

## Using Atorvastatin and L-Carnitine in Prevention of Pilocarpine-Induced Seizures: Animal Model Study

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### Abstract

<b>Background Objective</b>	Epilepsy is a common chronic neurological disorder characterized by recurrent unprovoked seizures. To investigate the possible antiepileptic effect of both atorvastatin and L-carnitine on seizure induced by pilocarpine.
<b>Methods</b>	Fifty male albino mice weighing between 30-35 gm were equally allocated into five groups (each group contained 10 mice) and were given one of the following; control group; distal water group (0.1 ml), diazepam group (1mg/kg), atorvastatin group (5 mg/kg) and L-carnitine group (300 mg/kg). All animals (except normal group) were injected with Pilocarpine hydrochloride (350 mg/kg) to induce generalized tonic-clonic seizures 30 minutes after the tested drugs had been administrated. The mean onset of seizure were determined as well as the mean serum concentration of electrolytes, glutathione (GSH) and malondialdehyde (MDA) were measured after seizure had been induced.
<b>Results</b>	Pilocarpine induced seizure at approximately 7 minutes after injection, while both atorvastatin and L-carnitine produced highly significant increase in mean onset of seizure $14 \pm 0.471$ and $14.5 \pm 0.909$ respectively as compared to that of D.W. group, also both drugs produced highly significant changes in mean serum concentration of electrolytes, GSH and MDA.
<b>Conclusion</b>	Atorvastatin and L-carnitine had antiepileptic effects against seizures induced by pilocarpine when used at applied doses.
<b>Key words</b>	Epileptic seizure, Atorvastatin and L-carnitine.

**List of Abbreviation:** eNOS = Endothelial nitric oxide synthase, FTI = Franeyltransferase inhibitor, GABA = Gamma-aminobutyric acid, GSH = Reduced glutathione, GTPase = Guanosine triphosphatase, HMG-CoA = 3-hydroxy-3-methylglutaryl-coenzyme A, H-Ras = Harvey rat sarcoma viral oncogene homolog, I.P = Intraperitoneal, MDA, Malondialdehyde, NMDA = N-methyl-D-aspartate, NO = Nitric oxide, PTZ = Pentylentetrazole, RhoA = Ras homolog gene family, member A, SEM = Standard error of mean.

### Introduction

Epilepsy is one of the major neurological diseases in humans and about one percent of the population is affected by some forms of epilepsy <sup>(1)</sup>. It is characterized by recurrent, unprovoked, paroxysmal episodes of brain

dysfunction manifesting as a large number of clinical phenomena <sup>(2)</sup>.

Epilepsy occurs due to many different cellular or biochemical changes such as alterations in ion channel function, neurotransmitter level (excitatory and inhibitory), neurotransmitter receptor function and energy metabolism, in addition to the body electrolytes, level of some trace elements, and membrane lipid peroxidation due to increase in free radicals or decrease in activities of antioxidant defense mechanisms all these may be causally involved in some forms of epilepsy and may increase the recurrence of seizures <sup>(3)</sup>.

Pilocarpine provides a useful animal model for studying epilepsy. In this model, the seizures induced by pilocarpine show the involvement of the cholinergic system in seizures and status epilepticus<sup>(4)</sup>. The activation of muscarinic receptors is the first step for seizure activity, while GABAergic and glutamatergic systems appear to mediate seizure propagation and/or maintenance in rodent epilepsy models<sup>(5)</sup>.

Atorvastatin is antihyperlipidemic agent that belongs to statins group and inhibits the first committed enzymatic step of cholesterol synthesis, in addition to its antioxidant activity<sup>(6)</sup>. While L-carnitine is a quaternary ammonium compound, it is biosynthesized from the amino acids lysine and methionine in both liver and kidney<sup>(7)</sup>. In living cells, it is required for the transport of free long-chain fatty acids from the cytosol into the mitochondria during the breakdown of lipids, in addition it has antioxidant effect<sup>(8)</sup>. These effects of both drugs are useful in terminating epilepsy.

The current study was performed to investigate the possible antiepileptic effects of both atorvastatin and L-carnitine against seizures induced by pilocarpine.

## Methods

This study was performed on fifty healthy male albino mice weighing between 30-35 gm, they were supplied by animal house of Al-Nahrain College of Medicine and were housed under good conditions and fed standard oxid palate with water ad libitum, and they were equally allocated into five groups (10 mice in each group):

- **Group 1: (Normal group):** This group served as normal control and takes no drug used to detect the normal values of serum electrolytes, GSH and MDA.
- **Group 2: (Distilled water group):** They were injected 0.1 ml of distilled water (I.P.) 30 mint. before pilocarpine injection, to induce epileptic seizures.
- **Group 3: (Diazepam group):** They were injected 1mg/kg of diazepam (I.P.) 30 mint.

before pilocarpine injection. This group served as positive control and was used to compare onset of seizure only with tested groups.

- **Group 4: (Atorvastatin group):** They were injected atorvastatin 5 mg/kg (I.P.) 30 mint. before pilocarpine injection.
- **Group 5: (L-Carnitine group):** They were injected L-carnitine 300 mg/kg (I.P.) 30 mint. before pilocarpine injection.

After giving the pilocarpine, each mouse was carefully evaluated by detecting the following parameters, which include the onset of the first seizure recorded by naked eyes. At the end of observations the blood samples were collected from survival mice for measuring other parameters which are the serum concentrations  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , GSH and MDA.

Statistical analysis was performed with the SPSS 19.0 statistical package for social sciences, data were expressed as mean  $\pm$  Standard error of mean (SEM), unpaired t-test at ( $P \leq 0.01$ ) and ( $P \leq 0.05$ ) for independent data was used<sup>(9)</sup>.

## Results

All the mice exhibited generalized limbic seizures after pilocarpine administration, at a latency of ( $7 \pm 0.394$ ) minutes; besides, it caused highly significant reduction in mean serum  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and GSH concentration, while it caused highly significant increase in mean serum concentration of  $\text{K}^+$  and MDA when compared to that of normal group.

Diazepam caused highly significant increase in mean onset of seizure; also diazepam had no effect on other parameters when compared with D.W group.

Both atorvastatin and L-carnitine resulted in highly significant increase and highly significant reduction in mean onset of seizure when compared to that of both D.W. and diazepam groups respectively, also both drugs resulted in a highly significant increase in mean serum concentration of  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and GSH with highly significant reduction and non-significant change in mean serum concentration of MDA and  $\text{K}^+$  respectively when compared to that of D.W group (Table 1).

**Table 1. Effect of Atorvastatin and L-Carnitine on Onset of Seizure and Serum Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, GSH and MDA Concentrations in Group I, II, and III in Pilocarpine-induced Seizure in Mice**

Group	Seizure Onset (minute) M ± SEM	Concentration				
		Sodium mmol/l M ± SEM	Potassium mmol/l M ± SEM	Calcium mg/dl M ± SEM	GSH mmol/l M ± SEM	MDA mmol/l M ± SEM
I	0	144.8 ± 2.958	5.47 ± 0.212	8.5 ± 0.306	1.13 ± 0.063	5.02 ± 0.103
II	7 ± 0.394	133.2 ± 1.781a**	8.11 ± 0.502a**	7.03 ± 0.157a**	0.8 ± 0.052a**	10.66 ± 0.265a**
III	18.6 ± 0.858b**	134.5 ± 1.45	7.78 ± 0.54	7.35 ± 0.34	0.85 ± 0.05	10.5 ± 0.28
IV	14 ± 0.471b**,c	157.8 ± 2.439a**,b	7.9 ± 0.436a**	9.37 ± 0.379b**	1.5 ± 0.158a*,b**	7.52 ± 0.442a**,b**
V	14.5 ± 0.909b**,c	148.3 ± 3.72b**	7.71 ± 0.281 a**	11.32 ± 0.198a**,b**	1.2 ± 0.076b**	5.09 ± 0.211b**

\*  $P < \leq 0.05$ , \*\*  $P = \leq 0.01$ , a = as compared to group I, b = as compared to group II, c = as compared to group III, n = (10/group)

## Discussion

Epilepsy is one of the most common neurological problems all over the world, being associated with paroxysmal discharge of cerebral neurons and is characterized by several symptoms including alterations of behaviors and consciousness sustained alteration in brain function<sup>(10)</sup>.

Statins are competitive HMG-CoA reductase inhibitors, the later are the rate limiting enzyme for synthesis of cholesterol and isoprenoids. Indeed, the inhibitory role of statins in cholesterol biosynthesis previously reported to inhibit glutamate receptor (NMDA) function in induction of status epilepticus and excitotoxicity<sup>(11,12)</sup>, this provide that statins possess NMDA antagonist-like effects, which would partially explain the anti-seizure and neuroprotective activity<sup>(13)</sup>.

Statins are also expected to exert their anti-seizure and anti-excitotoxic activities through inhibition of isoprenoid synthesis and interfering with small GTPase signaling. Indeed, recent reports have demonstrated that inhibition of H-Ras farnesylation by treatment with franeyltransferase inhibitor (FTI) can inhibit NMDA-mediated excitotoxicity in the rat brain<sup>(14)</sup>. These reports support the suggestion that statins may modulate Kainic acid mediated seizure activity and excitotoxicity by down-regulation of H-Ras isoprenylation.

The neuroprotective efficacies of statins also mediated by their anti-inflammatory activity through inhibition of isoprenylation of small GTPase (Ras and RhoA)<sup>(15)</sup>. A similar inhibitory

role of statins in inflammatory reactions was observed in Kainic acid treated rats.

In addition statins act as anticonvulsant substance by upregulation eNOS, may be pivotal in enhancing cerebral arterial vasodilator responses and decreasing the firing threshold<sup>(16)</sup>.

The neuroprotective and anticonvulsive actions of L-carnitine can be mediated by a reduction in lipid peroxidation levels and nitrite content levels. This reduction is possibly due to the modulatory activity of L-carnitine in the antioxidant enzymes (superoxide dismutase and catalase) in the hippocampus of adult rats<sup>(8)</sup>.

The neuroprotective effect of acetyl-L-carnitine may be due to at least three modes of action. First, acetyl-L-carnitine has been shown to maintain cellular membrane stability, it can also act as an antioxidant, scavenging harmful superoxide radicals. Second, preserve normal levels of nerve growth factor in brain tissue during aging. Third, acetyl-L-carnitine increases cerebral blood flow<sup>(17)</sup>.

L-carnitine has anticonvulsive effects through the suppression of c-fos gene expression in the brain of mice after single administration of PTZ which play an important role in the development of seizures<sup>(18,19)</sup>.

L-carnitine has neuroprotective and antioxidant effect by preventing glutamate neurotoxicity and neuronal death mediated by activation of NMDA receptor, L-carnitine decrease the affinity of glutamate for the NMDA receptor<sup>(20)</sup>, L-carnitine also prevent the formation of NO radical due to over activation of NMDA receptor,

since NO reduces the activity of antioxidant enzymes, leading to increased formation of superoxides and oxidative stress<sup>(21)</sup>. In addition L-carnitine elevates nigral levels of glutathione and GABA which plays an important role in its anticonvulsant effect<sup>(22)</sup>.

All the tested drugs had valid preventive effect against seizure induced by pilocarpine in mice when compared to that of diazepam.

Finally the aim of present study to find a new drugs or combination of drugs which are more potent with less adverse effects to be used in epilepsy after confirmation with clinical trials.

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### Author contribution

The authors designed the experiments, interpreted the results and drafted the manuscript. Uday did the technique of this work and conducted the writing of manuscript. Dr. Faruk supervised scientifically reviewed the manuscript.

### Conflict of interest

The authors declare no conflict of interest.

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### References

1. Manna F, El-Shamy KA, El-Shaikh KA, et al. Efficacy of fish liver oil and propolis as neuroprotective agents in pilocarpine epileptic rats treated with valproate. *Pathophysiology*. 2011; 699: 1-8.
2. Clark AM, Finkel R, Rey JA, et al. Lippincott's illustrated reviews. *Pharmacology*. 5<sup>th</sup> ed. New York: Lippincott Williams and Wilkins; 2012. p. 181-92.
3. Hamed SA, Abdellah MM, El-Melegy N. Blood levels of trace elements, electrolytes, and oxidative stress/antioxidant systems in epileptic patients. *J Pharmacol Sci*. 2004; 96: 465-73.
4. Nascimento VS, Oliveira AA, Freitas RM, et al. Pilocarpine-induced status epilepticus: monoamine level, muscarinic and dopaminergic receptors alterations in striatum of young rats. *Neurosci Lett*. 2005; 383: 165-70.
5. Freitas RM, Oliveira AA, Vasconcelo SMM, et al. Expression of muscarinic and dopaminergic receptors and monoamine levels frontal cortex of epileptic rats. *Pharmacol Biochem Behav*. 2006; 83: 302-6.
6. Hadi NR, Abdelhussein MA, Alhamami OMO, et al. Antioxidant effect of atorvastatin in type 2 diabetic patients. *Pharmacol Pharm*. 2010; 1(2): 53-9.
7. Steiber A, Kerner J, Hoppel C. Carnitine: a nutritional, biosynthetic, and functional perspective. *Mol Aspects Med*. 2004; 25(5-6): 455-73.
8. Zanelli SA, Solenski NJ, Rosenthal RE, et al. Mechanisms of Ischemic Neuroprotection by Acetyl-L-carnitine. *Ann NY Acad Sci*. 2005; 1053: 153-61.
9. Daneil WW. *Biostatistics: A foundation for analysis in the health sciences*. 9<sup>th</sup> ed. New York: Wiley; 2009. p. 89-92,102-3.
10. Freitas RM, Sousa FCF, Vasconcelos SMM, et al. Pilocarpine-induced status epilepticus in rats: lipid peroxidation level, nitrite formation, GABAergic and glutamatergic receptor alterations in the hippocampus, striatum and frontal cortex. *Pharmacol Biochem Behav*. 2004; 78: 327-32.
11. Pedroni SM, Gonzalez JM, Wade J, et al. Complement inhibition and statins prevent fetal brain cortical abnormalities in a mouse model of preterm birth. *Biochim Biophys Acta*. 2014; 1842(1): 107-15.
12. Rodríguez-Yáñez M, Agulla J, Rodríguez-González R, et al. Statins and stroke. *Ther Adv Cardiovasc Dis*. 2008; 2(3): 157-66.
13. Wang Q, Zengin A, Deng C, et al. High dose of simvastatin induces hyperlocomotive and anxiolytic-like activities: the association with the up-regulation of NMDA receptor binding in the rat brain. *Exper Neurol*. 2009; 216: 132-8.
14. Ruocco A, Santillo M, Cicale M, et al. Farnesyl transferase inhibitors induce neuroprotection by inhibiting Ha-Ras signalling pathway. *Eur J Neurosci*. 2007; 26: 3261-6.
15. Xiao H, Qin X, Ping D, et al. Inhibition of Rho and Rac geranylgeranylation by atorvastatin is critical for preservation of endothelial junction integrity. *PLoS ONE*. 2013; 8(3): e59233.
16. Shafaroodi H, Moezi L, Fakhrzad A, et al. The involvement of nitric oxide in the anti-seizure effect of acute atorvastatin treatment in mice. *Neurol Res*. 2012; 34(9): 847-53.
17. Onem G, Aral E, Enli Y, et al. Neuroprotective effects of L-carnitine and vitamin E alone or in combination against ischemia-reperfusion injury in rats. *J Surg Res*. 2006; 131(1): 124-30.
18. Zou X, Sadovova N, Patterson TA, et al. The effects of L-carnitine on the combination of, inhalation anesthetic-induced developmental, neuronal apoptosis in the rat frontal cortex. *Neuroscience*. 2008; 151: 1053-65.

19. Iryo Y, Matsuoka M, Igisu H. Suppression of pentylenetetrazol-induced seizures and c-fos expression in mouse brain by L-Carnitine. *J Occup Health.* 2000; 42: 119-23.
20. Zhang R, Hong Z, Wang T, et al. Neuroprotective effects of pre-treatment with L-carnitine and acetyl-L-carnitine on ischemic injury in vivo and in vitro. *Int J Mol Sci.* 2012; 13(2): 2078-90.
21. Siktar E, Ekinci D, Beydemir S, et al. Protective role of L-carnitine supplementation against exhaustive exercise induced oxidative stress in rats. *Eur J Pharmacol.* 2011; 668: 407-13.
22. Scafidi S, Fiskum G, Lindauer SL, et al. Metabolism of acetyl-L-carnitine for energy and neurotransmitter synthesis in the immature rat brain. *J Neurochem.* 2010; 114: 820-31.

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