Acta Biologica Szegediensis

Volume 65(2):247-252, 2021
Acta Biologica Szegediensis
http://abs.bibl.u-szeged.hu/index.php/abs

DOI:10.14232/abs.2021.2.247-252

ARTICLE

Investigation of efficacy of asenapine on passive avoidance learning and memory and oxidative stress in animal model of seizure-induced with pentylenetetrazole

Elham Farhadi[1], Naser Mirazi[1]*, Abdolkarim Hosseini[2]

[1]Department of Biology, Faculty of Basic Sciences, Bu-Ali Sina University, Hamedan, Iran
[2]Department of Animal Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran.

ABSTRACT Asenapine (ASE) has been used for treatment of bipolar disorder. There is also evidence that it may be useful in the treatment of neurodegenerative disorders. In this regard, the efficacy of ASE in an experimental model of seizure and memory impairment caused by seizures in rats has been investigated in the present study. Seizures in male Wistar rats (200-250 g) were induced by pentylenetetrazole (PTZ, 60 mg/kg, intraperitoneally (i.p.)), and the anticonvulsant effect of ASE (0.5 and 1 mg/kg, i.p.) was evaluated. The effect on memory was assessed using passive avoidance (PA) test in a shuttle box apparatus. After behavioral tests, the animals underwent deep anesthesia and were euthanized painlessly. Serum was isolated for oxidative stress assays (nitric oxide (NO), and glutathione (GSH)). Intraperitoneal injection of ASE decreased the mean number of myoclonic jerks and duration of generalized tonic clonic seizures (GTCS) and increased the mean latency of myoclonic jerk and GTCS compared to the PTZ group. Moreover, in the PA test, ASE caused a significant increase in retention latency (RL) and total time spent in the light compartment (TLC) compared to the PTZ group. Biochemical tests showed that ASE was able to significantly increase GSH serum levels and significantly reduce NO serum levels compared to the PTZ group. Overall, this study suggests the potential neuroprotective effects of ASE in a model of memory impairment caused by seizures via the mechanism of inhibition of the oxidative stress pathway.
Acta Biol Szeged 65(2):247-252 (2021)

KEY WORDS
asenapine
oxidative stress
passive avoidance learning
rat
seizure

ARTICLE INFORMATION
Submitted
08 October 2021
Accepted
28 November 2021
***Corresponding author**
E-mail: mirazi205@gmail.com

Introduction

Seizure is a condition in which nerve cells make sudden and simultaneous discharges and is often accompanied by changes in the network and neural function. The term epilepsy – one of the most common diseases in the world – is defined as the presence of two or more seizures (Katyayan and Diaz-Medina 2021). Along with seizures, epilepsy is also associated with several other comorbidities, including cognitive deficits, which are very common in patients with epilepsy (Suleymanova 2021). At present, most cases of epilepsy are treated or controlled with anti-epileptic drugs (AEDs), which, as has been shown, have limitations in performance, safety, and efficacy (Fattorusso et al. 2021).

Although there are many studies on how comorbidities with epilepsy develop, there is little information on how a generalized or acute seizure causes memory impairment associated with learning (Carter et al. 2017). In addition to memory impairment, studies have indicated that acute generalized seizures are associated with increased oxidative stress (OS) and the production of reactive oxygen species (ROS). There is ample evidence that OS plays a pivotal role in promoting seizures and epilepsy, causing membrane lipid peroxidation and depletion of antioxidant enzyme levels (Olowe et al. 2020).

Asenapine (ASE) is an antipsychotic drug developed to treat schizophrenia and bipolar disorder. ASE exhibits its pharmacological effects by acting on serotonergic, dopaminergic, alpha-adrenergic, and histaminergic receptors. Having these pharmacological properties, it has been shown to have anti-anxiety, anti-stress, sedative, and anti-depressant properties (Grossini et al. 2014; Marazziti et al. 2019; Marston et al. 2009; Vieta and Montes 2018). ASE has also been shown to improve phencyclidine-induced object recognition deficiency in rats due to its antagonistic properties on the dopamine d1 receptor (Snigdha et al. 2011).

To the best of our knowledge, so far, there has been no

report on the effect of ASE on the seizure process, as well as on the memory impairment associated with seizures in humans or animal models; likewise, how it affects OS is still unknown. Thus, this study was designed and performed to identify the neuroprotective properties of ASE in rats with seizure and consequent memory deficit induced by pentylenetetrazole (PTZ), and to investigate the possible antioxidant mechanisms that ASE may suggest.

Materials and methods

Animals

Locally bred male Wistar rats (8 weeks old, 200-250 g) were used in the present study. These rodents were kept in standard cages in the animal room under controlled conditions (room temperature 22 ± 2 °C and 12 h light/dark cycle). Standard food for rats (Pars Animal Feed Co., Iran) as well as water were made available to the animals in an unlimited manner. All the experiments were performed between 9 and 12 a.m. to reduce the effect of the light cycle on the susceptibility to seizures. Working with animals and the implementations of the experiments were completely done in accordance with the international ethical principles. The research protocol was also approved by the University's Animal Ethics Committee (IR.BASU.REC.1400.003).

Drugs and chemicals

Asenapine (ASE) (10 mg) was obtained from Hikma Pharma, Egypt. Pentylenetetrazole (PTZ) was purchased from Sigma Company as a crystalline, white powder.

Experimental design and treatment protocol

Twenty rats were randomly divided into four groups. Seizures were inducted by intraperitoneal (i.p.) injection of PTZ (60 mg/kg) dissolved in normal saline (Kumar et al. 2018). ASE was administrated at doses of 0.1 and 0.5 mg/kg (Huang et al. 2008). The volume of injections in all animals was constant at 0.5 ml. The test protocol used to evaluate the effect of ASE on the behavioral activities was as follows:

- -Group I (control group): Rats that received only normal saline.
- -Group II (PTZ group): Rats that received normal saline half an hour before the PTZ injection.
- -Group III (ASE 0.1 group): Rats that received asenapine (0.1 mg/kg) half an hour before the PTZ injection.
- -Group IV (ASE 0.5 group): Rats that received asenapine (0.5 mg/kg) half an hour before the PTZ injection.

Table 1. Racine's scale for pentylenetetrazole (PTZ)-induced seizure in rats.

Score	Behavioral manifestation
0	No behavioral sign
1	Ear and facial twitching
2	Head nodding and myoclonic jerks
3	Unilateral forelimb clonus with lorditic posture
4	Bilateral forelimb clonus with rearing and falling
5	Generalized tonic-clonic seizure (GTCS) with loss of postural tone

Behavioral evaluation of seizure manifestation

The motor behavior of the animals in each group was recorded in the plexiglass box (the box cleaned and dried before each test) and stored by a computer-connected camera for half an hour after the PTZ injection and was examined by researcher in a blind fashion. The latency and number of myoclonic jerks, and latency and duration of generalized tonic clonic seizure (GTCS) in animals was evaluated based on the stereotypical behavioral manifestations that were displayed after PTZ injections in six stages (Table 1) (Hosseini et al. 2021).

Evaluation of passive avoidance memory

The rate of memory recovery in animals was assessed by a shuttle box (Borj Sanaat Co., Iran) (Hosseini et al. 2021). The device consists of a dark and a light compartment, separated by a guillotine door. The floor of the dark chamber has steel rods that can transmit electric shock to the feet of the rats. Briefly, on the first day of the acquisition phase, each rat was placed separately in a clear compartment. After 30 s of habituation, the guillotine door was opened and initial latency (IL) was measured to enter the dark chamber. The rats that showed IL for more than 60 s were excluded from further analysis. When the rats entered the dark area, the guillotine door would quickly be closed, and an electric foot shock (75 V, 0.2 mA, 50 Hz) was applied to them for 3 s. The animal would be transferred to its cage 30 s after the electric shock and this operation was repeated 5 min later. The rats were shocked every time they put all four limbs in the dark side. The training would end when the animal stayed in the bright area for 120 consecutive seconds. The number of shocks (SN) was measured until acquisition. Twenty-four hours later, like before, retention latency (RL) as well as total light compartment (TLC) time was measured after seizure induction, but no electric shock was applied. Retention time was measured in 300 s.

Animal euthanasia and serum extraction

After behavioral tests, the animals underwent deep an-

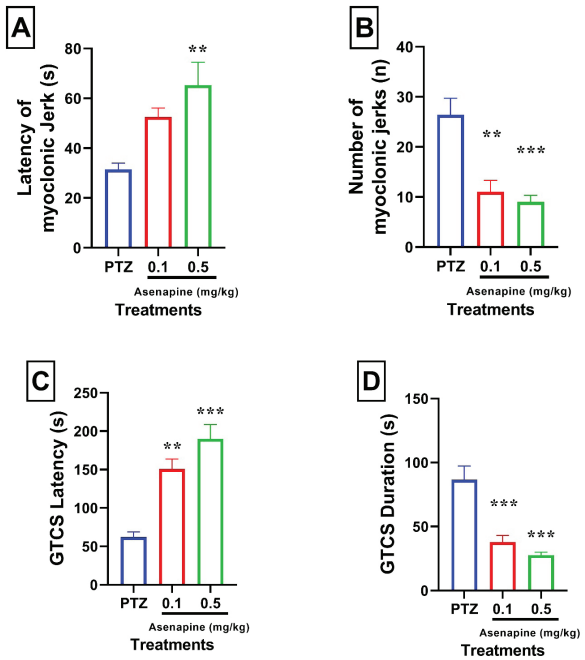

Figure 1. The effect of ASE (0.1, and 0.5 mg/kg, 30 min prior to testing) after PTZ treatment (60 mg/kg, i.p.) on latency of myoclonic jerk (**A**), number of myoclonic jerks (**B**), GTCS latency (**C**), and GTCS duration (**D**) in male Wistar rats. The data represents as the mean ± SEM (n = 5 rats per group). **p < 0.01; ***p < 0.001 significant difference between ASE (0.1 or 0.5 mg/kg) treatment with PTZ group.

ASE: asenapine, GTCS: generalized tonic clonic seizure, PTZ: pentylenetetrazole.

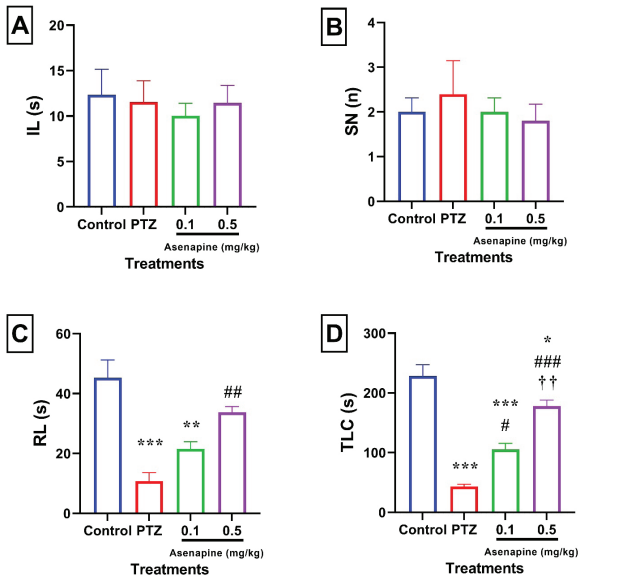

Figure 2. The effect of ASE (0.1, and 0.5 mg/kg, 30 min prior to testing) after PTZ treatment (60 mg/kg, i.p.) on IL (**A**), SN (**B**), RL (**C**), and TLC (**D**) in male Wistar rats. The data represents as the mean ± SEM (n = 5 rats per group). *p < 0.05; **p < 0.01; ***p < 0.001 significant difference between PTZ or ASE (0.5 or 1 mg/kg) treatment with control group. #p < 0.05; ##p < 0.01; ###p < 0.001 significant difference between ASE (0.1 or 0.5 mg/kg) treatment with PTZ group. ††p < 0.01 significant difference between ASE (0.1 mg/kg) treatment with ASE (0.5 mg/kg) group.

ASE: asenapine, IL: initial latency, PTZ: pentylenetetrazole, RL: retention latency, SN: shock number, TLC: total light compartment.

esthesia with ketamine and xylazine, and their blood was collected after cardiac puncture by a sterile syringe. The blood was allowed to clot for half an hour at room temperature and then the serums were separated by a centrifuge at 3000 rpm for 15 min and stored at -20 °C.

Measurement of oxidative stress markers

To measure GSH and NO oxidative stress indices, animal serum samples and conventional kits (Novin Navand Salamat Co., Iran) available in the market were used. The activity of GSH was measured according to the kit instructions at a wavelength of 340 nm by microplate reader (BioteTek ELx808, USA) and was reported in mU/mL. In addition, the amount of NO was measured according to the instructions of the kit and at a wavelength of 550 nm by the same microplate reader and was reported in terms of nmol/mL.

Statistical analysis of data

The results of the present study were shown as mean ± SEM. The normality of the data was tested by Shapiro-Wilk test. If the data were normal, then one-way ANOVA and Tukey's post hoc test were used to examine the differences between the groups. If the hypothesis of normality of the data was rejected, then non-parametric Kruskal-Wallis test and Dunn's test post hoc test were used to examine the differences between the groups. All statistical analyses were performed by GraphPad Prism software. In all analyses, the value of *P* was set at less than 0.05.

Results

The effect of ASE on the activity of PTZ-induced seizure

The effect of different treatments on the manifestations of PTZ-induced convulsive behavior is displayed in Fig. 1(A-D). Regarding the latency of myoclonic jerk, statistical analysis revealed a significant increase in the group IV, but not the group III, vs. group II (Fig. 1A). As shown in Fig. 1B, the number of myoclonic jerks in different groups was affected; the effect was a significant decrease in the group III and group IV compared to the group II. Regarding the GTCS latency, a significant increase was observed in the group III and group IV compared to the group II

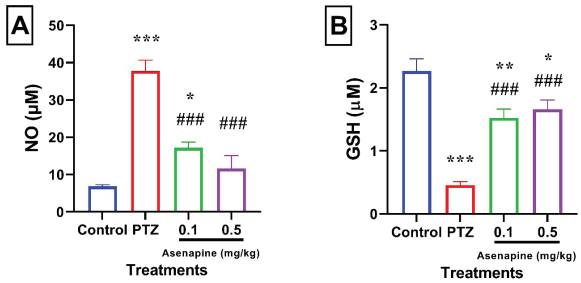

Figure 3. The effect of ASE (0.1, and 0.5 mg/kg, 30 min prior to testing) after PTZ treatment (60 mg/kg, i.p.) on serum level of NO (**A**), and GSH (**B**), in male Wistar rats. The data represents as the mean ± SEM (n = 5 rats per group). *p < 0.05; **p < 0.01; ***p < 0.001 significant difference between PTZ or ASE (0.1 or 0.5 mg/kg) treatment with control group. ###p < 0.001 significant difference between ASE (0.1 or 0.5 mg/kg) treatment with PTZ group.

ASE: asenapine, PTZ: pentylenetetrazole, NO: nitric oxide, GSH: glutathione.

(Fig. 1C). GTCS duration was significantly decreased in the group III and group IV vs. group II (Fig. 1D).

The effect of ASE on passive avoidance memory

There was no statistically significant difference between IL (Fig. 2A) and SN (Fig. 2B) in different treatment groups. However, RL in the PTZ group was significantly decreased compared to the control group, indicating memory impairment ($P < 0.001$). RL in the group III was not significantly different compared to the group II. On the contrary, in the group III, RL was increased significantly vs, group II ($P = 0.002$) (Fig. 2C). Regarding TLC time, the group II exhibited a significant decrease compared to the group I ($P < 0.001$). In both group III and group IV, TLC significantly increased vs. group II ($P = 0.010$ and $P < 0.001$, respectively) (Fig. 2D).

The effect of ASE on oxidative stress markers

As shown in Fig. 3A, there was a significant increase in serum NO levels in the group II compared to group I ($P < 0.001$); but in the group III and group IV a significant decrease was observed in serum NO level compared to the group II ($P < 0.001$). In case of serum GHS levels, the effects were similar (Fig. 3B).

Discussion

In the present study, the effect of ASE on seizures and PTZ-induced avoidance memory deficits in rats was investigated. The results revealed that ASE has a statistically significant effect on behavioral manifestation of PTZ-induced seizures. The PA test also showed that PTZ caused memory impairment resulting in reduced RL and TLC. The results of this study are in line with reports on memory impairment due to PTZ-induced seizures (Aghaie et al. 2021; Hosseini et al. 2021; Nagib et al. 2018). By inducing significant increase in RL and TLC compared to the PTZ group, ASE reversed the effect of PTZ-induced memory impairment, indicating its protective role against seizures and seizure-induced memory impairment. While no studies have been reported on the anticonvulsant effect or the effect on memory of ASE so far, there are some reports on the inhibitory effects of ASE on experimental models of psychosis or bipolar disorder (BD) as well as on the progression of underlying diseases associated with psychosis or BD (Marston et al. 2009; McLean et al. 2010; Snigdha et al. 2011).

Various mechanisms have been proposed for how PTZ injection causes seizures and the associated memory impairment. One of the most important factors in the development of seizures and resulting behavioral changes is OS and shift in the ROS level (Olowe et al. 2020). In the present study, the results showed a decrease in the GSH and an increase in NO serum levels following PTZ injection. The present study, similar to various prior studies, showed that the injection of PTZ in animal models increases OS and NO and decreases antioxidant levels (Hosseini et al. 2021; Kawakami et al. 2021; Kumar et al. 2018). Glutathione as an antioxidant helps protect cells against free radical damage. Glutathione is present inside cells in states of reduced GSH and oxidized GSSG. In healthy cells and tissues, more than 90% of total glutathione is in reduced form, and less than 10% as GSSG disulfide. The high concentration of GSH is due to the fact that the glutathione reductase enzyme (which transforms it from the oxidized state) is very active. Increase in GSSG to GSH is indicative of oxidative stress (Cárdenas-Rodríguez et al. 2014). NO is a molecular mediator which is made by the nitric oxide synthase (NOS) enzyme from L-arginine, oxygen, and NADPH and participates in vascular homeostasis by inhibiting vascular smooth muscle contraction, platelet aggregation, and leukocyte adhesion to endothelium, inflammation, thrombosis, immunity and neurotransmission. People with atherosclerosis, diabetes, and high blood pressure often manifest NO pathway disorders (Ghimire et al. 2017). NO has also been reported to be increased in PTZ-induced seizures (Kawakami et al. 2021). The effects of ASE on NO and GSH have not been reported in the status epilepticus condition, but in a previous study, ASE modulate inducible NOS (iNOS) and protected porcine coronary endothelial cells against oxidative stress by preventing ROS production and GSH loss (Grossini et al. 2014). Therefore, the positive effects of ASE on seizures as well as the improvement of memory impairment caused by seizures which were observed in the

present study can be partially ascribed to the antioxidant properties of the agent.

The present study for the first time examined the antioxidant effects of ASE, as well as its possible pathway. The elicited results could pave the way for future studies. Despite this strength, the present study had a limitation: this study did not investigate the molecular pathway or inflammatory cytokines due to the small number of samples and time constraints. This issue can be addressed in future research designs.

Conclusion

Overall, the present study provided evidence for the potential neuroprotective effects of the ASE drug. In addition, it was shown that ASE can be protective against oxidative stress induced during seizures. Therefore, a treatment strategy that could address the potential therapeutic effect of ASE with AEDs in the treatment of seizures as well as memory impairment associated with seizures calls for further research. Additional study designs are also needed to fully elucidate the mechanisms of anticonvulsant function as well as safety in their chronic use.

Acknowledgments

This study is financially supported by Bu-Ali Sina University. The research protocol was approved by the University's Animal Ethics Committee (IR.BASU.REC.1400.003). The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

Aghaie F, Shemshaki A, Rajabi M, Khatami P, Hosseini A (2021) Rapamycin alleviates memory deficit against pentylenetetrazole-induced neural toxicity in Wistar male rats. Mol Biol Rep 48(6):5083-5091.

Cárdenas-Rodríguez N, Coballase-Urrutia E, Pérez-Cruz C, Montesinos-Correa H, Rivera-Espinosa L, Sampieri A, 3rd, Carmona-Aparicio L (2014) Relevance of the glutathione system in temporal lobe epilepsy: evidence in human and experimental models. Oxid Med Cell Longev 2014:759293.

Carter AN, Born HA, Levine AT, Dao AT, Zhao AJ, Lee WL, Anderson AE (2017) Wortmannin attenuates seizure-induced hyperactive PI3K/Akt/mTOR signaling, impaired memory, and spine dysmorphology in rats. eNeuro 4:ENEURO.0354-16.2017.

Fattorusso A, Matricardi S, Mencaroni E, Dell'Isola GB, Di Cara G, Striano P, Verrotti A (2021) The pharmacoresistant epilepsy: An overview on existant and new emerging therapies Front Neurol 12:674483.

Ghimire K, Altmann HM, Straub AC, Isenberg JS (2017) Nitric oxide: what's new to NO? Am J Physiol Cell Physiol 312:C254-C262.

Grossini E, Gramaglia C, Farruggio S, Bellofatto K, Anchisi C, Mary D, Vacca G, Zeppegno P (2014) Asenapine increases nitric oxide release and protects porcine coronary artery endothelial cells against peroxidation. Vascul Pharmacol 60:127-141.

Hosseini A, Allahyari F, Azizi V (2021) Effects of *Tanacetum polycephalum* on passive avoidance learning and oxidative stress in epileptic model of memory impairment in the male Wistar rats. Adv Tradit Med 21:545–552.

Huang M, Li Z, Dai J, Shahid M, Wong EH, Meltzer HY (2008) Asenapine increases dopamine, norepinephrine, and acetylcholine efflux in the rat medial prefrontal cortex and hippocampus. Neuropsychopharmacology 33:2934-2945.

Katyayan A, Diaz-Medina G (2021) Epilepsy: Epileptic syndromes and treatment. Neurol Clin 39:779-795.

Kawakami Y, Murashima YL, Tsukimoto M, Okada H, Miyatake C, Takagi A, Ogawa J, Itoh Y (2021) The roles of dominance of the nitric oxide fractions nitrate and nitrite in the epilepsy-prone EL mouse brain. J Nippon Med Sch 88:189-193.

Kumar R, Arora R, Agarwal A, Gupta YK (2018) Protective effect of *Terminalia chebula* against seizures, seizure-induced cognitive impairment and oxidative stress in experimental models of seizures in rats. J Ethnopharmacol 215:124-131.

Marazziti D, Mucci F, Falaschi V, Dell'Osso L (2019) Asenapine for the treatment of bipolar disorder. Expert Opin Pharmacother 20:1321-1330.

Marston HM et al. (2009) Asenapine effects in animal models of psychosis and cognitive function. Psychopharmacology (Berl) 206:699-714.

McLean SL, Neill JC, Idris NF, Marston HM, Wong EH, Shahid M (2010) Effects of asenapine, olanzapine, and risperidone on psychotomimetic-induced reversal-learning deficits in the rat. Behav Brain Res 214:240-247.

Nagib MM, Tadros MG, Al-Khalek HAA, Rahmo RM, Sabri NA, Khalifa AE, Masoud SI (2018) Molecular mechanisms of neuroprotective effect of adjuvant therapy with phenytoin in pentylenetetrazole-induced seizures: Impact on Sirt1/NRF2 signaling pathways. Neurotoxicology 68:47-65.

Olowe R, Sandouka S, Saadi A, Shekh-Ahmad T (2020) Approaches for reactive oxygen species and oxidative stress quantification in epilepsy. Antioxidants (Basel) 9(10):990.

Snigdha S, Idris N, Grayson B, Shahid M, Neill JC (2011)

Asenapine improves phencyclidine-induced object recognition deficits in the rat: evidence for engagement of a dopamine D1 receptor mechanism. Psychopharmacology (Berl) 214:843-853.

Suleymanova EM (2021) Behavioral comorbidities of epilepsy and neuroinflammation: Evidence from experimental and clinical studies. Epilepsy Behav 117:107869.

Vieta E, Montes JM (2018) A review of asenapine in the treatment of bipolar disorder. Clin Drug Investig 38:87-99.