Ind. J. Pharm. Edu. Res, 2023; 57(2):418-422.
https://www.ijper.org

Original Article

Evaluation of the Effect of Pheophytin A on Electroshock-Induced Seizures in Experimental Animals

Qasem H Almuthaybiri[1,*], Eissa E Al-Rasheedi[2], Hossam A Elsisi[3]

[1]Medical City, Qassim University, SAUDI ARABIA.
[2]Pharco Pharmaceuticals Company, SAUDI ARABIA.
[3]Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, Buraydah, SAUDI ARABIA.

ABSTRACT

Background: An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. Approximately, 5–10% of the people have at least one kind of seizure, with more chance of occurrence in early childhood and late adulthood. There are many drugs used in seizures such as phenytoin and valproate sodium, so the main objective of current study was evaluated the efficacy of Pheophytin A compared to Valproate sodium on electroshock-induced seizures in experimental mice. **Materials and Methods:** The total number of 18 mice were used after divided into 3 groups in which each group was 6 mice. The first group, which is control, was received injection with distilled water. The second group was standard reference which received valproate sodium at dose of 300 mg/kg. The third group was test group which received 20 mg /kg of pheophytin A. Maximum electrical shocker was used to induce seizures. **Results:** The present study showed that pheophytin-A group had significantly reduced duration of seizures after 30 min of injection as compared to valproate sodium group. Valproate sodium produced more reduction in duration of seizures than pheophytin-A. **Conclusion:** The Pheophytin A showed antiseizure effect using maximal electrical shocker, so that it may be used in the future for treatment of seizures after further investigations.

Keywords: Seizures, Pheophytin A, Valproate sodium, Maximal electroshock seizure, *In vivo*.

Correspondence:
Qasem H Almuthaibri
Medical City, Qassim University,
SAUDI ARABIA.
Email id: qasem1511415@gmail.com

Received: 03-07-2022;
Revised: 27-09-2022;
Accepted: 16-12-2022.

INTRODUCTION

An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain.[1-5] According to the range of distribution of discharges, this massive brain action can have multiple manifestations, starting from dramatic convulsive activity to experiential phenomena not easily visible by a monitor. Although many factors can affect the incidence and frequency of seizures, ~5–10% of the people have at least one kind of seizure, with more chance of occurrence in early childhood and late adulthood.[6]

Classifications of seizures are focal seizures (which more described as having motor, sensory, autonomic, intellectual, or other manifestations), generalized seizures (Typically and atypically Absence, Tonic-clonic, clonic, Tonic, Atonic, Myoclonic).

Management of a patient affected with a seizure disorder is usually multi-aspects and should include treatment of causative conditions that induce or contribute to the seizures, avoidance of factors that precipitate seizure, prevent of recurrent seizures by prophylactic treatment with antiepileptic medications or sometime surgery, and clarifying a variety of psychological and social problems.[7,8]

The first anti-epileptic drug introduced was bromide, which was utilized in the late 19th span. Phenobarbital was the first synthesized agent approved as having anti-seizure action.[2]

There are many drugs used in seizures such as phenytoin, carbamazepine, lamotrigine, VPAs. Side effects differ between drugs such as gingival hyperplasia for phenytoin, carbamazepine causes aplastic anemia and agranulocytosis.[2] Although of availability of different classes and mechanisms of antiepileptic drugs, there are still >30% of patients suffered from medically refractory epilepsy and approximately 30-40% of all epileptic patients affected by numerous side effects and seizure resistance to the current AEDs.[9]

VPA is widely used in many types of seizures such as generalized tonic-clonic, simple or complex partial, absence and myoclonic seizures.[10] VPA is absorbed quickly and totally after oral ingestion. Peak concentration level in plasma compartment is noticed in 1-4 hr, although this can be deferred for couples of hours if the drug is ingested in enteric-coated formulation or is ingested with meals.[2] Side effects of VPA include dizziness, drowsiness, faintness, tremors, problems with motor or coordination, blurred vision, strabismus, hair loss, disorder in appetite and increased weight.[10]

DOI: 10.5530/ijper.57.2.52

The continuing use of herbal plants introduced in traditional medicines clarify their benefit in drug discovery. According to the historical evidence support, herbal medicines were used to treat convulsive seizures for decades.[11-15]

The widespread availability and use of herbal medicines increase the potential for adverse effects in the epilepsy patients.[16-18] Many complex extracts and single plant-derived compounds exhibit anti-inflammatory, neuroprotective, and cognition-enhancing activities that may be beneficial in the treatment of epilepsy.[19,20]

Many studies carried out to investigate the effect of different plants on induced seizures. In a study carried out in Malawi, they found that *M. discoidea* male leaves, *D. boehmii* roots, and *D. nitidula* leaves showed significant anti-seizure effects.[7] Few previous studies have demonstrated that some chlorophyll constitutes e.g.: phytol has anticonvulsant effect.[4]

Chlorophylls is the basic unit of plant energy systems during photosynthesis which found as examples in spirulina, spinach, and broccoli. Chlorophylls can be converted to pheophytin A by a reaction called chelate modification.[1] Chlorophyll a and its degradation products are valuable and abundantly available anti-inflammatory agents and promising for the development of phytomedicine or conventional medicine to treat inflammation and related diseases.[17]

Process of replacement of Mg^{2+} ions found in chlorophylls (Figure 1) by hydrogens gives pheophytin A (Figure 2), by using acidic solvent and heat. This process is known as pheophytinization.[14]

Rodent shocker is a known device used to induce electrical seizures in animals.[3] The objective of this research is to evaluate the efficacy of pheophytin A compared to VPA on electroshock-induced seizures.

Aim

The aim of this study was evaluated the probable effect of pheophytin A on electrically induced seizure in mice by rodent shocker instrument, then compared to VPA.

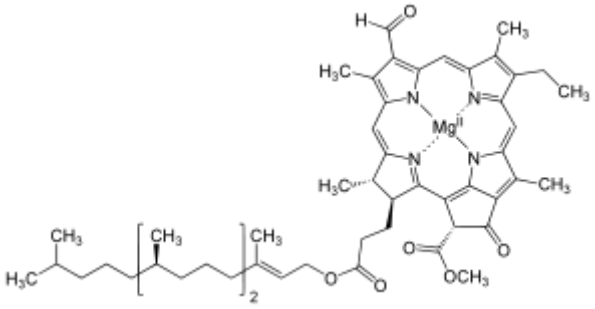

Figure 1: Chlorophylls.

MATERIALS AND METHODS

Extraction

Pheophytin A has been extracted from spinach leaves by using column chromatography.

Extraction procedure

3 g of freeze-dried spinach leaves with 3g of $MgSO_4$ was crushed by using mortar and pestle. Then, 3g of spinach mixture with 5 ml of 80% acetone were mixed in a test tube and Shaked for 5 min. The resulted liquid was transferred to a clean test tube by using a pipette. Four ml of petroleum ether and 1ml of water were added and again shaked for a minute. Then, the mixture was centrifuged for 1 min to separate the layers. Drying column to remove traces of water was prepared by placing small piece of cotton in the bottom of column, then 1 g and 3 g of sand and Na_2SO_4 were added, respectively. The green petroleum ether layer was eluted through the drying column to be collected in a clean test tube. Collected green pigment was covered and dried to be used in the column chromatography. Column chromatography was packed with 3g of alumina and one-half cm of sand. The extract was eluted through column by using hexane to induce movement of pigments. Three test tubes were used to collect yellow, intermediate, and green pigments. The green pigment was acidified by acetic acid to convert chlorophyll to pheophytin A. By using Beer's law, concentration of pheophytin A was calculated which was 2 mg /ml.[12]

Preparation of valproate sodium injection

4 tablets of 200 mg valproate sodium crushed and dissolved in 40 ml of distilled water and the result concentration was 20 mg/ml.

Rodent shocker

Figure 3 shows rodent shocker. In the maximal electroshock test (MES), mice of three groups received an electrical stimulus of 79 mA for 0.2 sec to induce maximal seizures of their hind limbs, with tonic extension as the end of the test.

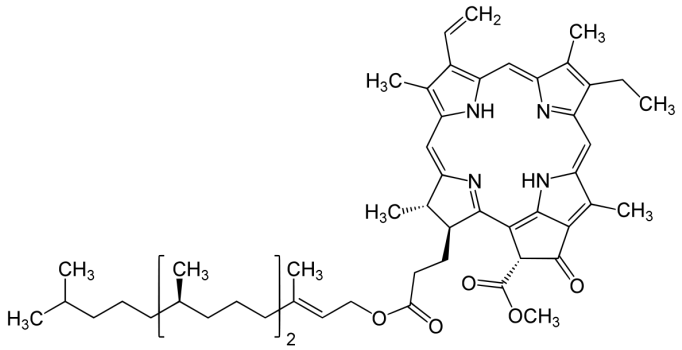

Figure 2: Pheophytin A.

Mice

Three groups of mice each group had 6 mice weighting 16-34 g utilized to receive electrical field to induce seizures. These three groups are: control group received distilled water injection, valproate sodium group, and pheophytin A group.

Rout of administration

The mice given the drug by IP injection.

Control group

Six mice weighted and exposed to electrical stimulation after thirty minutes of distilled water injection and the duration of seizures in seconds was reported for comparisons with other groups (Figure 4).

Valproate sodium group

Valproate sodium in dose of 300 mg/kg given to six weighted mice half hour before exposure to electrical stimulus. Then, electrical stimulus was given, and the duration of seizures reported to compare with other groups.

Pheophytin A group

Pheophytin A in a concentration of 2 mg/ ml was given in dose of 20 mg /kg to six weighted mice half hour before seizures induction. Then, the mice received electrical stimulus to report seizures duration and compared with other groups.

Figure 3: Rodent shocker.

Statistical analysis

The data were presented as Mean ±SD. The data of valproic acid, Pheophytin-A, and control group were analyzed by one-way ANOVA. The difference was considered significant at less than 0.05 level.

RESULTS

As shown in the Figure 5, Pheophytin-A significantly reduced the duration of electrically induced seizure by approximately 5 sec as compared to 18.83 sec of control group. It is well known that valproate sodium reduces the duration of seizures as it is apparent in this test. Valproate sodium more significantly reduced electroshock-induced seizure to 9.5 sec as compared to pheophytin-A which was 13.70 sec (Table 1). The statistical analysis results indicate the significance of Pheophytin A antiseizure action. Among the groups, mortality rate was 0% in Pheophytin A and valproic acid groups, while it was 16% in control group.

DISCUSSION

The results of the present study demonstrated that Pheophytin-A in dose of 20mg/kg had significant activity in reduction of seizure duration. However, it was less effective than valproate sodium.

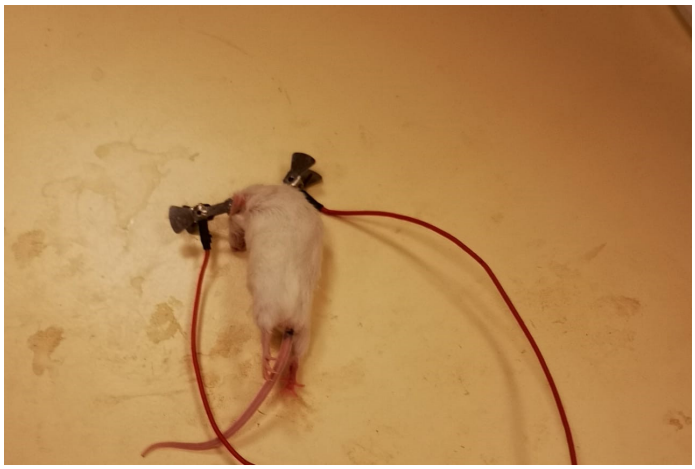

Figure 4: Measuring the duration of seizure.

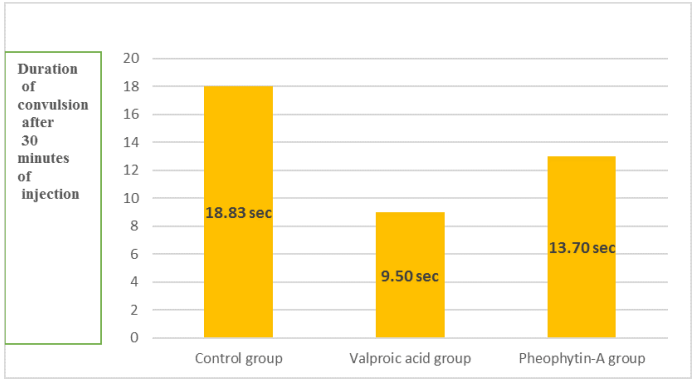

Figure 5: Effect of pheophytin-A extract compared to valproate sodium on electrically induced seizures after 30 min of injection.

Table 1: Comparison between the effect of pheophytin A and valproate sodium on electroshock in Induced seizure in mice.

Duration of seizure after 30 min	Valproate sodium treated group	Control group	Pheophytin-A treated group
	9.5000 ± 3.01662[A] Second	18.8333 ± 1.60208[B] second	13.7000 ± 0.86239[C] Second

* Value one presented as means duration of seizure in second ± Standard deviation.* Value with uncommon superscript capital letters is significantly different.

It reduced the duration of seizure 5 sec while valproate sodium reduced it by 9 sec when compared to control groups. Also, Pheophytin A was similar to valproic acid in preventing deaths which indicates anticonvulsant action.

The results of the present study are consistent with[4] who investigated the effects of phytol in pilocarpine-induced seizures, the pretreatment 30 min with phytol was able to decrease mortality percentage in the first hour in acute stage of seizures, and significantly decreased this percentage in a dose-dependent style ($p<0.05$), indicating an anticonvulsant action.

Our results are consistent with a study that investigated the effect of the antiepileptic drugs (AEDs), valproate sodium (VPA), htt ps://www.sciencedirect.com/topics/medicine-and-dentistry/l amotrigine (LTG), and https://www.sciencedirect.com/topics /medicine-and-dentistry/vigabatrin (VGB) on seizures in rats model in which epilepsy was induced by electrical stimulus of the amygdala. VPA was the most effective of the agents examined, reducing the mean seizure frequency by 83%. In the VPA group, the percentage of rats with a more than 50% reduction in seizure frequency was 100%.[10]

A seizure is a wide word that has been used to define uncontrollable muscle spasms. Some people use it instead of the word "seizure," although a seizure refers to an electrical disorder in the CNS.[9]

The causative factor is often not clear. Seizures can be induced by specific matters in the circulation, as well as infections in the organs like meningitis or encephalitis. A famous trigger in children is febrile seizures. Other potentials causes include celiac illness, head trauma, stroke or hypoxia in the brain. Also, the seizure can be triggered by genetic disorders or brain cancers. Seizures can also happen when the blood sugar concentration is very low and shortage of Vitamin B_6 (pyridoxine).

Traditional herbal medicine has a considerable benefit in the alleviation of seizures. Though herbal medicine is commonly used as antiepileptic therapy, there is a shortage of vigorous evidence for efficiency and safety of most herbs.[15] Some of the most frequently used herbs for epilepsy are: burning bush, groundsel, hydrocotyle, lily of the valley, mistletoe, mugwort, peony, skullcap, tree of heaven, and valerian.

CONCLUSION

We conclude that, Pheophytin-A extract at dose of 20 mg/kg possesses anticonvulsant activity using animal models. Further studies are required to support our results and to investigate safety profile.

ACKNOWLEDGEMENT

We would like to thank the college administration that provided the facilities and needs of our research.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

VPA: Valproate sodium; **HPLC:** High performance liquid chromatography; **MES:** Maximal electroshock seizure.

SUMMARY

Electroshock seizure is an experimental paradigm that induces synchronous neural discharges in the brain through artificial current input and is used to induce acute epileptic behaviors. Accumulated evidence implicates structures of the brainstem as being involved in both kinds of experimental seizures. Stimulation of the midbrain reticular formation induces motor seizures in animals. The current study showed the Pheophytin-A in dose of 20mg/kg had potentiation of antiepileptic activity in reduction of seizure duration. However, it was less effective than valproate sodium. It reduced the duration of seizure 5 sec while valproate sodium reduced it by 9 sec when compared to control groups. Also, Pheophytin A was like valproic acid in preventing deaths which indicates anticonvulsant action.

REFERENCES

1. Ahmad HR, Zia-ur-Rehman M, Sohail MI, Anwar ul Haq M, Khalid Hinnan AMA, *et al*. Effects of rare earth oxide nanoparticles on plants: Nanomaterials in plants, algae, and microorganisms. Academic Press; 2018;1239-75. doi: 10.1016/B978-0-12-811487 -2.00011-6.
2. Brunton LL, Lazo JS, Parker KL. Goodman and Gilman's the Pharmacologycal basis of therapeutics. 11th ed. New York: McGRAW-HILL. 2012.
3. Castel-Branco MM, Alves GL, Figueiredo IV, Falcão AC, Caramona MM. The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs. Methods Find Exp Clin Pharmacol. 2009;31(2):101-6. doi: 10.13 58/mf.2009.31.2.1338414, PMID 19455265.
4. Costa JP, Ferreira PB, De Sousa DP, Jordan J, Freitas RM. Anticonvulsant effect of phytol in a pilocarpine model in mice. Neurosci Lett. 2012;523(2):115-8. doi: 10.1016 /j.neulet.2012.06.055, PMID 22750154.
5. Fisher RS, 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 (IBE). Epilepsia. 2005;46(4):470-2. doi: 10.1111/j.0013-9580.2005.66104.x. PMID 15816939.

6. Goddard J, Turner A. Davidson's principles and practice of medicine. 23rd ed. Edinburgh: Churchill Livingstone. 2010.
7. Gwedela MNV, Terai H, Lampiao F, Matsunami K, Aizawa H. Anti-seizure effects of medicinal plants in Malawi on pentylenetetrazole-induced seizures in zebrafish larvae. J Ethnopharmacol. 2022;284:114763. doi: 10.1016/j.jep.2021.114763. PMID 34688800.
8. Kasper D, Fauci A, Hauser S, Longo D, Jemson J. Harrison's principles of internal medicine. 19th ed. New York: McGraw-Hill Education. 2015.
9. Zhu HL, Wan JB, Wang YT, Li BC, Xiang C, He J, *et al*. Medicinal compounds with antiepileptic/anticonvulsant activities. Epilepsia. 2014;55(1):3-16. doi: 10.1111/epi.12463, PMID 24299155.
10. Zeind CS, Caralho MG. Applied therapeutics: The clinical use of drugs. 11th ed. Philadelphia: Lippincott Williams and Wilkins. 2017.
11. Liu W, Ge T, Pan Z, Leng Y, Lv J, Li B. The effects of herbal medicine on epilepsy. Oncotarget. 2017;8(29):48385-97. doi: 10.18632/oncotarget.16801, PMID 28423368.
12. Nissinen J, Pitkänen A. Effect of antiepileptic drugs on spontaneous seizures in epileptic rats. Epilepsy Res. 2007;73(2):181-91. doi: 10.1016/j.eplepsyres.2006.10.003, PMID 17161937.
13. Pither RJ. Canning | Quality changes during canning. In: Encyclopedia of Food Science and Nutrition. Elsevier. 2003;845-51. doi: 10.1016/b0-12-227055-x/00163-2.
14. Piva DL, Lampman GM, Kriz GS, Engel RG. Introduction of Organic Laboratory Technique: A miroscale approach. 3rd ed. Saunders college publishing. New York. 1999.
15. Sahranavard S, Ghafari S, Mosaddegh M. Medicinal plants used in Iranian traditional medicine to treat epilepsy. Seizure. 2014;23(5):328-32. doi: 10.1016/j.seizure.2014.01.013, PMID 24525263.
16. Samuel I, Beale. Bioynthesis of chorophylls and Hemes: The Chlamydomonas Sourcebook. 2nd ed. Academic Press, Rhod island. 2009;2:731-98.
17. Song MX, Deng XQ. Recent developments on triazole nucleus in anticonvulsant compounds: A review. J Enzyme Inhib Med Chem. 2018;33(1):453-78. doi: 10.1080/14756366.2017.1423068, PMID 29383949.
18. Spinella M. Herbal medicines and epilepsy: The potential for benefit and adverse effects. Epilepsy Behav. 2001;2(6):524-32. doi: 10.1006/ebeh.2001.0281, PMID 12609386.
19. Subramoniam A, Asha VV, Nair SA, Sasidharan SP, Sureshkumar PK, Rajendran KN, *et al*. Chlorophyll revisited: Anti-inflammatory activities of chlorophyll a and inhibition of expression of TNF-α gene by the same. Inflammation. 2012;35(3):959-66. doi: 10.1007/s10753-011-9399-0, PMID 22038065.
20. Sucher NJ, Carles MC. A pharmacological basis of herbal medicines for epilepsy. Epilepsy Behav. 2015;52(B):308-18. doi: 10.1016/j.yebeh.2015.05.012, PMID 26074183.

Cite this article: Almuthaybiri QH, Al-Rasheedi EE, Elsisi HA. Evaluation of the Effect of Pheophytin A on Electroshock-Induced Seizures in Experimental Animals. Indian J of Pharmaceutical Education and Research. 2023;57(2):418-22.