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Cannabidiol Modulates Hippocampal Neuronal Morphology and Attenuates Reactive Astrogliosis in Animal Model of Epilepsy

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ABSTRACT

Intractable form of epilepsy is a major challenge worldwide, and despite an increasing number of medical therapies in the treatment of epilepsy, about one in three patients continues to experience seizures. This study evaluated the effects of cannabidiol on neuronal morphology and astrocytes in the hippocampus of epileptic rats. Adult Wistar rats (150-180 g) were grouped into acute, latent, and chronic phases of epilepsy. Each group was further subdivided into five subgroups A-E of eight rats each. Subgroups A, the control received corresponding vehicle volumes for the entire phases of epileptogenesis. Epilepsy was induced in subgroups B, C, D, and E by intraperitoneal administration of lithium chloride (127 mg/kg) 24 hours before pilocarpine (30 mg/kg). Seizures were allowed to last for 45 minutes and then terminated by diazepam (10 mg/kg). Subgroups C and D received 5 mg/kg and 10 mg/kg of cannabidiol respectively orally daily while subgroups E received 10mg/kg of sodium valproate. At the end of the three phases, the rats were sacrificed and their hippocampi were processed for histological and immunohistochemical assessment. Data were analysed using Analysis of Variance followed by the Newman-Keuls test for multiple comparisons. The Cornu Ammonis 1 and dentate gyrus across all phases of epilepsy showed loss of pyramidal neurons, dispersed granule cells, and glial fibrillary acidic protein expression in subgroups B. The expression was remediated by cannabidiol. The increase in degenerating neurons in Subgroup B was significantly reversed by cannabidiol across the three phases. The study concluded that cannabidiol ameliorated chemically induced epilepsy.

Keywords: cannabidiol, epilepsy, hippocampus, pyramidal neurons, astrocytes

INTRODUCTION

The complexity of the human brain and how it functions in the control of all the activities of the body make the body vulnerable to cases of brain injury, infections, or any damaging assault on the brain. The brain comprises billions of nerve cells called neurons which are also supported by glial cells of various types[1]. Neurons generate electrical impulses and messages to produce thoughts, feelings, and movement. Whenever the normal pattern of these impulses is disrupted, it leads to seizures[2]. Epilepsy is a brain disorder that is characterized by recurrent seizures[3]. Epilepsy can harm the brain, especially during development, and may be associated with cognitive impairment, neurobehavioral deficit, and psychiatric comorbidities that can combine to impair the quality of life severely [4,5]. Epilepsy affects people in every country of the world; it affects about 1% of the world's population with 80% living in developing countries [6].

Intractable form of epilepsy continues to be a major challenge worldwide, and despite an increasing number of medical therapies in the treatment of epilepsy, approximately one in three patients continue to have seizures[7]. *Cannabis sativa,* commonly called Indian hemp or marijuana and is popularly known in Nigeria as *kaya, wee-wee, igbo, oja, gbana, blau, kpoli* and *abana*[8] has been used for recreational and medicinal purposes[9]. This plant has been used to treat diseases since ancient times [9-11] and it has been reported to contain several phytocannabinoids among

which are tetrahydrocannabinol, cannabidiol, cannabigerol, tetrahydrocannabivarin (THCV). Tetrahydrocannabinol and cannabidiol are the major constituents of C*annabis sativa*[10,11]. Tetrahydrocannabinol is the major psychoactive ingredient and cannabidiol is the major non-psychoactive ingredient in cannabis. Both cannabis and tetrahydrocannabinol have been reported to be anticonvulsants with addictive tendencies in most animal models, and they can also be proconvulsants in some healthy animals [11].

Cannabidiol, one of the many constituents of the *Cannabis sativa* or marijuana plant, is receiving *a* soaring interest in the treatment of epilepsy, yet research on cannabidiol remains in infancy, mostly due to marijuana's status as a Schedule One drug in the United States, which limits the scope of medical research on it[7]. However, the lack of pure pharmacologically active compounds and legal restrictions have prevented clinical research and confined data on efficacy and safety to anecdotal reports[3]. Pure cannabidiol appears to be a candidate among phytocannabinoids to evaluate in patients with treatment-resistant epilepsy [12]. Cannabidiol's lack of tetrahydrocannabinol and the risks that are associated with addiction which are often found with the use of marijuana at a young age and the efficacy of cannabidiol in preclinical studies suggest that it could be a safe and effective drug for humans with epilepsy [13,14]. The hippocampus is a major part of the brain that is located in the medial temporal lobe, beneath the cortical surface. It plays important roles in behavioural, emotional, and memory processes (long-term memory and spatial navigation) [15]. It is composed of two regions: the dentate gyrus (DG) and the *Cornu Ammonis* (CA); later is subdivided into CA1, CA2, CA3, and CA4. Each of these regions is made up of the main cell layer which is the principal cell layer. The nerve cells of the principal cell layer of the DG are the granule cells and the nerve cells of the principal cell layer of the CA regions are the pyramidal cells. The granule and pyramidal cells are excitatory and inhibitory [16].

The hippocampus is often the site of epileptic seizures and hippocampal sclerosis is one of the most commonly visible types of tissue damage in temporal lobe epilepsy [17]. It is not clear, however, whether epilepsy is usually caused by hippocampal abnormalities or whether the hippocampus is damaged by the cumulative effects of seizures [18]. In experimental settings where repetitive seizures are artificially induced in animals, hippocampal damage is a frequent result, this may be because the hippocampus is one of very few brain regions where new neurons continue to be created throughout life [19].

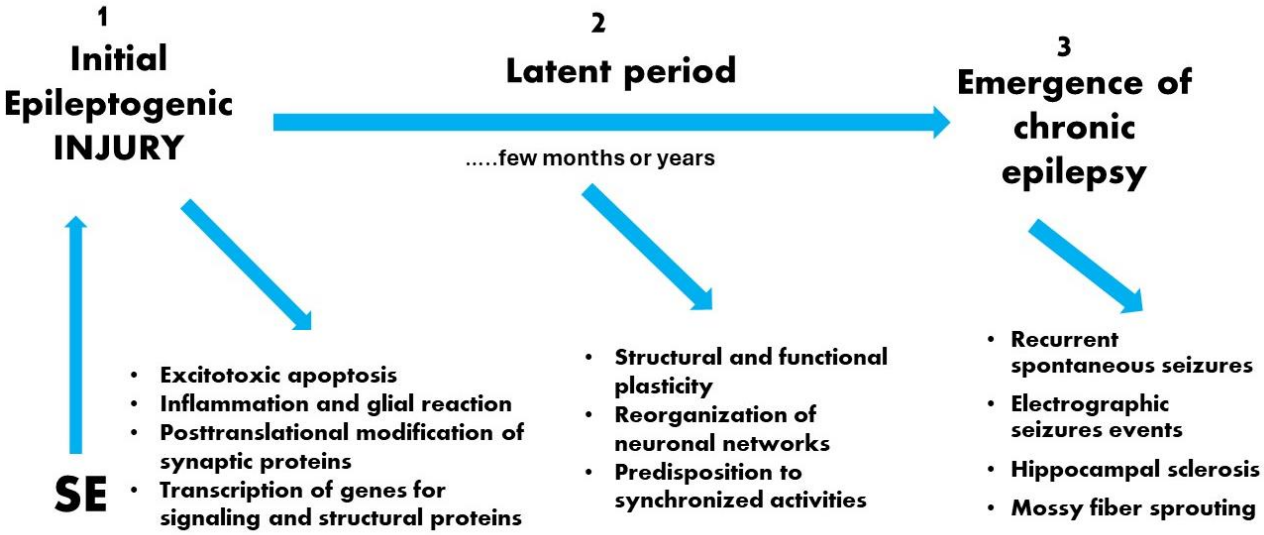

Figure 1: Diagrammatic profile of temporal lobe epilepsy progression [20]

The process of developing epilepsy, known as epileptogenesis, is complex and involves a combination of immediate and delayed events at the anatomical, molecular, and physiological levels. When a prolonged seizure causes damage to nerve cells, various signals are triggered, including inflammation, oxidative stress, cell death, new nerve cell formation, and changes in the connections between nerve cells [20]. These events gradually lead to both structural and functional alterations in the affected neurons. Over time, this ongoing process results in the reorganization of neural circuits, new nerve cell growth, and increased excitability, creating a hyperexcitability state that may persist for weeks, months, or even years (referred to as the latent period). Ultimately, these changes can culminate in the

occurrence of spontaneous recurrent seizures, marking the onset of epilepsy in susceptible individuals. Despite its significance, the precise understanding of epileptogenesis remains limited. The term "epileptogenesis" is used to describe the intricate changes in the brain that occur after an initial injury or insult, transforming a normal brain into one prone to recurring seizures [21]. The present study investigated the effects of cannabidiol on the neurons and astrocytes of the hippocampus of lithium chloride pilocarpine-induced epilepsy, especially the three phases of epilepsy in animal models.

MATERIALS AND METHODS

Animal care and management

One hundred and twenty (120) adult male Wistar rats (weighing between 150-180 g) were used for this study. Ethical approval was sought and obtained from the Health Research and Ethics Committee of the University with the ethical number: HREC NO: IPHOAU/12/1303. Rats were housed in clean plastic cages in a clean environment at room temperature. Rats in all groups were fed with standard laboratory rat feed and allowed access to water *ad libitum.* The rats were assigned into 3 groups 1, 2, 3 (Acute phase, Latent phase and Chronic phase of epilepsy) and each group was further subdivided into 5 groups (as shown in table 2) of 8 rats (group A, B, C, D, and E).

Animal treatments

Group A was the control and received a single dose of 0.5 ml of normal saline intraperitoneally and then 0.5 ml of 40% olive oil orally throughout administration (40% olive oil was used to dissolve cannabidiol). Epilepsy was induced in groups B, C, D, and E rats with pilocarpine (30 mg/kg, i.p.; Sigma-Aldrich Co. St Louis, USA) 24 hours after lithium chloride administration (127 mg/kg, i.p; Sigma-Aldrich Co., St. Louis, USA). Seizures were allowed to last for 45 minutes and then were terminated by the administration of diazepam (10 mg/kg, i.m.) to reduce rate of animal death. Only rats that displayed status epilepticus (stages 3– 5) were selected.[22] After the first seizure was achieved in all the groups, rats in groups C and D were administered orally 5 mg/kg and 10 mg/kg of cannabidiol respectively while group E was administered 10 mg/kg of sodium valproate a standard antiepileptic drug, and group B was left untreated. Administration of cannabidiol for the acute phase of epilepsy started on the second-day post-induction of status epilepticus and ended on the fourth day (2 days). Administration of cannabidiol for the latent phase of epilepsy started on the second-day post-induction of status epilepticus and ended on the 16th day (14 days). Administration of cannabidiol for the chronic phase of epilepsy started on the second-day post-induction of status epilepticus and ended on the 42nd day (40 days). All rats were handled following the guidelines for animal research as detailed in the guidelines for the care and use of laboratory animals. Lithium chloride was obtained from Sigma-Aldrich Co., St. Louis, USA, Pilocarpine hydrochloride was obtained from Sigma-Aldrich Co., St. Louis, USA, Diazepam (Made for F. Hoffmann-La Roche Ltd, Basel, Switzerland), Cannabidiol was obtained from Sigma-Aldrich Co., St. Louis, USA.

Table 1: Experimental design

GROUP 1: Acute Phase Day 2 to 4 post-induction of status epilepticus	**GROUP 2: Latent Phase** Day 2 to 16 post-induction of status epilepticus	**GROUP 3: Chronic Phase** Day 2 to 42 post-induction of status epilepticus
Group A: Control (Normal saline intraperitoneally)	Group A: Control (Normal saline intraperitoneally)	Group A: Control (Normal saline intraperitoneally)
Group B: Epilepsy was induced with 127 mg/kg LiCl (24 hrs before) 30 mg/kg Pilocarpine	Group B: Epilepsy was induced with 127 mg/kg LiCl (24hrs before) 30 mg/kg Pilocarpine	Group B: Epilepsy was induced with 127 mg/kg LiCl (24hrs before) 30mg/kg Pilocarpine Group
Group C: Epilepsy was induced with LiCl Pilocarpine and 5 mg/kg of cannabidiol	Group C: Epilepsy was induced with LiCl Pilocarpine and 5 mg/kg of cannabidiol	C: Epilepsy was induced with LiCl Pilocarpine and 5 mg/kg of cannabidiol
Group D: Epilepsy was induced with LiCl Pilocarpine and 10 mg/kg of cannabidiol	Group D: Epilepsy was induced with LiCl Pilocarpine and 10 mg/kg of cannabidiol	Group D: Epilepsy was induced with LiCl Pilocarpine and 10 mg/kg of cannabidiol
Group E: Epilepsy was induced with LiCl Pilocarpine and 10 mg/kg of sodium valproate	Group E: Epilepsy was induced with LiCl Pilocarpine and 10 mg/kg of sodium valproate	Group E: Epilepsy was induced with LiCl Pilocarpine and 10 mg/kg of sodium valproate

Racine's scale

Classification of seizure severity were confirmed according to the modified Racine's scale [22]: (1) mouth and facial movements, (2) head nodding, (3) forelimb clonus, (4) rearing with forelimb clonus, (5) rearing and falling.

Video recording

An observational system utilizing closed-circuit television cameras (CCTVs) or a webcam fitted into a laptop was used to monitor the behaviour of the animals after lithium chloride pilocarpine and cannabidiol administration. Input from CCTV/ webcam was managed on a PC and recorded by VCL software. Video of seizure behaviour was scored offline according to modified seizure severity scales (Racine's scale). The observation was done during the 12-hour day cycle to avoid disruption in the natural day/night cycle of the experimental animals.

Animal sacrifice

The rats (n=4) in each group were sacrificed by cervical dislocation, and the brains and two hippocampi were excised, weighed, and recorded. The remaining rats (n=4) in each group for histological and immunohistochemistry were anesthetized with diether and perfused intra-cardially with 100 mL phosphate buffered saline (PBS, 0.1 M pH 7.4), followed by 250 mL of neutral buffered formalin. The brains were excised and fixed in 10% neutral buffered formalin. One-millimeter-thick coronal brain slice was obtained at the level of the optic chiasma and processed via the paraffin embedding method.

Determination of brain weight and hippocampal weight

Brain weights were taken on a Pocket Digital Weighing Scale 5 g/0.01g ACL-PDS002 immediately after sacrifice. Relative brain weight was defined as the percentage ratio of brain weight to final body weight.

$$\% \text{ Relative brain weight} = \frac{\text{Brain weight (g)}}{\text{Final body weight (g)}} \times 100\%$$

Histological Studies

Following fixation, tissues were processed for rapid routine tissue processing and were stained for Haematoxylin and Eosin (H&E) for general histological appearance.

Immunohistochemical studies

Sections of the hippocampus were immunohistochemically stained for Neuronal nuclear protein (NeuN) a specific marker for neuron glial fibrillary acidic proteins (GFAP) to determine neuronal degeneration and demonstrations of astrocytic reactions respectively. Immunohistochemical studies for GFAP and Neuronal nuclear proteins (NeuN) were performed using the Novocastra™ NovoLink™ Polymer Detection System (Leica Biosystems, UK) and appropriate primary monoclonal antibodies.

The products were used in an immunohistochemical (IHC) procedure, and they allow for the qualitative identification by light microscopy of antigens in sections of formalin-fixed, paraffin-embedded tissue, via sequential steps with interposed washing steps. Endogenous peroxidase activity is neutralized using the Novocastra™ Peroxidase Block. This is followed by the application of the Novocastra™ Protein Block to reduce the non-specific binding of primary and polymer. The section is subsequently incubated with optimally diluted appropriate primary antibody. Novocastra™ Post Primary Block is used to improve penetration of the subsequent polymer reagent. Sections are further incubated with the substrate/chromogen, 3,3' - diaminobenzidine (DAB), prepared from Novocastra™ DAB Chromogen and NovoLink™ DAB Substrate Buffer (Polymer). Reaction with the peroxidase produces a visible brown precipitate at the antigen site.

All antibodies were Novocastra™ Liquid Mouse Monoclonal Antibodies (Leica Biosystems, UK) for the appropriate antigen (GFAP and NeuN) to be demonstrated.

Note: All antibodies used were diluted by 1/50 dilution using standard antibody diluent. Also, the procedure was carried out in a humidity chamber.

Photomicrography and image analysis

Stained sections were viewed with a Leica DM750 Digital Light microscope and digital photomicrographs were taken by an attached Leica ICC50 camera. Image Analysis and Processing for Java (Image J), public domain software sponsored by the National Institute of Health (USA), was used to analyze and quantify photomicrographs. Image J cell counter tool was used for the neuronal count to identify and quantify the number of intact and degenerating neurons in H&E stained sections. Photomicrographs of the stained sections were used for image analysis. For the evaluation of the neuronal count, a grid was superimposed on the photomicrograph with an area per point of 4000 μm^2.

To quantify neuronal degeneration, the following morphological criteria were considered: cell swelling and lysis, pyknosis, eosinophilic neurons, and vacuolated neurons.

Furthermore, immunohistochemically stained sections were also analysed for GFAP and Neuronal nuclear protein reactivity using the immunoratio plugin (as shown in Figure 2).

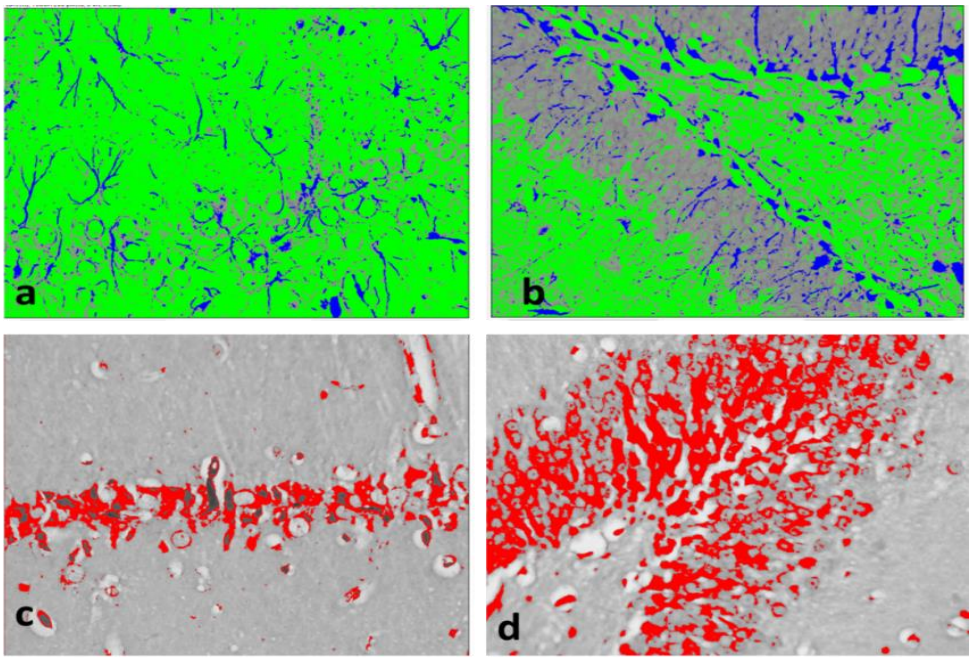

Figure 2: Immunoreactivity analysis of Immunohistochemical staining: a= GFAP reactivity in the CA1, b= GFAP reactivity in the dentate gyrus, c= Neuronal nuclear protein (NeuN) reactivity in the CA1 and d= Neuronal nuclear protein (NeuN) reactivity in the dentate gyrus of the Hippocampus

Statistical analysis

Data obtained from brain weight, hippocampal weight, and image analysis of photomicrograph and immunohistochemical studies of tissues were expressed as Mean ± Standard Error of Mean (S.E.M). Statistical significance was evaluated by one-way analysis of variance (ANOVA) using GraphPad Prism6 (GraphPad Inc. San Diego US). This was followed by Student Newan-Keuls (SNK) test for multiple comparisons. A value of $p < 0.05$ was considered to indicate significant difference between groups.

RESULTS

Brain weight during the acute, latent, and chronic phases of epilepsy

The results (Table 2) of the brain weight of the Wistar rats during the acute, latent, and chronic phases of epilepsy. In the acute phase, no significant difference was observed when the treated groups were compared with the control. This applies to the latent and chronic phases as well.

Table 2: Absolute Brain weight (g) during the acute, latent, and chronic phases of epilepsy.

Brain Weight	Acute Phase	Latent Phase	Chronic Phase
CONTROL	1.48±0.03	1.67±0.03	1.54±0.03
EPY ONLY	1.52±0.07	1.66±0.03	1.63±0.03
EPY+ LD of CBD	1.52±0.03	1.67±0.03	1.59±0.03
EPY+ HD of CBD	1.48±0.03	1.61±0.03	1.55±0.03
EPY+ SV	1.54±0.04	1.65±0.03	1.57±0.03

Key: Control, Epilepsy only (EPY ONLY), epilepsy + low dose of cannabidiol (EPY+LD of CBD), epilepsy + High dose of cannabidiol (EPY+HD of CBD), and epilepsy + sodium valproate (EPY+SV).

Relative brain weight during the acute, latent, and chronic phases of epilepsy

The results (Table 3) of the relative brain weight of the Wistar rats during the acute, latent, and chronic phases of epilepsy. There was no significant difference when the treated groups were compared with the control in the acute, latent, and chronic phases of epilepsy across all the groups.

Table 3: Relative brain weight (g) during the acute, latent, and chronic phases of epilepsy

Relative Brain Weight	Acute Phase	Latent Phase	Chronic Phase
CONTROL	1.18 ±0.11	0.89 ±0.04	0.89 ±0.04
EPY ONLY	1.30 ±0.09	0.88 ±0.03	0.83 ± 0.02
EPY+ LD of CBD	1.32±0.03	0.93±0.05	0.83 ±0.06
EPY+ HD of CBD	1.20 ±0.03	0.91 ±0.03	0.85 ±0.04
EPY+ SV	1.17 ±0.06	0.96 ±0.03	0.77 ±0.03

Key: A= Control, B= Epilepsy only (EPY ONLY), C= epilepsy + low dose of cannabidiol (EPY+LD of CBD), D= epilepsy + High dose of cannabidiol (EPY+HD of CBD) and E= epilepsy + sodium valproate (EPY+SV).

Hippocampal weight during the acute, latent, and chronic phases of epilepsy

The results (Table 4) of the hippocampal weight of the Wistar rats during the acute, latent, and chronic phases of epilepsy. There was no significant difference when the treated groups were compared with the control in the acute, latent, and chronic phases of epilepsy across all the groups. However, there was an observable increase in the control of the acute, latent, and chronic phases when compared with the treated groups across the three phases of the experiment.

Table 4: (Mean ± SEM) Hippocampal weight (g) during the acute, latent, and chronic phases of epilepsy.

Hippocampal Weight	Acute Phase	Latent Phase	Chronic Phase
CONTROL	0.23±0.09	0.21±0.04	0.27±0.03
EPY ONLY	0.18 ±0.02	0.14±0.01	0.15±0.01
EPY+ LD of CBD	0.19 ±0.02	0.12±0.02	0.15±0.01
EPY+ HD of CBD	0.15±0.01	0.11±0.02	0.14±0.02
EPY+ SV	0.18±0.01	0.11±0.01	0.18±0.03

Key: Control, Epilepsy only (EPY ONLY), epilepsy + low dose of cannabidiol (EPY+LD of CBD), epilepsy + High dose of cannabidiol (EPY+HD of CBD), and epilepsy + sodium valproate (EPY+SV).

Photomicrographs of H&E and immunohistochemistry of the hippocampus

Photomicrographs (Figure 3) of the general histoarchitecture of the hippocampus at low magnification (x 40) during the acute, latent, and chronic phases of epilepsy. The photomicrographs revealed all the layers of the hippocampus including the CA1, CA2, CA3, CA4, and the dentate gyrus (DG). The red arrow represents the dentate gyrus, the green arrow represents CA1 and the black arrow represents the CA4 region. General histoarchitecture (Figure 4) of the *Cornu Ammonis* 1 (CA1) region at 400 magnifications during the acute, latent, and chronic phases of epilepsy. This figure reveals a distinct tight arrangement of the CA1 in Figure 4:(1A, 2A, and 3A). When Figure 4: (1B, 2B, 3B) was compared with the control, it revealed dispersed pyramidal cells and this pattern of dispersion was more prominent in Figure 4(3B) which is the epilepsy-only group at the chronic phase of epilepsy. Figures 4 (1C, 2C, 3C) and 4 (1D, 2D, 3D) showed an improved pattern in the arrangement of the CA1 pyramidal cells compared to Figure 4 (1B, 2B, 3B)

Photomicrographs (Figure 5) of the CA1 of the hippocampus at 1000 magnification during the acute, latent, and chronic phases of epilepsy. The red arrow represents intact pyramidal neurons; the black arrow represents degenerating neurons (pyknotic neurons) and the green arrow represents vacuolated neurons. Figure 5 (1A, 2A,3A) which was the control group showed intact pyramidal neurons. When Figure 5(1B, 2B, 3B) were compared with Figure 5 (1A, 2A, 3A), they revealed several vacuolated, pyknotic, and neuronal loss and degenerating pyramidal neurons. The degenerating features observed in group B appeared to be most severe in Figure 5 (3B) which is the epilepsy-only group in the chronic phase. Examination of groups C, D, and E (the groups that received lithium chloride pilocarpine before the administration of low and high doses of cannabidiol revealed mild disruption and improved pyramidal neurons.

Photomicrographs (Figure 6) of the dentate gyrus of the hippocampus at the acute, latent, and chronic phases of epilepsy. Figure 6 (1A, 2A, 3A) showed intact granule cells of the dentate gyrus. Figure 6 (1B, 2B, 3B) revealed features of granule cell dispersion. The rate of the dispersion of the granules cells of the hippocampus was more extensive in the chronic phase Figure 6 (3B) of epilepsy when compared with the acute, Figure 6 (1B), and the latent, Figure 6 (2B) phases of epilepsy.

Photomicrograph (Figure 7) of the expression of GFAP in the CA1 of the hippocampus during the acute, latent, and chronic phases of epilepsy. GFAP was more expressed in the epilepsy only group, Figure 7 (1B, 2B, 3B) across the three phases of epilepsy and the expression appeared mild in Figure 7 (1C, 2C, 3C) and Figure 7 (1D, 2D, 3D) especially the low dose of CBD and epilepsy. Group B (epilepsy-only group) showed an increase in the size of astrocytes as well as astrocytic numbers.

Photomicrograph (Figure 8) of the expression of Neuronal nuclear antigen (NeuN) in the CA1 of the hippocampus during the acute, latent, and chronic phases of epilepsy. NeuN was more expressed in the control group and the rest of the treated groups compared to the epilepsy only group across the three phases of epilepsy.

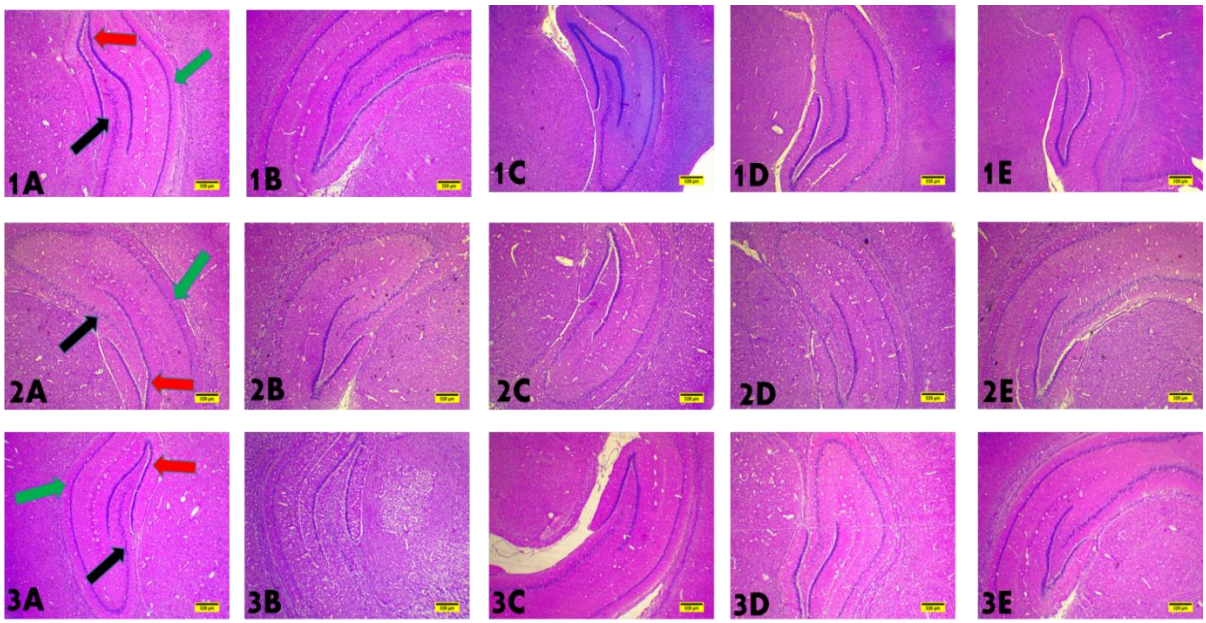

Figure 3: Photomicrographs of the hippocampus showing the general histoarchitecture of the hippocampus. This figure represents the Acute (1), Latent (2) and Chronic (3) Phases of epilepsy (A= Control, B= EPY ONLY, C= EPY +LD CBD, D= EPY+ HD CBD, E= EPY+ SV), Red arrow= dentate gyrus, green arrow= CA1 and the black arrow = CA4 (H&E x40) Scale bar = 328 µm.

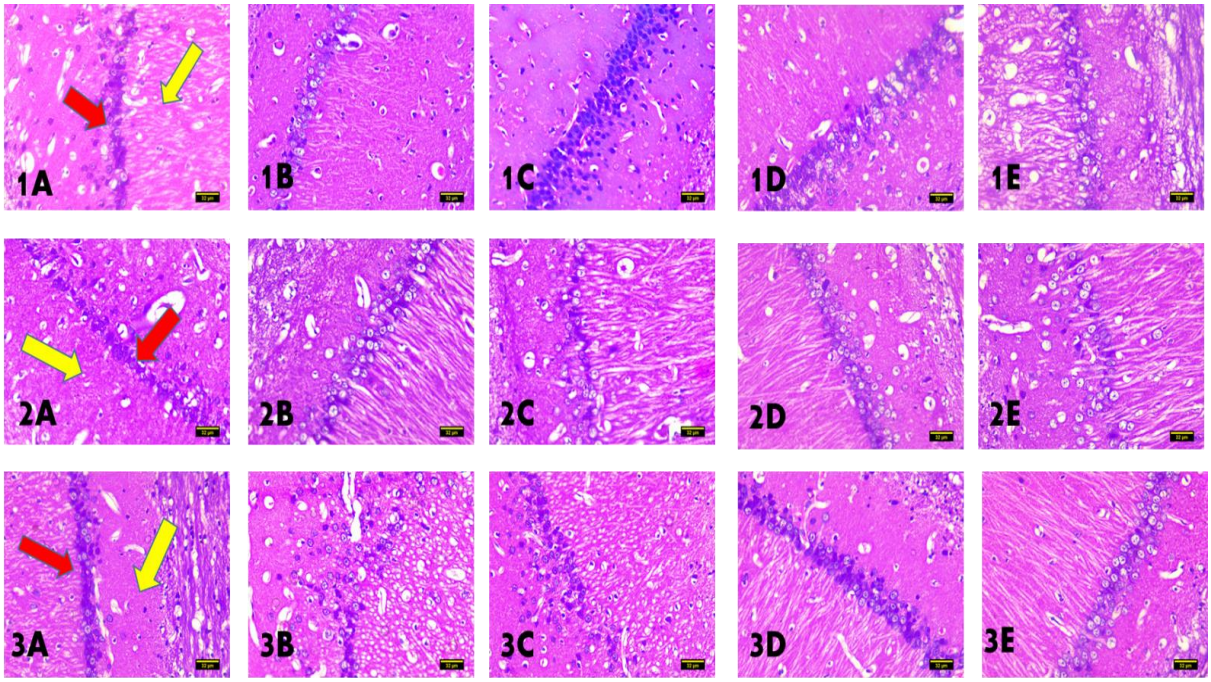

Figure 4: Photomicrographs of the CA 1 of the hippocampus showing the general histoarchitecture of the *Cornu Ammonis* 1 region. This figure represented the Acute (1), Latent (2) and Chronic (3) Phases of epilepsy (A= Control, B= EPY ONLY, C= EPY +LD CBD, D= EPY+ HD CBD, E= EPY+ SV. (H&E x400) Scale bar = 32 µm. Red arrow= pyramidal neuron pattern, yellow arrow= Neuropil.

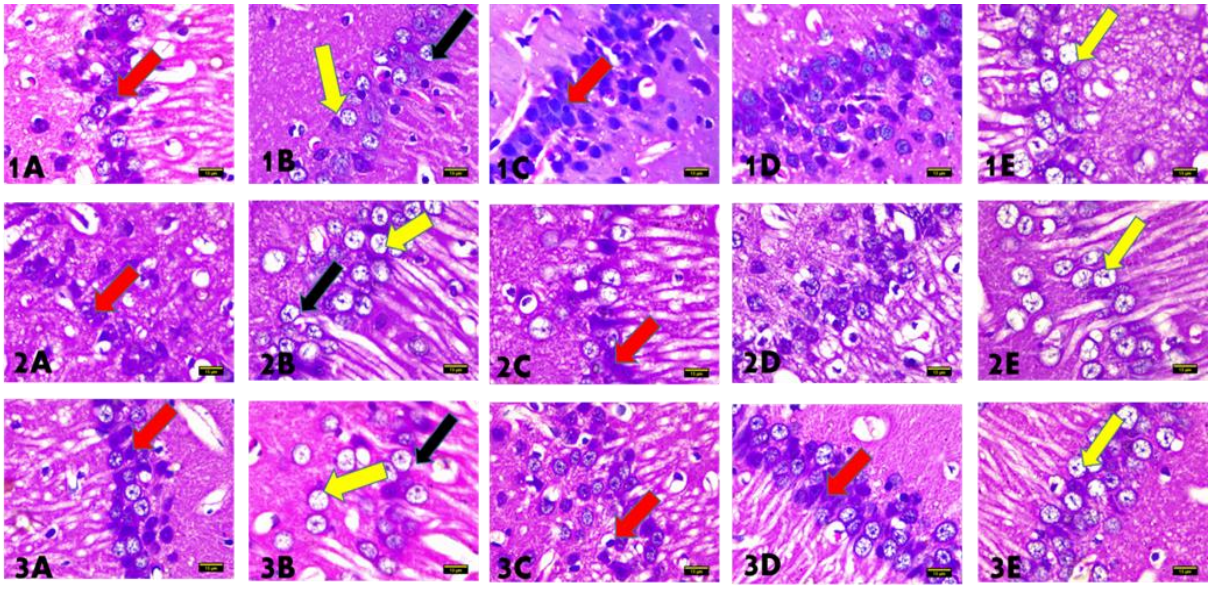

Figure 5: Photomicrographs of the CA 1 of the hippocampus showing the general histoarchitecture of the Cornu Ammonis 1 region. This figure represented the Acute (1), Latent (2) and Chronic (3) Phases of epilepsy (A= Control, B= EPY ONLY, C= EPY +LD CBD, D= EPY+ HD CBD, E= EPY+ SV. Red arrow= Intact neuron (pyramidal neuron), black arrow= degenerating neuron (pyknotic neuron) Yellow Arrow= vacuolated neuron (H&E x1000) Scale bar = 13 µm.

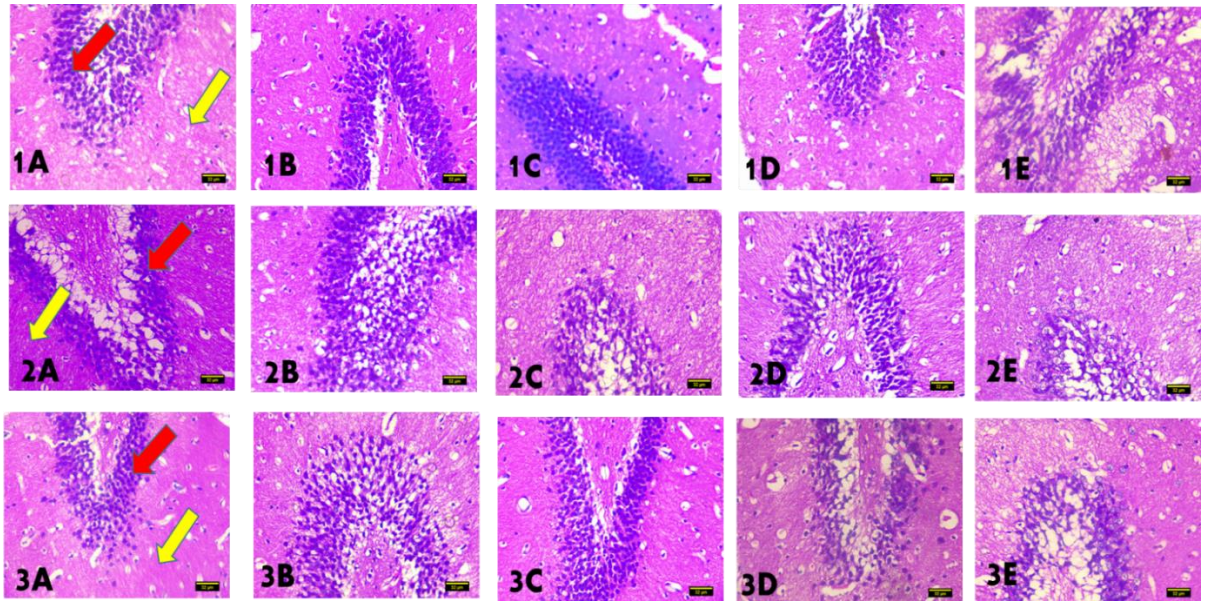

Figure 6: Photomicrographs of the Dentate gyrus (DG) of the hippocampus showing the general histoarchitecture of the Dentate gyrus region. This figure represents the Acute (1), Latent (2) and Chronic (3) Phases of epilepsy (A= Control, B= EPY ONLY, C= EPY +LD CBD, D= EPY+ HD CBD, E= EPY+ SV. Red arrow= granule cells pattern of the dentate gyrus, yellow arrow= Neuropil. (H&E x400) Scale bar = 32 µm.

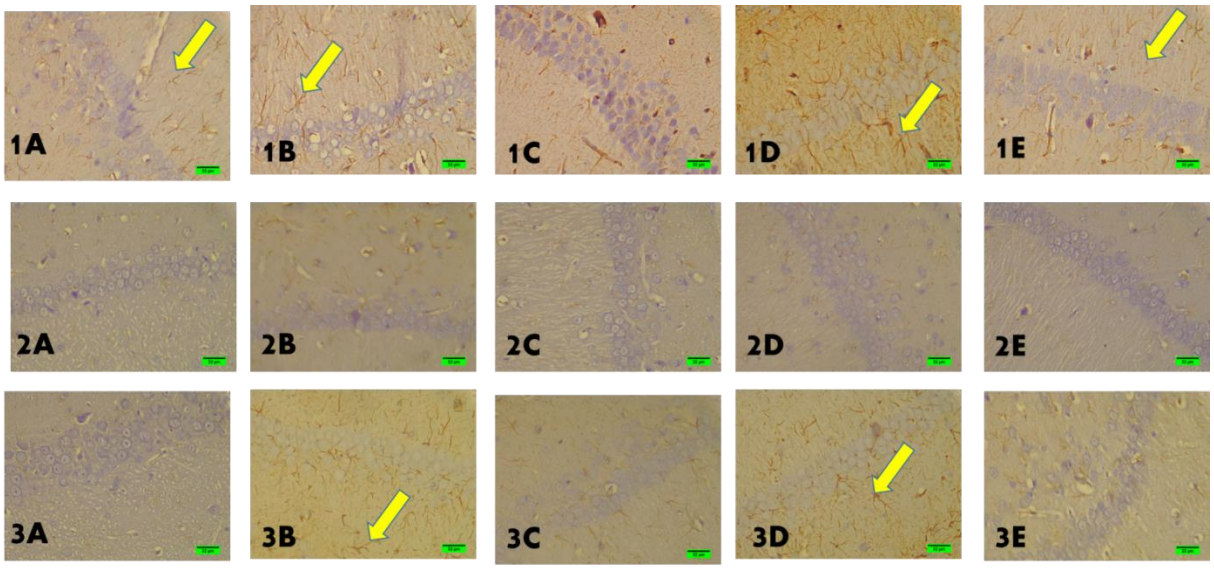

Figure 7: Photomicrographs of the CA 1 of the hippocampus showing the expression of astrocytes in the glial fibrillary acidic protein (GFAP) stained slides. This figure represented the Acute (1), Latent (2) and Chronic (3) Phases of epilepsy (A= Control, B= EPY ONLY, C= EPY +LD CBD, D= EPY+ HD CBD, E= EPY+ SV. Yellow arrow shows astrocytic cells (GFAP x400) Scale bar = 32 µm.

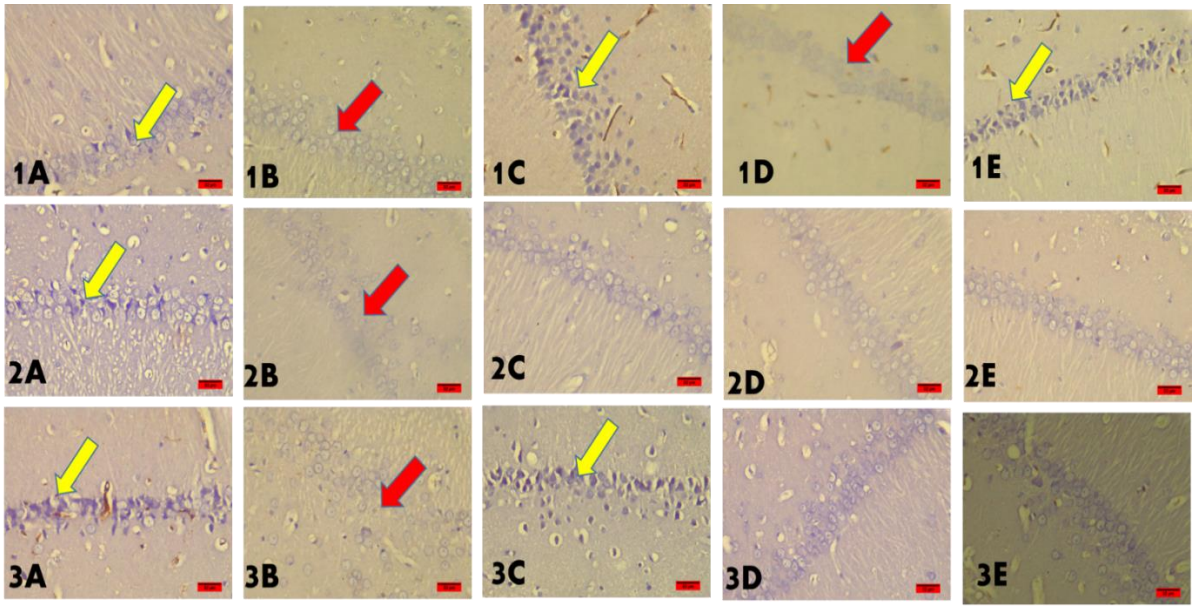

Figure 8: Photomicrographs of the *Cornu Ammonis* 1 of the hippocampus showing the expression of Neuronal nuclear antigen (NeuN) in the immunohistochemically stained slides. This figure represents the Acute (1), Latent (2) and Chronic (3) Phases of epilepsy (A= Control, B= EPY ONLY, C= EPY +LD CBD, D= EPY+ HD CBD, E= EPY+ SV. Red arrow=Unreactive NeuN in the pyramidal cells, Yellow arrow= Well expressed NeuN in the pyramidal cells (NeuNx400) Scale bar = 32 µm.

Image J analysis of the photomicrographs

Intact neurons in the hippocampal CA1 during the acute, latent, and chronic phases of epilepsy

The result (Figure 9A) of intact neurons in the hippocampal CA1 during the acute phase of epilepsy across the groups. The results showed a significant decrease ($p<0.05$) in the number of intact neurons in the epilepsy-only group compared with the control group. Also, the number of intact neurons in the epilepsy-only group was significantly lower ($p<0.05$) compared with epilepsy and low dose of cannabidiol and epilepsy and high dose of cannabidiol groups.

The result (Figure 9B) of intact neurons in the hippocampal CA1 during the latent phase of epilepsy across the groups. The result shows no significant difference in the number of intact neurons across the groups.

The result (Figure 9C) of intact neurons in the hippocampal CA1 during the chronic phase of epilepsy across the groups. The Epilepsy only group was significantly lower than the control group. Also, epilepsy and a low dose of cannabidiol and epilepsy at and high dose of cannabidiol were significantly higher ($p<0.05$) than the epilepsy-only group. Epilepsy and sodium valproate were significantly lower when compared with epilepsy and low dose of cannabidiol and epilepsy and high dose of cannabidiol groups.

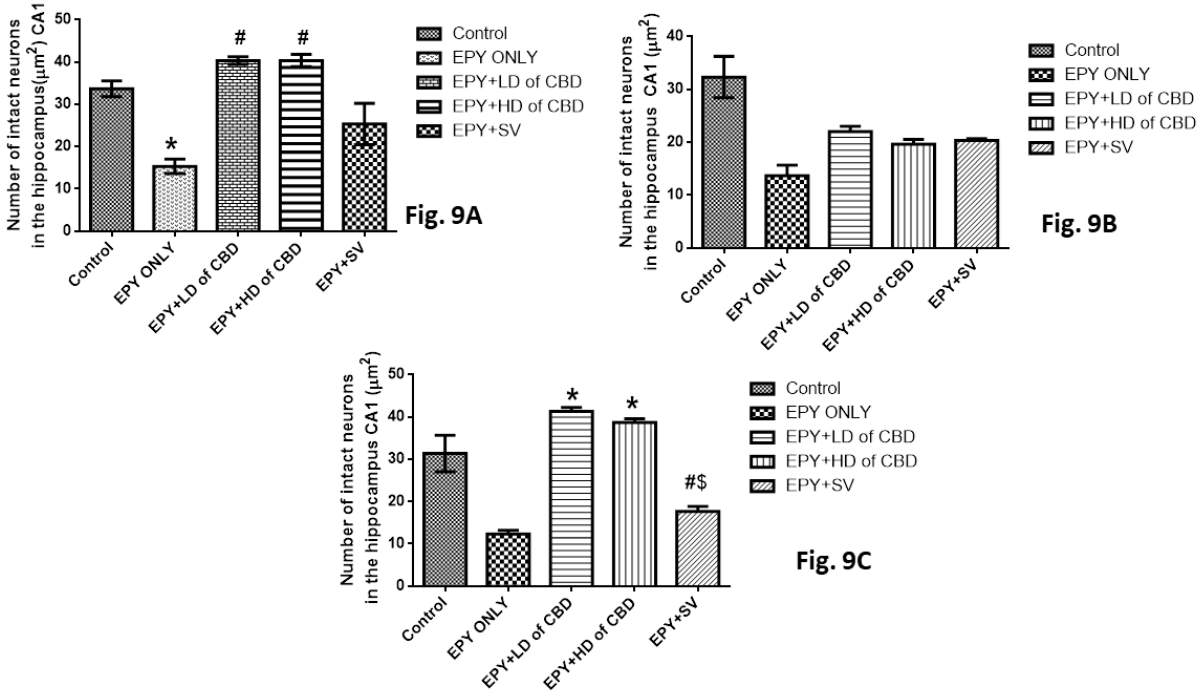

Figure 9: A, B, and C: Bar chart showing the number of intact neurons in the hippocampal CA1 during the acute, latent, and chronic phases of epilepsy across the groups($p<0.05$). *= significantly different from control # = significantly different from EPY ONLY

Key: Control, Epilepsy only (EPY ONLY), epilepsy + low dose of cannabidiol (EPY+LD of CBD), epilepsy + High dose of cannabidiol (EPY+HD of CBD), and epilepsy + sodium valproate (EPY+SV).

Degenerating neurons in the hippocampal CA1 during the acute, latent, and chronic phases of epilepsy

The result (Figure 10A) of degenerating neurons in the hippocampal CA1 during the acute phase of epilepsy across the groups. Epilepsy only group was significantly higher ($p<0.05$) than the control group. Also, epilepsy only group was significantly higher ($p<0.05$) than epilepsy and low dose of cannabidiol in the number of degenerating neurons. Epilepsy and high dose of cannabidiol and epilepsy and sodium valproate were significantly lower than the epilepsy-only group. The control was significantly lower ($p<0.05$) than epilepsy and high dose of cannabidiol and epilepsy and sodium valproate.

The result (Figure 10B) of degenerating neurons in the hippocampal CA1 during the latent phase of epilepsy across the groups. When the treated groups were compared with the control, they were significantly higher ($p<0.05$) than the control group in the number of degenerating neurons in the latent phase of epilepsy. Epilepsy only group was significantly higher ($p<0.05$) than epilepsy and low dose of cannabidiol. Also, epilepsy and high dose of cannabidiol and epilepsy and sodium valproate were significantly higher ($p<0.05$) than epilepsy and low dose of cannabidiol.

The result (Figure 10C) of degenerating neurons in the hippocampal CA1 during the chronic phase of epilepsy across the groups. The control was significantly lower ($p<0.05$) than the epilepsy-only group and epilepsy + high dose of cannabidiol and epilepsy + sodium valproate. Also, epilepsy only group and epilepsy + high dose of cannabidiol and epilepsy + sodium valproate were significantly higher ($p<0.05$) than the epilepsy + low dose of cannabidiol.

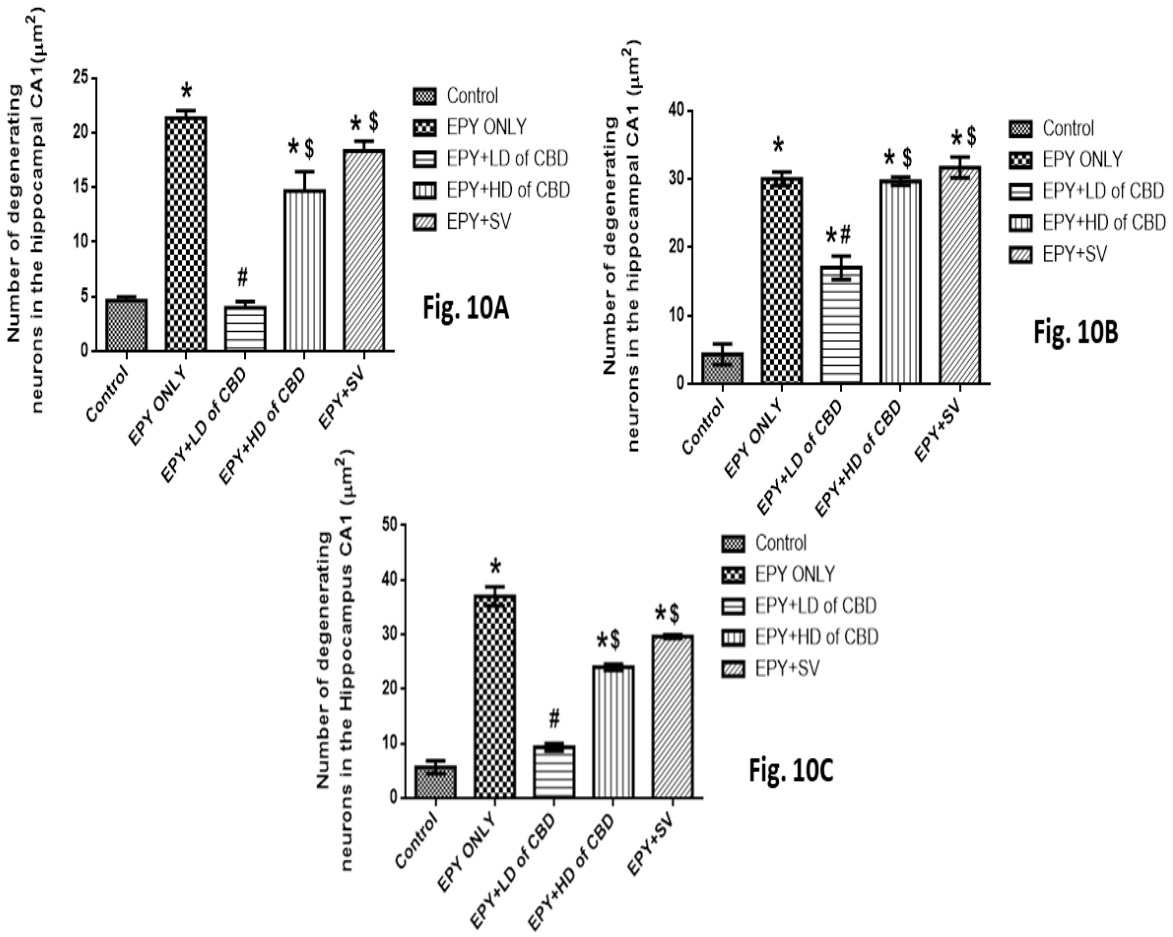

Figure 10: A, B, and C: Bar chart showing the number of degenerating neurons in the hippocampal CA1 during the acute phase of epilepsy across the groups ($p<0.05$). *= significantly different from control # = significantly different from epilepsy only group

$= significantly different from epilepsy + low dose of cannabidiol

Key: Control, Epilepsy only (EPY ONLY), epilepsy + low dose of cannabidiol (EPY+LD of CBD), epilepsy + High dose of cannabidiol (EPY+HD of CBD) and epilepsy + sodium valproate (EPY+SV).

Intact and degenerating neurons in the hippocampal CA1 during the acute, latent, and chronic phases of epilepsy

The result (Figure 11) of Intact and degenerating neurons in the hippocampal CA1 during (A) the acute phase, (B) the latent phase and (C) during the chronic phase of epilepsy across the groups.

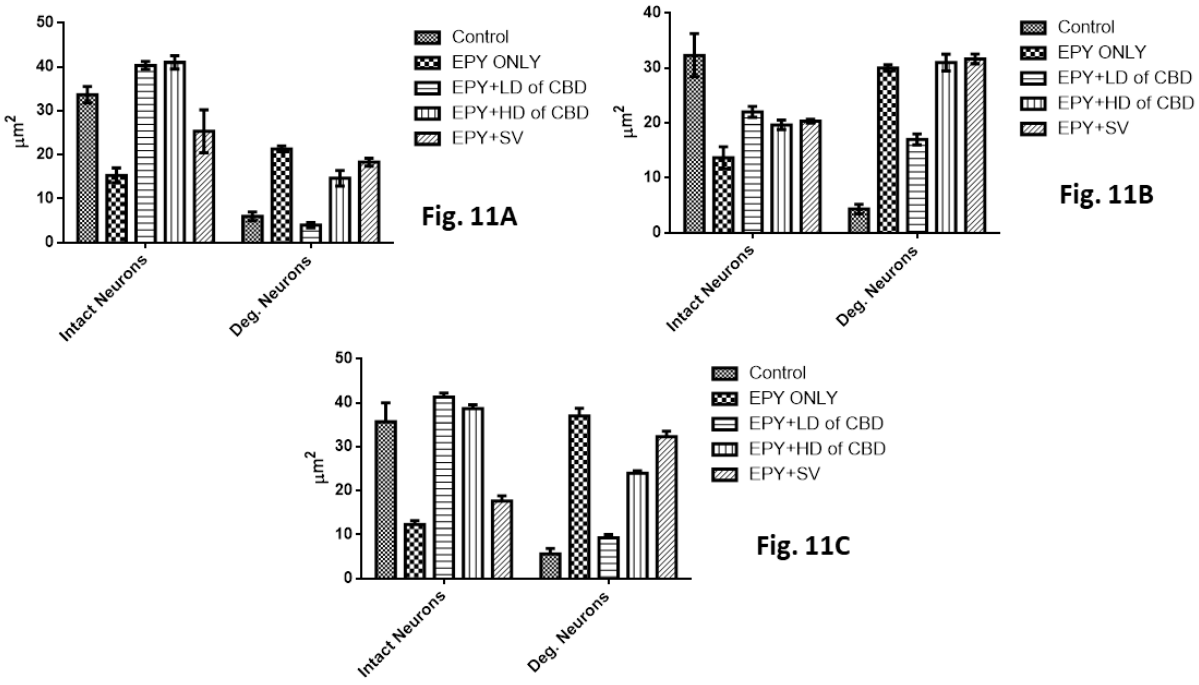

Figure 11: A, B and C: Bar chart showing the number of intact and degenerating neurons in the hippocampal CA1 during the acute phase of epilepsy across the groups.

Key: Control, Epilepsy only (EPY ONLY), epilepsy + low dose of cannabidiol (EPY+LD of CBD), epilepsy + High dose of cannabidiol (EPY+HD of CBD), and epilepsy + sodium valproate (EPY+SV).

GFAP (glial fibrillary acidic protein) immunoreactivity in the hippocampal CA1

The result (Table 5) of glial fibrillary acidic protein immunoreactivity in the hippocampal CA1 during the acute, latent, and chronic phases of epilepsy across the groups. No significant increase was observed across the group in all three phases of epilepsy. However, an observable increase was noticed in the epilepsy only group across the groups when compared with the control and the rest of the treated groups.

Table 5: Glial Fibrillary Acidic Protein Immunoreactivity in the hippocampal CA1

GFAP immunoreactivity in the CA1	Acute Phase	Latent Phase	Chronic Phase
CONTROL	149 ± 5.67	147 ± 3.53	144 ± 1.33
EPY ONLY	160 ± 4.26	163 ± 2.33	165 ± 2.65
EPY+ LD of CBD	150 ± 3.61	147 ± 1.45	144 ± 1.33
EPY+ HD of CBD	154 ± 3.61	148 ± 2.52	151 ± 1.45
EPY+ SV	157 ± 3.06	152 ± 4.70	150 ± 2.40

Key: Control, Epilepsy only (EPY ONLY), epilepsy + low dose of cannabidiol (EPY+LD of CBD), epilepsy + High dose of cannabidiol (EPY+HD of CBD), and epilepsy + sodium valproate (EPY+SV).

Glial fibrillary acidic protein immunoreactivity in the hippocampal dentate gyrus

The result (Table 6) of glial fibrillary acidic protein immunoreactivity in the hippocampal dentate gyrus during the acute, latent, and chronic phases of epilepsy across the groups. No significant increase was observed across the group in all three phases of epilepsy.

Table 6: Glial Fibrillary Acidic Protein Immunoreactivity in the Hippocampal dentate gyrus

GFAP Reactivity in the dentate gyrus	Acute Phase	Latent Phase	Chronic Phase
CONTROL	151 ± 2.33	154± 1.00	148 ± 1.86
EPY ONLY	153 ± 3.00	158± 3.38	160 ± 1.45
EPY+ LD of CBD	155± 0.33	148 ± 1.45	154 ± 0.66
EPY+ HD of CBD	147 ± 1.20	145 ± 2.08	145 ± 5.00
EPY+ SV	151± 1.20	145 ± 1.53	154 ± 0.57

Key: Control, Epilepsy only (EPY ONLY), epilepsy + low dose of cannabidiol (EPY+LD of CBD), epilepsy + High dose of cannabidiol (EPY+HD of CBD), and epilepsy + sodium valproate (EPY+SV).

Neuronal Nuclear Protein Immunoreactivity in the Hippocampal CA1

The results of Neuronal nuclear protein immunoreactivity in the hippocampal CA1 during the acute, latent, and chronic phases of epilepsy across the groups were presented in Table 7. No significant difference was observed in the acute phase of epilepsy across the group. In the latent phase, the control was significantly higher than the epilepsy only group. However, in the chronic phase, the control was significantly higher than the epilepsy only group and the epilepsy + high dose of cannabidiol. Epilepsy only group was significantly lower than epilepsy + low dose of cannabidiol and epilepsy + sodium valproate. Also, epilepsy + sodium valproate was significantly higher than the epilepsy + high dose of cannabidiol.

Table 7: Neuronal nuclear protein (NeuN) Immunoreactivity in the hippocampal CA1

NeuN immune Reactivity in the CA1	Acute Phase	Latent Phase	Chronic Phase
CONTROL	171 ± 4.18	184 ± 2.65	192± 1.73
EPY ONLY	167 ± 4.41	146 ± 0.88 *****	132± 4.63*****
EPY+ LD of CBD	164 ± 4.18	158± 6.17	162 ± 4.73**#**
EPY+ HD of CBD	173 ± 0.88	166 ± 4.04	152 ± 1.20*****
EPY+ SV	165 ± 3.18	163 ± 7.17	173 ± 2.65**#$**

* = significantly different from Control; # = significantly different from EPY + low dose of cannabidiol; $= significantly different from EPY + high dose of cannabidiol. Key: Control, Epilepsy only (EPY ONLY), epilepsy + low dose of cannabidiol (EPY+LD of CBD), epilepsy + High dose of cannabidiol (EPY+HD of CBD), and epilepsy + sodium valproate (EPY+SV).

Neuronal nuclear protein immunoreactivity in the hippocampal dentate gyrus

The result (Table 8) of Neuronal nuclear protein immunoreactivity in the hippocampal DG during the acute, latent and chronic phases of epilepsy across the groups. No significant difference was observed across the group in all the three phases of epilepsy.

Table 8: Neuronal nuclear protein Immunoreactivity in the hippocampal dentate gyrus

NeuN immunoreactivity in the dentate gyrus	Acute Phase	Latent Phase	Chronic Phase
CONTROL	168 ± 2.52	167 ± 4.51	180 ± 4.18
EPY ONLY	159 ± 0.57	171 ± 4.63	159 ± 0.57
EPY+ LD of CBD	177 ± 7.81	158 ± 5.86	162 ± 4.91
EPY+ HD of CBD	162± 6.44	166± 7.51	168 ± 5.17
EPY+ SV	156 ± 7.84	164± 12.5	165± 7.09

Key: Control, Epilepsy only (EPY ONLY), epilepsy + low dose of cannabidiol (EPY+LD of CBD), epilepsy + High dose of cannabidiol (EPY+HD of CBD), and epilepsy + sodium valproate (EPY+SV).

DISCUSSION

This study investigated the effects of cannabidiol (a non-psychoactive constituent of cannabis) on neuronal morphology and astrocytes in lithium chloride pilocarpine-induced epilepsy during the three phases of epilepsy which include acute, latent, and chronic phases. Investigations were carried out on brain and hippocampal weight and the astrocytes, granule cells, and pyramidal neurons of the hippocampus.

Jack [23] reported that, in the study of brain morphometry, it is accepted that a relationship exists between brain structure and function, both normal and abnormal. One descriptor of morphometric structure is volume. Abnormalities in hippocampal morphology, including unilateral or bilateral volume loss, are known to occur in epilepsy, Alzheimer's disease, and in certain amnestic syndromes[23]. In the present study, there was no significant difference in hippocampal weight when the treated groups were compared with the control in the acute, latent, and chronic phases of epilepsy across all the groups. However, we found an observable increase in bilateral hippocampal weight in the control when it was compared with the rest of the treated groups in the acute, latent, and chronic phases of epilepsy. The observable decrease in bilateral hippocampal weight in all the groups except the control may be attributed to hippocampal volume loss caused by the administration of lithium chloride pilocarpine to induce hippocampal damage in all these groups. Recurrent seizure has been reported to cause hippocampal damage and hippocampal volume loss [24].

Evidence from neuroimaging studies in patients with depression, as well as epilepsy, showed volume loss in the hippocampus. These findings parallel the hippocampal atrophy found in patients with temporal lobe epilepsy, who can have bilateral hippocampal atrophy [24]. The association between hippocampal damage and status epilepticus has been revealed in animal models of epilepsy. Lipid metabolism and myelin construction might be damaged during epilepsy and lead to brain white matter volume reduction [25].

Morphological changes in the hippocampus following the administration of lithium chloride pilocarpine have been reported by several authors [26-28]. Hippocampal sclerosis is a common pathologic finding in surgical specimens from patients with temporal lobe epilepsy [29]. In the present study, the photomicrograph of CA1 during the acute phase of epilepsy showed loss of pyramidal neurons and dispersed granule cells. These groups of animals in the acute phase only experience status epilepticus which was the first seizure before the onset of epileptogenesis. This is in agreement with the work of David and Brian [30] who reported that *status epilepticus* can directly cause neuronal death. Studies with animal models, pathology, and neuroimaging work in patients show that single or repeated brief seizures caused neuronal loss but they also indicated that neuron loss is not an inevitable consequence of a seizure [30]. The rats that received cannabidiol at low doses and high doses showed that cannabidiol prevented neuronal loss observed in the epilepsy only group. This could be a result of the fact that cannabidiol was able to alter the signaling pathway associated with cell death. This could be the mechanism to target seizure-induced neuronal death. If epileptic seizures cause neuronal death, therapeutic efforts to prevent all seizures from occurring become more important, our findings from the photomicrograph were also corroborated with the results obtained from the histomorphometry findings.

Studies in kindling models revealed that brief single seizures do not bring about the loss of cells. Thus, Bertram and Lothman reported reduced neuronal density after kindling which they attributed to tissue volume expansion [31] . The possible role of tissue volume changes and changes in neuronal morphology in reports of seizure-induced neuronal loss has been emphasized by numerous authors [32, 33]. Reports from some other studies [34-36] also failed to detect neuronal death after kindling in rats and mice. Thus, studies in which neuron counts were used as the principal measure of whether cell loss occurred are not in agreement as to whether brief seizures cause neuronal death. But in our present studies, the results of the neuronal cell count in the CA1 region of the hippocampus during the acute phase of epilepsy which is a model of single seizure showed a significant increase in the number of degenerating neurons in the epilepsy only groups compared to the control and epilepsy and low dose of cannabidiol. Our findings are in agreement with other studies that reported neuronal loss following a single seizure.

There has been considerable interest in granule cell reorganization in hippocampal sclerosis from the perspective of its potential contribution to pro-epileptogenic circuitry, reflection of interference with ongoing neurogenesis and correlation with memory disorders associated with epilepsy[37]. There is abundant evidence from experimental models that seizures influence rates of granule cell neurogenesis, with new neurons migrating to abnormal or ectopic positions and integrating into networks and acquiring pro-epileptogenic physiological properties [38,39].

Astrocytes are the most numerous cells populating the central nervous system (CNS) [40] . In healthy brain, astrocytes regulate neuronal growth and synapse formation and pruning, support neuro-signaling (e.g., by forming the so-called "tri-partite synapse"), regulate glutamate, potassium, and calcium release and uptake, and modulate synaptic potentiation and

learning [41]. Astrocytes serve as the primary glycogen storage site in the CNS, and they perform metabolite cleansing. Together with endothelial cells, they form the blood-brain barrier (BBB) providing selective protection to the brain [40] . Several studies have shown that astrocytes perform a series of complex functions that are beyond the uptake and recycling of neurotransmitters and the buffering of extracellular potassium [42, 43]. Our present study showed the expression of GFAP in the CA1 and the DG of the hippocampus during the acute, latent, and chronic phases of epilepsy and we also observed that GFAP was more expressed in the epilepsy only group across the three phases of epilepsy and the expression appeared mild in the cannabidiol treated group especially the low dose of cannabidiol and epilepsy. The rats in B (epilepsy-only group) showed an increase in the size of astrocytes as well as astrocytic numbers.

The reactive astrocytes in our study are in agreement with other studies that reported that astrocytes become more reactive in the epileptic brain and that they show changes in the expression of metabolic enzymes such as glutamine synthetase and adenosine kinase leading to modification of neuronal excitability [44,45]. Astrocytes also release glutamate through a Ca^{2+}-dependent mechanism that can synchronize neuronal firing and modulate neuronal excitability and synaptic transmission. The release of glutamate from astrocytes through a Ca^{2+}-dependent mechanism activated by receptors located at the presynaptic terminals may be through the activation of mGluR1 [46] or N-methyl-d-aspartate (NMDA) receptors [47]. The expression of GFAP observed in the photomicrograph plates in the CA1 and the DG of the hippocampus during the acute, latent, and chronic phases of the epilepsy was also corroborated with the results of the image J analysis which showed an observable increase in the epilepsy only group across the three phases, this further confirms astrocytic reactivity in the epileptic brain. Neuronal damage induced by seizure could be responsible for astrogliosis observed in this study. This finding is in agreement with the report of [48] who detected a significant elevation of CSF GFAP after seizures in children. Astrocytes together with microglia show reactive responses to various CNS insults such as infections, trauma, ischemia, and neurodegenerative disease. Reactive gliosis involves both phenotypic and molecular changes in astrocytes and microglia which sometimes results in scar formation [40]. Glia scar may have protective functions by limiting the spread of infections and neurodegeneration or may also produce ischemia-induced cellular rearrangement resulting in a higher degree of astrogliosis changing tissue structure and damaging neuronal connectivity [40] Inefficient astrocytic clearance of debris misfolded proteins brings about an accumulation of oxidative stress and chronic neuroinflammation has contributed significantly to neuronal degeneration, progressive cognitive loss, and motor disability [49]. However, pharmacological modulation of reactive astrocytes has been suggested as a major tool to blunt neuronal damage and slow the course of brain diseases[50]. Evidence from literature shows that many cannabinoids, including phytocannabinoids and endocannabinoids, can modulate reactive gliosis intensity leading to neuromodulatory, anti-inflammatory, and neuroprotective effects in both mature and immature brains [51]. In the present study, the expression of GFAP appeared suppressed in the groups that received cannabidiol following the induction of epilepsy with lithium chloride pilocarpine in all three phases of epilepsy. This could be a result of the ability of cannabidiol a non-psychoactive component of cannabis to modulate reactive astrocytes thereby suppressing astrogliosis and also astrocytes which is responsible for the control of balance between glutamate and GABA in the group that were treated with cannabidiol following epilepsy. Not much is known about the effects of cannabidiol on astrocytes in epileptic disorders [40]. The anti-epileptic activity of cannabidiol was recently described in rats with chronic Pentylenetetrazole-induced seizures[52]. The cannabidiol anti-epileptic effect was accompanied by decreased astrocyte hyperplasia (decrease in GFAP expression) along with decreased neuronal loss and decreased NMDA1 expression in the hippocampus [52].

CONCLUSION

The present study concluded that chemically induced epilepsy in rats resulted in significant cognitive deficit, increased seizure progression, and pathological changes and that cannabidiol showed some antiepileptic potential as seen in alteration in seizure progression, and amelioration of the pathological changes seen in the groups that received cannabidiol. Taken together, our results suggest that cannabidiol showed some antiepileptic potential for the management of epilepsy.

REFERENCES

1. Bear MF, Connors BW, Paradiso MA. Neuroscience: Exploring the Brain. Philadelphia, PA: Lippincott Williams & Wilkins; 2007.
2. Longo DL. Seizures and Epilepsy. In: Harrison's Principles of Internal Medicine. 2012.
3. Cilio MR, Thiele EA, Devinsky O. The case for accessing cannabidiol in epilepsy. Epilepsia. 2014;55(6):787-790.
4. Devinsky O, Vickrey BG, Cramer J**,** Perrine K, Hermann B, Meador K, et al. Development of

the quality of life in epilepsy inventory. Epilepsia. 1995; 36:1089-1104.
5. Donner EJ. Opportunity gained, opportunity lost treating pharmaco-resistant epilepsy in children. Epilepsia. 2013; 54:16-18.
6. Fisher R, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy. Epilepsia. 2005; 46:470-472.
7. Porcari GS, Fu C, Doll ED, Carter EG, Carson RB. Efficacy of Antisana preparation of cannabidiol for the treatment of epilepsy: Practical experience in a tertiary medical center. Epilepsy Behav. 2018; 80:240-246.
8. Tijani AA, Adekomi AD, Oyesomi TO, Fawole OB. Histoarchitectural organization of the visual system of male rats following oral administration of crude aqueous leaf extract of Cannabis sativa. Afr J Cell Pathol. 2014; 2:7-13.
9. Desalu ABO, Owolabi JO, Adeyemo FO, Olanrewaju AJ, Olatunji SY. Assessment of the effects of cannabis ingestion on lateral geniculate body and superior colliculus of Wistar rats. World J. 2017; 3:22-26.
10. Hosseinzadeh M, Nikseresht S, Khodagholi F, Naderi N, Maghsoudi N. Cannabidiol post-treatment alleviates rat epileptic-related behaviors and activates hippocampal cell autophagy pathway along with antioxidant defense in chronic phase of pilocarpine-induced seizure. J Mol Neurosci. 2016; 58:432-440.
11. Mechoulam R, Petersa M, Murillo-Rodriguezb E, Hanus L. Cannabidiol recent advances. Chem Biodivers. 2007; 4:1678-1692.
12. Cortesi M, Fusar-Poli P. Potential therapeutic effects of cannabidiol in children with pharmacoresistant epilepsy. Med Hypotheses. 2007; 68:920-921.
13. Evins AE, Green AI, Kane JM. Does using marijuana increase the risk for developing schizophrenia? J Clin Psychiatry. 2013; 74:8.
14. Mackie CJ, Leary-Barrett M, Al-Khudhairy N, Castellanos-Ryan N, Struve M, Topper L, et al. Adolescent bullying, cannabis use and emerging psychotic experiences: a longitudinal general population study. Psychol Med. 2013; 43:1033-1044.
15. Gray JA, McNaughton N. The Neuropsychology of Anxiety: An Enquiry into the Function of the Septo-Hippocampal System. 2nd ed. New York, NY: Oxford University Press; 2000.
16. Taupin P, Gage FH. Adult neurogenesis and neural stem cells of the central nervous system in mammals. J Neurosci. 2002; 69:745-749.
17. Chang BS, Lowenstein DH. Mechanisms of Disease. Epilepsy. N Engl J Med. 2003; 349:1257-1266.
18. Sloviter RS. The neurobiology of temporal lobe epilepsy: too much information, not enough knowledge. Comptes Rendus Biologies. 2005; 328:143-153.
19. Kuruba R, Hattiangady B, Shetty AK. Hippocampal neurogenesis and neural stem cells in temporal lobe epilepsy. Epilepsy Behav. 2009; 14:65-73.
20. Reddy DS, Kuruba R. Experimental models of status epilepticus and neuronal injury for evaluation of therapeutic interventions. Int J Mol Sci. 2013; 14:18284-18318.
21. Pitkanen A, Immonen RJ, Grohn OH, Kharatishvili I. From traumatic brain injury to posttraumatic epilepsy: What animal models tell us about the process and treatment options. Epilepsia. 2009; 50:21-29.
22. Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol. 1972; 32:281-294.
23. Jack CR. MRI-based hippocampal volume measurements in epilepsy. Epilepsia. 1994; 6:21-29.
24. Shamim S, Hasler G, Liew C, Sato S, Theodore WH. Temporal lobe epilepsy, depression, and hippocampal volume. Epilepsia. 2009; 50:1067-1071.
25. Berg AT, Zelko FA, Levy SR, Testa FM. Age at onset of epilepsy, pharmaco-resistance, and cognitive outcome: a prospective cohort study. Neurology. 2012; 79:1384-1391.
26. Freiman TM, Eismann-Schweimler J, Frotscher M. Granule cell dispersion in temporal lobe epilepsy is associated with changes in dendritic orientation and spine distribution. Exp Neurol. 2011; 229:332-338.
27. Bonilha L, Martz GU, Glazier SS, Edwards JC. Subtypes of medial temporal lobe epilepsy: influence on temporal lobectomy outcomes. Epilepsia. 2012; 53:1-6.
28. Ryufuku M, Toyoshima Y, Kitaura H, Zheng Y, Fu Y-J, Miyahara H, et al. Hypertrophy of hippocampal end folium neurons in patients with mesial temporal lobe epilepsy. Neuropathology. 2011; 31:476-485.
29. Tassi L, Meroni A, Deleo F. Temporal lobe epilepsy: neuropathological and clinical correlations in 243 surgically treated patients. Epileptic Disord. 2009; 11:281-292.
30. Henshall DC, Meldrum BS. Cell death and survival mechanisms after single and repeated brief seizures. In: Jasper's Basic Mechanisms of the Epilepsies. 2012:262-372.
31. Bertram EH, Lothman EW. Morphometric effects of intermittent kindled seizures and

limbic status epilepticus in the dentate gyrus of the rat. Brain Res. 1993; 603:25-31.
32. Guillery RW, August BK. Doubt and certainty in counting. Prog Brain Res. 2002; 135:25-42.
33. West MJ. Design-based stereological methods for counting neurons. Prog Brain Res. 2002; 135:43-51.
34. Khurgel M, Switzer RC 3rd, Teskey GC, Spiller AE, Racine RJ, Ivy GO. Activation of astrocytes during epileptogenesis in the absence of neuronal degeneration. Neurobiol Dis. 1995; 2:23-35.
35. Tuunanen J, Pitkanen A. Do seizures cause neuronal damage in rat amygdala kindling? Epilepsy Res. 2000; 39:171-176.
36. Watanabe Y, Johnson RS, Butler LS, Blinder DK, Spiegelman BM, Papaioannou VE, et al. Null mutation of c-fos impairs structural and functional plasticities in the kindling model of epilepsy. J Neurosci. 1996; 16:3827-3836.
37. Thom M. Hippocampal sclerosis in epilepsy: a neuropathology review. Neuropathol Appl Neurobiol. 2014; 40:420-543.
38. Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. J Neurosci. 1997; 17:3727-3738.
39. Shapiro LA, Ribak CE. Integration of newly born dentate granule cells into adult brains: hypotheses based on normal and epileptic rodents. Exp Brain Res. 2005; 48:43-56.
40. Kozela E, Juknat A, Vogel Z. Modulation of astrocyte activity by cannabidiol, a nonpsychoactive cannabinoid. Int J Mol Sci. 2017; 18:2-20.
41. Pereira A, Furlan FA. Astrocytes and human cognition: modeling information integration and modulation of neuronal activity. Prog Neurobiol. 2010; 92:405-420.
42. Halassa MM, Haydon PG. Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. Annu Rev Physiol. 2010; 72:335-355.
43. Haydon PG, Carmignoto G. Astrocyte control of synaptic transmission and neurovascular coupling. Physiol Rev. 2006; 86:1009-103.
44. Clasadonte J, Haydon PG. Astrocytes and epilepsy. In: Jasper's Basic Mechanisms of the Epilepsies. Oxford University Press, Inc; 2012:591-605.
45. Sontheimer H, Black JA, Waxman SG. Voltage-gated Na+ channels in glia: properties and possible functions. Trends Neurosci. 1996; 19:325-331.
46. Perea G, Araque A. Astrocytes potentiate transmitter release at single hippocampal synapses. Science. 2007; 317:1083-1086.
47. Jourdain P, Bergersen LH, Bhaukaurally K, Bezzi P, Santello M, Domercq M, et al. Glutamate exocytosis from astrocytes controls synaptic strength. Nat Neurosci. 2007; 10:331-339.
48. Gurnett CA, Landt M, Wong M. Analysis of cerebrospinal fluid glial fibrillary acidic protein after seizures in children. Epilepsia. 2003; 44:1455-1458.
49. Ridet JL, Malhotra SK, Privat A, Gage FH. Reactive astrocytes: Cellular and molecular cues to biological function. Trends Neurosci. 1997; 20:570-577.
50. Walter L, Stella N. Cannabinoids and neuroinflammation. Br J Pharmacol. 2004; 141:775-785.
51. Vázquez C, Tolón R, Pazos M, Moreno M, Koester EC, Cravatt BF, et al. Endocannabinoids regulate the activity of astrocytic hemichannels and the microglial response against an injury: in vivo studies. Neurobiol Dis. 2015; 79:41-50.
52. Mao K, You C, Lei D, Zhang H. High dosage of cannabidiol alleviates pentylenetetrazole-induced epilepsy in rats by exerting an anticonvulsive effect. Int J Clin Exp Med. 2015; 8:8820-8827.