Original Article

Piracetam as Neuroprotective, Anticonvulsant, and Anti-Anxiety Agent: An *In Vivo* Study on Ptz Epileptic Rats

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ABSTRACT

Epilepsy, a category of neurological disorder, is characterized by recurrent seizures. Epileptic seizures are characterized by sudden alterations in brain electrical activity. Piracetam is a derivative of cyclic aminobutyric acid that exerts neuroprotective effects. The objective of this study was to evaluate the neuroprotective, anticonvulsant, and anti-anxiety effects of piracetam in the pentylenetetrazole (PTZ) seizure rat model. To evaluate the anticonvulsant properties of piracetam in the PTZ seizure model, the experimental groups were administered piracetam at doses of 30 or 100 mg/kg. The positive control group was administered diazepam (2 mg/kg), while the negative control group was treated with only PTZ. The anti-anxiety effects were evaluated using the elevated plus maze and open field tests. Additionally, the antioxidant effects of piracetam on brain tissues were examined. The open field test results demonstrated a significant increase in the number of crossings over the line in the Piracetam (30 and 100 mg/kg) and diazepam groups, in comparison to the negative control group. In the plus maze test, the groups administered Piracetam demonstrated a greater tendency to spend time in the open arms than the control group. Furthermore, diazepam markedly elevated the time spent in the open arms in comparison to the negative control group. The histological results demonstrated structural alterations in hippocampal neurons. Additionally, the antioxidant test demonstrated that Piracetam possesses antioxidant properties when compared to the negative control group. Piracetam demonstrated anticonvulsant and neuroprotective effects in PTZ-induced epileptic rats, exhibiting the ability to inhibit or reduce the incidence of seizures. Additionally, it demonstrated anti-anxiety and sedative properties. The neuroprotective effects of Piracetam may be attributed to its ability to regulate neurotransmitter systems, including cholinergic, serotonergic, noradrenergic, and glutamatergic pathways. It can be posited that Piracetam may possess neuroprotective, anti-epileptic, anti-anxiety, and antioxidant properties in PTZ epileptic rats. Nevertheless, additional research is required to substantiate these findings.

Keywords: Antioxidant, Epilepsy, Piracetam, Plus Maze.

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1. Introduction

Epilepsy, a category of neurological disorder, is characterized by recurrent seizures (1, 2). Epileptic seizures are characterized by sudden alterations in brain electrical activity. Seizures are prone to recurrence and often have no underlying cause (3, 4). The precise mechanism underlying the occurrence of epileptic seizures remains unknown. However, it is widely accepted that excessive and aberrant nerve activity in the cerebral cortex represents the primary cause of such events (5, 6). Epilepsy may be caused by a number of factors, including brain damage, stroke, tumors, infections, or congenital abnormalities (7, 8). An electroencephalogram (EEG) is frequently employed as a diagnostic tool in the confirmation of epilepsy. The long-term risk of recurrent seizures is dependent on the area of the brain affected and the age of the individual. Seizures may vary in severity (10, 11). Piracetam is a cyclic aminobutyric acid derivative (GABA) with neuroprotective effects, which has recently been employed in the management of brain atrophy as a multifactorial therapy (12). Additionally, it is a nootropic agent that enhances learning and memory, increases memory resilience to disruptive conditions, and protects the brain from physical or chemical injuries (13, 14). Following oral administration, piracetam is rapidly absorbed and distributed to the majority of vital organs. It is able to pass through the blood-brain barrier and concentrate in the cerebellum, hippocampus, and choroid plexus gray matter (15). It is a cognitive enhancer that is used in numerous countries to treat cognitive impairment, brain damage, and dementia (16). Piracetam has been demonstrated to enhance prostacyclin production, which in turn results in the dilation of blood arteries and the prevention of platelet aggregation. In individuals experiencing acute cerebral ischemia, piracetam has also been demonstrated to enhance cerebral blood flow. Furthermore, Piracetam has demonstrated neuroprotective properties by reducing lipofuscin levels, which are indicative of neuronal membrane damage (16, 17). The objective of this research was to evaluate the neuroprotective, anticonvulsant, and anti-anxiety effects of Piracetam in the pentylenetetrazole seizure rat model.

2. Materials and Methods

2.1. Animals

Thirty-two adult male Wistar rats, with a weight range of 180 to 220 grams, were procured from the University of Tehran and divided into four groups, with eight rats in each group. The laboratory conditions were maintained at 28.2°C, with a 12:12 light-dark cycle, 65% humidity, and a standard rat diet. The experiments were conducted between 8 a.m. and 12 p.m. Each treatment group consisted of eight rats. All animal procedures were conducted in accordance with the guidelines set forth by the Human and Animal Ethics Committee of the UMA Local Committee (Approval No.: IR.UMA.REC.1400.086).

2.2. Chemicals and Drugs

The pentylenetetrazole (PTZ) was procured from Sigma Aldrich (USA), while the piracetam was obtained from Tolid Daru Pharmaceutical Co. (Tehran, Iran). The substances were dissolved in a saline solution. All medications were administered intraperitoneally at a dosage of 0.002 ml/g, in order to prevent the development of hypertension and excessive fluid retention. On a daily basis, the solutions of the aforementioned medications were freshly prepared.

2.3. Investigation of Pentylenetetrazole-Induced Seizures

A total of 32 rats were randomly divided into four groups, with each group comprising eight rats. Two groups were administered Piracetam at a dosage of either 30 or 100 mg/kg for a period of seven days. The positive control group was administered diazepam (2 mg/kg) half an hour prior to the PTZ injection (60 mg/kg). The negative control group was administered only PTZ. Following the administration of PTZ, each animal was placed in a separate clear Plexiglas cage and observed for a minimum of 30 minutes via video recording to document the occurrence of convulsive behaviors. Clonic seizures (raising and bilateral forelimb clonus) with the righting reflex maintained (Stage 4 of the Racine scale) and tonic-clonic seizures with the righting reflex lost (Stage 5 of the Racine scale) were both considered convulsive motions (18).

2.4. Open Field Test

This test is designed to assess behavioral responses, including motor activity and search behavior, as well as to measure anxiety. The apparatus is provided with a Plexiglass frame box with dimensions of 36×72×72 cm. An open box with a floor divided into 25 equal squares is utilized (6). The nine central squares are designated as the central region, while the remaining squares are classified as fringe portions (Figure 1). The animals were randomly assigned to one of the four corners of the device and permitted to explore for a period of five minutes. During this period, the mouse's behavior was documented via video recording. It is hypothesized that increases in the time spent in the center and the number of times the animal enters have anti-anxiety benefits and are also employed for search behavior. Additionally, the total distance traversed and the mean velocity were documented as indices of motor activity (6, 19).

2.5. Elevated Plus Maze Test

The elevated maze test is a behavioral assessment tool utilized to evaluate anxiety-related behaviors in animal models with central nervous system diseases. The maze apparatus was constructed in the shape of a plus sign and situated above the ground. The maze consisted of four arms and a central area, with two arms open (50 cm × 10 cm) and the other two arms closed (50 cm × 10 cm × 20 cm) (6, 20). The animal was permitted to explore the maze for a period of five minutes, during which it was able to move freely between the arms. The animal's behavior was recorded via video camera. At the conclusion of the test, the

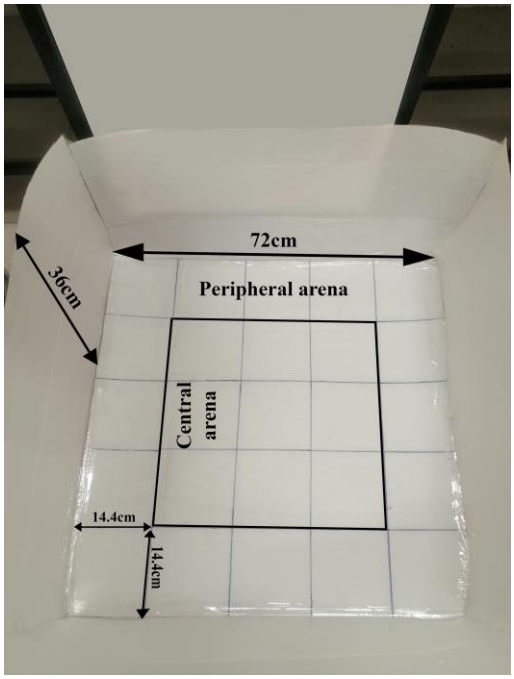

Fig 1. Open field test device used to assess behavioral responses.

proportion of time spent in the open arms relative to the closed arms will be calculated. In instances where the animal displays anxious behavior, it is observed that it tends to exhibit a preference for remaining in the closed arms (6, 21).

2.6. Antioxidant Test

2.6.1. Brain Tissue Homogenates Preparation

Following the combination of the brain tissue samples with cold phosphate buffer salt solution, homogenization was conducted using a mechanical homogenizer. The resulting homogenates were then subjected to centrifugation at 4°C for 10 minutes at a speed of 10,000×g. The resulting supernatants were subsequently stored in a freezer until subsequent biochemical analysis. TAS and TOS levels were quantified using commercial kits (Kia Zist assay kit, Iran) (22, 23).

2.6.2. Total Antioxidant Status (TAS)

The total antioxidant status was evaluated in accordance with the methodology previously described by Erel. To monitor the reaction rate of free radicals, the method employs the detection of the absorbance of colored dianisidyl radicals during free radical reactions, which are initiated by the production of hydroxyl radicals in the Fenton reaction. The results were expressed as micromolar Trolox equivalents per milligram of tissue protein (µmol Trolox Eq/mg protein) (24, 25).

2.6.3. Total Oxidant Status (TOS)

The concentration of tissue total oxidizable substances (TOS) was determined by the method of Earl. A change in absorbance is observed when ferrous ions are converted into ferric ions, which indicates the total oxidizable substance (TOS). The results were expressed as micromolar hydrogen peroxide equivalents per milligram of tissue protein (26, 27).

2.6.4. Oxidative Stress Index (OSI)

To assess the degree of oxidative stress, the OSI was determined by dividing the total oxidative status (TOS) by the total antioxidant status (TAS) and expressing the result as a percentage. The OSI was calculated according to the following formula: The OSI is calculated as follows:

OSI = [(TOS, mol H2O2 Eq/mg protein)/(TAS, mol Trolox Eq/mg protein) 100] (26, 28).

2.7 Histological Examination of the Hippocampus

The mice are initially anaesthetised with high doses of ketamine. Following confirmation of the mouse's demise through the absence of a pulse, respiration, heart rate, and response to toe pinching, the head was excised and brain tissue resection was performed (29). The tissue was subsequently subjected to a series of dehydration steps, including two hours in 10% formaldehyde, 24 hours in a new formaldehyde solution, 24 hours in 70% ethanol, one hour in 90% ethanol, and one hour in 100% ethanol. In some instances, the tissue was cleaned with xylene and molded in paraffin. Subsequently, the tissue was cut with a microtome to a thickness of 5 micrometers and stained with hematoxylin and eosin (30, 31).

2.8. Statistical Analysis

All data were analyzed with the SPSS 16 software (SPSS Inc., Chicago, IL, USA) and are presented as mean ± SEM, with a significance level of $P < 0.05$. One-way analysis of variance (ANOVA) was employed for the analyses. The Tukey post hoc test was employed to ascertain the existence of statistically significant differences between the groups.

3. Results

3.1. Pentylenetetrazole-Induced Seizures

The findings revealed that Piracetam at 30 and 100 mg/kg exhibited anticonvulsant properties and prolonged the latent period (stage 3 of the Racine scale) in comparison to the control group (Figure 2).

3.2. Open Field Test

A significant increase was observed in the Piracetam (30 and 100 mg/kg) and diazepam groups when compared to the control group (Figure 3A). Furthermore, the group treated with Piracetam (100 mg/kg) exhibited a significantly higher incidence than the diazepam group (Fig. 3B). The administration of Piracetam (30 and 100 mg/kg) resulted in a notable increase in the time spent in the center zone when compared to the control group (Figure 3B).

3.3. Plus Maze Test

The time spent in the open arms and the number of entries into this area was significantly greater in the Piracetam (30 and 100 mg/kg) and diazepam groups than in the control group (Fig. 4A and B). Furthermore, the time spent in the open arms was significantly longer in the Piracetam group (100 mg/kg) than in the diazepam-treated group (Figure 4A).

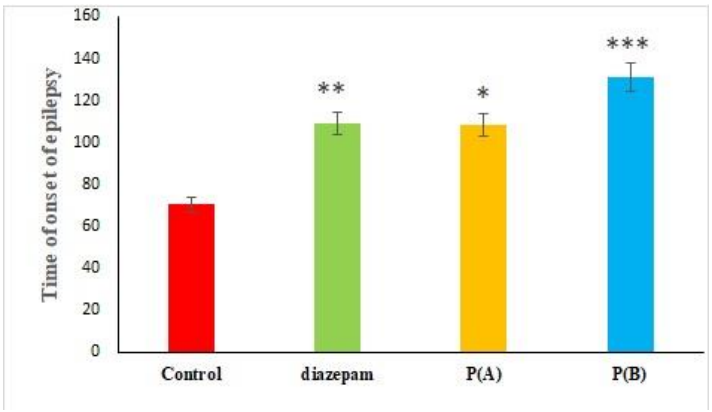

Figure 2. The protective effects of piracetam on the onset of epilepsy were found to be statistically significant when compared to the control group. (P < 0.05, P < 0.01, P < 0.001; Tukey's test), P(A): piracetam (30 mg/kg), P(B): (100 mg/kg).

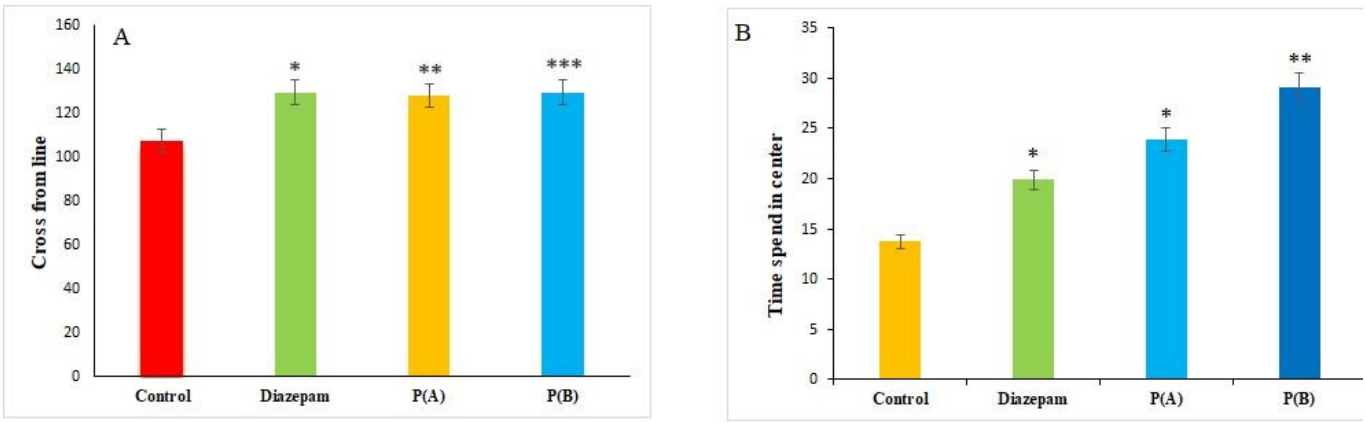

Figure 3. (A): Effect of Piracetam on Cross from Line. There were statistically significant differences in comparison to the control group (P < 0.05, P > 0.05). 01, P<0.001, Tukey's test, P(A): piracetam (30 mg/kg), P(B): (100 mg/kg), (B): effect of piracetam on time spent in the center. There were statistically significant differences in comparison to the control group (P < 0.05, P < 0.01, Tukey's test), with P(A) representing piracetam (30 mg/kg) and P(B) representing piracetam (100 mg/kg).

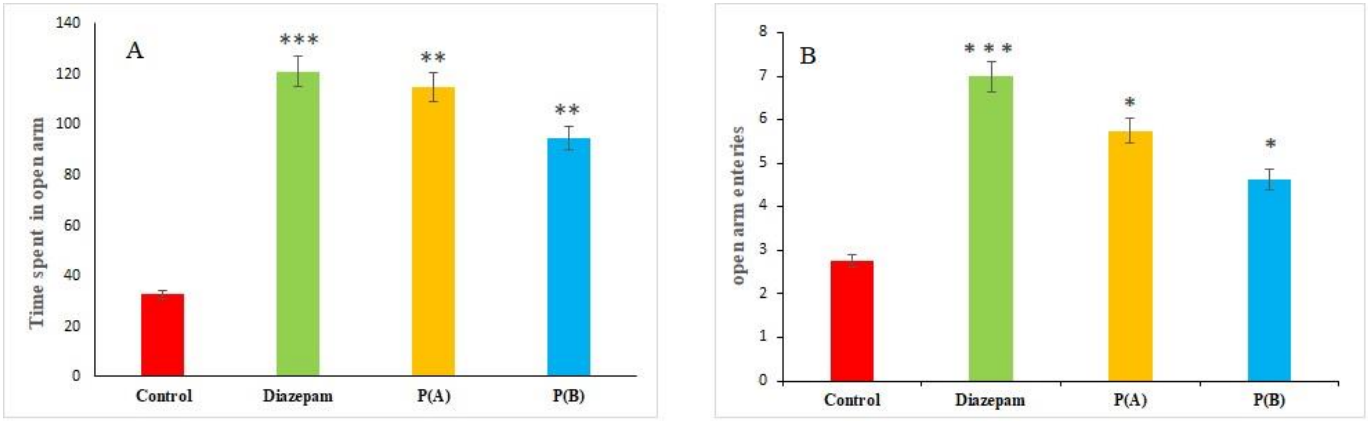

Figure 4. (A): The effect of piracetam on time spent in the open arm was statistically significant when compared to the control group (P<0.01, P<0.001, Tukey's test). The experimental groups were as follows: Group A: piracetam (30 mg/kg), Group B: piracetam (100 mg/kg). The effect of piracetam on open arm entries is also presented. There were statistically significant differences in comparison to the control group (P < 0.05, P < 0.001, Tukey's test), with P(A) representing the piracetam (30 mg/kg) group and P(B) representing the piracetam (100 mg/kg) group.

3.4. Biochemical Tests

The administration of Piracetam at doses of 30 and 100 mg/kg resulted in a notable reduction in brain TOS and OSI levels when compared to the control group (P <0.05, Table). Furthermore, administration of Piracetam (100 mg/kg) and diazepam resulted in a notable elevation in brain TAS levels when compared to the control group (p < 0.05, Table 1).

3.5. Histological Analysis

The results of the hematoxylin and eosin staining demonstrated that the normal hippocampus exhibited neurons with distinct morphology, homogeneous staining, and large nuclei. In contrast, hippocampal neurons in rats with unmanaged epilepsy exhibited cellular edema, nuclear degeneration, and cytoplasmic fragmentation, all of which are indicative of apoptosis (Figure 5). The negative control group demonstrated evidence of demyelination extension upon Luxol fast blue staining (Figure 6). Nevertheless, the administration of Piracetam and diazepam resulted in a notable reduction in demyelination when compared to the negative control group.

Table 1. Effect of Piracetam on oxidative stress in experimental groups compared to the negative control group. The data show the mean ± S.E.M. (n = 10); statistically significant differences from the negative control group (**P < 0.01, ***P < 0.001, Tukey's test).

Groups	TOS	TAS	OSI
Negative control	0.194 ± 0.02	0.057 ± 0.01	338.42 ± 8
Positive control	0.117 ± 0.08 **	0.076 ± 0.03	153.36 ± 18*
Piracetam 30 mg/kg	0.131 ± 0.02**	0.052 ± 0.02	252.92 ± 0.02
Piracetam 100 mg/kg	0.126 ± 0.07**	0.062 ± 0.01	210.18 ± 6*

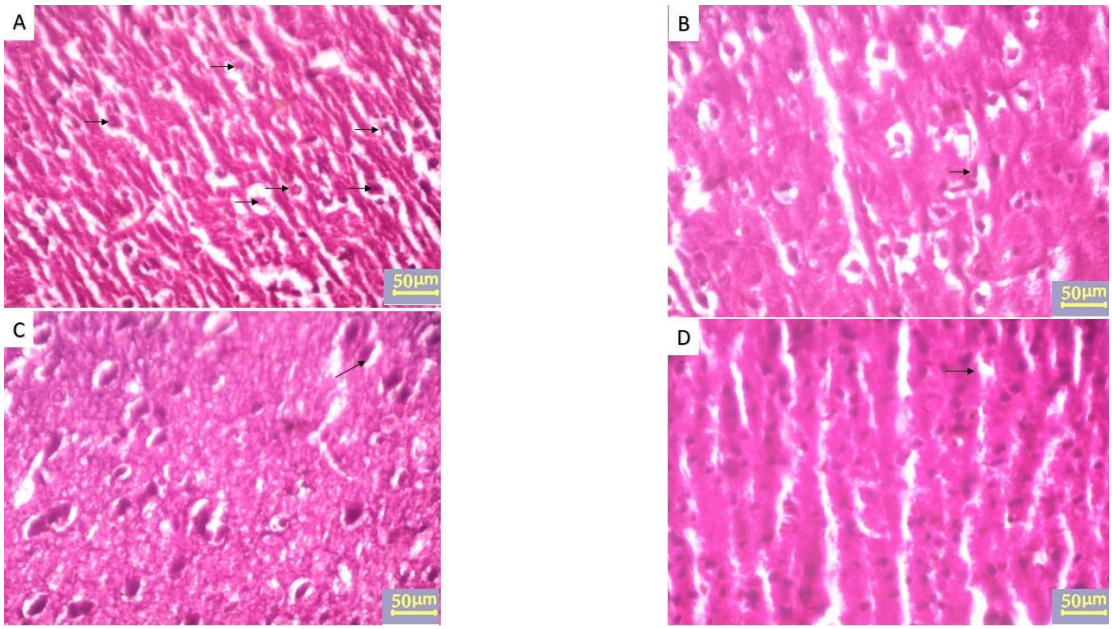

Figure 5. Effect of piracetam on neuronal injury following PTZ-induced seizures in rat. Parts of the rat hippocampus with staining Hematoxylin and Eosin. (black arrow) Neuronal damage in the hippocampus. (A): Negative control group, (B): Positive control group (diazepam), (C) Piracetam group (30 mg / kg), (D) Piracetam group (100 mg / kg).

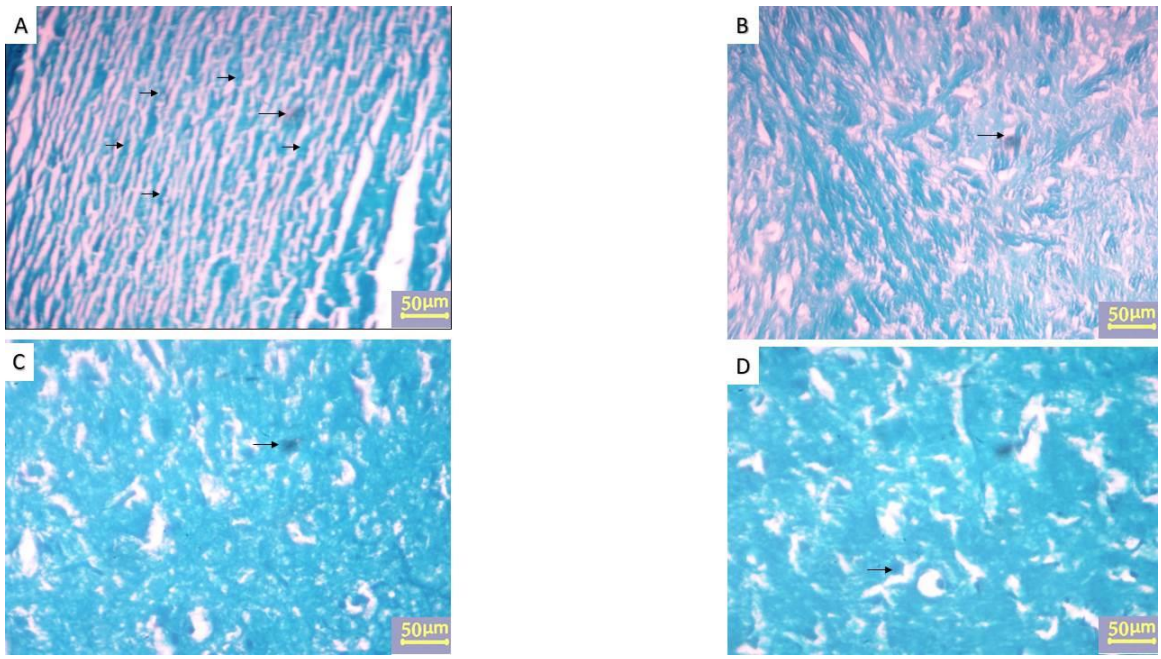

Fig 6. Effect of piracetam on neuronal injury following PTZ-induced seizures in rat. Parts of the rat hippocampus with staining Luxol fast blue. (black arrow) Neuronal damage in the hippocampus. (A): Negative control group, (B): Positive control group (diazepam), (C) Piracetam group (30 mg / kg), (D) Piracetam group (100 mg / kg).

4. Discussion

In light of the limitations of current pharmacological treatments for epilepsy, there is a pressing need for research into alternative or adjuvant therapies. Animal models are now frequently employed to study human symptoms, etiology, pathophysiology, and responses to anti-anxiety and anti-seizure medication (32, 33). The findings indicate that Piracetam exerts anticonvulsant and neuroprotective effects in PTZ-induced epileptic rats, with both doses demonstrating the capacity to inhibit or reduce the occurrence of seizures. Additionally, open field and elevated plus maze behavioral tests demonstrated that Piracetam exerts anti-anxiety and sedative effects. The precise anticonvulsant mechanism of action of Piracetam remains unclear. However, it is postulated that its effects on neurotransmitters may be responsible for this effect. The hypothesis is that Piracetam exerts its effects by acting on ion channels or ion carriers, thereby increasing neuronal stimulation (34-36). Piracetam has been demonstrated to enhance the functionality of the neurotransmitter acetylcholine receptor via muscarinic cholinergic receptors (ACh), which are implicated in memory processes (37, 38). Moreover, Piracetam may influence NMDA glutamate receptors, which are implicated in learning and memory processes (37). It can thus be surmised that Piracetam exerts significant influence over neurotransmission. It affects neurotransmitters, including cholinergic, serotonergic, noradrenergic, and glutamatergic systems (39). The modulation of these systems by Piracetam does not result from agonism or inactivation of the receptor, as the drug has no affinity for these receptors (7). It appears that Piracetam exerts its effects by increasing the number of presynaptic receptors and enhancing their functionality (7). The antioxidant tests demonstrated that seizures result in damage to the hippocampus and an increase in oxidative levels. Consequently, following the induction of epilepsy with PTZ, stress-inducing factors are observed to increase in brain tissue, resulting in the generation of reactive oxygen species that have the potential to damage the hippocampus (40). In this regard, Luo et al. demonstrated that Piracetam, which possesses anti-inflammatory properties, was able to decrease oxidative stress and neuronal damage caused by aluminum chloride in mice (26). Furthermore, Piracetam has been demonstrated to enhance the haloperidol-induced Parkinson's Disease (PD) rat model by modulating striatal dopamine release, thereby establishing its potential as a promising candidate for the management and control of PD. The primary mechanism through which Piracetam exerts its effects on PD is through its anti-inflammatory and antioxidant properties (41). In conclusion, the findings of this study indicate that Piracetam may possess neuroprotective, anti-epileptic, anti-anxiety, and antioxidant properties in PTZ epileptic rats. Nevertheless, additional research is required to substantiate these findings.

Limitations

It is recommended that future studies examine the nuclear factor erythroid 2-related factor 2 (Nrf 2), which is emerging as a regulator of cellular resistance to oxidants.

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Authors' Contribution
TK, AA and AAr performed the sampling and culture method, and drafted the manuscript. AA, TK and HH participated in the design of the study, performed the statistical analysis, and wrote the manuscript. All authors read and approved the final manuscript.

Ethics
We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest
The authors declare that they have no conflict of interest.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

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