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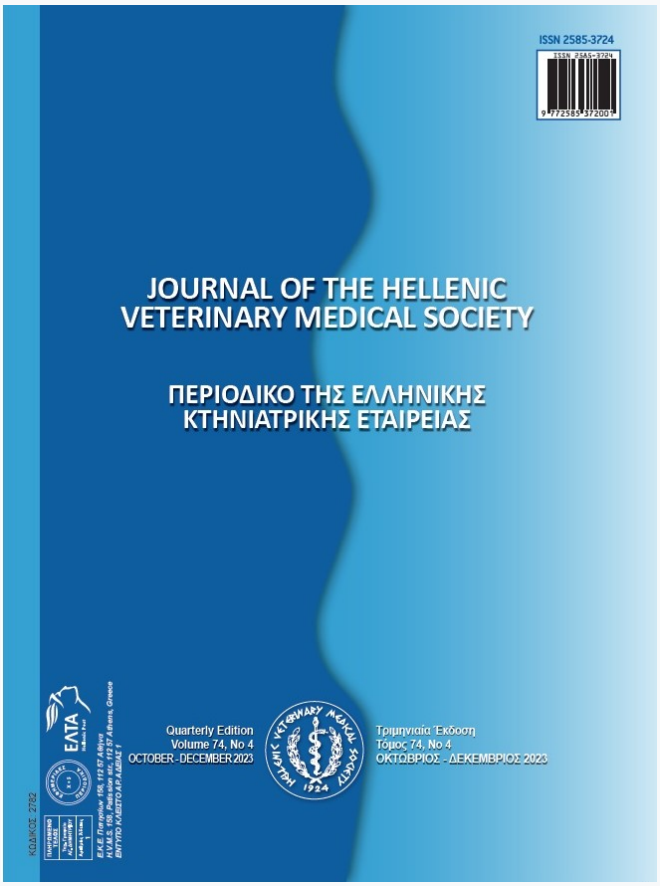

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Research article
Ερευνητικό άρθρο

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

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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.

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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 (PI3Kα). It is well known that the PIK3CA gene, which encodes the catalytic subunit (p110α) 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 ALPwere dissolved indimethyl 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 lorditic 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 prefilled with carbon dioxide (CO_2) 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 ± 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 latencytoS1 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 ALPas 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 toS3 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± 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 ($P<$

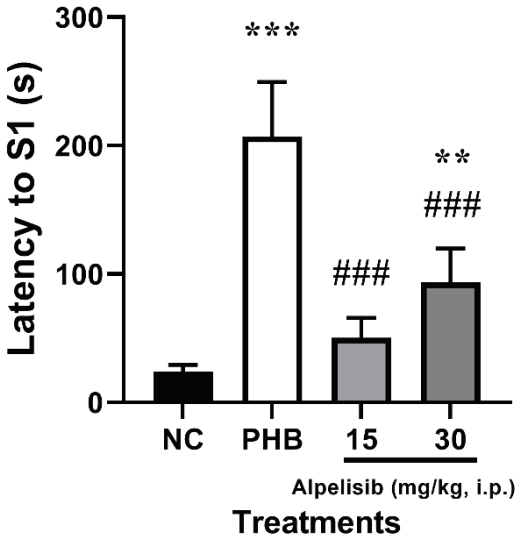

Fig. 1. The effect of ALP (15 and 30 mg/kg, i.p.) 30 min prior to PTZ exposure (70 mg/kg, i.p.) on latency to Stage 1 (S1) in male Wistar rat**s.**

Data are shown as mean ± 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.

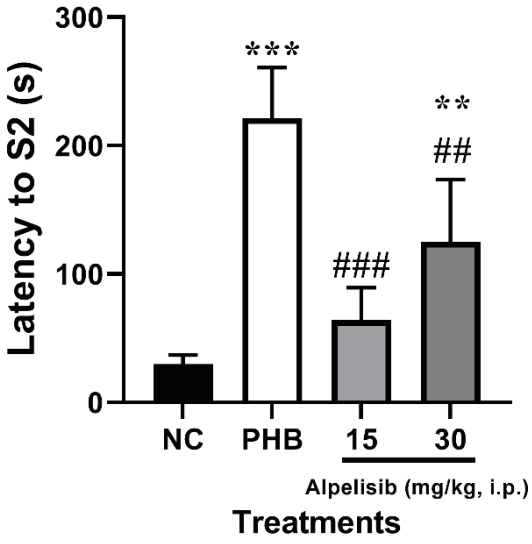

Fig. 2. The effect of ALP (15 and 30 mg/kg, i.p.) 30 min prior to PTZ exposure (70 mg/kg, i.p.) on latency of to Stage 2 (S2) in male Wistar rats.

Data are shown as mean ± 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 ± 19. 27 in NC group (Fig. 6). A marked decrease in the number was observed in experimental groups treated with ALPat 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 ± 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).

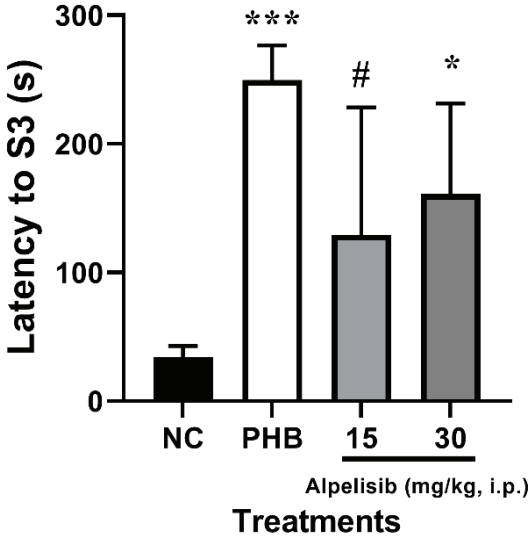

Fig. 3. The effect of ALP (15 and 30 mg/kg, i.p.) 30 min prior to PTZ exposure (70 mg/kg, i.p.) on latency to Stage 3 (S3) in male Wistar rats.

Data are shown as mean ± 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.

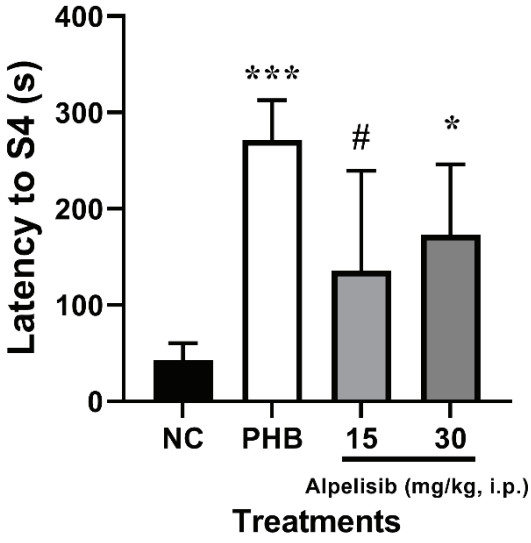

Fig. 4. The effect of ALP (15 and 30 mg/kg, i.p.) 30 min prior to PTZ exposure (70 mg/kg, i.p.) on latency of to Stage 4 (S4) in male Wistar rats.

Data are shown as mean ± 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.

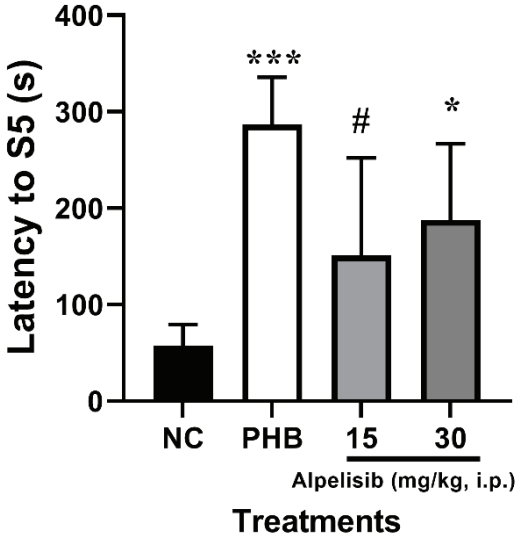

Fig. 5. The effect of ALP (15 and 30 mg/kg, i.p.) 30 min prior to PTZ exposure (70 mg/kg, i.p.) on latency to Stage 5 (S5) in male Wistar rats.

Data are shown as mean ± 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.

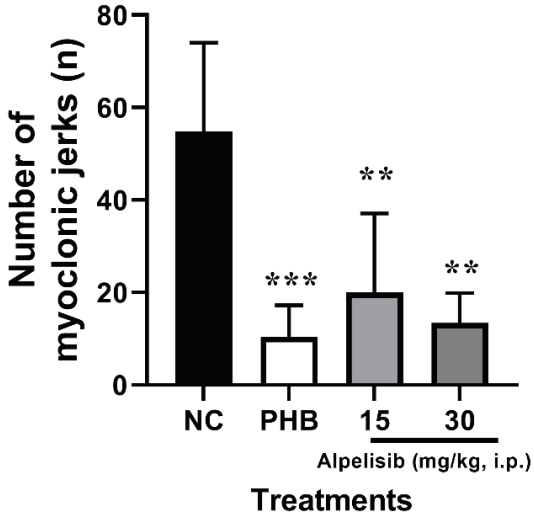

Fig. 6. The effect of ALP (15 and 30 mg/kg, i.p.) 30 min prior to PTZ exposure (70 mg/kg, i.p.) on number of myoclonic jerks in male Wistar rats.

Data are shown as mean ± 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.

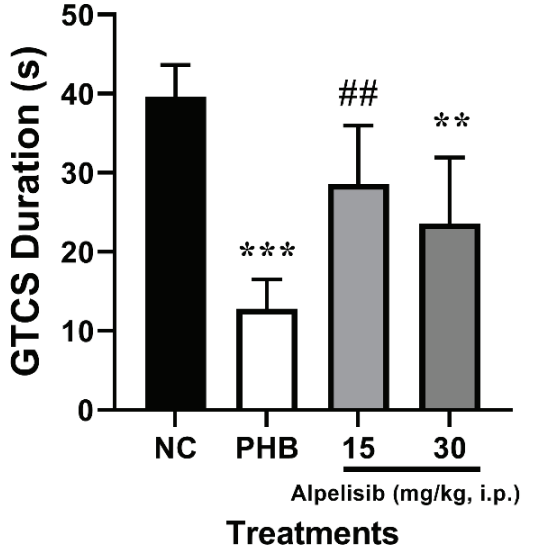

Fig. 7. The effect of ALP (15 and 30 mg/kg, i.p.) 30 min prior to PTZ exposure (70 mg/kg, i.p.) on GTCS duration in male Wistar rats.

Data are shown as mean ± 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.

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