These pathological changes contribute to the neurobehavioural co-morbidities commonly observed in epilepsy patients, such as memory deficits, anxiety, and impaired decision-making.

Most antiepileptic drugs work by enhancing GABA, blocking Na⁺ channels or inhibiting T-type Ca²⁺ channels [11]. Despite significant advances in anti-epileptic drug (AED) development, a substantial proportion of individuals with epilepsy (approximately 30%) remain refractory to existing pharmacological management; and continue to experience recurrent seizures despite optimal pharmacotherapeutic regimens [12]. Anti-epileptic drugs primarily target symptomatic seizure control by modulating neuronal excitability but do not address the underlying causes of epileptogenesis, nor do they offer neuroprotective or disease-modifying effects. Additionally, many AEDs are associated with adverse effects such as cognitive impairment, sedation, mood disturbances, and teratogenicity, which can significantly compromise quality of life and treatment adherence [13]. These limitations and complex neuropathological changes induced by status epilepticus underscore the urgent need to explore alternative therapeutic strategies, including natural remedies. One promising avenue lies in the investigation of medicinal plants with established ethnopharmacological use and potential neuroprotective properties. *Bryophyllum pinnatum* (commonly known as the "miracle leaf" or "life plant") is one such plant, traditionally used in African and Asian medicine for treating various ailments, including convulsions and inflammatory disorders [14].

Bryophyllum pinnatum belongs to the Crassulaceae family, it is a succulent medicinal plant widely used in traditional medicine across Africa, Asia, and South America. It has been employed in the management of several health conditions, including seizures, wounds, infections, hypertension, and kidney stones. The plant is rich in diverse bioactive compounds such as flavonoids (e.g. quercetin and kaempferol), alkaloids, tannins, saponins, terpenoids, and bufadienolides, which are believed to contribute to its pharmacological activities [15].

Experimental studies have reported that *Bryophyllum pinnatum* possesses notable anticonvulsant, anti-inflammatory, antioxidant, analgesic, and sedative properties [16,17]. In

seizure models, the plant extract has been shown to delay seizure onset and reduce severity, possibly by enhancing GABAergic neurotransmission and protecting against oxidative damage [18]. Its antioxidant activity is attributed to the enhancement of endogenous defense systems such as glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD), along with a reduction in lipid peroxidation levels [19]. Moreover, the plant demonstrates anti-inflammatory effects by downregulating pro-inflammatory cytokines such as TNF- α , IL-1 β , and IL-6, possibly through modulation of the NF- κ B and COX-2 pathways [19].

These properties are particularly relevant in the context of lithium-pilocarpine-induced status epilepticus, which is characterised by oxidative stress, neuroinflammation, and neuronal degeneration. The neuroprotective potential of *Bryophyllum pinnatum* in such a model could offer a promising alternative or adjunctive strategy to current antiepileptic drugs, many of which are limited by side effects and drug resistance. There is a clear research gap regarding the use of *Bryophyllum pinnatum* in lithium-pilocarpine model, and comprehensive preclinical evaluations are required to validate its efficacy and elucidate its mechanism of action in seizure-induced neuropathology.

2. Materials and Methodology

2.1 Chemicals and Drugs

Pilocarpine and Lithium Chloride were obtained from Sigma Aldrich, USA. The standard anti-epileptic drug (Sodium Valproate) was obtained from the Pharmacy unit of Babcock University Teaching Hospital, Ilishan Remo, Nigeria. Assay kits for MDA, TNF- α , Glutamate, Catalase, GABA, Acetylcholine were also gotten from Sigma Aldrich, USA.

2.2 Animals and Animal Welfare

Forty male Wistar rats, weighing between 120–150 g were procured from the Animal House Babcock University, Ilishan Remo, Ogun State, Nigeria. The rats were housed in well-aerated cages at room temperature environment under a 12-hour light/dark cycle. Animals had access to pelletized animal feed and clean water *ad libitum*.

2.3 Seizure

The method used by Olatunji et al. [20] was adopted for seizure induction. For seizure induction, the rats were intraperitoneally administered lithium chloride (127 mg/kg bw) first, followed by pilocarpine (30 mg/kg bw) after 24 hours.

2.4 Experimental Methods

Forty (40) male Wistar rats weighing between 120 - 150g was randomly assigned into 5 groups (A-E) of 8 animals each. All groups were fed standard diet throughout the experimental

period of 21 days. Group A, served as the control and was administered distilled water, group B received LiCl-P alone. Group C and D received LiCl-P and treated with BP at 250mg/kg bw and 500mg/kg bw respectively. Group E received LiCl-P and treated with sodium valproate at 200mg/kg bw. At the end of the administration period, on day 22, animals were exposed to behavioural paradigms (open-field and Y-maze). On day 23, animals were euthanised using di-ethyl ether, the brains were dissected; and the cerebral cortex excised, processed, sectioned at 5 μ m and stained using haematoxylin/eosin and Luxol fast blue. Homogenates of the cerebral cortex was used for the assessment of MDA, TNF- α , Glutamate, Acetylcholine, GABA, Catalase.

2.5 Behavioural Testing

Behavioural tests were conducted in the following sequence: Open field (line crossing, rearing, freezing frequency, centre square entries) and then the Y- maze. All neurobehavioural tests were recorded using a webcam camera connected through a cable from a laptop to the behavioural apparatus and later scored manually.

2.5.1 Open Field Test

The open field test was conducted according to the method originally described by Hall [21] to evaluate locomotor activity, anxiety-related behaviour, and exploratory tendencies in rats. The apparatus consisted of a square plywood arena (72 $\times$ 72 cm) with 36 cm high walls, painted white on the interior surfaces. The floor was marked with red lines to create 18 $\times$ 18 cm squares, with a centrally positioned green square (18 $\times$ 18 cm) sketched in the middle. A transparent Plexiglas sheet (72 $\times$ 72 cm) was placed over the floor surface. Each Wistar rat was placed individually in the centre of the arena and allowed to explore freely for 5 minutes. Between trials, the apparatus was cleaned with ethanol to eliminate olfactory cues from previous subjects. Behavioural parameters recorded included: (i) line crossing – number of times the animal crossed a grid line with all four paws; (ii) rearing – number of times the animal stood upright on its hind limbs; (iii) centre square entry – number of entries into the central square with all four paws (iv) freezing – number of episodes in which the animal remained completely immobile.

2.5.2 Y-Maze Test

The Y-maze test was employed to evaluate spatial working memory in rats, based on the innate tendency of rodents to explore a novel arm rather than return to a previously visited one. The apparatus consisted of three white-painted wooden arms (designated A, B, and C) arranged at 120° angles to each other. Each rat was placed at the centre of the maze and allowed to freely explore all three arms. An arm entry was recorded

when all four paws of the animal were positioned within an arm. The sequence and number of arm entries were recorded to calculate the percentage of spontaneous alternation, defined as successive entries into three different arms (a triad). Brain regions involved in this task include the hippocampus, septum, basal forebrain, and prefrontal cortex.

2.6 Sacrifice and Histological Analysis

At the end of the experiments, animals were sacrificed using diethyl-ether. The rat brains were carefully excised, blotted dry and weighed with AB204 Mettler Toledo weighing balance. The brains were fixed in 10% neutral buffered formalin by immersion. One-millimetre-thick coronal brain slices of the PFC were obtained and processed for routine paraffin embedding. Sections were stained with haematoxylin and eosin for general histoarchitecture demonstration of the PFC, and Cresyl fast Violet/Luxol fast Blue were used to show Nissl substance state and myelin sheath integrity.

2.7 Immunohistochemistry

Immunohistochemical analysis was carried out using a modified protocol based on Tascos *et al.* [22]. Serial sections (5 μ m) of the PFC were obtained from paraffin-embedded blocks, mounted on glass slides, and incubated on a hot plate at 70 °C for 1 h. The sections were deparaffinized in two changes of xylene, rehydrated through descending grades of ethanol, and rinsed in distilled water. Antigen retrieval was performed by heating the sections in 0.01 M citric acid buffer (pH 6.0) for 25 min, followed by gradual cooling under running tap water for at least 5 min. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 15 min, after which the sections were rinsed in phosphate-buffered saline (PBS). Non-specific binding was blocked using avidin for 15 min, followed by blocking of endogenous biotin with biotin solution for 15 min, with PBS washes between each step.

The sections were then incubated with the primary antibody (GFAP, 1:100 dilution) for the recommended duration, washed in PBS, and incubated with the secondary antibody (LINK) for 15 min. After another PBS wash, horseradish peroxidase (HRP) was applied for 15 min, followed by visualisation with 3,3'-diaminobenzidine (DAB) for 5 min. Excess DAB was removed with PBS washes, and the sections were counterstained with haematoxylin for 2 min, dehydrated in graded alcohols, cleared in xylene, and mounted with DPX. Images were captured using a Leica DM750 microscope equipped with a Leica ICC50 camera, and digital photomicrographs were archived for analysis.

2.8 Biochemical Tests

2.8.1 Evaluation of Lipid peroxidation

Lipid peroxidation levels were assayed using the malondialdehyde content which measures levels of

thiobarbituric acid reactive substance within biological samples. Coloured complexes are formed when free malondialdehyde combines with thiobarbituric acid reactive substance. These coloured complexes are then measured spectrophotometrically and expressed as μmol .

2.8.2 Tumour Necrosis Factor- α

Levels of Tumour necrosis factor- α were assessed using the enzyme-linked immunosorbent assay techniques with commercially available kits.

2.8.3 Determination of Catalase activity

Catalase activity was measured following the directions of the kit manufacturer.

2.8.4 Neurotransmitters

Prefrontal cortical slices were homogenised. The homogenates were then centrifuged and the supernatant were used to analyse the levels of acetylcholine, gamma aminobutyric acid (GABA), and glutamate with standard test kits.

2.9 Statistical Analysis

Data were analysed using Graph Pad Prism 6. Analysis of data was by One-way analysis of variance (ANOVA), and a post-hoc test (Tukey HSD) was used for comparisons. All results were expressed as mean $\pm$ S.E.M. and $p < 0.05$ was taken as the accepted level of significant difference from control.

3 Results

3.1 Effects of *Bryophyllum pinnatum* (BP) on horizontal locomotion

Figure 1 shows the effects of BP on open field horizontal locomotion measured as number of lines crossed. The frequency of line crossings varied significantly across experimental groups. The control group exhibited the highest locomotor activity, as reflected by a greater number of line crossings. In contrast, LiCl-P group showed a marked reduction in line crossings, indicating reduced locomotor activity. BP treated group had line crossings higher than the LiCl-P group but lower than the control group.

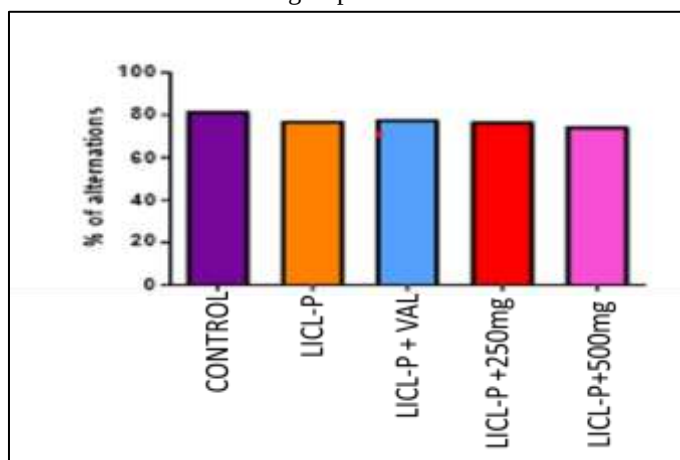


Figure 1: showing the frequency of the lines crossed by animals across all groups. Number of rats per treatment group =8. LiCl-P: Lithium

Chloride-Pilocarpine, BP: *Bryophyllum pinnatum*, LiCl-P-VAL: Lithium Chloride-Pilocarpine Sodium valproate

3.2 Effect of *Bryophyllum pinnatum* l on Rearing Behaviour

Figure 2 shows the effect of BP on open-field vertical locomotion measured as number of rears. The control group had the highest rearing frequency. The LiCl-P group had significantly fewer rearings, while BP treated groups showed increased rearing frequency compared to the LiCl-P group, suggesting improvement in exploratory and locomotor behaviour.

3.3 Effect of *Bryophyllum pinnatum* on Freezing Frequency

Figure 3 shows the effects of BP on open field test freezing frequency. The control group had the lowest scores. The LiCl-P group displayed a significant increase in freezing episodes, suggesting heightened anxiety-like behaviour or behavioural inhibition. Animals receiving BP treatment exhibited reduced freezing compared to the LiCl-P group, suggesting a potential anxiolytic effect.

3.4 Effect of *Bryophyllum pinnatum* on Centre Square Frequency

Figure 4 shows the effects of BP on open field test centre square frequency which is a measure inversely related to anxiety. Control group shows the highest frequency, reflecting low anxiety-like behaviour. The LiCl-P group showed a significant reduction in centre square entries, consistent with increased anxiety or reduced exploratory tendency. BP Treatment increased centre square entries compared to the LiCl-P group, suggesting an anxiolytic effect.

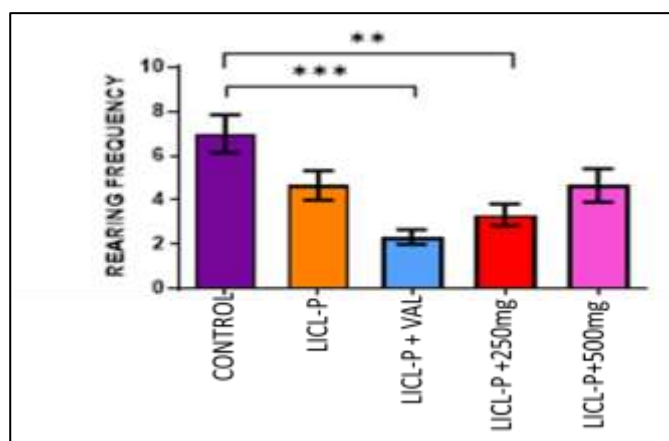


Figure 2: Effect of *Bryophyllum Pinnatum* on rearing frequency. Each bar represents Mean $\pm$ S.E.M, $p < 0.05$ vs. control, number of rats per treatment group =8. LiCl-P: Lithium Chloride-Pilocarpine, BP: *Bryophyllum Pinnatum*, LiCl-P-VAL: Lithium Chloride-Pilocarpine Sodium valproate

3.5 Effects of *Bryophyllum Pinnatum* on Y-maze Spatial

Working Memory

Figure 5 shows the effect of BP on Y maze spatial working memory measured as percentage alternation/5 minutes. Control group had the highest percentage indicating intact cognitive function. The LiCl-P group showed a marked reduction in alternation percentage, signifying impaired spatial memory. Animals treated with BP demonstrated higher alternation percentages than the LiCl-P group.

3.6 Effect of *Bryophyllum Pinnatum* on Prefrontal cortex histomorphology

Plates 1 (A–E), 2 (A–E), and 3(A–E) shows representative sections of the rat prefrontal cortex stained with haematoxylin and eosin (H&E), cresyl fast violet (CFV) and Luxol fast blue stains respectively. Examination of the H&E-stained sections from the control group (Plate 1A) revealed well-defined cortical layers, normal neuronal arrangement, intact pyramidal cells and no vacuolation or gliosis; while LiCl-P groups show neuronal loss, shrunken/pyknotic cells, vacuolation and disrupted architecture. The BP treated group showed some cell preservation, and reduced vacuolation. Examination of the CFV stained sections from the control group (Plate 2A) revealed strong, uniform staining, abundant Nissl bodies, while that of the LiCl-P group (2B) shows marked loss of Nissl substance (chromatolysis). In the BP treated group, the Nissl substance appears intact compared to the LiCl-P control. Examination of Luxol fast blue stained sections from the control group (Plate 3A) revealed dense, uniform myelin staining, while that from the LiCl-P group (2B) shows reduced myelin density, depicting demyelination. In the BP treated group, there is improvement in myelin density

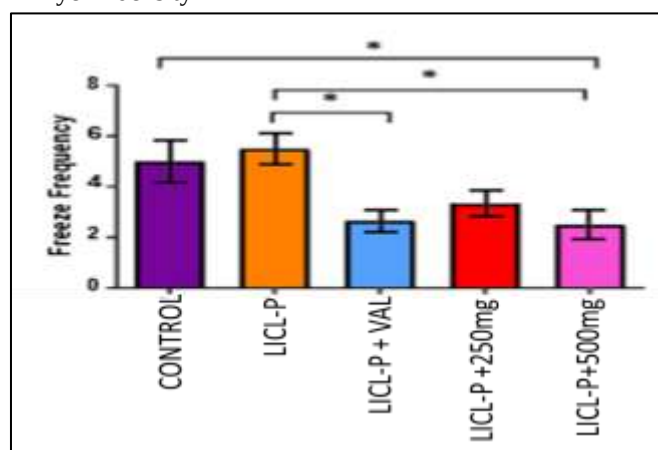


Figure 3: Effect of *Bryophyllum Pinnatum* on freezing frequency. Each bar represents Mean $\pm$ S.E.M, $p < 0.05$ vs. control, number of rats per treatment group =8. LiCl-P: Lithium Chloride-Pilocarpine, BP: *Bryophyllum Pinnatum*, LiCl-P-VAL: Lithium Chloride-Pilocarpine Sodium valproate

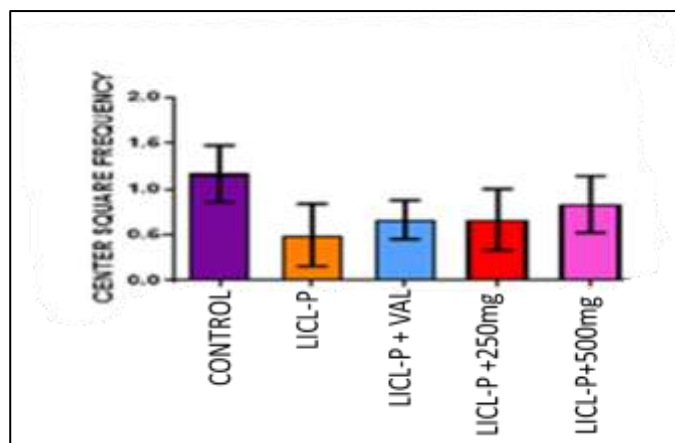


Figure 4: Effect of *Bryophyllum Pinnatum* on frequency of centre square entries. Each bar represents Mean $\pm$ S.E.M. Number of rats per treatment group=8. LiCl-P: Lithium Chloride-Pilocarpine, BP: *Bryophyllum Pinnatum*, LiCl-P-VAL: Lithium Chloride-Pilocarpine Sodium valproate

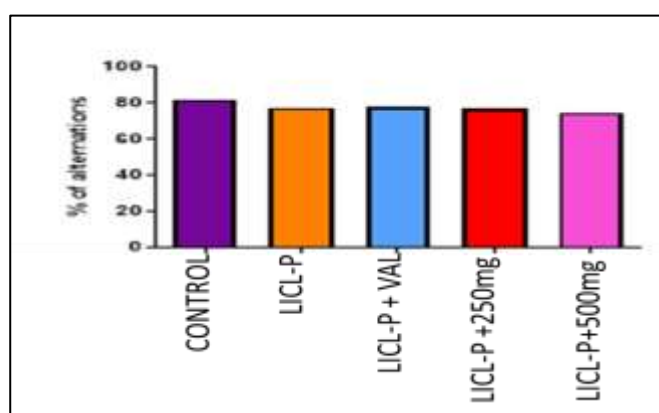


Figure 5: Effect of *Bryophyllum Pinnatum* on Y-maze spatial working memory. Each bar represents Mean $\pm$ S.E.M. Number of rats per treatment group =8. LiCl-P: Lithium Chloride-Pilocarpine, BP: *Bryophyllum Pinnatum*, LiCl-P-VAL: Lithium Chloride-Pilocarpine Sodium valproate

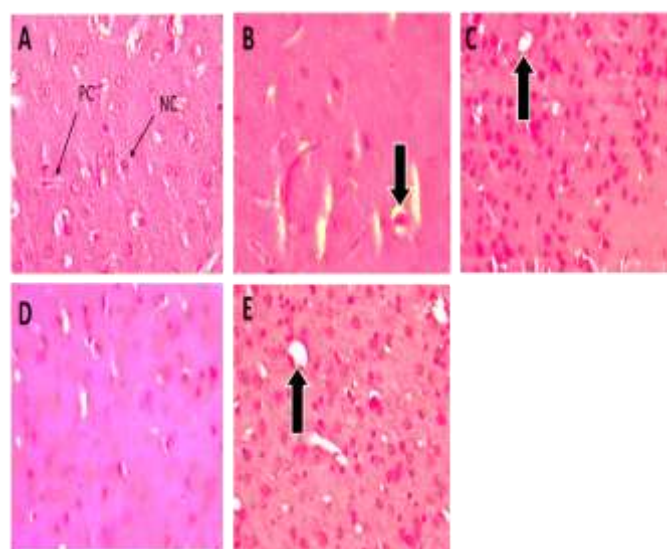


Plate 1: Effect of *Bryophyllum pinnatum* on pre-frontal cortex morphology of rats (haematoxylin and eosin stain) Photomicrographs

showed the presence of neuronal cells (NC), pyramidal cells (PC), and Vacuolated cells (Arrow head) Mag. X 400.

3.7 Effect of *Bryophyllum pinnatum* on Prefrontal cortex immunohistochemical assessment

Plates 4 (A–E), 5 (A–E), shows immunohistochemical sections of the rat pre-frontal cortex stained with GFAP and NeuN stains respectively. Examination of sections showed normal GFAP expression with fine, well-distributed astrocytic processes in the control group, while the LiCl-P group (4B) demonstrated marked upregulation of GFAP with intense cytoplasmic staining and hypertrophic astrocytic processes, indicating pronounced reactive astrogliosis. In BP treated groups, it showed moderate reduction of GFAP immunoreactivity compared to the LiCl-P group, with astrocytes appearing less hypertrophic and more closely resembling those in the control group. Plate 5 demonstrated NeuN expression with preserved neuronal distribution and morphology in the control group (5A); and intense nuclear staining indicating normal neuronal integrity. The LiCl-P group (5B) exhibited a marked reduction in NeuN-positive cells, particularly in the prefrontal cortex, consistent with neuronal loss and degeneration. The BP treated groups shows partially restored NeuN expression, with moderate neuronal preservation relative to the LiCl-P group.

3.8 Effects of *Bryophyllum pinnatum* on Neurotransmitter Levels

Figure 6 (A–C) shows the effects of BP on the glutamate level (6A), Acetylcholine (6B) and GABA (6C). Glutamate levels increased significantly in the LiCl-P only group compared with the normal control ($p < 0.05$), reflecting excitotoxic neuronal damage. Treatment with BP at both 250 mg/kg and 500 mg/kg significantly reduced glutamate levels in a dose-dependent manner, with the higher dose restoring values close to those of the normal control group. This indicates that BP mitigates glutamate excitotoxicity in the prefrontal cortex.

Acetylcholine levels (6B) declined significantly in the LiCl-P only group compared to the control group ($p < 0.01$). Administration of BP resulted in a significant and dose-dependent elevation in acetylcholine levels, with the higher dose showing near-restoration toward baseline. γ -aminobutyric acid (GABA) levels (6C) decreased significantly in the LiCl-P only group compared with normal controls ($p < 0.05$), indicating impaired inhibitory neurotransmission. Treatment with *Bryophyllum pinnatum* significantly increased GABA concentrations in a dose-dependent manner. The 500 mg/kg dose produced values that were comparable with those of the normal control group.

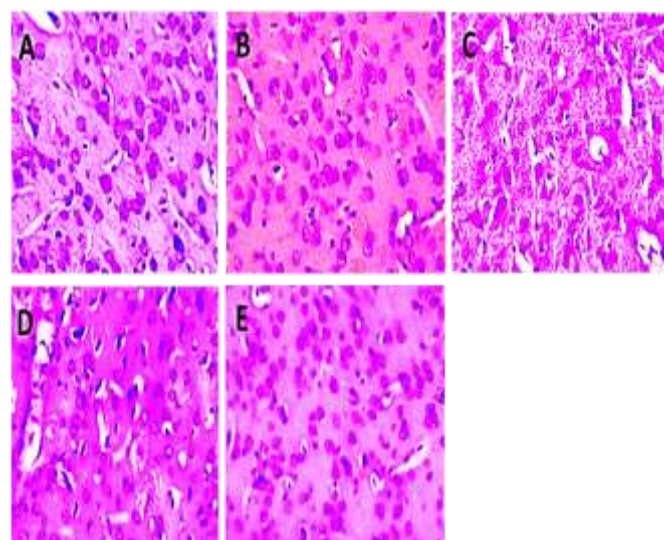


Plate 2: Effect of *Bryophyllum Pinnatum* on pre-frontal cortex morphology of rats (cresyl fast violet).

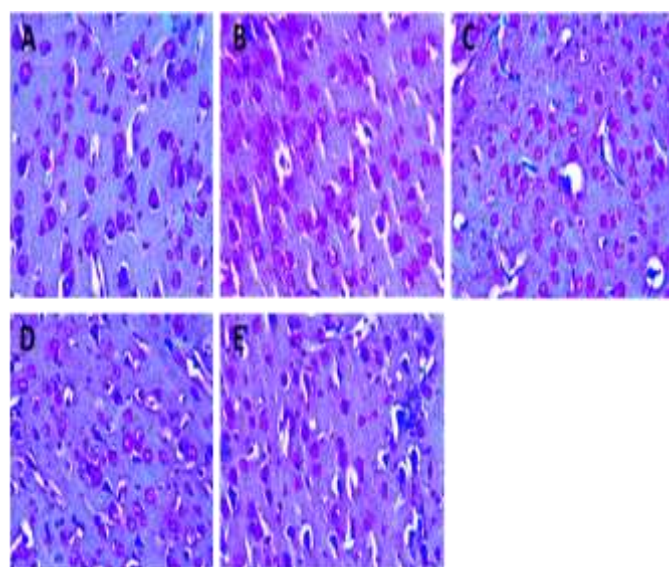


Plate 3: Effect of *Bryophyllum Pinnatum* on pre-frontal cortex morphology of rats (Luxol fast Blue).

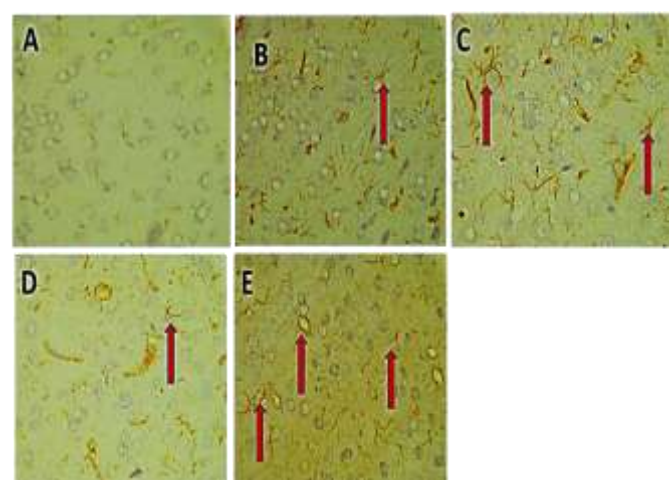


Plate 4: Effect of *Bryophyllum pinnatum* on prefrontal cortex morphology (GFAP)

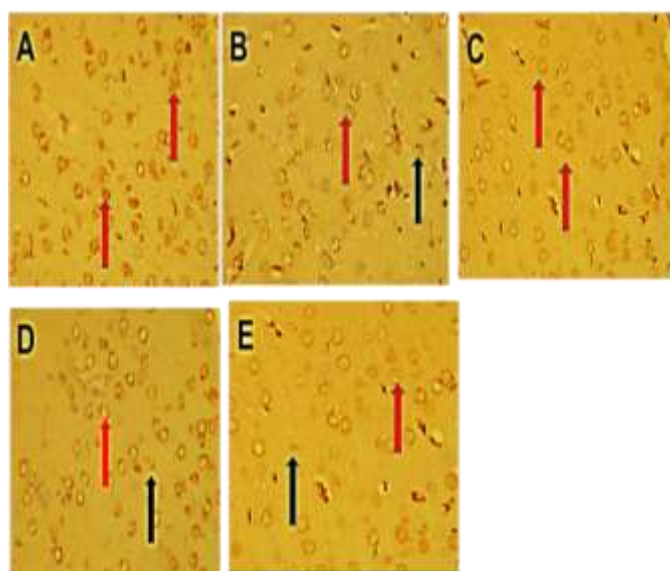


Plate 5: Effect of *Bryophyllum pinnatum* on prefrontal cortex morphology (NeuN)

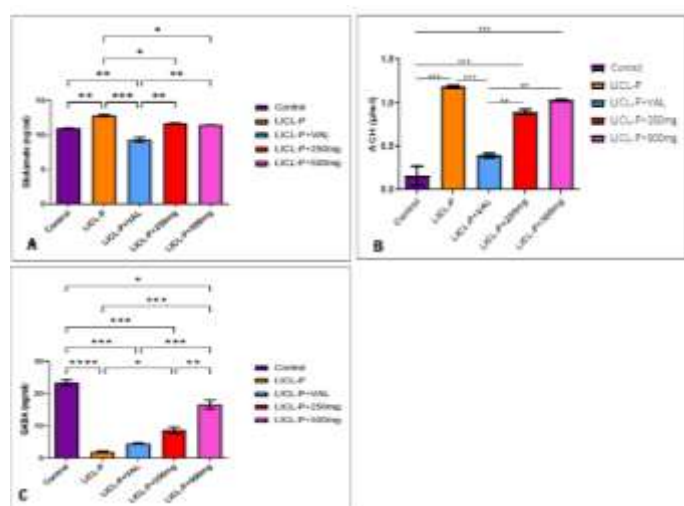


Figure 6 (A-C) shows the effects of BP on the glutamate level (6A), Acetylcholine (6B) and GABA (6C). Number of rats per treatment group =8. LiCl-P: Lithium Chloride-Pilocarpine, BP: *Bryophyllum Pinnatum*, LiCl-P-VAL: Lithium Chloride-Pilocarpine Sodium valproate

3.9 Effects of *Bryophyllum pinnatum* on Oxidative stress Markers and inflammatory cytokines

Figure 7 (A-C) shows the effects of BP on the Catalase activity (7A), Malondialdehyde (MDA) levels (7B) and Tumour necrosis factor (TNF)- α levels (7C). Catalase activity (6D) an important endogenous antioxidant enzyme, was significantly reduced in the LiCl-P only group compared to the normal control group ($p < 0.01$). Treatment with BP significantly elevated catalase activity in a dose-dependent fashion, suggesting enhancement of antioxidant defences. Malondialdehyde (7B), a biomarker of lipid peroxidation, was significantly elevated in the LiCl-P group relative to the normal control ($p < 0.05$). *Bryophyllum pinnatum* treatment significantly reduced MDA levels at both tested doses, with the higher dose showing a greater reduction.

Tumor necrosis factor-alpha (α), a pro-inflammatory cytokine, was markedly increased in the LiCl-P group compared to the normal group ($p < 0.01$), reflecting neuroinflammation associated with seizures. Treatment with BP significantly decreased TNF- α levels in a dose-dependent manner.

4 Discussion

The present study investigated the neuroprotective effects of *Bryophyllum pinnatum* (BP) on lithium-pilocarpine (LiCl-P)-induced prefrontal cortex alterations in a Wistar rat model of seizure disorder. Our results demonstrate that BP significantly ameliorated behavioural, biochemical, and histopathological impairments induced by LiCl-P, supporting its potential as a neuroprotective and anti-seizure agent.

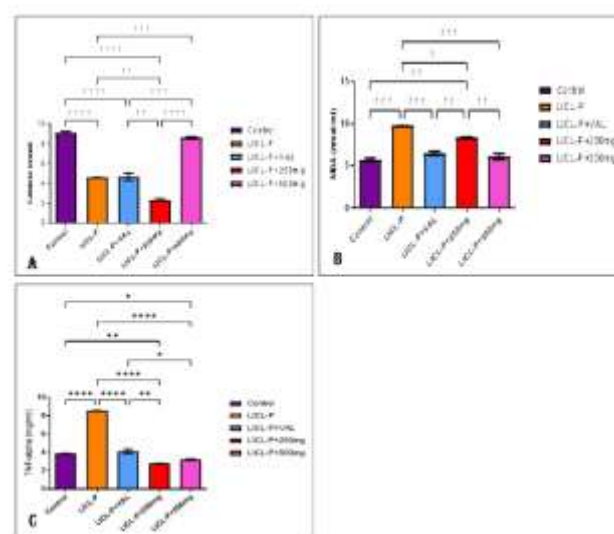


Figure 7 (A-C) shows the effects of BP on the Catalase activity (7A), Malondialdehyde levels (7B) and Tumour necrosis- α levels (7C). Number of rats per treatment group =8. LiCl-P: Lithium Chloride-Pilocarpine, BP: *Bryophyllum Pinnatum*, LiCl-P-VAL: Lithium Chloride-Pilocarpine Sodium valproate.

The open-field and Y-maze assessments revealed marked locomotor and cognitive deficits in LiCl-P-treated rats, as shown by reduced line crossings, rearing frequency, centre square entries, and percentage alternation, alongside increased freezing episodes. These findings align with earlier reports that pilocarpine-induced seizures impair exploratory behaviour, induce anxiety-like responses, and compromise working memory due to prefrontal cortical damage and hippocampal dysfunction [23–25]. Treatment with BP improved locomotor activity, exploratory behaviour, and memory performance in a dose-dependent manner, indicating that BP exerts anxiolytic and cognition-preserving effects, possibly through modulation of neurotransmitter systems and neuroinflammatory pathways.

LiCl-P administration significantly elevated glutamate while reducing GABA and acetylcholine levels, reflecting excitotoxicity, impaired inhibitory tone, and cholinergic dysfunction. These alterations are consistent with the

pathophysiological basis of epilepsy, characterised by an imbalance between excitatory and inhibitory neurotransmission [26]. *Bryophyllum pinnatum* treatment restored neurotransmitter levels toward baseline, suggesting that its phytoconstituents may enhance GABAergic signaling, mitigate glutamatergic hyperactivity, and support cholinergic function [27]. Similar modulatory effects have been reported in other seizure models treated with flavonoid- and alkaloid-rich extracts, underscoring the therapeutic relevance of *B. pinnatum* [28].

Oxidative stress and neuroinflammation play critical roles in seizure-induced neuropathology. In this study, LiCl-P significantly increased MDA and TNF- α levels, while reducing catalase activity, indicating lipid peroxidation, antioxidant depletion, and inflammatory activation [29]. These results are consistent with prior studies showing enhanced oxidative burden and cytokine upregulation in the pilocarpine model. *Bryophyllum pinnatum* treatment markedly attenuated MDA and TNF- α levels while restoring catalase activity, suggesting a strong antioxidative and anti-inflammatory effect. This protective action may be attributed to the plant's bioactive flavonoids (e.g., quercetin, kaempferol) and bufadienolides, which have documented antioxidant and NF- κ B inhibitory properties [27, 30].

Histological examination confirmed that LiCl-P induced neuronal loss, chromatolysis, vacuolation, and demyelination in the prefrontal cortex. Immunohistochemical analysis further revealed upregulation of GFAP (indicative of reactive astrogliosis) and reduced NeuN expression (reflecting neuronal degeneration). These findings are consistent with reports that seizures provoke glial activation and neuronal apoptosis in vulnerable brain regions [31, 32]. Treatment with BP preserved neuronal morphology, restored Nissl substance, enhanced myelin integrity, reduced GFAP overexpression, and partially restored NeuN immunoreactivity. These effects demonstrate that BP protects against seizure-induced neuronal and glial alterations, thereby preserving prefrontal cortical integrity.

The protective effects of BP, particularly at the 500 mg/kg dose, were comparable to sodium valproate, a standard antiepileptic drug. However, unlike conventional AEDs that mainly modulate ion channels and neurotransmission, BP exhibited multi-target actions, including antioxidant, anti-inflammatory, and neuroprotective mechanisms. This suggests that BP could serve as a potential adjunctive therapy, addressing both the symptomatic and neurodegenerative aspects of epilepsy.

Conclusion

This study demonstrates that *Bryophyllum pinnatum* confers significant neuroprotective effects against lithium pilocarpine

induced prefrontal cortex alterations in Wistar rats. The extract improved behavioural performance, restored neurotransmitter balance, reduced oxidative stress, suppressed neuroinflammation, and preserved neuronal integrity in a dose-dependent manner. Notably, the higher dose of *Bryophyllum pinnatum* (500 mg/kg) produced effects comparable to sodium valproate, underscoring its potential as a promising adjunct or alternative therapy for seizure-related neuropathology. These findings highlight the therapeutic relevance of *Bryophyllum pinnatum* as a multi-target agent capable of addressing both the symptomatic and neurodegenerative aspects of epilepsy. Further studies, particularly mechanistic and clinical investigations, are warranted to fully elucidate its efficacy, safety, and translational potential in the management of seizure disorders.

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Availability of data and materials

Data generated during and analysed during the course of this study are available from the corresponding author on request.

Declaration of ethical approval

Ethical approval (BUHREC069/19) for animal experimentation was obtained from the Babcock University Health Research Ethics Committee, guided by the guidelines for the care and use of laboratory animals.

Competing interests

None declared

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References

1. Anwar H, Khan QU, Nadeem N, Pervaiz I, Ali M, Cheema FF. Epileptic seizures. *Discoveries*. 2020;8(2):e110.
2. Wu Y, Liu D, Song Z. Neuronal networks and energy bursts in epilepsy. *Neuroscience*. 2015;287:175–86.
3. Fang M, Liu W, Tuo J, Liu M, Li F, Zhang L, Yu C, Xu Z. Advances in understanding the pathogenesis of post-traumatic epilepsy: a literature review. *Front Neurol*. 2023;14:1141434.
4. Lerche H, Shah M, Beck H, Noebels J, Johnston D, Vincent A. Ion channels in genetic and acquired forms of epilepsy. *J Physiol*. 2013 Feb;591(4):753–64.
5. Téllez-Zenteno JF, Hernández-Ronquillo L. A Review of the Epidemiology of Temporal Lobe Epilepsy. *Epilepsy Res Treat*. 2012 Dec 29;2012:1–5.
6. Jain S, Nirwan N, Agarwal NB, Vohora D. Methods for the Induction of Status Epilepticus and Temporal Lobe Epilepsy in Rodents: The Kainic Acid Model and the Pilocarpine Model. In: Vohora D, editor. *Experimental and Translational Methods to Screen Drugs Effective Against Seizures and Epilepsy* [Internet]. New York, NY: Springer US; 2021 [
7. Cavalheiro EA, Naffah-Mazzacoratti MG, Mello LE, Leite JP. The pilocarpine model of seizures. *Models Seizures Epilepsy*. 2006;433–48.
8. André V, Dubé C, François J, Leroy C, Rigoulot M, Roch C, Namer IJ, Nehlig A. Pathogenesis and Pharmacology of Epilepsy in the Lithium-pilocarpine Model. *Epilepsia*. 2007 Sep;48(s5):41–7.

9. Rusina E, Bernard C, Williamson A. The kainic acid models of temporal lobe epilepsy. *Eneuro* [Internet]. 2021;8(2). <https://www.eneuro.org/content/8/2/ENEURO.0337-20.202>
10. Sun H, Li X, Guo Q, Liu S. Research progress on oxidative stress regulating different types of neuronal death caused by epileptic seizures. *Neurol Sci*. 2022 Nov;43(11):6279–98.
11. Lingamaneni R, Hemmings Jr HC. Differential interaction of anaesthetics and antiepileptic drugs with neuronal Na⁺ channels, Ca²⁺ channels, and GABAA receptors. *Br J Anaesth*. 2003;90(2):199–211.
12. Riva A, Golda A, Balagura G, Amadori E, Vari MS, Piccolo G, Iacomino M, Lattanzi S, Salpietro V, Minetti C. New trends and most promising therapeutic strategies for epilepsy treatment. *Front Neurol*. 2021;12:753753.
13. Hirsch E, Schmitz B, Carreño M. Epilepsy, antiepileptic drugs (AEDs) and cognition: Epilepsy, AEDs and cognition. *Acta Neurol Scand*. 2003 Oct;108:23–32.
14. Kumar P, Malik S, Dubey KK. Bryophyllum Pinnatum: A Review on Medicinal Benefits and Potent Bioactive Molecules. *Curr Bioact Compd*. 2020 Oct 28;16(7):978–92.
15. Sharma G, Jangra A, Sihag S, Chaturvedi S, Yadav S, Chhokar V. Bryophyllum pinnatum (Lam.) Oken: unravelling therapeutic potential and navigating toxicity. *Physiol Mol Biol Plants*. 2024 Sep;30(9):1413–27.
16. Pal SK, Mohan P, Barua CC, Sarkar BK, Lahon LC. Analgesic, anti-inflammatory and local anesthetic activity of methanol extract of Bryophyllum pinnatum leaves. *J Entomol Zool Stud*. 2020;8(5):07–11.
17. Bakare LO, Abdullahi SM, Sadam M, Salaudeen AA, Ilyas MA. Evaluation of anticonvulsant properties of methanol aerial extract of Bryophyllum pinnatum (Lam.) Oken (Crassulaceae) in mice and chicks. *Niger J Pharm Res*. 2021;16(S):45–9.
18. Firdous SM, Mallik S, Paria B. Antioxidant Effects of Medicinal Plants for the Treatment of Epilepsy. In: Sindhu RK, Singh I, Arockia Babu M, editors. *Antioxidants* [Internet]. 1st ed. Wiley; 2025 <https://onlinelibrary.wiley.com/doi/10.1002/9781394270576.ch1>
19. Yemitan OK, Akinsuyi AO, Jewo PI, Oguntola JA, Olayemi SO. Toxicological and reversibility studies of Bryophyllum pinnatum Leaf extract on biochemical, peroxidation and histopathologic parameters in rodents. *J Complement Med Res* [Internet]. 2020;11(1).
20. Olatunji S.Y, Ogunnaike, P.O., Owolabi, J.O., Abijo, A.Z., Alabi, A.S., Adelodun, S.T., Olanrewaju, J.A. and Adelabi. A.S. (2021) Investigating the effects of allium sativum on the prefrontal cortex in lithium chloride pilocarpine-induced epilepsy in Wistar rat. *Nig. J. Neurosci*. 12(2):56-66. <http://doi.org/10.47081/njn2021.12.2/003>
21. Hall C. Drive and emotionality: factors associated with adjustment in the rat. *J Comp Psychol* 1934;27:89–108.
22. Seibenhener ML, Wooten MC. Use of the open field maze to measure locomotor and anxiety-like behavior in mice. *JoVE J Vis Exp*. 2015;(96):e52434.
23. Tascos NA, Parr J, Gonatas NK. Immunocytochemical study of the glial fibrillary acidic protein in human neoplasms of the central nervous system. *Hum Pathol*. 1982 May;13(5):454–8.
24. Pilocarpine-Induced Seizure - an overview | ScienceDirect Topics [Internet]. <https://www.sciencedirect.com/topics/medicine-and-dentistry/pilocarpine-induced-seizure>
25. Kohek SRB, Foresti ML, Blanco MM, Cavarsan CF, Silva CS da, Mello LE. Anxious profile influences behavioral and Immunohistological findings in the pilocarpine model of epilepsy. *Front Pharmacol*. 2021;12:640715.
26. Grötcke I, Hoffmann K, Löscher W. Behavioral alterations in the pilocarpine model of temporal lobe epilepsy in mice. *Exp Neurol*. 2007 Oct;207(2):329–49.
27. Barker-Haliski M, White HS. Glutamatergic mechanisms associated with seizures and epilepsy. *Cold Spring Harb Perspect Med*. 2015;5(8):a022863.
28. Waris A, Ullah A, Asim M, Ullah R, Rajdoula MdR, Bello ST, Alhumaydi FA. Phytotherapeutic options for the treatment of epilepsy: pharmacology, targets, and mechanism of action. *Front Pharmacol*. 2024 May 24;15:1403232.
29. Adelakin LA, Fabiya OS, Okonjo E, Olatunji SY, Olanrewaju JA, Owolabi JO. Kainic Acid Epilepsy Model in Wistar Rats: The Comparative Ameliorate Use of Bryophyllum pinnatum, Ketogenic Diet and Carbamazepine on the Frontal Lobe Cortex with Selected Behavioral Parameters. *J Sci Res Rep*. 2022 Apr 9;28–46.
30. Łukawski K, Czuczwar SJ. Oxidative Stress and Neurodegeneration in Animal Models of Seizures and Epilepsy. *Antioxidants*. 2023 May 5;12(5):1049.
31. Rabidas SS, Prakash C, Tyagi J, Suryavanshi J, Kumar P, Bhattacharya J, Sharma D. A Comprehensive Review on Anti-Inflammatory Response of Flavonoids in Experimentally-Induced Epileptic Seizures. *Brain Sci*. 2023 Jan 5;13(1):102.
32. Eraković V, Zupan G, Varljen J, Laginja J, Simonić A. Lithium plus pilocarpine induced status epilepticus--biochemical changes. *Neurosci Res*. 2000 Feb;36(2):157–66.
33. Fan J, Shan W, Yang H, Zhu F, Liu X, Wang Q. Neural Activities in Multiple Rat Brain Regions in Lithium-Pilocarpine-Induced Status Epilepticus Model. *Front Mol Neurosci* [Internet]. 2020 J;12://www.frontiersin.org/journals/molecular-neuroscience/articles/10.3389/fnmol.2019.00323/full

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