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NEUROPROTECTIVE EFFECTS OF *CURCUMA LONGA* ON PENTYLENETETRAZOLE-INDUCED NEUROTOXICITY ON THE CEREBRUM AND HIPPOCAMPUS OF ALBINO WISTAR RATS

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ABSTRACT: *Epilepsy is a neurological disorder characterized by recurrent seizures, and current anti-epileptic drugs often have side effects. Curcuma longa, commonly known as turmeric, has been traditionally used for various medicinal purposes, including the treatment of neurological disorders. This study investigates the neuroprotective effects of Curcuma longa in pentylenetetrazol (PTZ)-induced epilepsy in albino rats. Group one received normal feed (control). Group two received PTZ to induce epilepsy intraperitoneally. Group three received PTZ with feed. Group four received a low dose of Curcuma longa with PTZ, and Group five received a high dose of Curcuma longa with PTZ. The experiment lasted for 28 days, during which seizure severity and neurotoxicity were assessed. The results revealed that PTZ administration significantly increased seizure severity and frequency, as well as oxidative stress and neuroinflammatory markers. Treatment with Curcuma longa extract, particularly at the low dose and high dose, significantly reduced oxidative stress and neuroinflammation and improved neuroprotection. These findings suggest that Curcuma longa extract may have potential neuroprotective effects and anticonvulsant properties, making it a promising candidate for adjunctive therapy in epilepsy management.*

KEYWORDS: Epilepsy, *Curcuma longa*, Pentylenetetrazol, Seizure, Neuroprotection, Anticonvulsant.

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INTRODUCTION

Pentylenetetrazol (PTZ), also known as pentylenetetrazol, leptazol or metrazol, is a drug with a complex chemical structure formerly used as a circulatory and respiratory stimulant. PTZ was first synthesized in 1894 by Andreas von Baeyer and Arthur Pinner (Vogt *et al*., 2022). High doses cause convulsions, as discovered by a Hungarian-American neurologist (Dhikav & Anand, 2007). It has been used in convulsive therapy, and was found to be effective primarily for depression, but side effects such as uncontrolled seizures were difficult to avoid (Bello *et al.,* 2022). However, it soon became evident that PTZ induces seizures in animals, leading to its adoption as a research tool in neuropharmacology to study seizure phenomena and to identify pharmaceuticals that may control seizure susceptibility. For instance, researchers induce kindling in animal models by administering repeated sub convulsive doses of PTZ (Bello *et al.*, 2022). PTZ primarily acts as a gamma-aminobutyric acid (GABA) receptor antagonist (Nicoll, 1988). GABA is the primary inhibitory neurotransmitter in the central nervous system. By blocking GABA receptors, PTZ disrupts inhibitory neurotransmission, leading to increased neuronal excitability and, ultimately, seizures (Ishaku *et al.*, 2023).

Epilepsy is a chronic brain condition that causes seizures, which are abnormal activities in the brain that can lead to temporary loss of consciousness or uncontrollable movement (Fisher *et al.*, 2014). Epileptic seizures, as well as chronic use of most anti-epileptic drugs, predisposes to cognitive impairment. *Curcuma longa* has been reported to possess antioxidant, anticonvulsant as well as neuroprotective potentials (Wu *et al*., 2024).

Curcuma longa, popularly known as turmeric, is native to India, and now extensively cultivated in the tropical and subtropical regions of South and Southeast Asia, including China, Indonesia, and India, and some areas of Africa, such as Nigeria, with a warm and wet tropical climate. ‘Curcuma’ is derived from its Arabic name (kurkum) or the Hebrew name (karkom), which means yellow. The term *longa* comes from the elongated shape of its rhizome (Iweala *et al.,* 2023). It is a rhizomatous herbaceous perennial plant used in folk medicine for the treatment, prevention, and management of various illnesses, such as epileptic seizures (Iweala *et al.,* 2023). It has been noted for its coloring, flavoring, and digestive properties since ancient times. *Curcuma longa* has been reported to possess antioxidant, anticonvulsant, and neuroprotective potentials. Hence, this study was conducted to evaluate the effect of *Curcuma longa* against neurotoxicity seizures, cognitive impairment, and oxidative stress in pentylenetetrazol-induced kindling in rats.

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MATERIALS AND METHODS

Materials

The materials used for this research were in accordance with Gupta *et al.* (2019). They include animal cages with bedding, food (standard rat chow), water bottle, *Curcuma longa L*. extract, pentylenetetrazole (PTZ), normal saline, standard antiepileptic drug (diazepam), spectrophotometer, centrifuge, microscope (light and fluorescence), autoclave, incubator, vortex mixer, pipettes and micropipettes, animal weighing balance, gloves, mask, lab coat, biohazard waste disposal bags, and animal sacrifice equipment (e.g., CO2 chamber).

Plant Authentication, Extraction, and Storage

We got the plant from Monday Market, Maiduguri and the authentication was done by a taxonomist called Dr. Cletus A. Ukwubile at the Faculty of Pharmacy, University of Maiduguri, Borno State, Nigeria. The plant extraction was carried out in accordance with Kumar *et al*. (2019). The turmeric seeds were washed and dried (air drying) to 491 mg and combined with 3 litres of distilled water. It was strained and filtered after 24 hours. The liquid was filtered and evaporated until the volume was decreased. The extract was dried in an oven until it was ready for use.

Preparation of *Curcuma longa L.*

Curcuma longa was washed and cut into smaller pieces and dried. The dried portions were ground into fine powder; 491 g of the powder was macerated in maceration equipment with 3000 ml of distilled water for 24 hours and left to settle for an hour before being decanted and evaporated; and the dried residue was scraped off (Kumar *et al.*, 2019).

Chemicals

Pentylenetetrazole (PTZ) was purchased from Sigma Aldrich in St. Louis in USA. Standard antiepileptic drug diazepam was obtained from the Faculty of Pharmacy, University of Maiduguri, Maiduguri, Borno State. The anesthesia, including Ketamine used for the sacrifice, was also obtained from the Faculty of Pharmacy, University of Maiduguri, Borno State. The ethanol and distilled water were purchased from the university teaching hospital and the human anatomy histology laboratory.

Experimental Design

The rats were randomly divided into 5 groups of 7 rats each. Group one (1) served as the normal control group and was given normal animal feeds with distilled water orally by gavage. Group two (2) was the positive control and was given diazepam 5 mg/kg intraperitoneally one hour prior to 35 mg/kg of PTZ subcutaneously. Group three (3) was the negative control and was given normal animal feed with 35 mg/kg of pentylenetetrazol. Group four (4) served as the low dose group and was administered with 250 mg/kg of the extract orally one hour prior to 35 mg/kg of PTZ subcutaneously. Group five (5) was the high dose group and was administered with 500 mg/kg of the extract orally one hour prior to 35 mg/kg of PTZ subcutaneously. The animals were observed

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for one hour after PTZ injection and the latency to seizure and the seizure were recorded for 28 days (14 injections).

Evaluation of Seizure Activity

The seizure activity was measure using the modified racing scale as follows:

Stage 0: No behavioural response

Stage 1: Restlessness, ear and vibrissae

Stage 2: Head nodding, head clonus, and myoclonic jerks

Stage 3: Unilateral forelimb clonus

Stage 4: Rearing with bilateral forelimb clonus

Stage 5: Generalized tonic-clonic seizure with falling (Azizi *et al.*, 2020).

Kindling is a neurobiological phenomenon characterized by the progressive development of seizure activity in response to repeated sub-convulsive electrical stimulation of the brain (Goddard *et al.*, 1969). This process, often likened to the gradual buildup of fire from a small spark, involves a series of changes in brain function that ultimately lead to spontaneous seizures.

Animal Sacrifice

Animal sacrifice is a critical step in experimental research, ensuring humane treatment and minimizing animal suffering. The animals were made to fast for 8–12 hours to minimize gastrointestinal contents. Anesthesia (ketamine) was used to reduce stress and pain, followed by the brain tissue collection (of the brain), for histopathological studies.

Brain Homogenization

The brain tissues were homogenised in phosphate buffered saline at 4^0C so as to maintain the biochemical integrity of the tissue.

Histological Analysis

At the end of the experiment, the rats were humanely sacrificed, and their brains were quickly removed and fixed in neutral buffered saline for 48 hours. The cerebrum and hippocampus were then dissected, processed for paraffin embedding, and cut into 5 μm thick coronal sections. The sections were stained with Hematoxylin and Eosin (H&E) for general histological examination. The H&E-stained sections were examined under a light microscope for histopathological changes in the cerebrum, including neuronal damage, gliosis, and inflammation.

Statistical Analysis

The data were analyzed using the Statistical Package for Social Sciences (SPSS) software version 23. The results were expressed as mean ± standard error of mean (SEM). One-way analysis of

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variance (ANOVA) was used to compare the means of different groups. Post-hoc Tukey's test was used to determine the significance of differences between groups. The level of significance was set at $p < 0.05$.

RESULTS

Effect of Curcuma Longa on the Activities of Malondialdehyde (MDA) as a Biomarker of Oxidative Stress in Brain Homogenate of PTZ-kindled Rats

The result of this experiment shows an increase and a decrease in all groups. The graph indicates that Control Group MDA levels were relatively low in the control group. Co-treatment with PTZ and DZP significantly reduced MDA levels compared to the PTZ-only group. This shows that DZP has an antioxidant effect, mitigating the oxidative stress caused by PTZ-induced seizures. The MDA levels were significantly elevated in the PTZ-treated group compared to the control group ($P<0.05$), indicating increased lipid peroxidation and oxidative stress. Treatment with both low-dose and high-dose turmeric extract significantly reduced the MDA levels compared to the PTZ-treated group ($P<0.05$), suggesting the antioxidant and neuroprotective effects of turmeric extract.

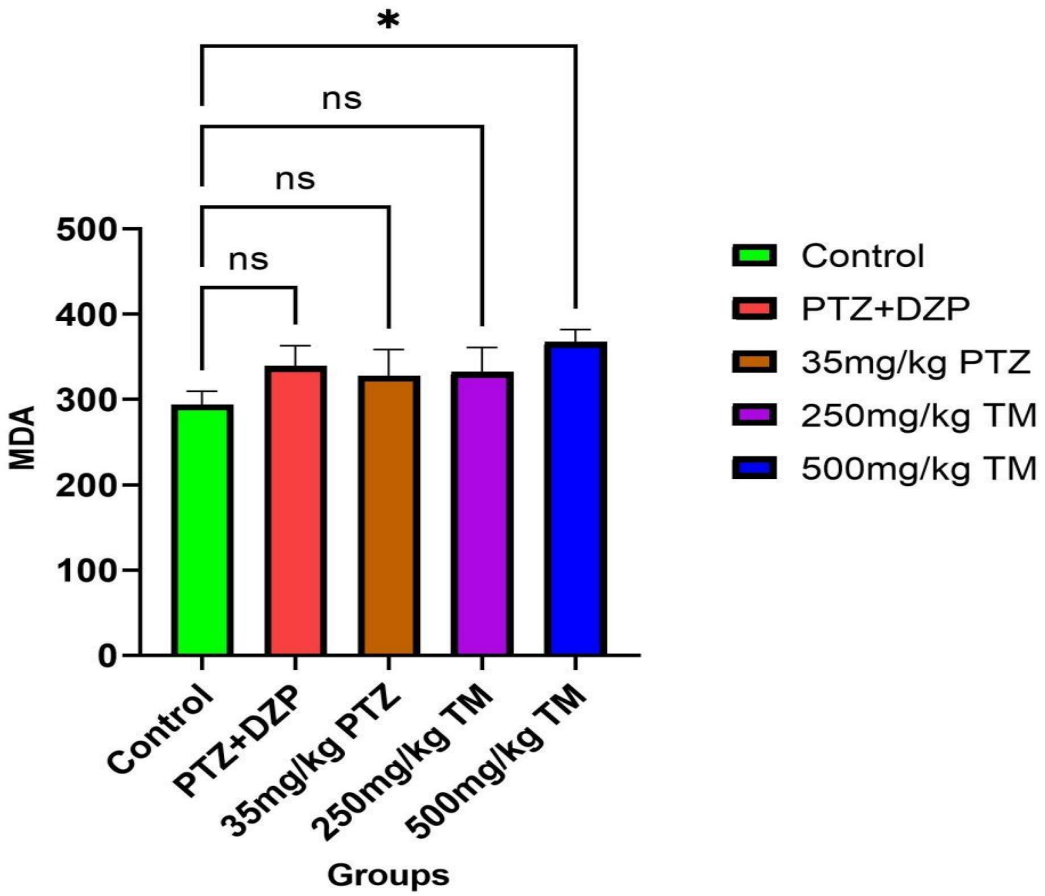

Figure 1 showing the effects of an aqueous extract of *Curcumin longa L*. on MDA as an oxidative stress biomarker in the brains of albino wistar rats.

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Effect of *Curcuma longa* on Superoxide Dismutase (SOD) as a Biomarker of Oxidative Stress in Brain Homogenate of PTZ-kindled Rats

A partial significant difference in the normal control and the PTZ/DZP group indicated an increased level of SOD activity in the normal control and a decrease in the PTZ/DZP group. A significant difference in the normal control group and the 35 mg/kg PTZ group indicated an increased level of SOD activity in the normal control and a decrease in 35 mg/kg PTZ. A significant difference in the normal control and the 250 mg/kg TM group indicated an increased level of SOD activity in the normal control group and a decrease in the 250 mg/kg TM group. No significant difference in the normal control group and the 500 mg/kg TM group indicated an increase in SOD activity in both groups.

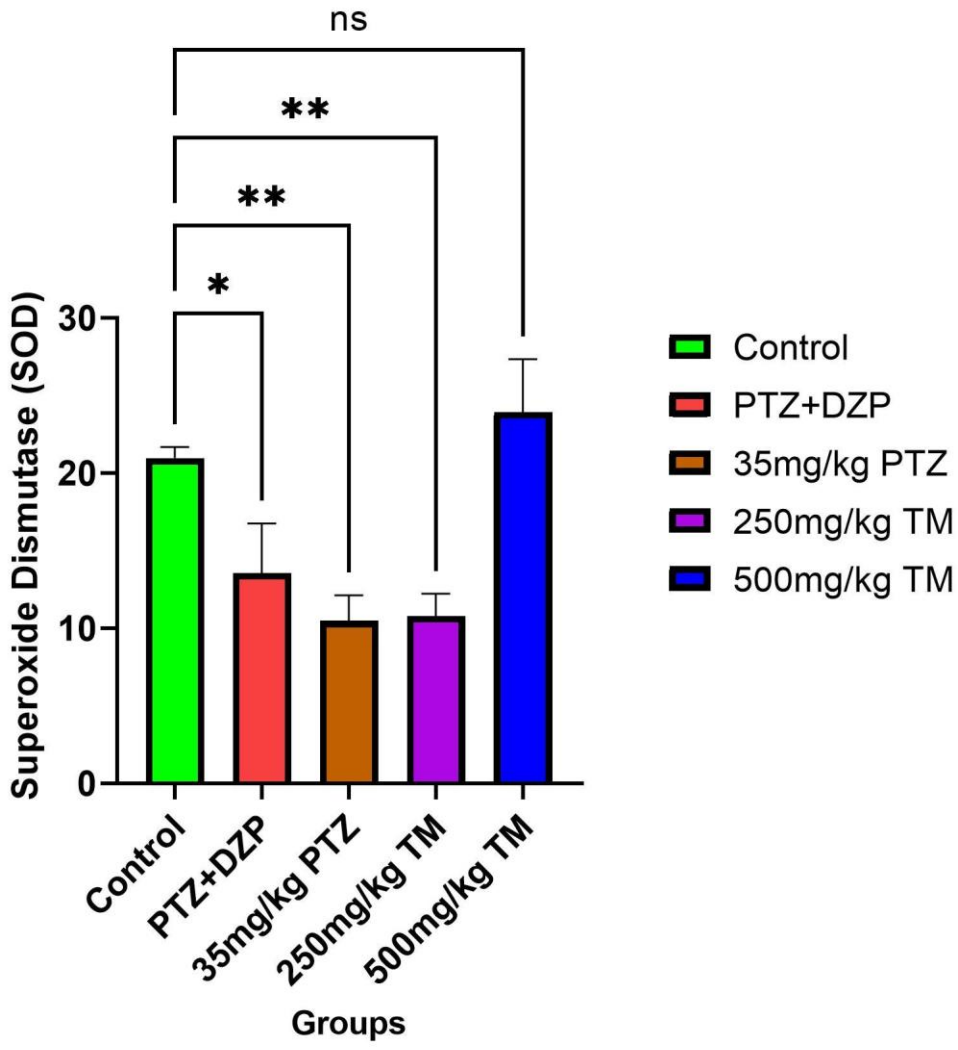

Figure 2 shows the effects of aqueous extract of *Curcumin longa L*. extract on MDA as an oxidative stress biomarker in the brains of albino Wistar rats.

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Histological Results

Effect of *Curcumin longa L.* Extract on the Histology of the Cerebral Cortex of PTZ-kindled Wistar Rats

In Group one (1) [normal control], the normal histology of the cerebrum is seen with neurons and astrocytes in the affected area. Group two (2) [PTZ and DZP] shows that there is a healing process ongoing, and the proliferation of astrocytes is seen more; the microglial cells are also present, and cells with enlarged nuclei and cytoplasm are also seen. In Group three (3) [PTZ only], some neurons appear to be distorted and shrunken, which is an initiation of cell injury and cell death; cell polarity is also seen as a result of glial cells' proliferation. The low dosage group shows that there is healing and regeneration taking place at the nephrons, and the degeneration is not much. The high-dose group shows some regeneration, but it is not as effective as the low-dose group. The morphology is almost like the diazepam group, which does not have as much effect as the low dose.

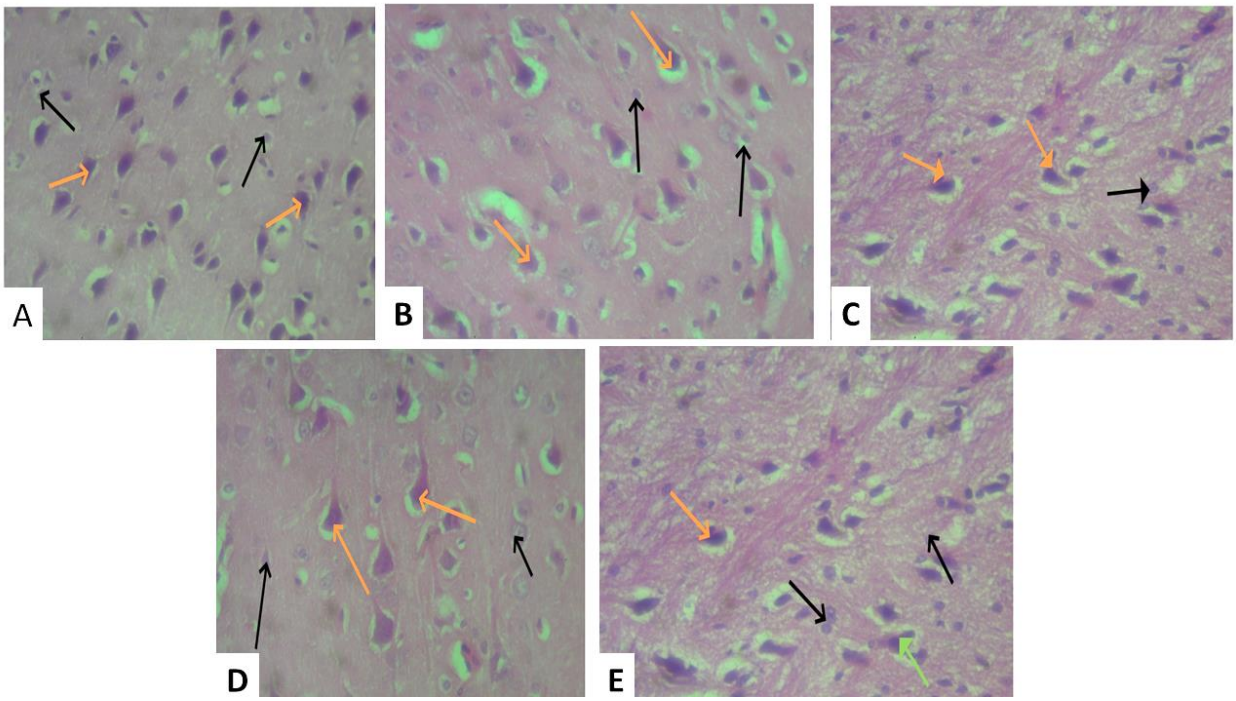

Figure 3 showing the histology of cerebrum of the control group (1): undisrupted histology, Diazepam; (2): with regeneration and degeneration caused by PTZ, PTZ group only; (3): with degeneration and gliosis, low dosage (250 mg\kg) group; (4): with regeneration of neurons, glial cells and no inflammation and high dose (500 mg\kg) group; (5): with degeneration caused by PTZ (H and E). Orange arrow: neurons. Black arrows: neuroglial. Mag. 200x.

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Effect of the *Curcuma longa L.* Extract on the Histology of Hippocampus CA1 Region of Albino Wistar Rats

Composite photomicrograph of the rat's CA1: The photomicrography of the temporal lobe of the normal control shows normal cells, and the photomicrography of CA1 of rats that received 35 mg/kg PTZ + DZP shows normal cells. Figure 4A: Photomicrograph of the temporal lobe of a normal control shows normal cells of CA1 of rats. Figure B shows a photomicrograph of CA1 of rats that received diazepam 5 mg/kg, followed by 35 mg/kg PTZ. Figure 4C shows photomicrography of CA1 of rats that received only 35 mg/kg PTZ. Figure D shows photomicrograph of CA1 region of rats that received 250 mg/kg of *C. longa*, followed by 35 mg/kg PTZ showing normal cells, while Figure 4E shows photomicrography of CA1 of rats that received 500 mg/kg of *C. longa*, followed by 35 mg/kg PTZ which shows normal cells**.**

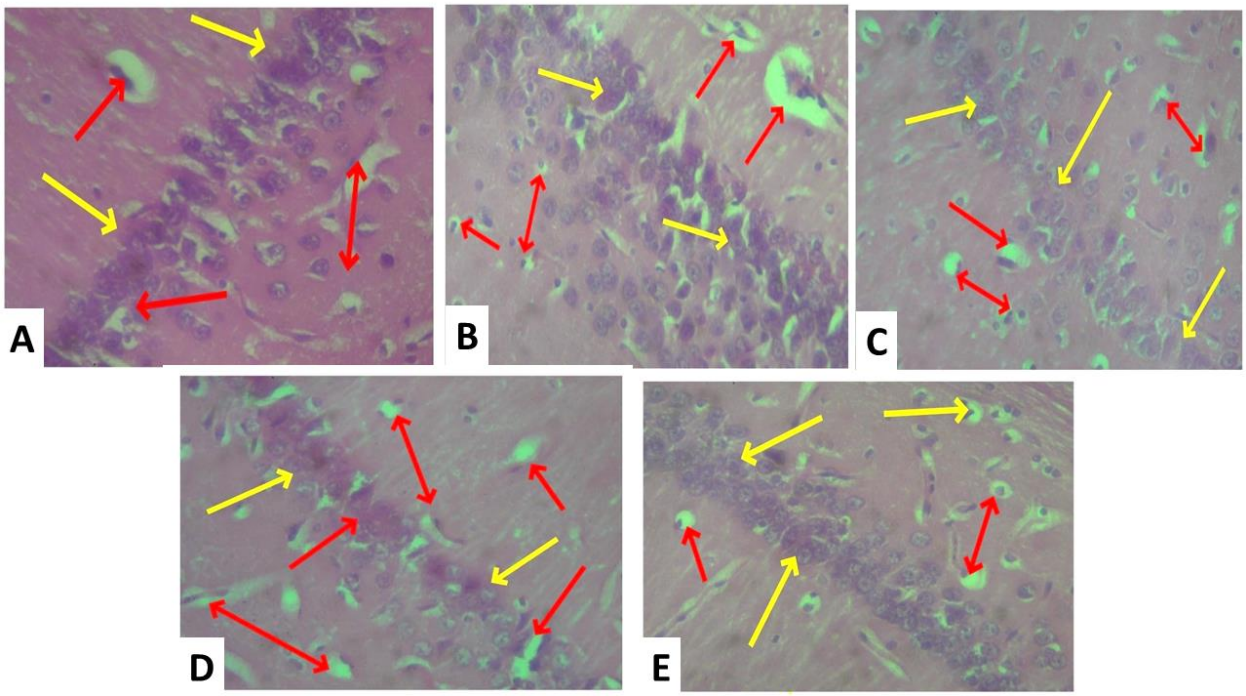

Figure 4 showing the histology of hippocampus CA1 of the control group; (1): undisrupted histology, Diazepam; (2): with regeneration and degeneration caused by PTZ, PTZ group only; (3): with degeneration and gliosis, low dosage (250 mg\kg) group; (4) with regeneration of neurons, glial cells and no inflammation and high dose (500 mg\kg) group; (5) with degeneration caused by PTZ (X200, H and E). Orange arrow: neurons. Red arrow: neuroglial. Mag. 200x.

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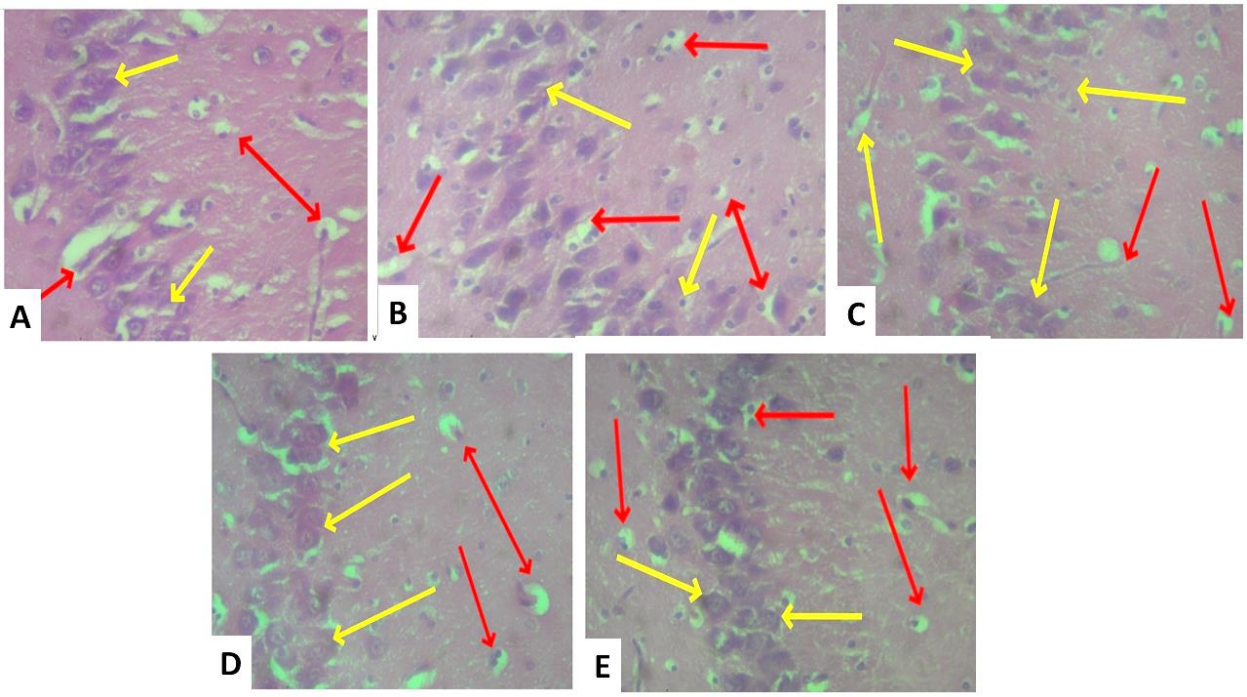

Figure 5 showing the histology of hippocampus CA3 of control group (1): undisrupted histology, Diazepam; (2): with regeneration and degeneration caused by PTZ, PTZ group only; (3): with degeneration and gliosis, low dosage (250 mg\kg) group; (4) with regeneration of neurons, glial cells and no inflammation and high dose (500 mg\kg) group; (5) with degeneration caused by PTZ (X200, H and E). Orange arrow: neurons. Red arrows: neuroglial. Mag. 200x

DISCUSSION

During the routine physical observation of the animals, the control group (group one) animals were observed to be hyper active throughout the 42 days of studies, while the animals in the group that was administered 5 ml of diazepam before 35 mg of PTZ in H_2O (group 2) only, and those treated with *Curcuma Longa L* (group 4 and 5), were seen to have restlessness and use their forelimbs to scratch their faces, which became apparent after the seventh injection. Also, unilateral and bilateral forelimb clonus was seen after the twelfth injection; this observation was most especially vivid in the 35 mg of PTZ only (group 2), where the animals showed restlessness and ear vibration after the fifth injection. The epileptic seizure manifestation increased, and kindling was completed after the fourteenth injection. The low-dose and high-dose treated groups (group 4 and 5, respectively) displayed progress in physical activities during the period of treatment. This is in agreement with the research carried out by Kulkarni and Dhir (2010), which proves that our extract had an anticonvulsant effect.

The present study investigated the effects of turmeric extract on oxidative stress in a rat model of seizures induced by pentylenetetrazole (PTZ). The results showed that PTZ-induced seizures significantly increased malondialdehyde (MDA) levels, indicating enhanced lipid peroxidation

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and oxidative stress. This result is in congruence with the findings of Bello *et al.* (2022) that also reported an increased level of MDA in the brains of rats that are kindled with PTZ. Co-treatment with diazepam (DZP) and PTZ significantly reduced MDA levels compared to the PTZ-only group, suggesting that DZP has an antioxidant effect, mitigating the oxidative stress caused by PTZ-induced seizures. This finding is consistent with previous studies, which have reported the antioxidant properties of DZP (Mohammadi *et al*., 2018; Nkwingwa *et al*., 2023). Findings from this study also reveal lower levels of SOD in all the groups when compared with the control, except for the high dose group. This result is also in agreement with the findings of Bello et al. (2022) that reported a lower level of SOD in the brains of PTZ-kindled rats. Treatment with both low-dose and high-dose turmeric extracts significantly reduced MDA levels compared to the PTZ-treated group, suggesting the antioxidant and neuroprotective effects of turmeric extract. This is in contrast with that of a study by Mohammadi *et al*. (2018), which investigated the effects of diazepam (DZP) on oxidative stress and lipid peroxidation in a rat model of seizures induced by pentylenetetrazole (PTZ). The results showed that DZP treatment increased MDA levels and oxidative stress in the brain, which was accompanied by a decrease in antioxidant enzymes.

In Figure C, neurodegeneration is more obvious, and the histomorphology of the cerebrum is altered. In Figure D, the low dosage group shows a fast healing process and a good architecture of the histomorphology of the cerebrum. In Figure E, the high dose shows a slight recovery as to that of Figure B (Kumar *et al*., 2018).

These findings are consistent with previous studies, which have reported the antioxidant and anti-inflammatory properties of turmeric extracts (Kumar *et al.*, 2018; Kim *et al.*, 2024). The neuroprotective effects of turmeric extract may be attributed to its ability to scavenge free radicals and reduce oxidative stress (Kaur *et al*., 2024).

CONCLUSION

In conclusion, the present study provides evidence for the antioxidant and neuroprotective effects of turmeric extract in reducing oxidative stress and seizures induced by PTZ. These findings suggest that turmeric extract may be a potential therapeutic agent for the management of epilepsy and other neurodegenerative disorders.

Authors' Contributions

Hasiya Sule Buba, Abubakar Muhammad Bello and Luqman Adepoju Hassan designed the experiment. Wusa Makena and Sani Hyedima Garba carried out the experiment. Foluso Olamide Ojo and Fatsuma Buba Jajere analysed the data. Hasiya Sule Buba, Luqman Adepoju Hassan, and Foluso Olamide Ojo drafted and edited the manuscript. All authors read the final version of the manuscript.

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Conflict of Interest

Authors declared none.

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