

PAPER • OPEN ACCESS

The effect of auricular electro-stimulation as compared with diazepam in seizure attack reduction in epilepsy rat model induced by pilocarpine

To cite this article: Noor Hadi Farhan and Hasan Abo almaali 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **571** 012044

View the [article online](#) for updates and enhancements.



IOP | ebooks™

Bringing you innovative digital publishing with leading voices to create your essential collection of books in STEM research.

Start exploring the collection - download the first chapter of every title for free.

The effect of auricular electro-stimulation as compared with diazepam in seizure attack reduction in epilepsy rat model induced by pilocarpine

Noor Hadi Farhan and Hasan Abo almaali

**Department of clinical laboratory science. College of Pharmacy, Karbala University,
Karbala, Iraq.**

hadin87@yahoo.com

Abstract

The efficacy of electro stimulation in epilepsy remained to be approved due to the diversity and complex effect which induced by this alternative management. So that, the objective of the study was to demonstrate the anticonvulsant methods of electro stimulation of ear points in rat with lab experimental seizure. To achieve this goal, the epilepsy rats' model was prepared by intra peritoneal injection of pilocarpine. Seizure rats, would be stimulated electrically, it simulated as ear-point electrical stimulation with rats that treated with diazepam as a pharmacological treatment. The results revealed that the convulsion behaviours were improved in comparison to control group, but the beneficial effect was lesser than diazepam treated rat group. Basing on the results that obtained, it suggested that the electrical stimulation of certain ear-point had weak effect as anti-epilepsy, that might be get involved in decreasing the epilepsy attacks as an attempt to use a non-pharmacological management to improve epilepsy in combination with other stimulation points or longer periods.

1.Introduction

Epilepsy is the neurological disorder that affecting about fifty million persons in the worldwide [1]. It consist of repeated seizures resulted from exaggerated and non-controlling electrical activity of the brain. Although there were effective pharmacologic and neurosurgical managements used for treating the epilepsy seizures, the management of epilepsy that were intractable medically would remain as a difficult problem [2]. Most patients with epilepsy were controlling successfully by using antiepileptic drugs. However, approximately 20–40% of patients are refractory to the available pharmacological treatments [3]. Vagus nerve stimulation (VNS) shows an alternative, promising neurosurgical method to treat the patients suffering from refractory epilepsy [4, 5].

The VNS known as a type of neuronal modulation. It is originated to



modify the action of how brain cells work through induced an electrical stimulated pulses at certain areas that concerned with seizure attacks. The vagal nerve is a part of the autonomic nervous system (ANS), which could control the functions of the body which are not under the voluntary control like breathing and heart rate [6]. Vagal nerve was able to send the information from brain to various areas in the body and vice versa that made it able in controlling seizure attacks through increasing the blood flow in certain areas in brain, it raised the amounts of some brain markers which called neurotransmitters that had important role in controlling seizures and causing changes in electroencephalogram [EEG] patterns during the epilepsy seizures [7].

2. Materials and Methods

In total, 24 rats with no specific sex difference were needed in seizures induction and survival observation, so both females and males' data were taken with average weight 200–250 g, were used.

All experiments were conducted and approved by the ethical committee in Karbala University/ college of pharmacy.

2.1. Experimental Protocol

Animals were divided into 4 groups: 1) Control group. 2) Epilepsy only group. 3) Diazepam with epilepsy group. 4) Electro-stimulation with epilepsy group.

Each group included 8 animals. In the control group, all rats received an identical volume of normal saline (IP) instead of drug treatments. Animals in seizure groups were injected once weekly with pilocarpine [intra-peritoneal injection] as epilepsy induced drug. This study was last for four weeks.

2.2 Induction of epilepsy in rats

Rats were administered an Intra-Peritoneal injection [IP] of pilocarpine at dose 300 mg/ kg; until the onset of seizure attacks appeared, which characterised by continuous seizures observed at least one stage 3–5 seizure noticed as forelimb clonus, falling and rearing according to the scale of Racine [8]. Rats developing seizure attacks were treated with diazepam at dose 10 mg/kg [intra-peritoneal injection] after ninety minutes of induced seizure to reduce the duration of Status Epilepticus. A dose of (5 mg/kg) diazepam can be repeated after 30 minutes if seizures are persisted.

2.3 Electro stimulation of vagus nerve

This group were treated daily for four weeks by using vagus stimulation with electrical sparks that getting by with voltage of 800 volt/pulse with frequency of 20 pulse/minute, this procedure designated in current study.

2.4 Pharmacological treatment group

Rats were administered diazepam as antiepileptic drug at dose of 10 mg/kg [IP] weekly 15 minutes before pilocarpine dose administration.

2.5 Statistical analysis

Data were evaluated by one way ANOVA using SPSS version [21], values were significant at $p < 0.05$.

3. Results

Experimental observations to the rat groups after seizure induction by pilocarpine were noticed and seizure attacks were recorded, the number of seizure attacks approved significant difference between treatment groups which mean that diazepam treating group as well as the electrostimulation treating group showed significant difference when compared with epilepsy group as indicated in table [1] and figure [1] respectively. However, the average of seizure duration showed no difference between treatment groups when compared with the epilepsy group as shown in table [2] and figure [2].

Table 1. showed the mean of seizures attacks number for each treatment group by using one way ANOVA.

	1 st week	2 nd week	3 rd week	4 th week	Sig.
Epilepsy group	1.8	2	3.5	5	0.02*
Diazepam with epilepsy group	1.5	1.7	2.8	2.8	0.001*
Electro stimulation with epilepsy group	1	1.3	2.8	2.6	0.003*

*Significant when $P < 0.05$

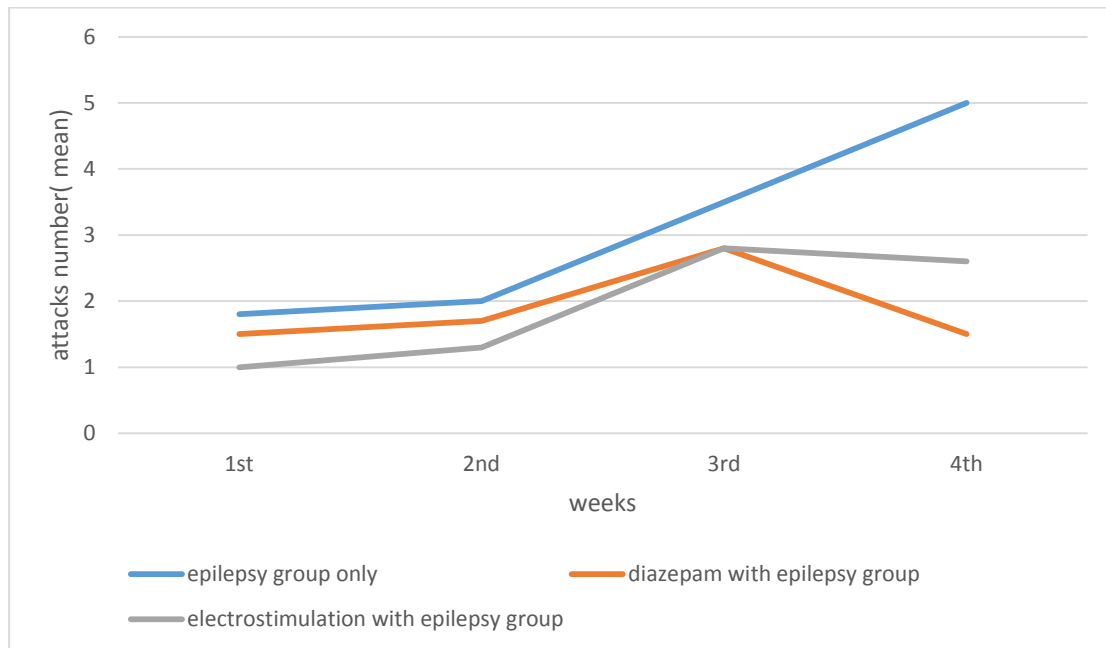


Diagram (1) showed the mean of seizure attacks number in epilepsy, diazepam with epilepsy state and electro stimulation with epilepsy state respectively after four weeks.

Table :2 showed seizure duration (sec.) for each treatment group by using one way ANOVA.

	1 st week	2 nd week	3 rd week	4 th week	Sig.
Epilepsy group	22	30	29	45	0.208
Diazepam with epilepsy group	19	17	13	9	0.05
Electro stimulation with epilepsy group	12	11	20	25	0.396

Significant difference when $P < 0.05$

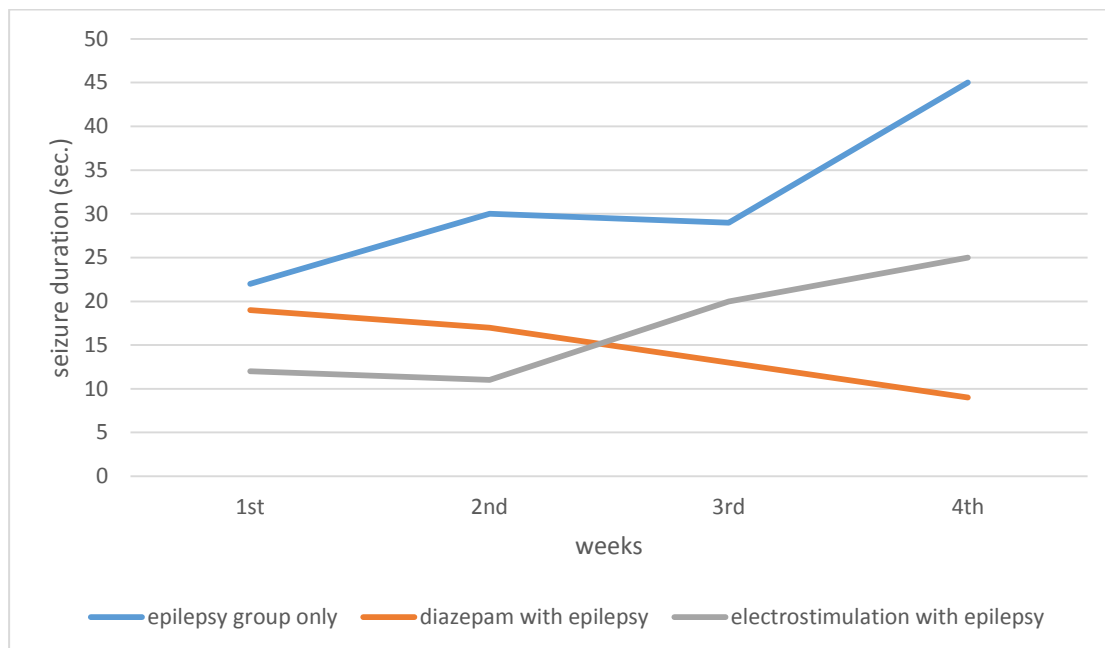


Diagram 2: showed seizure duration in each treatment after first, second, third and fourth weeks respectively. Mean of duration time was calculated as second/attack for each treatment group.

4. Discussion

Patients with drug resistant seizure were majorly showed various adverse effects of using pharmacologic drugs, in addition to failure of limiting epilepsy attacks could have devastated psychological, health and economic consequences, which included accidents, cognitive and deterioration in behaviour. [9].

However, the current study done to find out the possible alternatives of pharmacological antiepileptic drugs and reduce the seizure attacks and reduce the attack duration of time. On the other hand, VNS is an original synergistic therapy which had become available commercially available for resistant epilepsy [10]. US Food and Drug Administration FDA was approving the clinical uses of vagal nerve induction consequently with ongoing studies and preclinical researches about expanding the use of VNS to new applications [11]. The vagal nerve could provide an expensive innervation network for the viscera afferent and efferent signals made it able to play a key role as an interface pathway between the central nervous system CNS circuits and the brain stem with autonomic control circuitry [12]. Additionally, VNS exerts antiepileptic or anti- epileptogenic effect possibly through neuro modulation of release of noradrenaline from locus coeruleus; induced profound changes in

brain blood flow; immunomodulation or anti neuroinflammation; change EEG brain functional connectivity; and modulation of adenosine system and DNA methylation [13]

The current study, pilocarpine was used to induce epilepsy like symptoms according to the recent researches findings which raised the possible effect of pilocarpine to induce seizure attacks due to the direct activation of cholinergic system in the brain that branched from the primary pro-inflammatory effects of pilocarpine including the peripheral and induced the Status Epilepticus (SE) [14]. The pharmacological treatment that used diazepam as antiepileptic drug and compared with electro stimulation, both are showed to reduce the seizure attack number as compared with epilepsy state induced by pilocarpine only while the seizure duration of each attack showed no significant difference between treatment groups.

Our study was greed with many researched that approved the synergistic effect of electro stimulation as found in Netherland study which concluded that vagus nerve stimulation to the treating rat is a clinically relevant animal model because it is a chronic epilepsy model that responded to electro stimulation with effects that are comparable to the effects of vagus nerve stimulation in patients with epilepsy. Additionally, this study would demonstrate that rats treated with VNS could be used to evaluate the VNS mode of action and the effective reduction of seizure severity and decrease the seizure induction of cardiac rhythm changes [15].

An American study indicate that VNS had significant reduction of the severity of pentylenetetrazole which induce seizures in rat groups as comparable with their no VNS as control group of seizure severity. There was no significant difference in the existed efficiency that depended on side of stimulation. These data would indicate that the right side of VNS in rats is the same efficacy as the left sided VNS [16]

Another study observed that Electro Acupuncture (EA) had anti-seizure effect at different sites of the auricle in rats. Rats would divide randomly into model, ear outer margin, ear apex, earlobe, cavitas cochae, cymba-concha groups according to area of stimulation. These five EA treatment groups were compared and noticed that that both the effects of cymba-concha and cavitas-conchae groups were significantly effective more than ear apex, earlobe and ear outer margin groups in elevating the latency of first seizure of the epilepsy, and suppressing the features of epileptic behaviors and the duration of seizures.

EA of cavitas conchae and cymba concha had a good effects in decreasing the seizures of epilepsy that can be associated with auricular branch of vagal nerve [17]. On the other hand, a Korean study concluded that vagus nerve stimulation by acupuncture at HT8 could decrease mRNA expression of TNF- α which indicate the ability to inhibit hippocampal cell death and suppress kainic acid induced epilepsy with inflammatory consequences that suggest a possible role for acupuncture in the treatment of epilepsy [18]. Additionally, a Chinese study found out that the acute anti-seizure duration of vagal nerve stimulation had no significant difference with electro-acupuncture at the auricular concha [AC]. Electro-acupuncture at AC was convenient, effective, and low-cost, that can be used as a synergistic therapy for epilepsy [19]. A study about electro acupuncture (EA) showed significantly reduction of times of the spontaneous recurrent attacks of seizure. Furthermore, EA had significant elevation in the expression of GAD₆₇ mRNA in DG of the granule cell layer, neither of the two sham controls indicated a significant effect on recurrent seizure or the expression of GAD₆₇ mRNA in granule cell layer or hilus. The data suggested that EA possessed some curative management on rats with seizure epilepsy, associated with changes of GAD₆₇ mRNA level in region of stimulation [20]. Another study of electrical stimulation in ear point had noticed that the convulsion behaviors were improved and the amounts of somatostatin, glutamine and aspartic acid in hippocampus of seizure rats and showed significant reduction. The amounts of taurine, glycine, and GABA had been elevated that suggested the electrical stimulation of ear point had anti epilepsy effects and related to the inhibition of somatostatin contents, aspartic acid and glutamine, while the contents of glycine, taurine and GABA elevated in hippocampus of seizure rat [21].

5. Conclusion:

1. Pilocarpine induced seizure attacks in all treatment groups
2. Both electro stimulation and diazepam administration in epilepsy groups were able to reduce the number of attacks for the four weeks of treatment period as compared with epilepsy control group but had no significant difference in the duration of each attack between treatment groups.

6. Recommendation:

1-This experimental study suggested for farther neurochemical markers measurements in all study groups.

2- Histopathology examinations were recommended to find out the histological changes that occurred due to different treatment groups.

References:

- [1]. Kwan P, Brodie MJ. Early Identification of Refractory Epilepsy. New England Journal of Medicine. Massachusetts Medical Society; 2000 Feb 3; 342[5]:314–9. <http://dx.doi.org/10.1056/nejm200002033420503>
- [2] Liu C-H, Lin Y-W, Tang N-Y, Liu H-J, Hsieh C-L. Neuroprotective Effect of Uncaria rhynchophyllain Kainic Acid-Induced Epileptic Seizures by Modulating Hippocampal Mossy Fiber Sprouting, Neuron Survival, Astrocyte Proliferation, and S100B Expression. Evidence-Based Complementary and Alternative Medicine. Hindawi Limited; 2012:1–11. <http://dx.doi.org/10.1155/2012/194790>
- [3] Vonck K, Thadani V, Gilbert K, Dedeurwaerdere S, De Groote L, De Herdt V, et al. Vagus Nerve Stimulation for Refractory Epilepsy: A Transatlantic Experience. Journal of Clinical Neurophysiology [Internet]. Ovid Technologies [Wolters Kluwer Health]; 2004 Jul;21[4]:283–9. <http://dx.doi.org/10.1097/01.wnp.0000139654.32974.4e>
- [4] BOON P, VONCK K, REUCK J, CAEMAERT J. Vagus nerve stimulation for refractory epilepsy. Seizure. Elsevier BV; 2001 Sep; 10 [6]:448–55. [http://dx.doi.org/10.1016/s1059-1311\[01\]90626-0](http://dx.doi.org/10.1016/s1059-1311[01]90626-0)
- [5] Johnson RL, Wilson CG. A review of vagus nerve stimulation as a therapeutic intervention. Journal of Inflammation Research. Dove Medical Press Ltd.; 2018 May; Volume 11:203–13. <http://dx.doi.org/10.2147/jir.s163248>
- [6] R. Talati, J. M. Scholle, O. P. Phung et al., “Efficacy and safety of innovator versus generic drugs in patients with epilepsy: a systematic review,” Pharmacotherapy; 2012, vol. 32, pp. 314–322. <https://doi.org/10.1002/j.1875-9114.2012.01099.x>
- [7] Racine RJ. Modification of seizure activity by electrical stimulation: II.

Motor seizure. *Electroencephalography and Clinical Neurophysiology*. Elsevier BV; 1972; 32[3]:281–94. <http://dx.doi.org/10.1016/0013-4694> [72]90177-0.

[8] Uthman BM. Vagus Nerve Stimulation for Seizures. *Archives of Medical Research*. Elsevier BV; 2000; 31[3]:300–3. [http://dx.doi.org/10.1016/s0188-4409\[00\]00060-6](http://dx.doi.org/10.1016/s0188-4409[00]00060-6)

[9] Berthoud HR, Neuhuber WL, Functional and chemical anatomy of the afferent vagal system. *Auton Neurosci*. 2000 Dec 20; 85[1-3]:1-17. [https://doi.org/10.1016/s1566-0702\[00\]00215-0](https://doi.org/10.1016/s1566-0702[00]00215-0)

[10] Qing Gao¹, Guoming Luan, Mechanisms of Vagus Nerve Stimulation for Epilepsy and Associated Comorbidities. Review Article - *Neuropsychiatry* [2017] Volume 0, Issue 0. <https://doi.org/10.4172/neuropsychiatry.1000s1003>

[11] Marchi N, Oby E, Batra A, Uva L, De Curtis M, Hernandez N, Van Boxel-Dezaire A, Najm I, Janigro D, In vivo and in vitro effects of pilocarpine: relevance to ictogenesis. *Epilepsia*. 2007; 48[10]:1934-46. <https://doi.org/10.1111/j.1528-1167.2007.01185.x>

[12] Rijkers K¹, Aalbers M, Hoogland G, van Winden L, Vles J, Steinbusch H, Majoie M. Acute seizure-suppressing effect of vagus nerve stimulation in the amygdala kindled rat. *Brain Res*. 2010 Mar 10; 1319: 155-63. <https://doi.org/10.1016/j.brainres.2010.01.014>

[13] Krah SE, Senanayake SS, Handforth A. Right-sided vagus nerve stimulation reduces generalized seizure severity in rats as effectively as left-sided. *Epilepsy Res*. 2003; 56[1]:1-4. [https://doi.org/10.1016/s0920-1211\[03\]00122-0](https://doi.org/10.1016/s0920-1211[03]00122-0)

[14] Lenz M, Ben Shimon M, Deller T, Vlachos A, Maggio N. Pilocarpine-Induced Status Epilepticus Is Associated with Changes in the Actin-Modulating Protein Synaptopodin and Alterations in Long-Term Potentiation in the Mouse Hippocampus. *Neural Plasticity*. Hindawi Limited; 2017; 2017:1–7. <http://dx.doi.org/10.1155/2017/2652560>

[15] Krah S. Vagus nerve stimulation for epilepsy: A review of the peripheral mechanisms. *Surgical Neurology International*. Medknow; 2012; 3[2]:47. Available from: <http://dx.doi.org/10.4103/2152-7806.91610>

[16] Deniz Sahin, Deniz Sahin, Gilbay Mimal, Vagus Nerve Stimulation Suppresses Generalized Seizure Activity and Seizure-Trigged Postictal

Cardiac Rhythm Changes in Rats Physiological research / Academia Scientiarum Bohemoslovaca ; 2009; 58[3]:345-50

[17] Ru-Peng Liu, Pei-Jing Rong, Pei-Jing Rong, Zhan-Xia Huang, Effect of electroacupuncture stimulation of auricular concha region at different time-points on behavior changes in depression rats, *Acupuncture research*, 2012; 37[2]:131-5

[18] Wei He, Pei-Jing Rong, Liang Li, Hui Ben, Bing Zhu, Gerhard Litscher. Auricular Acupuncture May Suppress Epileptic Seizures via Activating the Parasympathetic Nervous System: A Hypothesis Based on Innovative Methods; *Evid Based Complement Alternat Med.*; 2012: 615476. doi: 10.1155/2012/615476.

[19] Seung-Tae, Kim Ah Reum, Doo Seung-Nam Kim, Seung-Nam Kim et al. Acupuncture suppresses kainic acid-Induced neuronal death and inflammatory events in mouse hippocampus, *The Journal of Physiological Sciences*, 2012; 62[5]:377-83. doi: 10.1007/s12576-012-0216-9

[20] Jianjun Guo, Jianhua Li, Wenbin Fu, Wentao Ma, Zhenhua Xu, Mingquan Yu, Jian Song, The effect of electro acupuncture on spontaneous recurrent seizure and expression of GAD67 mRNA in dentate gyrus in a rat model of epilepsy. *Brain Research*, 2008, Vol. 1188, Pages 165–172. <https://doi.org/10.1016/j.brainres.2007.10.017>

[21] Shu, Jia, Liu, Rong Yu, Huang, Xian Fen; The Effects of Ear Point stimulation on the Contents of somatostatin and amino acid neurotransmitters in brain of rat with experimental seizure, *Acupuncture & Electro-Therapeutics Research*, Vol. 29, Numbers 1-2, 2004, pp. 43-51[9]. <https://doi.org/10.3727/036012904815901498>.