

# Effects of Levetiracetam in Acute and Kindling Model of Epilepsy Through Adenosinergic Pathway in Mice: Possible Involvement of Adenosine A1 Receptor

Muhammad Jamal

International Center for Chemical and Biological Sciences

Muhammad Azam

International Center for Chemical and Biological Sciences

Shabana Usman Simjee ([✉ shabana.simjee@iccs.edu](mailto:shabana.simjee@iccs.edu))

International Center for Chemical and Biological Sciences

---

## Research Article

**Keywords:** Levetiracetam, Adenosinergic pathway, Seizure, Kindling model, Epilepsy

**Posted Date:** June 3rd, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-564229/v1>

**License:**  This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

## Abstract

**Objective:** The present study aimed to explore the possible levetiracetam mechanisms of action in the adenosine signaling systems using the PTZ-induced acute seizure and the PTZ-induced kindling model of epileptogenesis.

**Method:** In acute model, male mice received caffeine (non-specific adenosine receptor antagonist), or dipropylcyclopentylxanthine (DPCPX) (specific A1 receptor antagonist) prior to levetiracetam. After 30 minutes, a convulsant dose of PTZ (100 mg/kg) was administered to determine whether caffeine or DPCPX have any antagonistic effects on anti-seizure activity of levetiracetam by analyzing the onset of first myoclonic jerk (FMJ), generalized clonic seizures (GCS) and percent mortality.

The chronic PTZ-induced kindling model was set to assess the gene expression changes in adenosine A1 receptor, inwardly-rectifying potassium channel (Kir3.2) and equilibrative nucleoside transporter-1 (ENT-1) through RT-qPCR. Data were analyzed using Origin statistical software version 8.5 and represented as Mean ± SEM.

**Results:** In acute study, we found that caffeine (100 mg/kg) and DPCPX (25 mg/kg) reversed the anti-seizures effects of the levetiracetam significantly by reversing the percent protection and shortening the delay in the FMJ and onset of GCS in animals. In kindling model of epileptogenesis, it was found that levetiracetam increased the gene expression of adenosine A1 receptor and Kir3.2 in the brain. Furthermore, levetiracetam significantly reduced the gene expression of ENT-1 in the brain that supposed to enhance the extracellular adenosine in the brain.

**Conclusion:** Based on these results, it can be concluded that in addition to its action on SV2A vesicular protein, levetiracetam also prevent epileptogenesis by acting on the adenosine pathway in the CNS.

## Introduction

Epilepsy is the most serious and debilitating neurological disorder characterized by recurrent intermittent seizures that affects more than 65 million population globally (Thurman, Beghi et al. 2011). It has been reported that first seizure takes months to years after the occurrence of brain insults and this time period is known as seizure-free interval or latent period (Wong 2009). Various changes occurs during the latent period after the acute insult that later in life leads to the formation of epileptic circuit. These changes include the gliosis, neurodegeneration, mossy fibers sprouting, dendritic remodeling, blood brain barrier damage, neuro-inflammatory mediators production and reorganization of synaptic connections (Pitkänen and Lukasiuk 2011). The insult-induced transformation of non-epileptic brain to epileptic, ultimately leading to epilepsy is known as epileptogenesis (Herman 2002). The time period between brain insult and onset of first epileptic seizures may provide an opportunity during which an appropriate treatment stop or modify the epileptogenesis caused by brain insult (Löscher and Brandt 2010).

Classical antiepileptic drugs (AEDs) have various central and peripheral adverse effects that caused the non-compliance of the therapy by the patients. Novel drugs were developed with less adverse effects and broader range of effectiveness in epileptic patients. Among the novel AEDs, levetiracetam has been considered as the safest drug in term of adverse effects profile(Briggs and French 2004). Levetiracetam has been used for epilepsy as monotherapy (Del Bianco et al., 2019, Tabrizi et al., 2019), adjuvant therapy and prophylactically for the prevention of post-traumatic seizures (Chen, Kuo et al. 2018). Recently, LEV has been used effectively for the management of neonatal seizures(Arican, Gencpinar et al. 2018) and neuropathic pain and hyperalgesia (Ardid, Lamberty et al. 2003). It has been found further that the anti-hyperalgesic effects of levetiracetam were due to its action on the adenosine A1 receptor, and pharmacological blockade of A1 receptor by caffeine or DPCPX at the periphery weakened the effects of this drug. However, the antiepileptic effects of levetiracetam through adenosine pathway was not reported previously. Hence, the current study focuses on evaluating the effects of levetiracetam on the adenosine A1 receptor in the CNS.

Adenosine is an endogenous anticonvulsant agent present in the brain that regulate hyper-excitation of the neurons by regulating directly on its receptors or indirectly by modulating the release of other neurotransmitters (Boison 2007). Adenosine acts on its receptor subtypes i.e. A1, A2a, A2b and A3 adenosine receptors. It has been reported that antiepileptic effect of adenosine is through its action on inhibitory A1 receptors(Gouder et al. 2003, Fredholm et al. 2005). Adenosine A1 coupled to  $G_{i/o}$  GPCR that causes the inhibition of the adenylyl cyclase and cAMP and reduces the influx of voltage-gated sodium channels at the presynaptic terminal. This further leads to the inhibition of the glutamate release from the nerve terminals(Kosmowska et al. 2020). Similarly, the postsynaptic hyperpolarization by A1 receptors occurred by activation of G-protein coupled inwardly-rectifying potassium channels that lower the hyperexcitability of neurons at postsynaptic terminals(Hill et al. 2020, Spanoghe et al. 2021). Furthermore, studies showed that the Kir3.2 channels are involved in the action of A1R that causes hyperpolarization of the neurons and alleviate the seizure severity. Studies showed that Kir3.2 channels knock-out mice are more susceptible to seizures (Signorini et al. 1997).

Since, the effects of levetiracetam in the periphery for anti-hyperalgesic activity were evaluated through adenosine A1 receptor. We analyzed the antiepileptic effects of levetiracetam through modulation of adenosinergic pathway in acute and kindling model of epilepsy.

## Methodology

### Chemicals

Pentylenetetrazole,1,3-dipropyl-8-cyclopentylxanthine (DPCPX), caffeine and Diazepam were purchased from Sigma Aldrich (USA). Levetiracetam(obtained from Helix Pharmaceutical, Pakistan). All drugs were diluted in distilled water except DPCPX, which was dissolved in 5% DMSO. The test compounds were given intraperitoneally with dosing volume of about 10ml/kg of the body weight.

## **Animals**

Male NMRI mice weighing between 18-22 g were used in this study. Animals were provided with food and water *ad libitum*. Experiments were performed in the day time and were conducted according to the institutional ethical guidelines and after getting approval from the Institutional Animal Care and Use Committee (IACUC) (license number 2018-006).

### **Acute PTZ- induced seizure model**

Animals (n = 8) were brought to the experimental room one hour prior to the start of the experiment. They were grouped as shown in table 1. Onset of first myoclonic jerk (FMJ), generalized clonic seizures (GCS) and percent mortality were recorded after the administration of PTZ 110 mg/kg.

### **PTZ-induced Kindling model of epileptogenesis.**

Animals (n = 8) were grouped as shown in the table 2. The seizure scoring was recorded over a period of 30 minutes after the administration of PTZ45mg/kg on each alternate day. Scores were noted according to the Racine's scale([Racine 1972](#)) i.e. score 1 (mouth and facial twitching), score 2 (slight body jerks), score 3 (tonic seizures with tail erection), score 4 (tonic clonic seizure with posture on one side of the body) and score 5 (tonic clonic seizure with inability of the animal to maintain posture). Animals were considered kindled when 3 consecutive occasion of score 4/5 appeared in the animals of PTZ group. A total of 13 injections were required to kindle all the animals in PTZ-treated group. Twenty four hours after the completion of kindling, animals were anesthetized by administration of ketamine/xylazine cocktail. Cardiac perfusion was performed with 1x PBS. Animals were decapitated by guillotine apparatus. Brain was removed carefully; hippocampus and cortex were isolated and processed for the extraction of total RNA.

### **RNA Isolation**

Isolation of RNA from hippocampus and cortex was done through trizol reagent. Approximately 30-40 mg of tissue was placed in 2 ml eppendorf tube, and 1 ml of trizol reagent was added. The collected tissue was homogenized by using homogenizer. For the isolation of RNA, 0.2ml of chloroform was added in each tube containing homogenized tissue in trizol. The content was incubated for 2-3 minutes at room temperature, and centrifuged for 12000g for duration of 15 minutes. After centrifugation, the upper aqueous layer containing RNA was isolated in the new RNase free tube, and 0.5 ml isopropanol was added for precipitation of RNA. Incubate for 10 minutes, and then centrifuged for 10 minutes at 12000 x g. A pellet of RNA will be settled down at the base of the tube. The supernatant was discarded carefully leaving the RNA pellet inside the tube. For the purification of RNA pellet, 1 ml of 75% ethanol was added to the tube and after brief vertex, centrifuged the tubes at 7500g for 5minutes. Remove the supernatant and air dry the RNA pellet inside the tube. Pellet was dissolved in 20-40 $\mu$ l nuclease free water.

### **Quantification of RNA**

The total RNA in each sample was quantified with Nanodrop. First the reading was adjusted to zero by nuclease free water. Purity of the isolated RNA was investigated through the value of A260:A280 in nanodrop. If the value of this ratio falls within the range of  $2\pm0.25$  then the samples were found to be free from contamination. Samples were stored at  $-20^{\circ}\text{C}$  until used for the synthesis of cDNA.

### **cDNA synthesis**

Following RNA isolation from brain tissue samples cDNA were synthesized from isolated RNA samples using cDNA synthesis kit (Invitrogen, cDNA Synthesis kit 1622). To an RNase free tube 1  $\mu\text{g}$  of total RNA1, 1  $\mu\text{l}$  10X reaction buffer with  $\text{MgCl}_2$ , and 1  $\mu\text{g}$  DNase-1 were mixed and the final volume was made up with nuclease free water to 10  $\mu\text{l}$ . The mixture was incubated at  $37^{\circ}\text{C}$  on water bath for a duration of 30 minutes. After incubation 1  $\mu\text{l}$  50mM EDTA was added to the tube and incubated at  $65^{\circ}\text{C}$  for 5 minutes. To this mixture 1  $\mu\text{l}$  of Oligo dT<sub>18</sub> was added. The volume of this mixture became 12  $\mu\text{l}$ . To this mixture the following reagents were added in the indicated order, 4  $\mu\text{l}$  of 5X reaction buffer, 1  $\mu\text{l}$  Ribolock RNase inhibitor, 2  $\mu\text{l}$  dNTP mixture and 1  $\mu\text{l}$  reverse transcriptase were added. The final volume of the mixture in the tube became 20  $\mu\text{l}$ . Incubate for 60 minute at  $42^{\circ}\text{C}$  followed by  $70^{\circ}\text{C}$  for a duration of 5 minutes.

### **RT-qPCR reaction**

RT-qPCR reactions were prepared in low profile PCR tubes. For amplification of the cDNA SYBR™ Green PCR Master Mix (ThemoFisher™, UK) was used according to the protocol of the manufacturer. 1  $\mu\text{l}$  cDNA, 0.5  $\mu\text{l}$  forward and 0.5  $\mu\text{l}$  reverse primer, 3  $\mu\text{l}$  DFW and 5  $\mu\text{l}$  SYBR green mixture were added the tube in triplicate. Reactions were placed in the RT-qPCR machine. The protocol was set at  $95^{\circ}\text{C}$  (hotstart) for 10 minutes followed by 40 cycles of  $95^{\circ}\text{C}$  for 15s(denaturation),  $60^{\circ}\text{C}$  for 15s(annealing), and  $72^{\circ}\text{C}$  for 30s(extension) and melting curve analysis between  $65^{\circ}\text{C}$  to  $95^{\circ}\text{C}$  (With 5s increment). After the completion of the PCR, data was exported to excel file and analyzed in Origin 8.5 statistical software.

### **Expression of Gene**

Quantification of genes were done by using  $\Delta\Delta\text{Ct}$  method. Initially, the  $\Delta\text{Ct}$  values of the samples were calculated by subtracting the Cq value of GAPDH of the respective samples from Cq values of the samples.  $\Delta\text{Ct}$  value of all other groups were subtracted from the  $\Delta\text{Ct}$  value of the control group to obtain  $\Delta\Delta\text{Ct}$  values.

### **Statistical Analysis**

Results obtained were presented as Mean  $\pm$  SEM. Data were analyzed through One-Way ANOVA using OriginLab (version 8.5) statistical software. Where \*, \*\* and \*\*\* represented  $P$ value  $< 0.05$ ,  $< 0.01$  and  $< 0.001$  respectively.

## **Results**

## **Caffeine and DPCPX attenuated the anti-seizure activity of levetiracetam in acute PTZ-induced seizure model**

In the acute PTZ-induced seizure model, it was observed that the administration of caffeine (100 mg/kg) or DPCPX (25 mg/kg) significantly reduces the anti-seizure activity of LEV (200mg/kg). The onset of first myoclonic jerk (FMJ) in LEV was observed at  $146.66 \pm 6.76$  s which was significantly ( $P < 0.001$ ) delayed response compared to the PTZ control group in which the FMJ occurred at  $48.33 \pm 3.75$  s (Fig. 1). It was interesting to find that administration of caffeine (100mg/kg) or DPCPX (25mg/kg) prior to the LEV (200mg/kg) treatment, reverses the anti-seizure effects of LEV by reducing the delay of the FMJ to  $76 \pm 14.52$  s and  $100 \pm 16.32$  s respectively, as compared to the LEV 200mg/kg ( $146.66 \pm 6.76$  s,  $P < 0.05$ , Fig.1).

Furthermore, LEV (200mg/kg) followed by PTZ 100mg/kg did not produce any mortality and confers 100% protection as compared to the PTZ control group. It was also observed that caffeine (100mg/kg) and DPCPX (25 mg/kg) completely abolished the percent protection caused by LEV as shown in table-4. Similarly, table-4 also shows that administration of PTZ leads to the onset of generalized clonic seizure (GCS) at  $66.66 \pm 6.00$  s. On the other hand, LEV prevented the onset of GCS remarkably in mice administered with acute convulsive dose of PTZ. Caffeine (100mg/kg) and DPCPX (25mg/kg) reversed the effects of LEV by producing the GCS in  $115 \pm 16.52$  s and  $142.33 \pm 12.17$  s respectively, as shown in table-4.

### **Effects of levetiracetam in PTZ-induced kindling model**

It was observed that the repeated administration of subconvulsive dose of PTZ (45 mg/kg, i.p.) on alternate day gradually increased the seizure scores to 4-5 (clonic-tonic seizures) in mice (Fig. 2). Diazepam 7.5mg/kg did not produce seizures in any animal of the group. Furthermore, LEV (200mg/kg, i.p.) significantly ( $P < 0.001$ , Fig. 2) reduced the seizure score in PTZ-induced kindled animals. Interestingly, none of the animals achieved a score above 3, thus showed a significant antiepileptogenic activity.

### **Effect of levetiracetam on the gene expression of adenosine A1R in kindling model**

Adenosine A1R has been one of the initial pathways involved in the regulation of the neuronal hyperexcitation. It was found that LEV 200mg/kg increased the fold change mRNA expression in adenosine A1R in the RT-qPCR by  $1.76 \pm 0.144$  and  $1.45 \pm 0.124$  times in the hippocampus and cortex respectively, as compared to the PTZ-treated group ( $n = 8$ , Fig. 1,  $P < 0.05$ ) in kindling model of epilepsy. Furthermore, it was also observed that LEV 200mg/kg only group decreased the fold change of A1R in mice hippocampal and cortical region. Results also revealed insignificant upregulation of A1R expression in the hippocampus and cortex in diazepam-treated group in mice (Fig 1,  $P < 0.05$ ). Data obtained in the RT-qPCR inferred that levetiracetam showed its antiepileptic effects in PTZ-Induced kindling model by acting on the adenosine A1 receptor by up-regulating the A1 receptor mRNA expression in the hippocampal and cortical regions.

## Gene expression of Kir3.2 in the brain of PTZ-kindled mice receiving treatment of levetiracetam

Adenosine A1 receptor mediated potassium inwardly-rectifying channels (Kir3.2) play a key role in regulation of neuronal over-excitation. Figure 4 shown that LEV (200 mg/kg, i.p.) prevented the PTZ-induced seizures in kindling model by increasing the mRNA expression of the Kir3.2 in hippocampus (fold change:  $2.089 \pm 0.214$ ) and cortex (fold change:  $1.57 \pm 0.058$ ), as compared to the PTZ-treated group ( $n = 8$ , Fig.4,  $**P < 0.01$ ). Furthermore, diazepam 7.5mg/kg has shown little effect on the mRNA expression of the Kir3.2 in cortical region (fold change  $1.18 \pm 0.126$ ) while in the hippocampus it significantly ( $*P < 0.05$ ) increased the gene expression of Kir3.2 (fold change:  $1.55 \pm 0.129$ ) in kindling model of epilepsy.

### Effects of levetiracetam on the gene expression of ENT-1

Figure 5 shows increased mRNA expression of ENT1 in PTZ-induced kindling model of epilepsy in hippocampal (fold change:  $1.7452 \pm 0.27117$ ) and cortical region (fold change:  $2.08532 \pm 0.30613$ ) in RT-qPCR ( $n = 8$ ,  $P < 0.05$ ) as compared to the saline treated group. The levetiracetam treatment was found to significantly reduced the fold change of mRNA expression of ENT1 in hippocampus (fold change:  $0.57783 \pm 0.12722$ ) and cortex ( $1.02139 \pm 0.07938$ ) in comparison to the PTZ-induced kindling group in mice ( $P < 0.05$ ). When compared with PTZ control group, diazepam treatment was found to decrease the ENT1 expression however this reduction was non-significant both in cortex and hippocampus.

## Discussion

Conventional AEDs have various adverse effects and therefore efforts are made to develop novel drugs with less side effects and broader range of effectiveness in epileptic patients. Among the novel AEDs, levetiracetam has been considered as the safest drug in term of adverse effects profile(Briggs and French 2004). It is also known that levetiracetam partially protect seizures in animals in acute seizure models and demonstrated a significant suppression of seizures in kindling model of epilepsy(Klitgaard 2001). Previously, studies showed that levetiracetam acts peripherally on A1 receptor to produce its anti-hyperalgesic effects and this effects was antagonized by the adenosine receptor antagonists caffeine (non-selective A1 and A2 receptor antagonist) and DPCPX (selective A1R antagonist)(Micov, Tomić et al. 2010). Effects of levetiracetam on adenosinergic pathway in the CNS were not investigated completely. Therefore, in this study we evaluated the antiepileptic action of levetiracetam through adenosinergic pathway specifically through its action on A1 receptors.

Adenosine A1R suppressed the hyperexcitability of the neuronal network through two mechanisms i.e. by inhibiting the release of the neurotransmitters from the presynaptic terminals and by hyperpolarizing the postsynaptic terminal. The hyperpolarizing effects of A1R was because of the activation of GPCR-linked potassium inwardly rectifying channels (Kir3.2). These channels are also activated by other receptors such as GABA-B receptors. It was observed that the effects of GABA mediated inhibition through Kir3.2 are not that pronounced than the ones due to the activation of A1R. Furthermore, for the optimal effects of Kir3.2 channels presence of adenosine were required (Hill, Hickman et al. 2020).

The current study was divided into two main phases' i.e. acute phase and chronic kindling phase. In acute study, it was found that antiepileptic activity of levetiracetam was antagonized by the caffeine and DPCPX which inferred that levetiracetam may partially acts on the adenosine receptor pathway to alleviate seizures. To further confirm the results of acute study, the changes in the A1R and its associated GIRK2 channels and ENT1 mRNA expression were investigated in the kindling model of epileptogenesis. It was found that levetiracetam increased the expression of A1R and its associated Kir3.2 mRNA expression in kindling and parallel decrease in the mRNA expression of ENT1 in the hippocampus and cortex.

Adenosine A1 receptor density in TLE in human subjects were reported to be increased (Angelatou, Pagonopoulou et al. 1993) or decreased (Glass, Faull et al. 1996) in temporal neocortical region of the brain. However, it has been reported in animal models that A1 receptor density decreased in hippocampal pyramidal neurons in rats (Ochiishi et al. 1999, Ekonomou et al. 2000, Rebola et al. 2003). It has been found that A1R knockout mice leads to the development of status epilepticus after the cerebral injury (Kochanek, Vagni et al. 2006). Here we have found that adenosine A1 receptor density decreased in cortical and hippocampal region of the brain isolated from the control PTZ group. However, treatment of levetiracetam caused a gradual increase in the gene expression of adenosine A1 receptor in the brain of the mice that results in the reduction of the seizure due to their inhibitory tone on the neurons caused by activation of adenosine A1 receptors. Previously it has been shown that knock-out of Kir3.2 channels increased seizure susceptibility in animals (Signorini et al. 1997). It was known that GABA-B receptor-mediated hyperpolarization may also be due to the activation of Kir3.2 channels. There are other studies giving evidence that levetiracetam does not act on the GABA receptors (De Smedt et al. 2007) thus indicating that the activity of levetiracetam mediated action on the Kir3.2 is due activation of A1 receptor in the brain.

It has also been revealed that the expression of ENT1 increased in patients suffering from epilepsy and also in animal models of epilepsy and pharmacological inhibition of ENT1 transporters in epileptic animal models decreased the severity of seizures (Ho et al. 2020). Data of the present study revealed that the increased expression of the ENT1 mRNA seen in the kindling model of epilepsy is ameliorated by levetiracetam. It may be hypothesized that increase in ENT1 expression could be due to the seizure induced astrogliosis and levetiracetam decreased the gene expression of ENT1 by preventing astrogliosis in animal model (Devinsky et al. 2013). Therefore, it can be suggested that levetiracetam prevent the hallmark feature of epilepsy i.e. astrogliosis.

## Conclusion

Based on the present experimental findings, it can be concluded that levetiracetam increases the adenosine A1 receptor and Kir3.2 channel mRNA expression both in hippocampus and cortical regions of the brain and therefore it reduces the seizure scores. Adenosine A1 receptor activation has been known for preventing the hyperpolarization of neurons through Kir3.2 channels located at postsynaptic terminals while at presynaptic terminal adenosine A1 receptor decreased the release of glutamatergic

neurotransmitters. However, prior administration of adenosine receptor antagonist reverses the anticonvulsant effects of levetiracetam. Further we suggest that by reducing the mRNA expression of ENT-1, levetiracetam enhances the extracellular level of adenosine. Increase in adenosine A1 receptor and down regulating ENT-1 supposed to cause the antiepileptogenic effects of levetiracetam through adenosinergic pathway.

## Declarations

### Ethics declarations

The authors declare that all data were generated in-house and that no paper mill was used.

**Ethical approval:** This research was approved by the Animal Research Ethics Committee of the ICCBS, University of Karachi (license number 2018-006). Procedures involving animals and their care were carried out in conformity with international guidelines for the use of laboratory animals (NIH Guide for the Care and Use of Laboratory Animals, NIH Publications No. 85-23, 1985).

**Consent to Participate:** Not applicable

**Consent to Publish:** The final content of this paper was read and approved by all authors for publication.

**Conflict of interest:** Authors declare no conflict of interest.

**Funding:** The authors have nothing to declare in funding.

**Competing Interests:** The authors declare that they have no competing interests.

**Availability of data and materials:** Backup of data generated in this study are stored in both laboratory and authors' computer which will be available upon request.

### Authors information:

### Affiliations

1. E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan

Muhammed Jamal , Shabana Usman Simjee

2. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan

Muhammad Azam, Shabana Usman Simjee

### Contributions

MJ and SUS conceived and designed the project. MJ and MA conducted the experiments. MJ, MA and SUS contributed to interpretation of data. MJ wrote the manuscript. All authors read and approved the manuscript. The authors declare that all data were generated in-house.

### Corresponding author

Correspondence to Shabana Usman Simjee

## References

1. Angelatou F, Pagonopoulou O, Maraziotis T, Olivier A, Villemure JG, Avoli M, et al. (1993) Upregulation of A1 adenosine receptors in human temporal lobe epilepsy: a quantitative autoradiographic study. *Neurosci Lett* 163: 11-14. [https://doi.org/10.1016/0304-3940\(93\)90217-9](https://doi.org/10.1016/0304-3940(93)90217-9)
2. Ardid D, Lamberty Y, Alloui A, Coudore-Civiale MA, Klitgaard H, Eschalier A (2003) Antihyperalgesic effect of levetiracetam in neuropathic pain models in rats. *Eur J Pharmacol* 473: 27-33. [https://doi.org/10.1016/S0014-2999\(03\)01933-2](https://doi.org/10.1016/S0014-2999(03)01933-2)
3. Arican P, Gencpinar P, Cavusoglu D, Dundar NO (2018) Levetiracetam monotherapy for the treatment of infants with epilepsy. *Seizure* 56: 73-77. <https://doi.org/10.1016/j.seizure.2018.02.006>
4. Boison D (2007). Adenosine as a modulator of brain activity. *Drug News Perspect* 20: 607-611. <https://doi.org/10.1358/dnp.2007.20.10.1181353>
5. Briggs DE, French JA (2004) Levetiracetam safety profiles and tolerability in epilepsy patients. *Expert Opin Drug Saf* 3: 415-424. <https://doi.org/10.1517/14740338.3.5.415>
6. Chen Y-H, Kuo T-T, Huang EY-K, Hoffer BJ, Chou Y-C, Chiang Y-H, et al. (2018) Profound deficits in hippocampal synaptic plasticity after traumatic brain injury and seizure is ameliorated by prophylactic levetiracetam. *Oncotarg* 9: 11515. <https://dx.doi.org/10.18632%2Foncotarget.23923>
7. De Smedt T, Raedt R, Vonck K, Boon P (2007) Levetiracetam: the profile of a novel anticonvulsant drug—part I: preclinical data. *CNS Drug Rev* 13: 43-56. <https://doi.org/10.1111/j.1527-3458.2007.00004.x>
8. Del Bianco C, Placidi F, Liguori C, Mari L, Ulivi M, Ornello R, et al. (2019) Long-term efficacy and safety of lacosamide and levetiracetam monotherapy in elderly patients with focal epilepsy: A retrospective study. *Epilepsy & Behav* 94: 178-182. <https://doi.org/10.1016/j.yebeh.2019.02.022>
9. Devinsky O, Vezzani A, Najjar S, De Lanerolle NC, Rogawski MA (2013) Glia and epilepsy: excitability and inflammation. *Trends Neurosci* 36: 174-184. <https://doi.org/10.1016/j.tins.2012.11.008>
10. Ekonomou A, Sperk G, Kostopoulos G, Angelatou F (2000) Reduction of A1 adenosine receptors in rat hippocampus after kainic acid-induced limbic seizures. *Neurosci Lett* 284: 49-52. [https://doi.org/10.1016/S0304-3940\(00\)00954-X](https://doi.org/10.1016/S0304-3940(00)00954-X)
11. Fredholm BB, Chen J-F, Cunha RA, Svensson P, Vaugeois J-M (2005) Adenosine and brain function. *Int Rev Neurobiol* 63: 191-270.

12. Glass M, Faull R, Bullock J, Jansen K, Mee E, Walker E, *et al.* (1996) Loss of A1 adenosine receptors in human temporal lobe epilepsy. *Brain Res* 710: 56-68. [https://doi.org/10.1016/0006-8993\(95\)01313-X](https://doi.org/10.1016/0006-8993(95)01313-X)
13. Gouder N, Fritschy JM, Boison D (2003) Seizure suppression by adenosine A1 receptor activation in a mouse model of pharmacoresistant epilepsy. *Epilepsia* 44: 877-885. <https://doi.org/10.1046/j.1528-1157.2003.03603.x>
14. Herman ST (2002) Epilepsy after brain insult: targeting epileptogenesis. *J. Neurol* 59: S21-S26. DOI: [https://doi.org/10.1212/WNL.59.9\\_suppl\\_5.S21](https://doi.org/10.1212/WNL.59.9_suppl_5.S21)
15. Hill E, Hickman C, Diez R, Wall M (2020) Role of A1 receptor-activated GIRK channels in the suppression of hippocampal seizure activity. *J Neuropharmacol* 164: 107904. <https://doi.org/10.1016/j.neuropharm.2019.107904>
16. Ho S-Y, Chen I-C, Chang K-C, Lin H-R, Tsai C-W, Lin C-J, *et al.* (2020) Equilibrative nucleoside transporters-1 inhibitors act as anti-epileptic agents by inhibiting glutamatergic transmission. *Front Neurosci* 14. <https://dx.doi.org/10.3389%2Ffnins.2020.610898>
17. Klitgaard H (2001) Levetiracetam: the preclinical profile of a new class of antiepileptic drugs? *Epilepsia* 42: 13-18. <https://doi.org/10.1111/j.1528-1167.2001.00003.x>
18. Kochanek PM, Vagni VA, Janesko KL, Washington CB, Crumrine PK, Garman RH, *et al.* (2006) Adenosine A1 receptor knockout mice develop lethal status epilepticus after experimental traumatic brain injury. *J Cereb Blood Flow Metab* 26: 565-575. <https://doi.org/10.1038%2Fsj.jcbfm.9600218>
19. Kosmowska B, Ossowska K, Konieczny J, Lenda T, Berghauzen-Maciejewska K, Wardas J (2020) Inhibition of excessive glutamatergic transmission in the ventral thalamic nuclei by a selective adenosine A1 receptor agonist, 5'-chloro-5'-deoxy-( $\pm$ )-ENBA underlies its tremorolytic effect in the harmaline-induced model of essential tremor. *J Neurosci* 429: 106-118. <https://doi.org/10.1016/j.neuroscience.2019.12.045>
20. Löscher W, Brandt C (2010) Prevention or modification of epileptogenesis after brain insults: experimental approaches and translational research. *Pharmacol Rev* 62: 668-700. doi:10.1124/pr.110.003046
21. Micov A, Tomić M, Popović B, Stepanović-Petrović R (2010) The antihyperalgesic effect of levetiracetam in an inflammatory model of pain in rats: mechanism of action. *Br J Pharmacol* 161: 384-392. <https://doi.org/10.1111/j.1476-5381.2010.00877.x>
22. Ochiishi T, Takita M, Ikemoto M, Nakata H, Suzuki SS (1999) Immunohistochemical analysis on the role of adenosine A1 receptors in epilepsy. *Neuroreport* 10: 3535-3541.
23. Pitkänen A, Lukasiuk K (2011) Mechanisms of epileptogenesis and potential treatment targets. *The Lancet Neurol* 10: 173-186. [https://doi.org/10.1016/S1474-4422\(10\)70310-0](https://doi.org/10.1016/S1474-4422(10)70310-0)
24. Racine RJ (1972) Modification of seizure activity by electrical stimulation: II. Motor seizure. *Electroencephalogr Clin Neurophysiol* 32: 281-294. [https://doi.org/10.1016/0013-4694\(72\)90177-0](https://doi.org/10.1016/0013-4694(72)90177-0)
25. Rebola N, Coelho JE, Costenla AR, Lopes LV, Parada A, Oliveira CR, *et al.* (2003) Decrease of adenosine A1 receptor density and of adenosine neuromodulation in the hippocampus of kindled

- rats. Eur J Neurosci 18: 820-828. <https://doi.org/10.1046/j.1460-9568.2003.02815.x>
26. Signorini S, Liao YJ, Duncan SA, Jan LY, Stoffel M (1997) Normal cerebellar development but susceptibility to seizures in mice lacking G protein-coupled, inwardly rectifying K<sup>+</sup> channel GIRK2. Proc Natl Acad Sci 94: 923-927.
27. Spanoghe J, Larsen LE, Craey E, Manzella S, Van Dycke A, Boon P, *et al.* (2021) The Signaling Pathways Involved in the Anticonvulsive Effects of the Adenosine A1 Receptor. Int J Mol Sci 22: 320. <https://doi.org/10.3390/ijms22010320>
28. Tabrizi N, Zarvani A, Rezaei P, Cheraghmakani H, Alizadeh-Navaei R (2019) Levetiracetam in genetic generalized epilepsy: A prospective unblinded active-controlled trial. Epilepsy Res 157: 106214. <https://doi.org/10.1016/j.eplepsyres.2019.106214>
29. Thurman DJ, Beghi E, Begley CE, Berg AT, Buchhalter JR, Ding D, *et al.* (2011) Standards for epidemiologic studies and surveillance of epilepsy. Epilepsia 52: 2-26. <https://doi.org/10.1111/j.1528-1167.2011.03121.x>
30. Wong M (2009) The window of epileptogenesis: looking beyond the latent period. Epilepsy Curr 9: 144-145. <https://doi.org/10.1111%2Fj.1535-7511.2009.01322.x>

## Tables

**Table 1: Groups of Acute PTZ-induced seizure model**

<b>Groups</b>	<b>Treatment</b>
Group-I	PTZ (110 mg/kg, i.p)
Group-II	LEV (200 mg/kg) followed by administration of (PTZ 110 mg/kg, i.p.) after 30 minutes.
Group-III	Caffeine (100 mg/kg i.p.) followed by LEV 200 mg/kg and then after 30 minutes administration of PTZ (110mg/kg) was done.
Group-IV	DPCPX (25 mg/kg i.p.) followed by LEV and then after 30 minutes PTZ (110 mg/kg) was administered.

**Table 2: Groups of PTZ-induced kindling model**

<b>Groups</b>	<b>Treatment</b>
Group 1	Saline control group (only normal saline was administered to these animals)
Group 2	Saline 10 ml/kg + PTZ (45 mg/kg i.p every alternate day)
Group 3	LEV 200 mg/kg i.p. + PTZ (45 mg/kg i.p every alternate day)
Group 4	LEV (200 mg/kg i.p) only
Group 5	Diazepam 7.5 mg/kg i.p + PTZ (45 mg/kg i.p every alternate day)

**Table 3: Primers sequence of A1R, ENT1 and Kir3.2.**

S. No.	Primer	Forward and Reverse Strand Sequence	Product Size
1	GAPDH	(F) AACTTTGGCATTGTGGAAGG (R) ACACATTGGGGTAGGAACA	223
2	Adenosine A1R	(F) GCCCGGAAATGTACTGGTGA (R) GGCAGGTGTGGAAGTAGGTC	170
3	ENT1	(F) AGCCAGACAGGGCTCGATA (R) GTGACTGGTTGTCATGGCTC	103
4	Kir3.2 (GIRK2)	(F) GACAAACCCAGCATGCACAA (R) TTAGAGGGCCAGCAGTCAAG	198

**Table 4: GCS and percent mortality in acute PTZ-induced seizure model.**

Group	Onset time of GCS (sec)	% Mortality
PTZ 110 mg/kg	66.66 ± 6.009	100 %
LEV 200 mg/kg + PTZ 110 mg/kg	No GCS	0 (No mortality)
CAF 100 mg/kg +LEV 200 mg/kg + PTZ 110 mg/kg	115 ± 16.52	100 %
DPCPX 25 mg/kg + LEV 200 mg/kg + PTZ 110 mg/kg	142.33 ± 12.170	100 %

Data is represented as Mean ± SEM of n = 8 animals / group.

## Figures

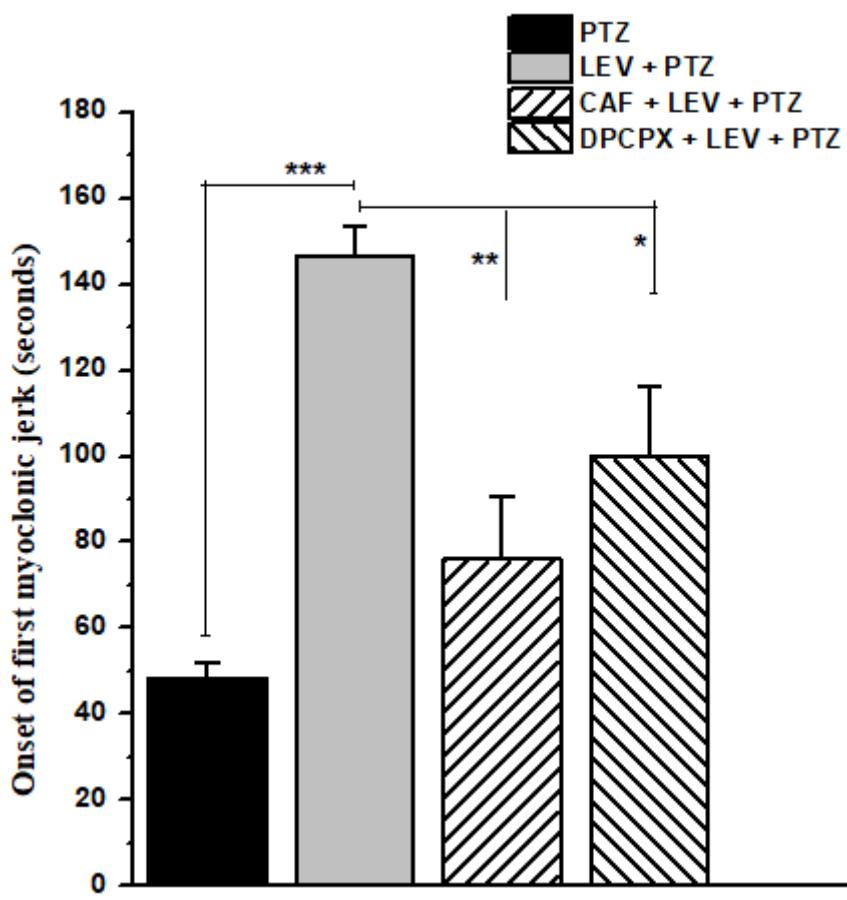
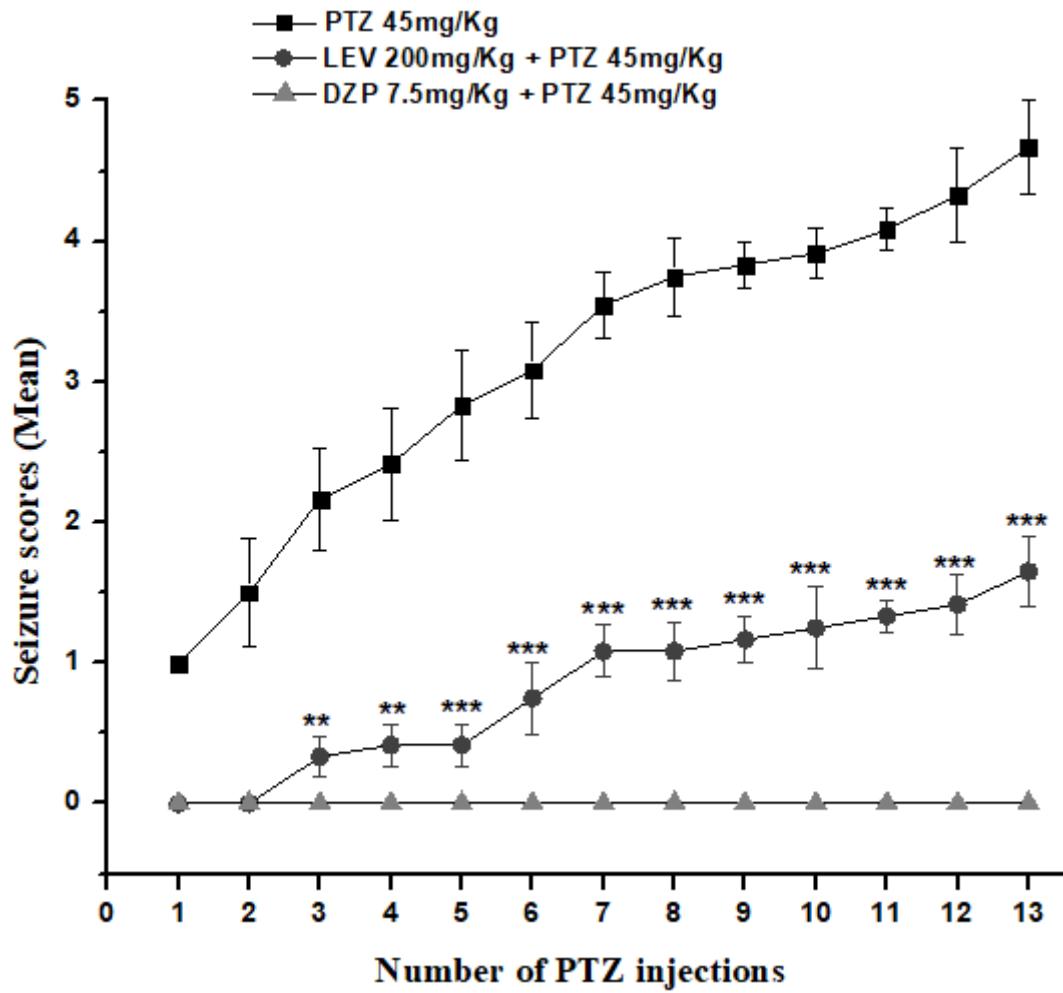


Figure 1

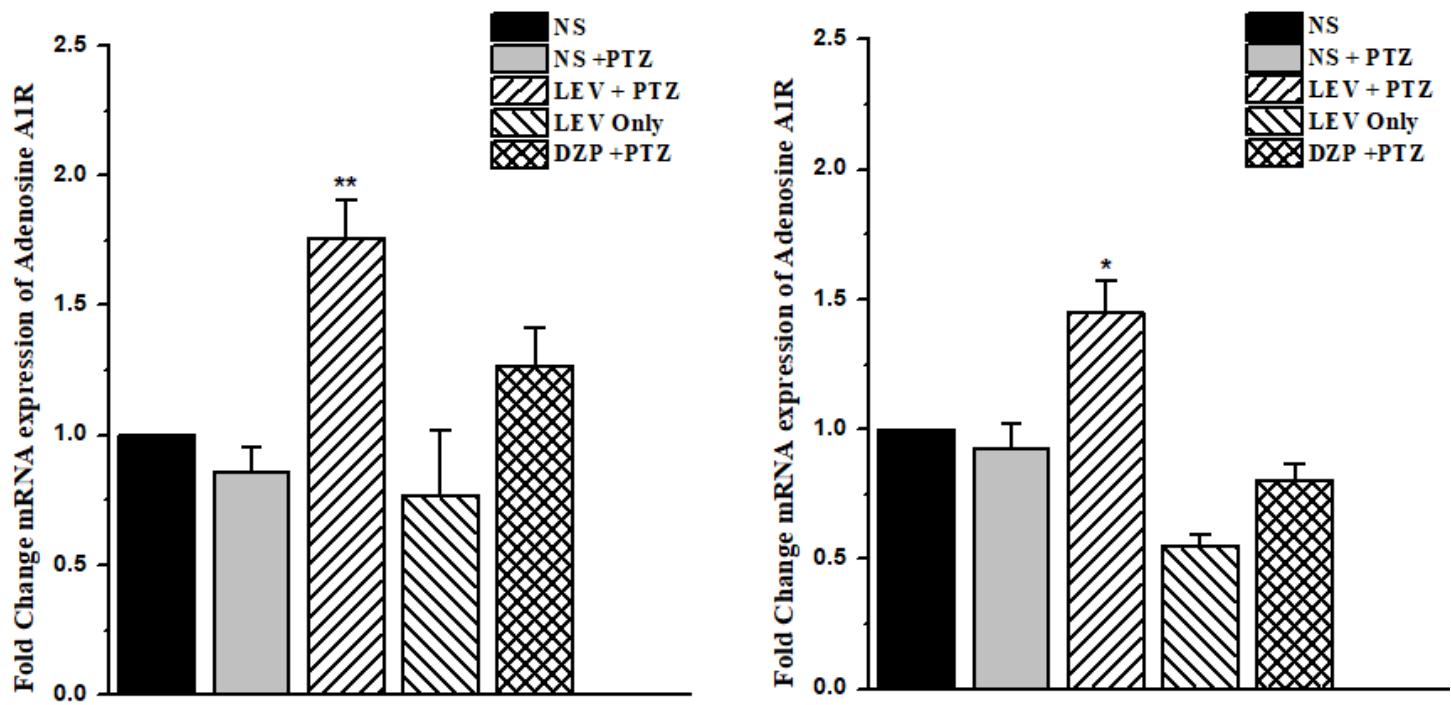
Caffeine and DPCPX attenuated the LEV-mediated delayed-onset of first myoclonic jerk (FMJ) in acute PTZ-induced seizure model. Effect of levetiracetam on the onset of FMJ and attenuation of this effect by caffeine and DPCPX. Data is shown as mean  $\pm$  SEM, where n = 8, \*, \*\*, \*\*\* represents P < 0.05, P < 0.01 and P < 0.001 respectively.



**Figure 2**

Seizure scores in PTZ-induced kindling model of epilepsy. Levetiracetam significantly reduced the mean seizure score in the PTZ-induced kindling. Data is represented as Mean  $\pm$  SEM and analyzed through One-way ANOVA using Origin statistical software. \*\*\*P< 0.001 is considered significant vs. PTZ-treated group.

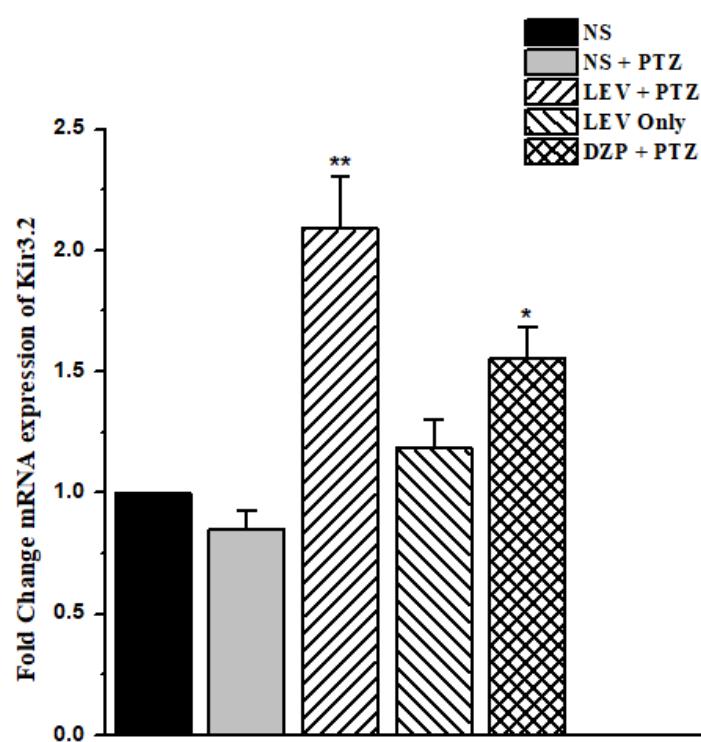
**(a) Hippocampus (b) Cortex**



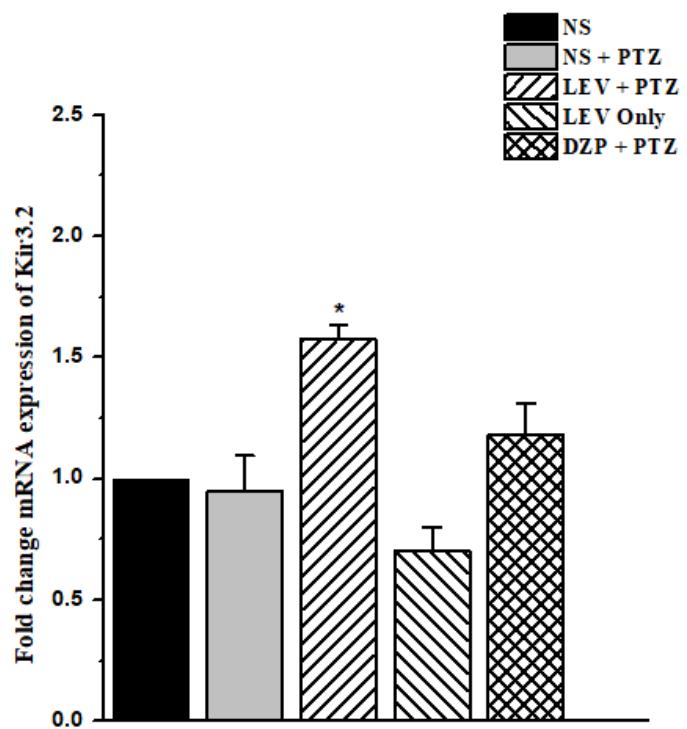
**Figure 3**

Levetiracetam increased the gene expression of adenosine A1 receptor in PTZ-induced kindling in mice. Changes in the gene expression of the A1 receptor in the kindling model is shown both in (a) hippocampus; and (b) cortex. Data were shown as Mean  $\pm$  SEM and analyzed through One-way ANOVA followed by post hoc Tukey's test. \* $P < 0.05$ , \*\* $P < 0.01$  considered as significant as compared to the PTZ group.

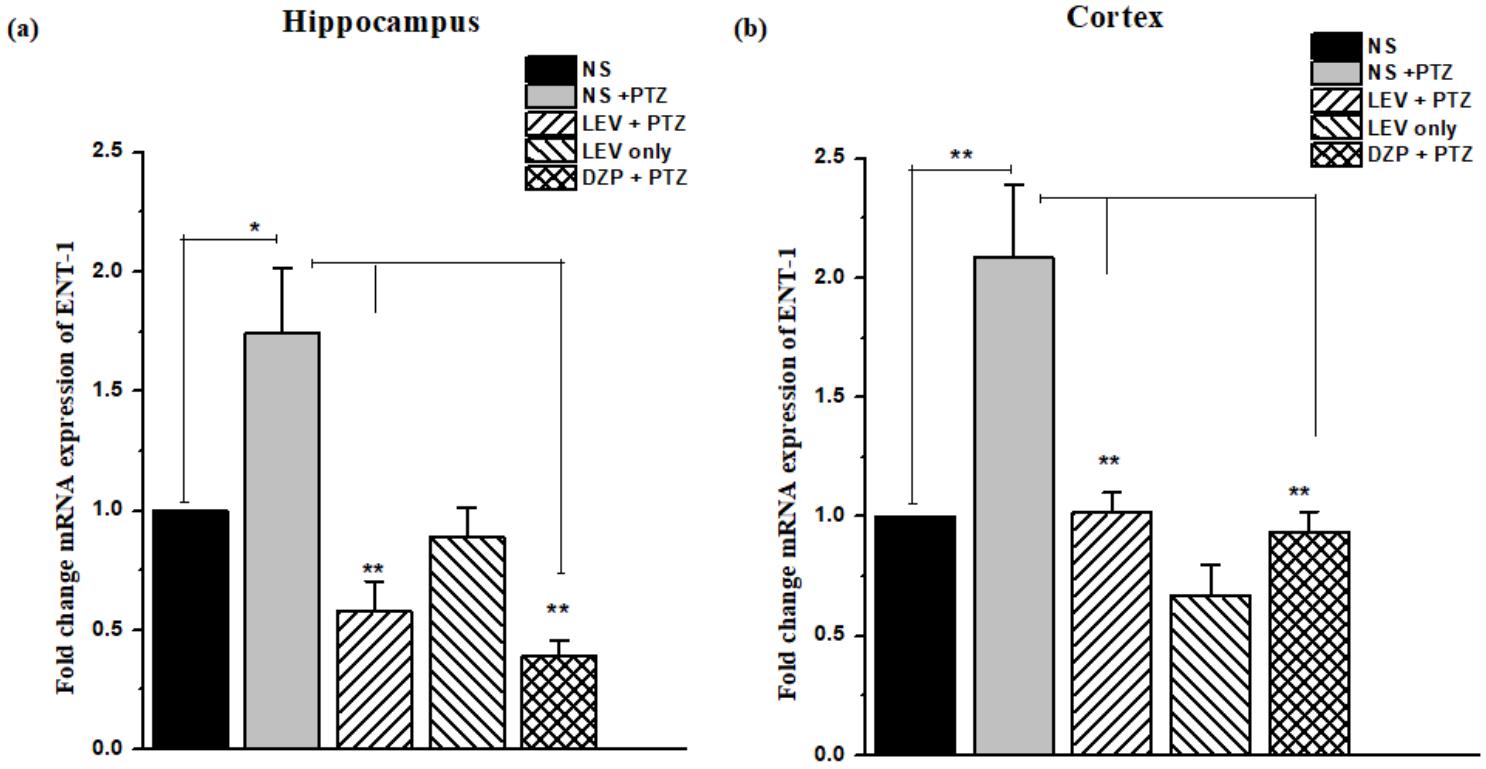
(a)

**Hippocampus**

(b)

**Cortex****Figure 4**

Levetiracetam increased the gene expression of Kir3.2 in kindling.(a) the Kir3.2 gene expression changes in the hippocampus and, (b) gene expression changes in the cortex following treatment with levetiracetam (200 mg/kg). Data were represented as Mean  $\pm$  SEM and analyzed through One-way ANOVA followed by post hoc Tukey analysis. \*P< 0.05, \*\*P < 0.01 were considered as significant vs. PTZ group.



**Figure 5**

Gene expression of ENT-1 following levetiracetam treatment. (a) The expression of ENT-1 in the hippocampus, and (b) gene expression changes in the cortex. Data is represented as Mean  $\pm$  SEM and analyzed through One-way ANOVA followed by post hoc Tukey analysis. Where \*\* & \* denote  $P < 0.01$  and  $P < 0.05$  respectively.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Original dataset of the article.xlsx](#)