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*A Rostamkhani, N Mirazi, A Hosseini*

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## Effect of alpelisib, a selective phosphatidylinositol-3-kinase inhibitor, on seizure development in a rat pentylenetetrazole model

A. Rostamkhani<sup>1</sup> , N. Mirazi<sup>1,\*</sup> , A. Hosseini<sup>2</sup> 

<sup>1</sup>Department of Biology, Faculty of Basic Sciences, Bu-Ali Sina University, Hamedan, Iran

<sup>2</sup>Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran

**ABSTRACT:** Epilepsy is a neurological disease that results from an abnormality in the brain's activity. Phosphatidylinositol-3-kinase (PI3K) signaling pathway has played a crucial role in epilepsy pathogenesis. Alpelisib (ALP) is a selective inhibitor of PI3K. We examined the ability of ALP to treat pentylenetetrazole (PTZ)-induced convulsions in a rat model. Male Wistar rats (200-250 g, 8 weeks old) were injected intraperitoneally (IP) with ALP at different doses of 15 and 30 mg/kg, or vehicle 30 min prior to PTZ (70 mg/kg, IP) treatment. Racine's scale was used to assess behavioral seizures. The results showed that pretreatment with ALP prolonged the seizure stages according to the Racine scale, significantly decreased the duration of general tonic-clonic seizure (GTCS) and reduced the number of myoclonic jerks ( $P < 0.05$ ). In conclusion, based on results it was shown that ALP inhibited the development of PTZ-induced seizure in the experimental animal.

**Keywords:** Epilepsy; Alpelisib; Phosphatidylinositol-3-kinase; Seizure; Pentylenetetrazole.

*Corresponding Author:*

Naser Mirazi, Department of Biology, Faculty of Basic Sciences, Bu-Ali Sina University, Hamedan, Iran  
E-mail address: [mirazi205@gmail.com](mailto:mirazi205@gmail.com); [mirazi@basu.ac.ir](mailto:mirazi@basu.ac.ir)

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## INTRODUCTION

Epilepsy occurs as a result of an imbalance in excitatory and inhibitory brain signals, characterized by periodic seizures (Fisher et al. 2014). In addition to the excito-inhibitory mechanism, other mechanisms are involved in generation of epileptic seizures such as nonsynaptic electrical transmission through gap junctions, the involvement of non-neuronal glial cells such as astrocytes and microglia in triggering seizures, and oxidative stress causing metabolic dysfunction (Dudek et al. 1998; Devinsky et al. 2013; Pearson-Smith and Patel 2017). Conventional antiepileptic drugs function to either prolong the blocking of excitation or induce the inhibition of neurotransmission by acting on synaptic neuronal signals. The exact epileptogenic mechanism precipitating seizures remains a mystery of all research conducted so far on epilepsy. As well, the available AEDs offer only symptomatic relief without indicating factors that result in the cessation of epileptic seizures (Zeng et al. 2009a, b). Some patients remain resistant to conventional AEDs therapy with increasing incidence of seizure as well as associated conditions such as memory impairment, depression, anxiety-like behavior, etc., which reduces the quality of life of the patients. Several biomarkers of epilepsy (genetic, structural, functional, electrophysiological, and neuroinflammatory) have been identified as potential targets for improved outcome, but they come with their own set of challenges which have to be addressed with rationality (Pitkänen et al. 2016). Consequently, ongoing efforts are being made to identify the exact molecular pathways at play in epileptogenic processes, with a view to developing more effective agents for better epilepsy treatment (Kobow et al. 2012; Laxer et al. 2014).

The phosphatidylinositol-3-kinase (PI3K) pathway has gained significant attention in the last decade as a molecular target for various diseases. PI3K belongs to the family of lipid kinases, which are responsible for a number of cellular functions including development, proliferation, metabolism, and differentiation (Engelman et al. 2006). This pathway is activated when growth factors (e.g., epidermal growth factor, insulin-like growth factor, human epidermal growth factor) get attached to it or receptor tyrosine kinases bind to it (Kapeller and Cantley 1994). In the progression of epilepsy, PI3K phosphorylates the serine/threonine protein kinase (AKT) to activate the mechanistic target of rapamycin (mTOR) pathway and its downstream genes (Berdichevsky et al. 2013). The use of specific inhibitors has demonstrated an ability

to suppress epilepsy resulting from hyperactivation of mTOR (Huang et al. 2010). For this reason, inhibiting the mTOR pathway is a good treatment for epilepsy (Citraro et al. 2016). Furthermore, PI3K activation and subsequent cellular processes require phosphorylation for its activation (Cohen et al. 1990). Class I PI3Ks (which are composed of two subunits, p110 and p85), have been extensively studied in diseases that alter the normal function of the body (Vanhaesebroeck et al. 2016).

The PI3K inhibitor alpelisib (ALP) is being studied for treating breast cancer, specifically targeting PI3K alpha (PI3K $\alpha$ ). It is well known that the PIK3CA gene, which encodes the catalytic subunit (p110 $\alpha$ ) of PI3K, is frequently mutated or amplified in solid tumours, including approximately 40 % of breast cancers; therefore, it is an attractive therapeutic target. In response to PI3K activation, several intracellular enzymes necessary for cellular proliferation, survival, and motility are recruited into the cell (Markham 2019). Research has shown that treatments with PI3K inhibitors during embryogenesis caused neuronal death and initiated apoptosis in primary cultures, despite being essential to brain development and neuroprotection (Chen et al. 2017; Dai et al. 2012). Based on the literature, it is evident that PI3K inhibitors, such as ALP, act both as neuroprotectants and apoptosis inducers. However, as an antiepileptic, yet neuroprotective and pro-apoptotic mTOR inhibitor, rapamycin possesses a similar profile (Ding et al. 2015; Saqcena et al. 2015), thus justifying the use of ALP in our study.

This study examined the anticonvulsant potential of ALP in a rat model of pentylenetetrazole (PTZ)-mediated seizures.

## MATERIALS AND METHODS

### Drugs and chemicals

The PTZ was obtained from Sigma Aldrich, USA. Phenobarbital sodium (PHB) was procured from Chemidarou Pharmaceutical Company, Iran and used as a comparison to conventional anticonvulsant drugs. ALP was obtained from Gilead Sciences, Inc, Canada. PTZ was prepared as a 1% v/w solution in saline. PHB and ALP were dissolved in dimethyl sulfoxide (DMSO) (60 mg/mL) and stored at -20 °C.

### Animal maintenance and ethical consideration

We maintained eight-week-old adult male Wistar rats (*Rattus norvegicus*) (200-250 g) in standard cages

with a temperature of 20-24 °C and a light-dark cycle of 12:12 h, with access to fresh water and food. All methods were carried out in accordance with relevant guidelines and regulations, the ethics of working with animals were observed throughout the whole study in accordance with the Animal Research Reporting In Vivo Experiments (ARRIVE) guidelines and the research protocol was also approved by the Bu-Ali Sina University's Research Ethics Committee (IR. BASU. REC. 1400. 055). In current study the sample size was twenty rats ( $n = 5$  per group) which is adequate to reproducibly calculate the variability and statistical differences between the groups as shown by previous works (Ye-wei et al. 2015; Santos et al. 2017; Panahi et al. 2020).

#### **PTZ-mediated seizures in rats**

The animals were randomly assigned into 4 groups via simple randomization ( $n = 5$ /group) as follow:

- Negative control group (NC): In this group, there were adult male rats administrated with vehicle (DMSO) diluted 100-fold with distilled water)30 min prior to PTZ (70 mg/kg) exposure.
- Positive control group (PHB): In this group, there were adult male rats administrated with PHB (30 mg/kg) 30 min prior to PTZ (70 mg/kg) exposure.
- Experimental group 1 (ALP15): In this group, there were adult male rats administrated with ALP (15 mg/kg) 30 min prior to PTZ (70 mg/kg) exposure.
- Experimental group 2 (ALP30): In this group, there were adult male rats administrated with ALP (30 mg/kg) 30 min prior to PTZ (70 mg/kg) exposure.

Throughout the study, fresh solutions were administered intraperitoneally (IP) at a constant volume of 4 mL/kg. The doses of PTZ, PHB, and ALP were chosen based on previous studies on experimental animal models (Azizi et al. 2018; Wang et al. 2021; Hedges et al. 2021). Also, previous studies at this concentration of DMSO did not show any negative physiological effects via IP route (George et al. 2020; Alsuwaidi et al. 2017). A 30 min cut-off time was used to determine the behavior of each rat individually after exposure to PTZ. A 5-point scale was used to evaluate the convulsive behavior caused by PTZ as, Stage 1: ear and facial twitching; Stage 2: head nodding and myoclonic jerks; Stage 3: unilateral forelimb clonus with lordotic posture; Stage 4: bilateral forelimb clonus with rearing and falling; and Stage 5: generalized tonic-clonic

seizure (GTCS) with loss of postural tone (Hosseini et al. 2022). We recorded each rat's seizure activity with the help of video tracking software (SMART V3. 0, Panlab, Barcelona) connected to a camera mounted at the center of the chamber. The latency to each stage (S1-5), number of myoclonic jerks, and GTCS duration was also recorded in a blind fashion with help of two independent observer. The rats were euthanized 24 h after the last treatment using a chamber pre-filled with carbon dioxide (CO<sub>2</sub>) gas with a concentration of 70% which, is a common and safe method for euthanizing (Conlee et al. 2005).

#### **Statistical analysis**

All the results were expressed as mean  $\pm$  standard deviation. Testing the normality of data was done using Shapiro-Wilk test. Given to the normal distribution of the data the statistical significant difference in behavioral parameters among different groups was examined by one-way analysis of variance followed by Tukey's post hoc test using GraphPad Prism version 9. 4. 0 for Windows (GraphPad Software, San Diego, California USA). Statistical significance was defined as  $P < 0.05$  for the results

## **RESULTS**

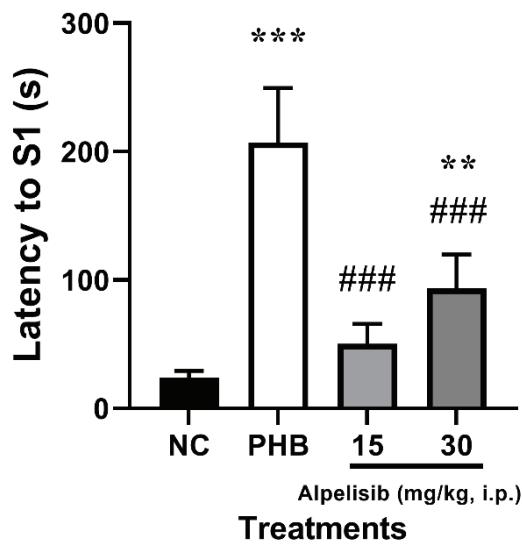
#### **Effect of ALP on PTZ-mediated seizures in adult rats**

The latency to S1 was significantly ( $P = 0.003$ ) increased in the rats treated with 30 mg/kg of ALP as compared to NC group [ $F_{(3,16)} = 47.45, P < 0.001$ ]. However, insignificant ( $P = 0.405$ ) change was observed at 15 mg/kg of ALP as that of NC group (Fig. 1).

PHB group showed significant ( $P < 0.001$ ) increase in latency to S2 as compared to NC group of rats [ $F_{(3,16)} = 30.83, P < 0.001$ ]. The latency to S2 was significantly ( $P = 0.002$ ) increased in the group pre-exposed to ALP at dose of 30 mg/kg followed by PTZ (Fig. 2).

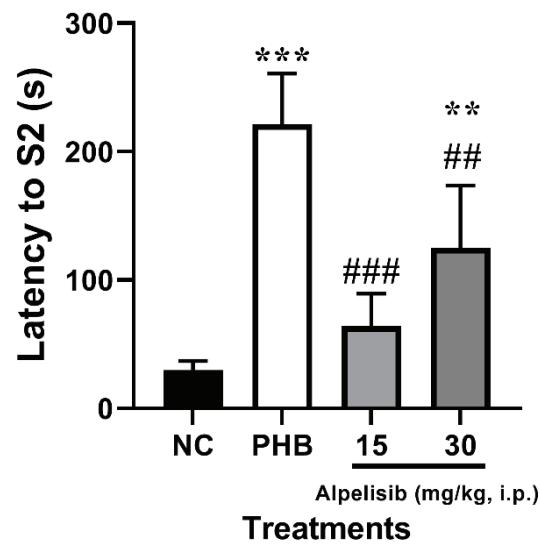
The seizure latency to S3 was significantly ( $P = 0.025$ ) increased in the rats treated with 30 mg/kg of ALP as compared to NC group [ $F_{(3,16)} = 10.16, P < 0.001$ ]. However, insignificant ( $P = 0.114$ ) change was observed at 15 mg/kg of ALP as that of NC group (Fig. 3).

The latency to S4 seizures onset in rats was  $42.80 \pm 17.60$  s following 70 mg/kg PTZ exposure in NC group (Fig. 2). The latency to S4 was significantly increased in the groups of rats pre-treated with PBH ( $P <$



**Fig. 1.** The effect of ALP (15 and 30 mg/kg, i.p.) 30 min prior to PTZ exposure (70 mg/kg, i.p.) on latency to Stage 1 (S1) in male Wistar rats.

Data are shown as mean  $\pm$  SD of  $n = 5$  rat/group. \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  significant difference vs. NC group. #\*\* $P < 0.001$  significant difference vs. PBH group. ALP: Alpelisib, PBH: Phenobarbital sodium, NC: Negative Control, PTZ: Pentylenetetrazole.



**Fig. 2.** The effect of ALP (15 and 30 mg/kg, i.p.) 30 min prior to PTZ exposure (70 mg/kg, i.p.) on latency of to Stage 2 (S2) in male Wistar rats.

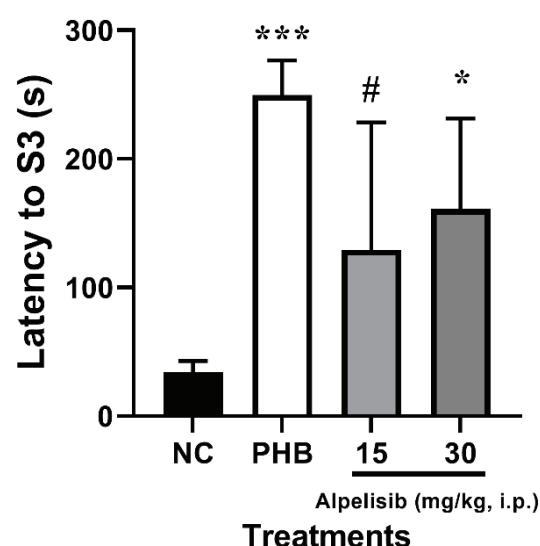
Data are shown as mean  $\pm$  SD of  $n = 5$  rat/group. \*\* $P < 0.01$ , and \*\*\* $P < 0.001$  significant difference vs. NC group. #\*\* $P < 0.01$ , and #\*\* $P < 0.001$  significant difference vs. PBH group. ALP: Alpelisib, PBH: Phenobarbital sodium, NC: Negative Control, PTZ: Pentylenetetrazole.

0.001), however significantly ( $P = 0.033$ ) change was only observed at 30 mg/kg (ALP group) as compared to NC group [ $F_{(3,16)} = 9.91, P < 0.001$ ] (Fig. 4).

The latency to S5 was significantly ( $P < 0.001$ ) increased following PTZ exposure in rat of PHB group as compared to NC group [ $F_{(3,16)} = 9.27, P < 0.001$ ]. The increased latency to S5 was found to be significantly ( $P = 0.041$ ) increased in 30 mg/kg ALP pre-incubated group as compared to NC group (Fig. 5).

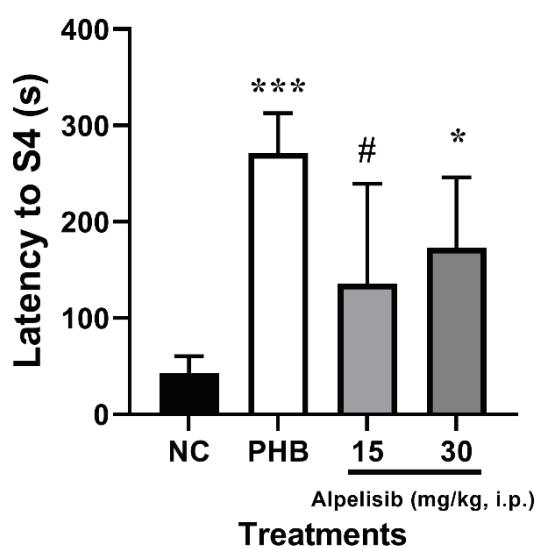
The number of myoclonic jerks was found to be  $54.80 \pm 19.27$  in NC group (Fig. 6). A marked decrease in the number was observed in experimental groups treated with ALP at doses of 15 and 30 mg/kg as compared to NC group ( $P = 0.005$  and  $P = 0.001$ ; respectively) [ $F_{(3,16)} = 11.18, P < 0.001$ ].

GTCS duration showed a significant change in the tested adult rats between groups [ $F_{(3,16)} = 16.08, P < 0.001$ ]. The GTCS duration significantly ( $P = 0.004$ ) reduced to  $23.60 \pm 8.35$  s in groups treated with 30 mg/kg of ALP, as compared to NC group. However, insignificant ( $P = 0.055$ ) change was observed at 15 mg/kg of ALP as that of NC group (Fig. 7).



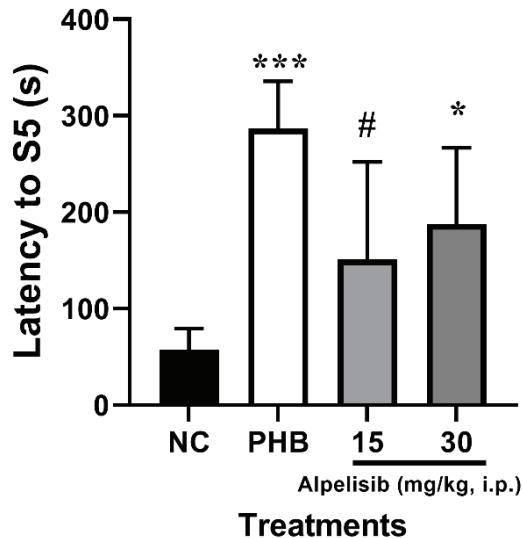
**Fig. 3.** The effect of ALP (15 and 30 mg/kg, i.p.) 30 min prior to PTZ exposure (70 mg/kg, i.p.) on latency to Stage 3 (S3) in male Wistar rats.

Data are shown as mean  $\pm$  SD of  $n = 5$  rat/group. \* $P < 0.05$ , and \*\*\* $P < 0.001$  significant difference vs. NC group. # $P < 0.05$  significant difference vs. PBH group. ALP: Alpelisib, PBH: Phenobarbital sodium, NC: Negative Control, PTZ: Pentylenetetrazole.



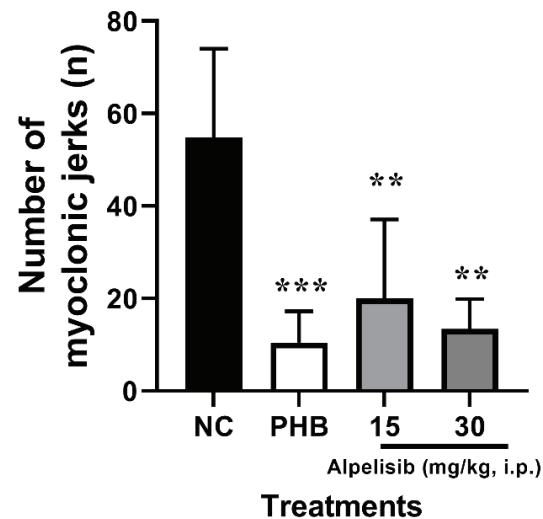
**Fig. 4.** The effect of ALP (15 and 30 mg/kg, i.p.) 30 min prior to PTZ exposure (70 mg/kg, i.p.) on latency of to Stage 4 (S4) in male Wistar rats.

Data are shown as mean  $\pm$  SD of  $n = 5$  rat/group.  $^*P < 0.05$ , and  $^{***}P < 0.001$  significant difference vs. NC group.  $^{\#}P < 0.05$  significant difference vs. PBH group. ALP: Alpelisib, PBH: Phenobarbital sodium, NC: Negative Control, PTZ: Pentylenetetrazole.



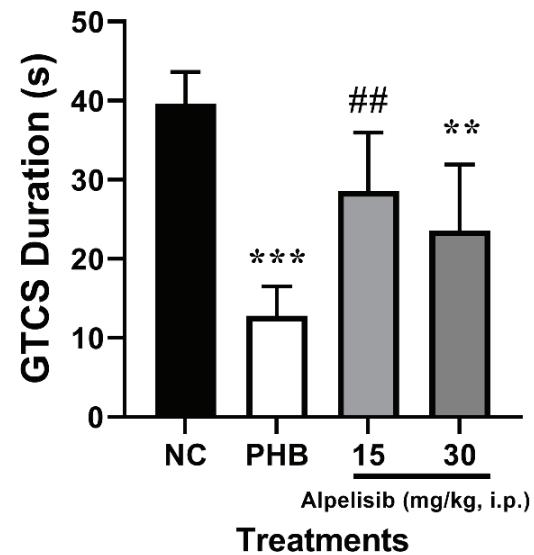
**Fig. 5.** The effect of ALP (15 and 30 mg/kg, i.p.) 30 min prior to PTZ exposure (70 mg/kg, i.p.) on latency to Stage 5 (S5) in male Wistar rats.

Data are shown as mean  $\pm$  SD of  $n = 5$  rat/group.  $^*P < 0.05$ , and  $^{***}P < 0.001$  significant difference vs. NC group.  $^{\#}P < 0.05$  significant difference vs. PBH group. ALP: Alpelisib, PBH: Phenobarbital sodium, NC: Negative Control, PTZ: Pentylenetetrazole.



**Fig. 6.** The effect of ALP (15 and 30 mg/kg, i.p.) 30 min prior to PTZ exposure (70 mg/kg, i.p.) on number of myoclonic jerks in male Wistar rats.

Data are shown as mean  $\pm$  SD of  $n = 5$  rat/group.  $^{**}P < 0.01$ , and  $^{***}P < 0.001$  significant difference vs. NC group. ALP: Alpelisib, PBH: Phenobarbital sodium, NC: Negative Control, PTZ: Pentylenetetrazole.



**Fig. 7.** The effect of ALP (15 and 30 mg/kg, i.p.) 30 min prior to PTZ exposure (70 mg/kg, i.p.) on GTCS duration in male Wistar rats.

Data are shown as mean  $\pm$  SD of  $n = 5$  rat/group.  $^{**}P < 0.01$ , and  $^{***}P < 0.001$  significant difference vs. NC group.  $^{##}P < 0.01$  significant difference vs. PBH group. ALP: Alpelisib, PBH: Phenobarbital sodium, NC: Negative Control, GTCS: generalized tonic clonic seizure, PTZ: Pentylenetetrazole.

## DISCUSSION

In the present study, we examined ALP's anticonvulsant properties in an experimental model for the first time. In the group that was pre-incubated with ALP, the latency to Racine's stages was significantly higher than in the NC group. A significant reduction in the number of myoclonic jerks was noted following pre-treatment with ALP at a dose of 15 or 30 mg/kg, compared with the NC group. Following treatment with 15 or 30 mg/kg of ALP, our results also revealed that GTCS duration decreased significantly in adult rats.

The use of PTZ, a common chemoconvulsant, causes hyperlocomotor activity in rats and seizures. PTZ exposure induces epileptic seizures by provoking hyperlocomotion, behavioural seizures and electrophysiological parameters (electrical events occurring in the brain) (Baraban et al. 2005). In studies, conventional antiepileptic drugs have been shown to reduce locomotion in rats with PTZ models and decrease seizure severity (Azizi et al. 2018; Hosseini et al. 2022). In our study, we also found that rats that were pre-treated with 15 or 30 mg/kg of ALP showed longer latency to Stages 1-5 seizures when compared to the NC group. Following ALP treatment, there was a decrease in the number of the myoclonic jerks and the duration of GTCS associated with an increase in seizure activity due to PTZ. The results of this study support the anticonvulsant effects of ALP.

It has been extensively explored how PI3K/AKT/mTOR participate in various neurological disorders, including epilepsy (Crino 2015; Mazumder et al. 2019a). Furthermore, it has been demonstrated that hyperactivation of this pathway causes further seizure generation and propagation (Pene et al. 2002; Dai et al. 2012; Xiao et al. 2016). The PI3K/AKT signaling cascade has also been shown to exert neuroprotective effects in animal models, however (Chen et al. 2017). According to scientific literature, PI3K is necessary for AKT phosphorylation in order to activate mTOR, the master regulator of cellular processes, when activated by growth factors and receptor tyrosine kinases (Laplante and Sabatini 2012; Mazumder et al. 2016). Literature indicates that mTOR plays an important role in epilepsy and can be controlled through its action (Galanopoulou et al. 2012). Several therapeutic

interventions for epilepsy, especially in cases of acquired epilepsy, have made use of mTOR inhibitors. There are, however, a number of adverse side effects associated with these inhibitors, including rapamycin (Zeng et al. 2009a, b; Mazumder et al. 2016). Therefore, research is continuing to identify mTOR inhibitors that are effective and safe for treating epilepsy. PI3K/AKT/mTOR pathway involvement in epilepsy disease is demonstrated in the present study by the anticonvulsant action of a selective PI3K inhibitor ALP. An mTOR pathway activation study in rats with acute seizures induced by PTZ supports these findings (Zhang and Wong 2012). Accordingly, PI3K expression increased following acute exposure to PTZ, thereby activating AKT and mTOR downstream genes.

Considering ALP's selectivity toward PI3K class I targets, it might also change the central signaling pathway, altering the normal cellular physiology in this manner (Markham 2019). For these results to be consolidated, more studies are needed. The complex mechanism of hyperactivation of PI3K/AKT/mTOR in rodents associated with epilepsy has been linked to altered gene expression earlier in the study (Meng et al. 2013; Mazumder et al. 2019b). By inhibiting PI3K using ALP, our study showed anticonvulsant effects on animals that generated and progressed seizures. There are a number of limitations to the present study. We did not measure the biochemical and molecular components of PI3K/AKT/mTOR pathway, which are important in the pathology of epilepsy because of limited funding and time constraints, yet our results could pave the way for future studies.

## CONCLUSION

Based on our findings, ALP, a PI3K inhibitor, reduced PTZ-mediated seizures in adult rats. ALP may inhibit PI3K/AKT/mTOR pathways, which contributes to its anticonvulsant effect. ALP has proven to be a more effective therapeutic intervention for epilepsy based on our study's results. The safety and efficacy of this drug in other epilepsy models must, however, be determined through other studies.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## REFERENCES

Alsuwaidi AR, George JA, Almarzooqi S, Hartwig SM, Varga SM, Souid AK (2017) Sirolimus alters lung pathology and viral load following influenza A virus infection. *Respir Res* 18 (1):136. doi:10.1186/s12931-017-0618-6

Azizi V, Allahyari F, Hosseini A (2018) The antiepileptic and neuroprotective effect of the Buxus hyrcana Pojark hydroethanolic extract against the pentylenetetrazole induced model of the seizures in the male rats. *Int J Neurosci* 128 (10):913-919. doi:10.1080/00207454.2018.1443925

Baraban SC, Taylor MR, Castro PA, Baier H (2005) Pentylenetetrazole induced changes in zebrafish behavior, neural activity and c-fos expression. *Neuroscience* 131 (3):759-768. doi:10.1016/j.neuroscience.2004.11.031

Berdichevsky Y, Dryer AM, Saponjian Y, Mahoney MM, Pimentel CA, Lucini CA, Usenovic M, Staley KJ (2013) PI3K-Akt signaling activates mTOR-mediated epileptogenesis in organotypic hippocampal culture model of post-traumatic epilepsy. *J Neurosci* 33 (21):9056-9067. doi:10.1523/jneurosci.3870-12.2013

Chen S, Liu Y, Rong X, Li Y, Zhou J, Lu L (2017) Neuroprotective Role of the PI3 Kinase/Akt Signaling Pathway in Zebrafish. *Front Endocrinol (Lausanne)* 8:21. doi:10.3389/fendo.2017.00021

Citraro R, Leo A, Constanti A, Russo E, De Sarro G (2016) mTOR pathway inhibition as a new therapeutic strategy in epilepsy and epileptogenesis. *Pharmacol Res* 107:333-343. doi:10.1016/j.phrs.2016.03.039

Cohen B, Liu YX, Druker B, Roberts TM, Schaffhausen BS (1990) Characterization of pp85, a target of oncogenes and growth factor receptors. *Mol Cell Biol* 10 (6):2909-2915. doi:10.1128/mcb.10.6.2909-2915.1990

Conlee KM, Stephens ML, Rowan AN, King LA (2005) Carbon dioxide for euthanasia: concerns regarding pain and distress, with special reference to mice and rats. *Lab Anim* 39 (2):137-161. doi:10.1258/0023677053739747

Crino PB (2015) mTOR signaling in epilepsy: insights from malformations of cortical development. *Cold Spring Harb Perspect Med* 5 (4). doi:10.1101/cshperspect. a022442

Dai R, Xia Y, Mao L, Mei Y, Xue Y, Hu B (2012) Involvement of PI3K/Akt pathway in the neuroprotective effect of Sonic hedgehog on cortical neurons under oxidative stress. *J Huazhong Univ Sci Technolog Med Sci* 32 (6):856-860. doi:10.1007/s11596-012-1047-x

Devinsky O, Vezzani A, Najjar S, De Lanerolle NC, Rogawski MA (2013) Glia and epilepsy: excitability and inflammation. *Trends Neurosci* 36 (3):174-184. doi:10.1016/j.tins.2012.11.008

Ding K, Wang H, Wu Y, Zhang L, Xu J, Li T, Ding Y, Zhu L, He J (2015) Rapamycin protects against apoptotic neuronal death and improves neurologic function after traumatic brain injury in mice via modulation of the mTOR-p53-Bax axis. *J Surg Res* 194 (1):239-247. doi:10.1016/j.jss.2014.09.026

Dudek FE, Yasumura T, Rash JE (1998) 'Non-synaptic' mechanisms in seizures and epileptogenesis. *Cell Biol Int* 22 (11-12):793-805. doi:10.1006/cbir.1999.0397

Engelman JA, Luo J, Cantley LC (2006) The evolution of phosphatidylinositol 3-kinases as regulators of growth and metabolism. *Nat Rev Genet* 7 (8):606-619. doi:10.1038/nrg1879

Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, Engel J, Jr., Forsgren L, French JA, Glynn M, Hesdorffer DC, Lee BI, Matherne GW, Moshé SL, Perucca E, Scheffer IE, Tomson T, Watanabe M, Wiebe S (2014) ILAE official report: a practical clinical definition of epilepsy. *Epilepsia* 55 (4):475-482. doi:10.1111/epi.12550

Galanopoulou AS, Gorter JA, Cepeda C (2012) Finding a better drug for epilepsy: the mTOR pathway as an antiepileptic target. *Epilepsia* 53 (7):1119-1130. doi:10.1111/j.1528-1167.2012.03506.x

George JA, Alshebli Z, Alneyadi A, Al Mukhaini N, Al-Salam S, Sudhadevi M, Souid AK, Alsuwaidi AR (2020) Idelalisib induces apoptosis in the lymphoid tissues and impairs lung function in mice. *J Chemother* 32 (2):88-97. doi:10.1080/1120009x.2019.1708153

Hedges CP, Boix J, Jaiswal JK, Shetty B, Shepherd PR, Merry TL (2021) Efficacy of Providing the PI3K p110 $\alpha$  Inhibitor BYL719 (Alpelisib) to Middle-Aged Mice in Their Diet. *Biomolecules* 11 (2). doi:10.3390/biom11020150

Hosseini A, Allahyari F, Azizi V (2022) Effect of Stachys lavandulifolia on Pentylenetetrazole-Induced Seizures, Passive Avoidance Learning, and Oxidative Stress in Male Wistar Rats. *Arch Epilepsy* 28 (2):53-58

Huang X, Zhang H, Yang J, Wu J, McMahon J, Lin Y, Cao Z, Grunenthal M, Huang Y (2010) Pharmacological inhibition of the mammalian target of rapamycin pathway suppresses acquired epilepsy. *Neurobiol Dis* 40 (1):193-199. doi:10.1016/j.nbd.2010.05.024

Kapeller R, Cantley LC (1994) Phosphatidylinositol 3-kinase. *Bioessays* 16 (8):565-576. doi:10.1002/bies. 950160810

Kobow K, Auvin S, Jensen F, Löscher W, Mody I, Potschka H, Prince D, Sierra A, Simonato M, Pitkänen A, Nehlig A, Rho JM (2012) Finding a better drug for epilepsy: antiepileptogenesis targets. *Epilepsia* 53 (11):1868-1876. doi:10.1111/j.1528-1167.2012.03716.x

Laplante M, Sabatini DM (2012) mTOR signaling in growth control and disease. *Cell* 149 (2):274-293. doi:10.1016/j.cell.2012.03.017

Laxer KD, Trinka E, Hirsch LJ, Cendes F, Langfitt J, Delanty N, Resnick T, Benbadis SR (2014) The consequences of refractory epilepsy and its treatment. *Epilepsy Behav* 37:59-70. doi:10.1016/j.yebeh.2014.05.031

Markham A (2019) Alpelisib: First Global Approval. *Drugs* 79 (11):1249-1253. doi:10.1007/s40265-019-01161-6

Mazumder AG, Kumari S, Singh D (2019a) Anticonvulsant action of a selective phosphatidylinositol-3-kinase inhibitor LY294002 in pentylenetetrazole-mediated convulsions in zebrafish. *Epilepsy Res* 157:106207. doi:10.1016/j.epilepsyres.2019.106207

Mazumder AG, Padwad YS, Singh D (2016) Anticancer Mammalian Target of Rapamycin (mTOR) Signaling Pathway Inhibitors: Current Status, Challenges and Future Prospects in Management of Epilepsy. *CNS Neurol Disord Drug Targets* 15 (8):945-955. doi:10.2174/1871527315666160615022203

Mazumder AG, Patial V, Singh D (2019b) Mycophenolate mofetil contributes to downregulation of the hippocampal interleukin type 2 and 1 $\beta$  mediated PI3K/AKT/mTOR pathway hyperactivation and attenuates neurobehavioral comorbidities in a rat model of temporal lobe epilepsy. *Brain Behav Immun* 75:84-93. doi:10.1016/j.bbi.2018.09.020

Meng XF, Yu JT, Song JH, Chi S, Tan L (2013) Role of the mTOR signaling pathway in epilepsy. *J Neurol Sci* 332 (1-2):4-15. doi:10.1016/j.jns.2013.05.029

Panahi Y, Kiani Fard D, Feyzi F (2020) Effect of Oral Methylphenidate on the Experimental Epileptiform Activity in Male Rats. *J Arak Uni Med Sci* 22 (6):252-261. doi:10.32598/jams.22.6.5959.1

Pearson-Smith JN, Patel M (2017) Metabolic Dysfunction and Oxidative Stress in Epilepsy. *Int J Mol Sci* 18 (11). doi:10.3390/ijms18112365

Pene F, Claessens YE, Muller O, Viguié F, Mayeux P, Dreyfus F, Lacombe C, Bouscary D (2002) Role of the phosphatidylinositol 3-kinase/Akt and mTOR/P70S6-kinase pathways in the proliferation and apoptosis in multiple myeloma. *Oncogene* 21 (43):6587-6597. doi:10.1038/sj.onc.1205923

Pitkänen A, Löscher W, Vezzani A, Becker AJ, Simonato M, Lukasiuk K, Gröhn O, Bankstahl JP, Friedman A, Aronica E, Gorter JA, Ravizza T, Sisodiya SM, Kokaia M, Beck H (2016) Advances in the development of biomarkers for epilepsy. *Lancet Neurol* 15 (8):843-856. doi:10.1016/s1474-4422(16)00112-5

Santos AC, Temp FR, Marafiga JR, Pillat MM, Hessel AT, Ribeiro LR, Miyazato LG, Oliveira MS, Mello CF (2017) EP2 receptor agonist ONO-AE1-259-01 attenuates pentylenetetrazole- and pilocarpine-induced seizures but causes hippocampal neurotoxicity. *Epilepsy Behav* 73:180-188. doi:10.1016/j.yebeh.2017.03.033

Saqcena M, Patel D, Menon D, Mukhopadhyay S, Foster DA (2015) Apoptotic effects of high-dose rapamycin occur in S-phase of the cell cycle. *Cell Cycle* 14 (14):2285-2292. doi:10.1080/15384101.2015.1046653

Vanhaesebroeck B, Whitehead MA, Piñeiro R (2016) Molecules in medicine mini-review: isoforms of PI3K in biology and disease. *J Mol Med (Berl)* 94 (1):5-11. doi:10.1007/s00109-015-1352-5

Wang Q, Lan X, Zhao Z, Su X, Zhang Y, Zhou XY, Xu RA (2021) Characterization of Alpelisib in Rat Plasma by a Newly Developed UPLC-MS/MS Method: Application to a Drug-Drug Interaction Study. *Front Pharmacol* 12:743411. doi:10.3389/fphar.2021.743411

Xiao Z, Peng J, Gan N, Arafat A, Yin F (2016) Interleukin-1 $\beta$  Plays a Pivotal Role via the PI3K/Akt/mTOR Signaling Pathway in the Chronicity of Mesial Temporal Lobe Epilepsy. *Neuroimmunomodulation* 23 (5-6):332-344. doi:10.1159/000460254

Ye-wei X, Rong W, Xun-tai M, Shan Z, Qian C, Shi-hua H, Fu-qun M, Xiao-ming X (2015) Cognitive impairment and spontaneous epilepsy in rats with malformations of cortical development. *Seizure* 33:29-34. doi:10.1016/j.seizure.2015.10.005

Zeng LH, Rensing NR, Wong M (2009a) Developing Antiepileptogenic Drugs for Acquired Epilepsy: Targeting the Mammalian Target of Rapamycin (mTOR) Pathway. *Mol Cell Pharmacol* 1 (3):124-129. doi:10.4255/mcpharmacol.09.16

Zeng LH, Rensing NR, Wong M (2009b) The mammalian target of rapamycin signaling pathway mediates epileptogenesis in a model of temporal lobe epilepsy. *J Neurosci* 29 (21):6964-6972. doi:10.1523/jneurosci.0066-09.2009

Zhang B, Wong M (2012) Pentylenetetrazole-induced seizures cause acute, but not chronic, mTOR pathway activation in rat. *Epilepsia* 53 (3):506-511. doi:10.1111/j.1528-1167.2011.03384.x