# Phenytoin attenuates seizure severity and hippocampal derangement more than levetiracetam or phenytoin-levetiracetam adjunctive treatment in electrically-convulsed male BALB/c Mice

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**Abstract**

This study evaluated the anticonvulsant and neuroprotective efficacy of individual or adjuvant treatment of two different antiepileptic drugs (AEDs) phenytoin (PHT) and levetiracetam (LEV) against maximal electric shock in mice model.

Twenty-five male BALB/c mice, randomized into five groups (n=5) received treatment as follows: groups 1 and 2 received intraperitoneal (i.p) administration of normal saline (0.1 ml), while groups 3-5 received a therapeutic dose of PHT (50 mg/kg BW) or LEV (50 mg/kg/day BW), or combination of PHT (25 mg/kg) and LEV (25 mg/kg) at sub-therapeutic doses. Thirty minutes later, the mice in groups 2-5 were challenged with maximal electroshock, while various indices of convulsion were determined and the histomorphological profile of the hippocampus was investigated. Data were analyzed using descriptive and inferential statistics with the help of graph pad prism software. The results were presented as mean ± SEM in the graph or table. The level of significance was taken at $p < 0.05$.

There was a decrease ($p = 0.0001$) in the duration of tonic flexion, tonic limb extension, clonic convulsion, stupor, and righting reflex in all the drug-treated groups compared with positive control. Also, the duration of clonic convulsion significantly decreased in the PHT ($p = 0.0003$) and PHT + LEV treated mice compared with LEV treated. There was significant ($p = 0.0001$) damage in the neuronal hippocampal tissue of the untreated mice.

The anticonvulsant efficacy and neuroprotective effects of these treatment drugs are in this order: PHT > PHT + LEV > LEV > normal saline.

**Keywords:** Seizure; Anticonvulsants; Phenytoin, Levetiracetam, Adjunctive-treatment

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## Introduction

"A seizure is a paroxysmal alteration of neurologic function brought about by the excessive, hypersynchronous discharge of neurons in the brain. An epileptic seizure is a term used to differentiate a seizure caused by abnormal neuronal firing from a nonepileptic event".[1] However, an enduring predisposition to generate recurrent and unprovoked seizures is referred to as epilepsy.[2]In the Global Burden of Disease 2010 study, epilepsy is one of the most common chronic neurologic disorders, affecting almost 70 million people; this was ranked fourth among 220 health conditions in terms of disability weight.[3] This neurological dysfunction affects individuals of any age and ethnicity [4]. Generally, the risk of developing epilepsy is 3.9%, with males having a slightly higher percentage.[5] In industrialized countries, 34% of people develop epilepsy [6]; while the risk is higher in resource-poor countries.[7] Epilepsy has deleterious effects on social, vocational, physical, and psychological well-being.[8]

Treatment with anticonvulsant drugs also known as antiepileptic drugs (AEDs) is one of the best approaches to epilepsy management, with a record of seizure freedom in about two-thirds of patients treated with conventional AED, e.g., valproic acid, carbamazepine, phenytoin (PHT), and the several others.[9] PHT has been one of the most widely used medications in the treatment of both partial and generalized seizures. Since its synthesis in the year 1908 by the German chemist Heinrich Biltz, marketed under the trade-name Dilantin, PHT has been the predominant medication for the treatment of epilepsy for over seven decades. However, with the introduction of numerous newer AED medications (such as fosphenytoin, gabapentin, topiramate, levetiracetam, and many others), with fewer adverse effects, better pharmacokinetic profiles, better patient tolerability, and proven efficacy, the role of phenytoin as a treatment of choice in epilepsy has become uncertain.[10]Data from the previous study have shown that PHT reduces inward sodium movement by binding to inactivated voltage-gated channels after depolarization and modifying their sodium



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permeability with a resultant increase in the inactivation (or refractory) period of frequently firing neurons.[11] PHT also appears to diminish the amplitude of the action potential and slow neuronal conduction, both likely related to sodium channel inhibition.[12] Phenytoin gives an impression to be a drug of enriched pharmacology or a 'dirty drug' as it has an affinity for several targets in the family of voltage-gated sodium channels, the L-type calcium channel, and some extra receptors such as the GABA-A receptor.[13] Therefore, phenytoin is more than a broad-acting ion channel blocker only.[14]More than 15 second-generation AEDs introduced since the 1990s, among which is levetiracetam, expanding opportunities to tailor treatment for each patient. However, they have not substantially altered the overall seizure-free outcomes, as only 20% are seizure-free; the rest remain refractory.[15]

The most relevant LEV mechanism of action is through binding to the synaptic vesicle protein SV2A.[16] The SV2A binding affinity of LEV derivatives correlated strongly with their binding accord to the brain, as well as with their ability to protect against seizures in the audiogenic mouse model[17]; similar to the report of Kaminski *et al*[18] in the mouse corneal kindling model and the GAERS rat model of generalized absence epilepsy. The specific effect of LEV binding to SV2A appears to be a reduction in the rate of vesicle release.[19] LEV has other mechanisms of action that likely play a comparatively smaller role: reversing the inhibition of neuronal GABAand glycine-gated currents by the negative allosteric modulators, zinc, and ß-carbolines[20], and partial depression of the N calcium current [21]. However, the mechanisms of action have not yet helped identify a specific clinical efficacy profile for LEV.[22]

However, the number of AEDs approved for the adjunctive treatment of refractory partial-onset seizures has increased dramatically in the past two decades, intending to provide better seizure control and improved safety and tolerability profile relative to older AEDs[23]. Surprisingly, there is a dearth of experimental literature on the efficacy and neuroprotective effects of adjunctive AEDs treatment using different AEDs with varying mechanisms of action such as PHT-LEV adjunctive treatment hence, this study.

## Materials and Methods

Animal management

A total of twenty-five male BALB/c mice (25–30g) were obtained from the animal holdings of the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. These animals were housed in clean plastic cages with access to standard laboratory chow and drinking water ad libitum. The experimental protocol compliedwith the regulations of the Health Research Ethics Committee, Institute of Public Health, Obafemi Awolowo University, Ile –Ife and the ARRIVE guidelines and the U.K. Animals (Scientific Procedures) Act, 1986. [24]

### Drugs and animal treatment

Phenytoin and levetiracetam (Sigma, USA) were freshly prepared and dissolved in physiological saline. The animals were randomized into five groups (n-5). Group I and II (negative and positive control respectively) received normal saline (0.2 ml/day intraperitoneally [i.p.]); group III received PHT (50 mg/kg i.p.) [25]; group IV received LEV (50 mg/kg i.p.) [26]; while group V received the adjunctive treatment of PHT (25 mg/kg i.p.) with LEV (25 mg/kg i.p.). The treatments were carried out once between 9:00 and 9:30 am.

### Induction of seizures by maximal Electric Shock

To determine the anticonvulsant efficacy of the drugs either as an individual or adjunctive treatment, 30 minutes after drug administration, animals in groups II-V were challenged for seizure at maximal electric shock (MES) at a current of 150 mA, a pulse width of 0.5 at 100 Hz for a duration of 2 s using electroconvulsiometer (Ugo Basile, Italy).[27] Each mouse was observed for the total time (in seconds) taken to experience the markers of the seizure (i.e., tonic flexion, tonic hindlimb extension, clonic convulsion, stupor, and righting reflex), and eventually, the percentage mortality.

### Histological studies

At the end of the administration, animals were sacrificed by decapitation, while the brain was rapidly and carefully excised. The brain tissue was preserved with 10% neutral buffered formalin followed by paraffin wax embedding, sectioned on a rotary microtome at 6µm thickness, and stained using hematoxylin and eosin (H & E) according to the method of Olaibi*et al.*[28]

### Immunohistochemistry of the Hippocampus

The expression of a neuronal nuclear protein (i.e., nuclear factor erythroid 2-related factor 2 count) within the CA 3 of the hippocampus was carried out using Nrf2 according to the method of Kai-Liang *et al.* [29]

### Photomicrography and image analysis

Stained hippocampal sections were subjected to microscopic investigation under a Leica DM750 digital light microscope, and digital photomicrographs were obtained via the attached Leica ICC50 camera. Moreover, the H&E-stained photomicrographs were imported into Image J software (NIH-sponsored public domain image analysis software). Neurons showing degenerating features and counted using the Image J

cell counter tool as earlier described in the method of Onaolapo et al.[30]

**Statistical analysis**

Data were analyzed using descriptive and inferential statistics with the help of graph pad prism (Version 5.03, GraphPad Inc.) software. The results were expressed as mean ± SEM, using a one-way analysis of variance (ANOVA), followed by Student Newman-Keuls post hoc analysis and presented in a graph or table. The level of significance was set at $p<0.05$.

**Result**

Effects of phenytoin, levetiracetam, and phenytoin-levetiracetam adjunctive treatment on the markers of convulsion in male Albino mice.

There was a decrease ($p = 0.0001$) in the period of tonic flexion (S) in all the drug-treatment groups compared with the positive control. The period of tonic flexion also decreased significantly in the PHT-treated mice among the drug-treated groups. However, there

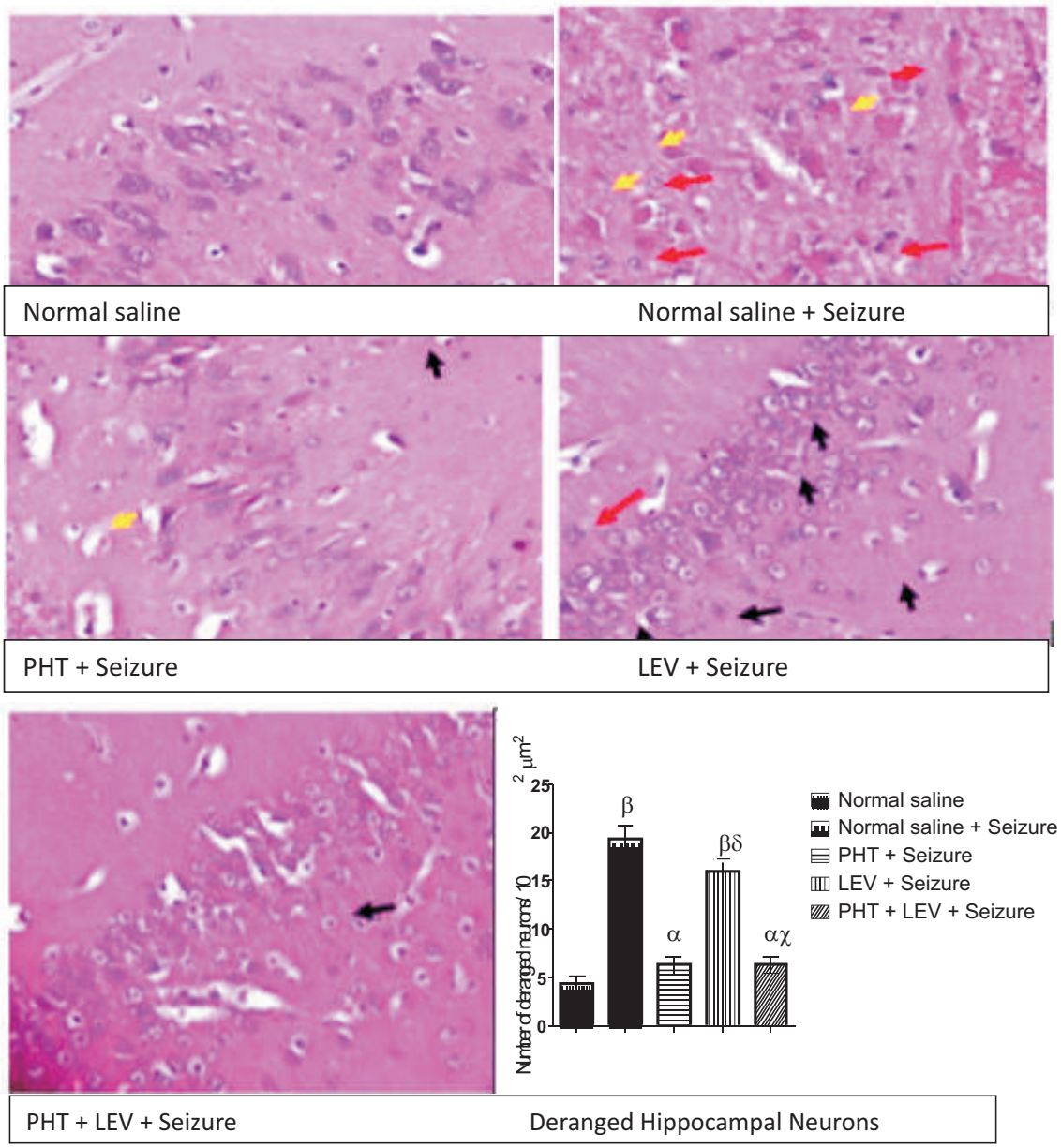


Figure 1 The hippocampal histomorphology and neuronal morphometry following maximal electric shock in the phenytoin, levetiracetam, and phenytoin-levetiracetam adjunctive treated mice

*Yellow arrow: severe neuronal chromatolysis; red arrow: severe neuronal vacuolation; black arrow: mild neuronal granulation; PHT: Phenytoin; LEV: Levetiracetam; Following image J analysis, the number of damaged neurons increased significantly ($p = 0.0001$) following seizure activities compared with non-convulsed mice, while the damage decreased significantly ($p = 0.0003$) in the PHT, and PHT + LEV compared LEV treated mice.*

Table 1 Effects of phenytoin, levetiracetam, and phenytoin-levetiracetam adjunctive treatment on the indices of convulsion in male Albino mice

| Treatment Groups | Tonic Flexion (S) | Tonic Hind-Limb Extension (S) | Clonic Convulsion (S) | Righting Reflex (S) | Stupor (S) | Percentage Mortality (%) |
|---|---|---|---|---|---|---|
| Control | 44.50 ± 3.20 | 15.50 ± 2.25 | 19.7 ± 0.88 | 27.0 ± 1.53 | 151 ± 10.70 | 100 |
| PHT | 0.50 ± 0.30$^{\alpha}$ | 0.25 ± 0.25$^{\alpha}$ | 2.67 ± 0.67$^{\alpha}$ | 1.33 ± 0.33$^{\alpha}$ | 6.20 ± 1.16$^{\alpha}$ | 0 |
| LEV | 0.30 ± 1.31$^{\alpha\beta}$ | 3.75 ± 0.75$^{\alpha\beta}$ | 17.3 ± 1.45$^{\beta}$ | 20.3 ± 1.45$^{\alpha\beta}$ | 71.0 ± 10.30$^{\alpha\beta}$ | 60 |
| PHT + LEV | 15.70 ± 2.19$^{\alpha\beta}$ | 9.00 ± 1.18$^{\alpha\beta\delta}$ | 6.00 ± 2.08$^{\alpha\delta}$ | 5.67 ± 1.45$^{\alpha\beta\delta}$ | 29.2 ± 8.00$^{\alpha\beta\delta}$ | 20 |

*The anticonvulsant efficacy of PHT, LEV, and PHT + LEV adjunctive treatment was evaluated and compared 30 minutes after the drug (s) was administered. Maximal electric shock (MES) was kindled in groups II-V with the aid of an electroconvulsiometer. α: compared with the control (p = 0.0005); β: compared with PHT (p =0.0005); δ: compared with LEV (p < 0.0035).*

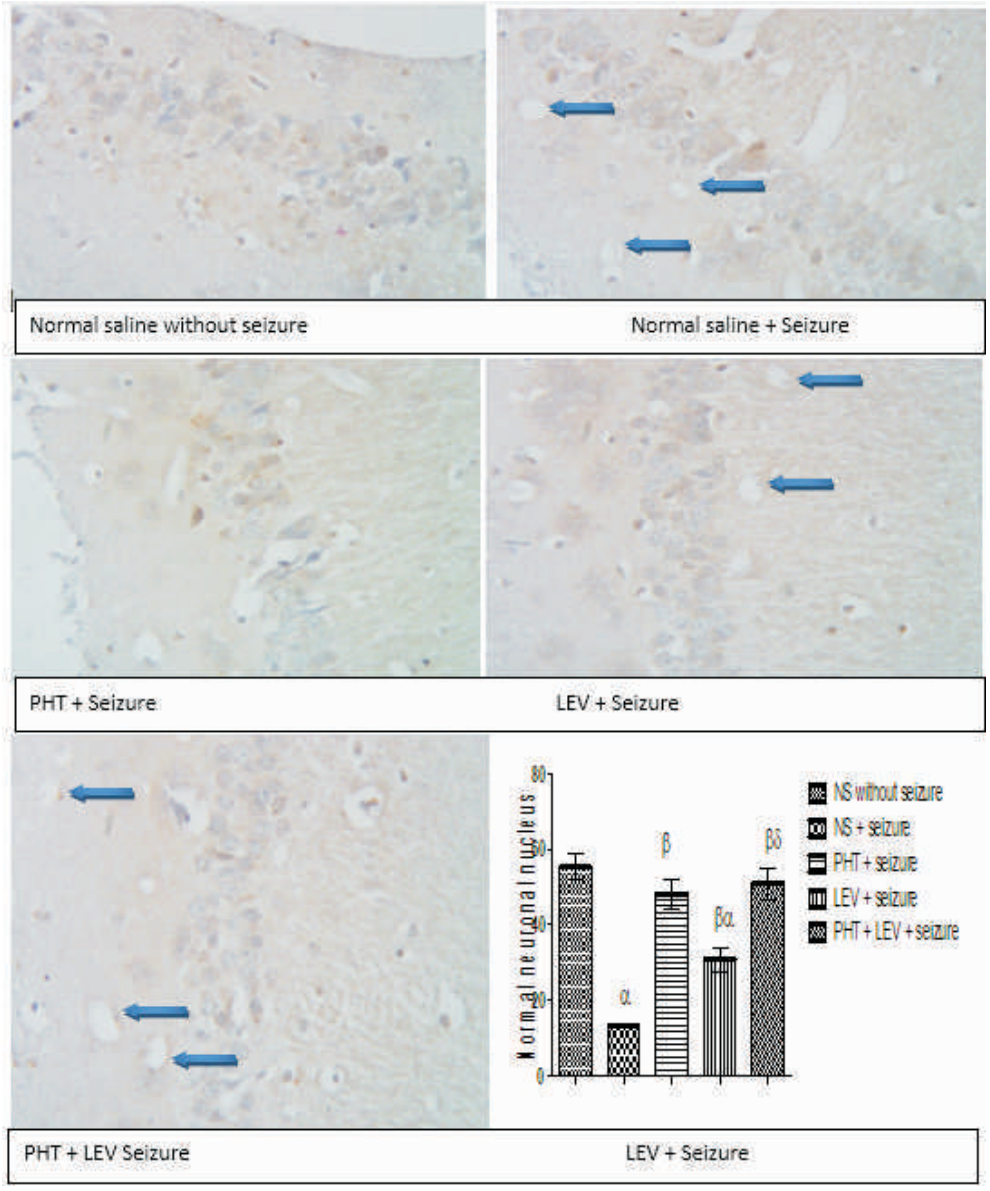


Figure 2 The hippocampal expression of nuclear factor erythroid 2-related factor 2 count following maximal electric shock in the phenytoin, levetiracetam, and phenytoin-levetiracetam adjunctive treated mice *Blue arrow: neuronal vacuolation; a: decrease compared with control (p = 0.0001); β: increase compared with normal saline + seizure (p = 0.0001); δ: increase compared with LEV treatment group*

was no significant difference in the period of tonic flexion between LEV and PHT + LEV adjunctive treatment (t=2.26 df=5) (Table 1).

The duration of tonic hind-limb extension (S) decreased significantly (p = 0.0001) across the treatment groups compared with the positive control.

The duration of tonic hind limb extension also decreased (p = 0.0001) in the PHT-treated mice relative to the LEV and PHT + LEV adjunctive-treated mice. Also, the period of tonic hind-limb extension increased significantly in the PHT + LEV adjunctive treated mice compared with the LEV treated (t=3.51 df=7) (Table 1). The period of clonic convulsion decreased significantly (p = 0.0001) in the PHT and PHT + LEV adjunctive treatment, while the LEV treatment had no significant (p = 0.2417) effect compared with the positive control. Moreover, among the drug-treated groups, the period of clonicconvulsion increased significantly (p = 0.0011) in the LEV-treated mice, there was no significant difference between PHT and PHT + LEV adjunctive treatment (t=1.52 df=4) (Table 1).

A significant (p = 0.0001) reduction in the duration of stupor was observed served across the drug-treatment groups compared with the positive control. However, the period of stupor exhibition by the mice increased significantly (p = 0.0001) in the LEV-treated mice compared with the PHT and

PHT + LEV adjunctive treated mice (Table 1). The time lag between the end of clonic convulsion and the on-set of righting reflexes decreased significantly (p = 0.0001) across the drug-treatment groups relative to the positive control, while this was significantly increased (p = 0.0002) in the LEV-treated mice compared with other treatment groups (Table 1). The percentage mortality decreased in the PHT, PHT + LEV adjunctive treatment mice, and the treated mice, while the percentage of mortality was total in the normal-saline treated (Table 1).

The hippocampal histomorphology and neuronal morphometry following maximal electric shock in the phenytoin, levetiracetam, and phenytoin-levetiracetam adjunctive-treated mice

Following the seizure activities, neurons within the Cornusammonis 3 (CA3) of the positive control (normal saline-treated + seizure) showed scattered and disorganized neuronal cells in addition to severe neuronal chromatolysis (yellow arrow) and vacuolation (red arrow). There was a moderate chromatolysis in the PHT + seizure representative mouse. The hippocampus of LEV + seizure appeared with signs of neuronal granulation (black arrow), while the PHT + LEV + seizure representative mouse showed mild neuronal granulation (black arrow) (Figure 1).

The hippocampal expression of nuclear factor erythroid 2-related factor 2 count following maximal electric shock in the phenytoin, levetiracetam, and phenytoin-levetiracetam adjunctive treated mice

In this study, there was a significant (p = 0.0001) decrease in the hippocampal expression of Nrf2 in the positive control (normal saline), and LEV treatment groups following an electrically-induced seizure. (Figure 2).

**Discussion**

Electroshock-induced seizures are among the most studied models of electrical stimulation, owing to the comprehension of the complex mechanisms underlying epileptogenesis, seizure generation in temporal lobe epilepsy, and other forms of epilepsy, which cannot be investigated in-depth in clinical studies with humans. As a result, the use of appropriate animal models is essential.[31] Findings in this study confirmed the anticonvulsant activities of the duo drugs either as an individual or adjunctive treatment. In this study, neither the newer AED, LEV, nor PHT + LEV adjunctive treatment decimated the markers of seizure-like the conventional PHT alone treatment. This finding is in contrast to the report of Bansal *et al.*[32] that LEV was efficacy is at pal with PHT. The difference between the two studies is attributable to the disparity in the study design. However, Lee [33] concluded that no new AED is more potent than old AEDs. In this study, there was a significant decrease both in the period of sustained flexion of the upper limb and the hind-limb extension across the treatment groups relative to the untreated mice (positive control).

This finding is in tandem with the report of Abend *et al*,[34] that tonic seizures are sudden in their inception and characterized by a tonic extension of the head, trunk, or extremities that lasts for several seconds. A suggestive indication is that these drugs (either as individual or adjuvant) inhibit appendicular muscle activities, which may be due to the involvement of the closure of

voltage-gated ion channels such as sodium and calcium ions. This attribute was evident in a significant decrease in all the indices of seizure stemming from the tonic flexion, tonic extension, clonic convulsion, stupor, and a decline in the duration of righting reflex. Findings from this study revealed that at the tail end of the episode of seizure, there was a significant increase in the righting reflex (latency to the adjustment in the orientation of the body taken out of its normal upright position, including response to external stimulus) in the normal saline, and LEV treatment groups relative to the PHT treated mice. However, this is a condition similar to Todd's paralysis in humans (a neurological abnormality characterized by temporary limb weakness or hemiplegia, which typically occurs following a seizure) .[35] The duration of Todd's paralysis can range from minutes to days, depending on the seizure types or whether the patient has experienced cortical structural damage, diffuse signs of cerebral paralysis such as stupor and coma to localized signs of neurological deficit such as hemiplegia, chemosensory disorder, and hemianopia.[36] Therefore, a significant increase in the duration of stupor and righting reflex in this present study, especially in the normal saline and LEV-treated mice suggest neurological damage with evidence of increased percentage mortality sequela to

tonic-clonic seizures.

In this study, hippocampal degeneration of neurons characterized by chromatolysis and neuronal granulation increased significantly following positive control, and LEV-treated mice relative to the PHT treatment group. A previous study has shown that neuronal damage following a seizure is attributable to hyper-excitotoxicity, hypoxia, hypotension, pulmonary aspiration, hyperthermia, rhabdomyolysis, and metabolic acidosis.[36] Chromatolysis is a reactive change that occurs in the cell body of damaged neurons, involving the dispersal and redistribution of Nissl substance (rough endoplasmic reticulum and polyribosomes) to meet the increased demand for protein synthesis such as is required to regenerate axons .[37] This finding suggests that brain damage after the seizure is attributable to the unmet metabolic demand during the hyper excitation of the CNS neurons.

In conclusion, the anticonvulsant and neuroprotective effects of these drugs are in this order: PHT > PHT + LEV > LEV. Therefore, conventional AED, PHT remains most efficacious compared with either adjunctive treatment or newer AED. A further immunohistochemical investigation is necessary on the hippocampus and striatum following chemical and electrically-induced seizures.

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**Authors Contribution**

OS: Conceptualization, visualization, methodology, formal analysis, validation, writing the original and final draft of the manuscript; GO: resources; OS, KI, OO, and TA: investigation, data curation, project administration; GO and AO: Supervision, review, and editing.

**References**

1. Stafstrom, C.E., Carmant, L (2015) Seizures, and epilepsy: an overview of neuroscientists. *Cold Spring Harbor perspectives in medicine* 5(6): a022426.

2. Fisher, R.S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J.H., Elger, C.E, et al (2014) A practical clinical definition of epilepsy Epilepsia 55(4):475–482.

3. Reichard, A, Stolzle, H, Fox, M.H (2011) Health disparities among adults with physical disabilities or cognitive limitations compared to individuals with no disabilities in the United States. *Disabil Health J.* 4(2):59-67.

4. Beghi, E (2020) The Epidemiology of Epilepsy. *Neuroepidemiology* 54:185-191.

5. Hesdorffer, D.C., Logroscino, G., Benn, E.K., Katri, N., Cascino, G., and Hauser, W.A (2011. Estimating risk for developing epilepsy: a population-based study in Rochester, Minnesota. *Neurology* 76(1):23-27.

6. Sirven, J.I (2015) Epilepsy: A Spectrum Disorder. Cold Spring Harbor perspectives in medicine 5(9), a022848.

7. Espinosa-Jovela, C., Toledanob, R., Aledo-Serrano, A., García-Morales, I., Gil-Nagel, A (2018) Epidemiological profile of epilepsy in low-income populations. *Seizure* 56: 67–72.

8. Perucca, P., Scheffer, I.S., Kiley, M (2018) The management of epilepsy in children and adults. *The Medical Journal of Australia* 208 (5): 226-233.

9. Landmark, C.J., Fløgstad, I., Syvertsen, M., Baftiu, A., Enger, U and Koht, J (2019) Treatment and challenges with antiepileptic drugs in patients with juvenile myoclonic epilepsy. *Epilepsy and Behavior* 98: 110–116.

10. Das, R.R., Griesemer, D.A., Kothare, S.V (2013) The role of phenytoin in the treatment of localization-related epilepsy: an international internet-based survey of neurologists and epileptologists. *ISRN Neurol*. 2013:613456.

11. Ruiz, M.D., Kraus, R.L (2015) Voltage-Gated Sodium Channels: Structure, Function, Pharmacology, and Clinical Indications. *Journal of Medicinal Chemistry* 58, 18: 7093– 7118.

12. Cook, A.M and Bensalem-Owen, M.K (2011) Mechanisms of action of antiepileptic drugs. *Therapy* 8(3):307–313.

13. Rogawski, M.A., Löscher, W., and Rho, J.M (2016). Mechanisms of Action of Antiseizure Drugs and the Ketogenic Diet. *Cold Spring Harbor Perspectives in Medicine* 6(5), a022780.

14. Hesselink, J.M.K (2017) Phenytoin: a step-by-step insight into its multiple mechanisms of action—80 years of mechanistic studies in neuropharmacology. *Journal of Neurology* 10.1007/s00415-017-8465-4.

15. Thurman, D.J., Beghi, E and Begley, C.E (2011) Standards for epidemiologic studies and surveillance of epilepsy. *Epilepsia* 52(7), 2– 26.

16. Lynch, B.A., Lambing, N., Nocka, K., Kensel-Hammes, P., Bajjalieh, S.M., Matagne, A., Fuks, B (2004) The synaptic vesicle protein SV2A is the binding site for the antiepileptic drug levetiracetam. *Proceedings of the National Academy of Sciences of the United States of America* 101(26): 9861–9866.

17. Löscher, W., Gillard, M., Sands, Z.A., Kaminski, R.M, and Klitgaard H (2016) Synaptic Vesicle Glycoprotein 2A Ligands in the Treatment of Epilepsy

and Beyond. *CNS Drugs* 30: 1055– 1077.
18. Kaminski, R.M., Matagne, A., Leclercq, K., Gillard, M., Michel, P., Kenda, B., Klitgaard, H (2008) SV2A protein is a broad-spectrum anticonvulsant target: A functional correlation between Phenytoin-levetiracetam treatment in convulsion protein binding and seizure protection in models of both partial and generalized epilepsy. *Neuropharmacology* 54(4), 715–720.
19. Wood, M.D and Gillard, M (2017) Evidence for a differential interaction of brivaracetam and levetiracetam with the synaptic vesicle 2A protein. *Epilepsia* 58(2):255–262.
20. Rigo, J.M., Hans, G., Nguyen, L., Rocher, V., Belachew, S., Malgrange, B. et al (2002) The anti-epileptic drug levetiracetam reverses the inhibition by negative allosteric modulators of neuronal GABA- and glycine-gated currents. *British Journal of Pharmacology* 136(5):659-672.
21. D'Onofrio, S., Mahaffey, S., Garcia-Rill, E (2017) Role of calcium channels in bipolar disorder. *Current Psychopharmacology* 6(2): 122–135.
22. Abou-Khalil, B (2008) Levetiracetam in the treatment of epilepsy. Neuropsychiatric disease and treatment 4(3), 507–523.
23. Slater, J., Chung, S., Huynh, L., Duh, M.S., Gorin, B., McMicken, C. et al (2018) Efficacy of antiepileptic drugs in the adjunctive treatment of refractory partial-onset seizures: Meta-analysis of pivotal trials. *Epilepsy Research* 143: 120.129122.
24. Guidance on the Operation of the Animals [Scientific Procedures] Act (1986). The ARRIVE guidelines and the U.K. Animals (Scientific Procedures). EU Directive 2010/63/EU for animal experiments.
25. Soysal, H., Unur, E., Duzler, A., Karaca, O, and Ekinci, N (2011) Effects of intraperitoneal administration of the phenytoin on the skeletal system of rat fetuses. *Seizure* (20),187–193.
26. Talos, D., Chang, M and Kosaras, B (2013) Antiepileptic effects of levetiracetam in a rodent neonatal seizure model. *Pediatric Research* 73: 24–30.
27. Osuntokun, O. S., Olayiwola, G., Oriare, A. K., Odeniran, H. T. and Ayoka, A. O. (2021) The activities of the central nervous system following ethyl acetate extract of Mucuna pruriens seed administration in male BALB/c mice. *Nig. J. Neurosci*. 12(3):92-99.
28. Olaibi, O.K., Osuntokun, O.S, and Ijomone O.M (2014). Effects of chronic administration of gabapentin and carbamazepine on the histomorphology of the hippocampus and striatum. *Annals of Neurosciences* 21(2), 57-61.
29. Kai-Liang, W.u., Yu-Qing, L.i., Tabassum, A., Wei-Yang, L (2010) Loss of Neuronal Protein Expression in Mouse Hippocampus After Irradiation. *Journal of Neuropathology and Experimental Neurology* 69 (3): 272-280
30. Onaolapo, O.J., Onaolapo, A.Y., Akanmu, M.A and Olayiwola, G (2016). Evidence of alterations in brain structure and antioxidant status following 'low dose' monosodium glutamate ingestion. *Pathophysiology* 23: 147-156.
31. Kandratavicius, L., Balista P.A., Lopes-Aguiar C., Ruggiero, R.N., Umeoka, E.H., Garcia-Cairasco, N. et al (2014) Animal models of epilepsy: use and limitations. *Neuropsychiatric disease and treatment* (10): 1693–1705.
32. Bansal, S., Blalock, D., Kebede, T (2014) Levetiracetam versus (fos) phenytoin for seizure prophylaxis in pediatric patients with intracranial hemorrhage. Journal of Neurosurgery and Pediatrics 13:20.
33. Lee, S.K (2014) Old versus New: Why Do We Need New Antiepileptic Drugs? *Journal of epilepsy research* 4(2): 39–44.
34. Abend, N.S., Jensen. F.E., Inder, T.E and Volpe, J.J (2017) Chapter 12 - Neonatal Seizures. Volpe's Neurology of the (6);275-321.e14.
35. Xu, S.Y., Li, Z.X., Wu, X.W., Li L., Li, C.X (2020) Frequency and Pathophysiology of Post-Seizure Todd's Paralysis. *Medical science monitor: international medical journal of experimental and clinical research* 26: e920751.
36. Mastriana, J., Pay, J.L., Taylor, R.S (2019) Todd paresis. StatPearls; Treasure Island (FL).
37. Hantson, P.E (2015). Toxicant-induced seizure. In: Brent J. et al (eds) Critical Care Toxicology. *Springer*, Cham 2017; http://doi.org/10.1007/978-3-319-17900-17. 34.