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Research Article

Ketogenic Diet Mitigates Hippocampal CA3 Degeneration in Male Wistar Rats with PTZ-Induced Kindling Seizures

***Danladi J.[1,5], Suleiman Y. A.[2,5], Ugochukwu S. O.[1], Lawal A.A.[3], Abdullahi I.[1], Dzekashu B. B.[1], Sule H., Jahun A. S.[4], Salifu B. U.[1], and Muhammad S. F.[3]**

[1]Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Basic Medical Sciences, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Human Anatomy, Abubakar Tafawa Balewa University, Bauchi, Nigeria
[3]Department of Human Anatomy, Federal University, Lafia, Nasarawa, Nigeria
[4]Department of Human Anatomy, Federal University Dutse, Jigawa, Nigeria
[5]Molecular Genetics and Infectious Disease Laboratory, Abubakar Tafawa Balewa University, Bauchi, Nigeria
[6]Department of Biochemistry, Faculty of Sciences, Bayaro University, Kano, Nigeria
****Corresponding Author's email*: jibrin.danladi00@gmail.com**

ABSTRACT
The ketogenic diet (KD) is a high-fat, low-carb diet used to treat neurological illnesses, inborn metabolic abnormalities, and pharmaco-refractory epilepsy. The pathophysiology of KD is unknown. The PTZ-kindling (PTZK) 35 mg/kg every other day model was utilised to study KDs' effects on epilepsy. Thirty adult male Wistar rats were divided into six groups of five rats each. Group 1, control (standard feed); Group 2 PTZK; Group 3 PTZK + standard feed and levetiracetam (10 mg/kg); Group 4, PTZK + palm-kernel-oil-KD; Group 5, PTZK + castor-oil-KD; and Group 6, PTZK + olive-oil-KD. To promote kindling, the PTZ i.p. dose was increased progressively (5 mg/kg) after the fourth injection. Animals were fed their diets for 14 days after 21 days of Kindling induction. Ketone bodies' (KB) and levetiracetam's binding-affinity to free fatty acid receptor 2 and 3 (FFAR2 and FFAR3) were determined by molecular docking *in silico*. To assess memory, Y-maze was used. The Unbiased designed-based stereological approach assessed hippocampi CA3 neurons, and the Feulgen reaction examined DNA-fragmentation. The KB has a stronger binding-affinity to FFAR2 and FFAR3 than levetiracetam *in silico* analysis. The KD groups (4, 5, and 6) had a seizure score of 3 compared to the PTZK group (2)'s 4. KD (4) has reversed memory loss. The PTZK group (2) had fewer pyramidal layer CA3 neurons and higher DNA-breakage than the KD groups (4, 5, and 6). The study shows that KB binding to FRR2 and FFR3 may improve seizure resistance, short-term memory, and neurodegenerative safety.

Keywords: DNA fragmentation; Epilepsy; Ketogenic diets; Kindling; PTZ

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INTRODUCTION

Epilepsy is a multifaceted neurological condition marked by recurring, spontaneous seizures, reflecting an enduring predisposition to abnormal neuronal activity (Aliyu *et al.,* 2014; Stafstrom and Carmant 2015). Epilepsy affects an estimated 50 million people globally, it imposes a significant burden, extending beyond seizure control to encompass a range of debilitating neuropsychiatric comorbidities such as anxiety, depression, and cognitive impairments,

which often persist independently of seizure frequency and remains a major global public health problem (Feigin, 2022; Arulsamy, 2024). The pathophysiology of epilepsy is rooted in a fundamental imbalance between excitatory and inhibitory neurotransmission (Engelborghs *et al.,* 2000; Mastrangelo, 2021; Bryson *et al.,* 2023). This imbalance stems from a confluence of factors, including excitotoxicity mediated by excessive glutamate, compromised GABAergic inhibition, and dysregulated ion channels (Shao *et al.,* 2019; Hadjighassem *et al.,* 2024; Hammer, 2025).

Furthermore, chronic neuroinflammation, involving activated glial cells and the release of pro-inflammatory mediators, and oxidative stress contribute significantly to neuronal hyperexcitability and progressive damage within the epileptic brain (Vezzani *et al.,* 2011; Vezzani *et al.,* 2016; Pickard *et al.,* 2025). In light of these issues, extensive research has been conducted over the years to further the understanding of this condition and to identify therapeutic solutions for affected individuals. Therefore, there is need for adequate and reliable experimental models to be achieved, although researchers have developed numerous methodologies for the experimental investigation of epilepsy, including both in vitro and in vivo models. The epilepsy model emulates human characteristics through three primary induction methods: surgical intervention to create epileptiform lesions, electrical induction utilizing electrodes, and chemical substance induction (Erkeç and Arihan, 2015; Samokhina and Samokhin, 2018).

The Pentylenetetrazol (PTZ) kindling model in rodents provides a robust experimental platform for investigating therapeutic and preventive interventions (Goel *et al.,* 2015). PTZ, a GABA-A receptor antagonist, induces progressive kindling, leading to a chronic epileptic state characterized by established seizures, behavioural deficits, and neuropathological changes in structures like the hippocampus (Kandratavicius *et al.,* 2014; Kandratavicius *et al.,* 2015). PTZ interacts with the $GABA_A$ receptor complex by inhibiting the benzodiazepine, picrotoxin, and GABA binding sites. Blocking these three sites inhibits the activation of the $GABA_A$ receptor, hence obstructing the influx of chloride ions into neurones and resulting in the cessation of GABAergic inhibition (Samokhina and Samokhin, 2018; Erkeç and Arihan, 2015). The blockade of GABAergic inhibition would lead to increased hyperexcitation, mainly caused by the excessive activation of glutamatergic signalling, which would facilitate the gradual onset of epileptogenesis and eventually result in epilepsy (Samokhina and Samokhin, 2018; Erkeç and Arihan, 2015). This model allows for the assessment of interventions administered before and after the disease is established, thereby evaluating their capacity to therapeutically reverse or ameliorate existing neurological dysfunction, a critical and underexplored area in epilepsy research (Löscher, 2017).

A brain region critically implicated in epilepsy, particularly temporal lobe epilepsy, is the hippocampus. This structure, vital for learning and memory, is highly susceptible to seizure-induced damage, leading to phenomena like hippocampal sclerosis (HS) (Pitkänen and Lukasiuk, 2011). HS is characterized by profound neuronal loss, especially in the vulnerable CA3 subfield, accompanied by reactive gliosis and aberrant synaptic reorganization such as mossy fiber sprouting. The neuronal damage in the CA3 region is strongly correlated with cognitive deficits prevalent in epilepsy patients (Vezzani *et al.,* 2011; Liu *et al.,* 2018). While current antiepileptic drugs effectively suppress seizures for many, they largely lack the capacity to reverse these established pathological changes or address the underlying disease progression, leaving a significant patient population with intractable seizures and persistent comorbidities (Kwan *et al.,* 2011).

In the search for more comprehensive therapies, the ketogenic diet (KD) has re-emerged as a powerful non-pharmacological intervention. Originating in the 1920s as a dietary mimic of therapeutic fasting, KD is a high-fat, low-carbohydrate regimen that induces ketosis, wherein the brain utilizes ketone bodies as its primary fuel source (Mychasiuk and Rho, 2017; Rho, 2017; Masino and Rho, 2019; Rho *et al.,* 2019; Choi *et al.,* 2025). Beyond its established anticonvulsant properties in refractory epilepsy, particularly in pediatric populations, growing evidence points to KD's broader neurotherapeutic potential, including anti-inflammatory, antioxidant, and neuroprotective effects that could mitigate existing brain damage (Pinto *et al.,* 2018; Yang *et al.,* 2019; Gough *et al.,* 2021; Pietrzak *et al.,* 2022; Dyńka *et al.,* 2022; Ildarabadi *et al.,* 2024; Monda *et al.,* 2024). Thus, the present study sought the ameliorative effect of three KDs on model of PTZ-kindling in Wistar rat.

MATERIALS AND METHODS

Ketogenic diets formulation: The three ketogenic fats; Palm kernel oil (lauric acid 45%, myristic acid 16%and oleic acid 15%), Castor oil (recinoleic acid 85 – 95%, oleic acid 2 – 6%), Olive oil (oleic acid 55 – 83%,

palmitic acid 7 – 20%, linoleic 3.5 – 21%), were purchased from Samaru market in Zaria Kaduna State. All of the ketogenic diets were prepared in the laboratory by mixing specific percentage of fat/oils, proteins and carbohydrate (Table 1) This product comprises a custom-formulated powdered dietary blend that includes nutrients, fibre, minerals, and vitamins (Table 2). The premixes for the KDs and the ready-to-use control diet were acquired from vital feed, Zaria, Kaduna State, Nigeria.

Table 1: Comparison of carbohydrate, lipid, and protein compositions in conventional and ketogenic diets

Diet	Carbohydrate (%)	Fat (%)	Protein (%)
Standard	52.20	7.00	15.25
Ketogenic	5.66	86.19 (PKO, CTO, OLO)	8.15

(Arsyad *et al.,* 2020)

PKO: Palm kernel oil, CTO: Castor oil, OLO: Olive oil.

Table 2: Standard diets and ketogenic diets formulation for 1kg.

Standard diets formulation		ketogenic diets formulation	
Ingredients	Quantity/g	**Ingredients**	Quantity/g
Maize	522	**Maize**	50
Groundnut	35	**Groundnut**	250
Soybeans	35	**Soybeans**	250
Soybeans meal	100	**Soybeans meal**	80
Groundnut cake	52.5	**Palm kernel**	100
Lysine	50	**Lysine**	1
Methionine	50	**Methionine**	1
Wheat hoofers	50	**Wheat hoofers**	1
Limestone	20	**Limestone**	1
Salt	20	**Salt**	1
Bone Ash	30	**Bone Ash**	1
Premix(grower)	20	**Premix(grower)**	13
Enzymes	5.5	**Enzymes**	1
CTO, PKO and OLO	10ml	**CTO, PKO and OLO**	250 ml

Molecular Docking of Ketone Bodies and Standard Drug Ligands

The 3D structures of Acetoacetate (PubChem CID: 6971017), Acetone (PubChem CID: 180), β-Hydroxybutyrate (PubChem CID: 3541112), and Levetiracetam (PubChem CID: 5284583) were retrieved from the PubChem database and were saved in SDF format. Preparation of ligands was carried out using PyRx v0.8; ligands were imported into PyRx and energy minimized using the MMFF94 force field with steepest descent followed by conjugate gradient algorithms. Hydrogen atoms were added automatically at physiological pH, and Gasteiger charges were assigned before the conversion of the SDF to PDBQT format for docking.

The crystal structures of FFAR2 (PDB ID: 8J24), and FFAR3 (PDB ID: 8J20) were retrieved from the RCSB PDB database in PDB format. Protein preparation was performed in PyMOL v3.1.6.1 by isolating chain A, removing water molecules, ions, and other non-protein residues, and deleting co-crystallized ligands. Polar hydrogens were added, and the structures were subjected to gentle energy minimization using Open Babel (PyRx backend) to relieve steric clashes while maintaining crystallographic geometry.

Molecular docking was carried out using AutoDock Vina (PyRx v0.8). Grid boxes were defined around the binding pockets identified from co-crystallized ligands, with an additional 5–8 Å margin. Docking parameters were set to an exhaustiveness of 16, an energy range of 4 kcal/mol, and 8 output poses per ligand. Each ligand (acetoacetate, acetone, β-hydroxybutyrate, and levetiracetam) was docked separately against each protein target (FFAR2 and FFAR3).

Animal Welfare and Ethic

Thirty young adult albino female Wistar rats (Rattus norvegicus), weighing approximately 90-100 grams, were purchased from the Faculty of Pharmaceutical Sciences, Ahmadu Bello University (ABU), Zaria. The rats were moved and maintained in standard rat plastic cages containing sawdust bedding, wooden sticks, and nests at room temperature and subjected to a natural light-dark cycle at the animal facility at

the Department of Human Anatomy, Ahmadu Bello University Zaria. Rats were allowed regular free access to water and food during the period of acclimatization for 2 weeks before the commencement of the experiment. Animals were treated in accordance with the guidelines of the Ahmadu Bello University, Zaria committee on Animal Use and Care (Approval No: ABUCUAC/2025/105). With all efforts, animal suffering was minimized to reduce the number of animals used for this experiment.

PTZ Kindling

PTZ (Sigma Aldrich, St. Louis, USA) was used to induce kindling. PTZ was injected intra-peritoneally (ip) at a dose 35 mg/kg (subconvulsive dose). PTZ dose was increased (5 mg/kg) in subsequent injection to encourage the progression of kindling (Shimada and Yamagata, 2018).

Experimental animals

Adult male Wistar rats weighing 90-100 grams were reared at ambient temperature and exposed to a natural light-dark cycle in the animal facility of the Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria, Nigeria. Rats received water and food ad libitum. Rats were divided into six (6) groups, each containing five (5) Wistar rats per group. Group 1 control (standard-feed); Group 2 PTZ-kindling group (35 mg/kg) every other day; Group 3 PTZ-kindling every other day with standard feed + levetiracetam (10 mg/kg); Group 4 PTZ- kindling (35 mg/kg) every other day + palm kernel oil-KD; Group 5 PTZ- kindling (35 mg/kg) every other day + castor oil-KD; and Group 6 was the PTZ-kindling (35 mg/kg) every other day + olive-KD. After the fourth PTZ i.p injection, PTZ dose was increased (5 mg/kg) in subsequent injection for every other day to encourage the progression of kindling. After the induction of kindling for 21 days, animals were fed with their respective diets for14 days (Bough and Rho, 2007).

Post-injection, rats were observed in clear chambers for 30 minutes, with seizure severity scored using a modified Racine's scale (Racine, 1972):

i. Stage 0: No seizure activity.
ii. Stage 1: Immobility, prone posture.
iii. Stage 2: Head nodding, myoclonic twitches.
iv. Stage 3: Unilateral forelimb clonic seizures.
v. Stage 4: Rearing, generalized clonic seizures, tonic posture.
vi. Stage 5: Tonic-clonic seizures with loss of balance and falling.

After kindling seizure induction, Wistar rats were exposed to standard feed, ketogenic diets (PKO, CTO, OLO), and standard antiepileptic drug administration for 14 days. The Wistar rats were weighed before the kindling seizure induction and at an interval of 5 days during the seizure induction and treatment.

Y-maze Behavioral Evaluation

Behavioral tests were performed after the 35-day experimental period (i.e., after the 21-day PTZ kindling and subsequent 14-day dietary/drug intervention), during the light phase (09:00 AM to 03:00 PM) in a controlled, quiet environment. Rats were habituated to the testing environment for 1 hour before commencement of behavioral test. Equipment was cleaned with 70% ethanol between trials to eliminate odor cues. A video tracking system recorded all activities.

The Y-maze test is widely used to assess spatial working memory and exploratory behavior in rodents. This behavioural assessment was executed according to the outlined methodology (Maurice *et al.,* 1996). The Y-shaped maze apparatus of brown wooden box with three identical arms (A, B, C) spaced 1200 mm apart (each measuring approximately 40 cm in length, 10 cm in width, and 15 cm in height) arranged at an angle of 120° from each other (Figure 1). The trial begins by placing a Wistar rats in the center and allowed to explore for 5 minutes each. The apparatus was cleaned with 70% ethanol between trials to eliminate olfactory cues. The following parameters were analyzed to evaluate spatial memory performance: (1) Number of arm entries (an indicator of locomotor activity) (2) Percentage of successful spontaneous alternation behavior (SAB).

Spontaneous Alternation = (Number of Alternations / (Total Arm Entries - 2)) × 100

Figure 1: Y-shaped Maze with Wistar Rat Placed in It

Animal Euthanasia and Brain Procurement

At the end of the experiment, within twenty-four hours (24 h), animals were sacrificed by decapitation. The animal skulls were dissected, brain were collected and immersed in 10% buffered formalin at 4 ^{o}C for 48 hours and further processed for morphological assessment. The brain tissues were grossed, dehydrated in a graded percentage of alcohol (70%, 80%, 100%), cleared in xylene, infiltrated in molten wax, and then embedded to form a block.

Morphological Analysis of Hippocampus CA3

Brain tissue processing and Thionine staining for cell number estimation

The processed tissue was sectioned to sizes of 10 microns using a rotary microtome, and every 1 of 20 sections was collected and stained with 2.5% thionine. The sections were dewaxed using xylene, followed by rehydration in a descending grade of alcohol, and then the section was dipped in water and stained with thionine. The stained section was dehydrated with ascending graded alcohol, cleared with xylene, and mounted with DPX mountant and then left to dry at room temperature for further stereological analysis.

Stereological Analysis

Using an unbiased design-based stereological method (West 2002; Schmitz and Hof, 2005; West and Gundersen, 1990), a thionine-stained section, and a physical dissector, hippocampi CA3 pyramidal neurons in the pyramidal cell layer was estimated from every 1 of 20 thionine (2.5%) stained sections. The region of interest was viewed and captured under a light microscope. A counting frame was superimposed on the captured region of interest, and the number of cells was counted by following the counting rule (neurons within the counting frame and pyramidal neurons touching the dash lines were counted).

Feulgen Reaction for DNA fragmentation: Every 20th 8-micron-thick section was dewaxed in xylene, rehydrated in descending graded alcohol, and dipped ten times in distilled water before washing in a cold-prepared 1N HCL, followed by incubation at 60 degrees centigrade. Then the sections were later washed in cold-prepared 1N HCl before being directly placed in Schiff's reagent solution, followed by washing in tap water, potassium metasulfite, counterstaining with light green, washing in distilled water, and then dehydrating and clearing in xylene. Then it was mounted with DPX. Sections were allowed to dry at room temperature and viewed and captured under the microscope using an OMAX camera at X400. For DNA fragmentation analysis using ImageJ optical density, one section at a time was uploaded into ImageJ to determine the optical density, it involved color deconvolution, light Feulgen stain with the appreciated cell of interest with stained nucleus and washed cytoplasm, then the mean gray value MGV. The mean values were calculated using the formula log10(255/MGV).

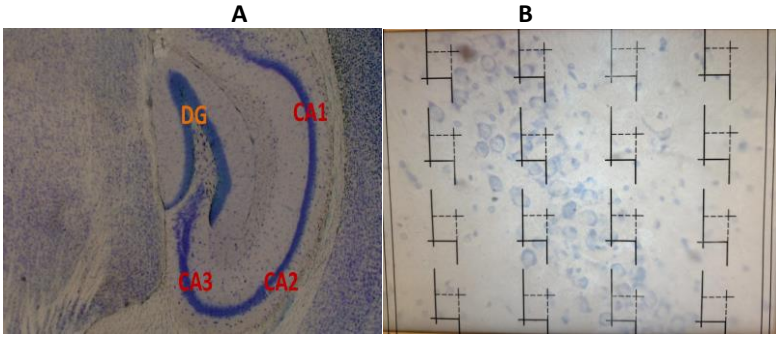

Figure 2: Photomicrographs of thionine-stained sections showing: (A) hippocampus CA 1-3 and dentate gyrus. (B) Counting frame super-imposed on CA3 pyramidal cell layer.

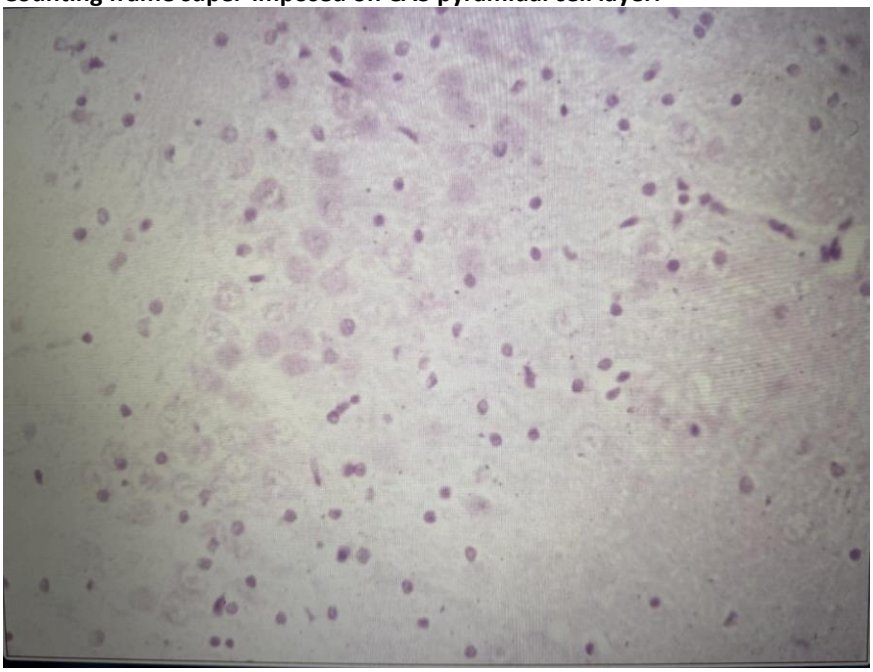

Figure 3: Feulgen-stained CA3 region showing stained nuclei intensity

Data Analysis

The data was analyzed using GraphPad Prism version 8.4.3; the data was presented as mean ± standard error of mean (SEM). One-way and two-way ANOVA were used appropriately, followed by the *Turkey post-hoc* test for multiple comparisons between groups. Results were deemed significant when $p<0.05$.

RESULTS

Molecular Docking of ketone bodies and standard drug ligand

The docking analysis of FFAR2 with the test ligands revealed that levetiracetam demonstrated a strong binding affinity of −4.4 kcal/mol (Figure 4D). Among the ketone bodies, acetoacetate (−3.8 kcal/mol) (Figure 4A) showed slightly higher affinity than β-hydroxybutyrate (−3.6 kcal/mol) (Figure 4B), while acetone (−2.7 kcal/mol) (Figure 4C) displayed the weakest binding. The moderate interactions of Acetoacetate and β-hydroxybutyrate suggest favorable but less stable binding compared to Levetiracetam, likely reflecting partial compatibility within the receptor's orthosteric site. The weak binding of acetone may be due to its small size and limited hydrogen bond potential.

For the FFAR3 receptor, levetiracetam recorded a higher binding affinity of −5.1 kcal/mol (Figure 5D), indicating better stabilization within the binding pocket. Among the ketone bodies, β-hydroxybutyrate (−4.0 kcal/mol) (Figure 5B) bound slightly stronger than Acetoacetate (−3.8 kcal/mol) (Figure 5A) and Acetone (−2.6 kcal/mol) (Figure 5C). The improved affinity of β-hydroxybutyrate likely arises from its hydroxyl group enabling polar contacts with residues within the transmembrane domain. The lower affinities of acetone and acetoacetate imply less

optimal spatial accommodation within the receptor cavity.

Neurobehavioral Analysis

Effect of different kindling models on short-term memory: Y-maze spontaneous alternation showed significant difference across the groups. F (5, 18) =2.870, p =0.0446. The MEC and MSC group showed significant decrease to MN (p=0.0446). The MP group also shows an increased mean value compared to MEC and MSC group (p=0.0446) (Figure 6).

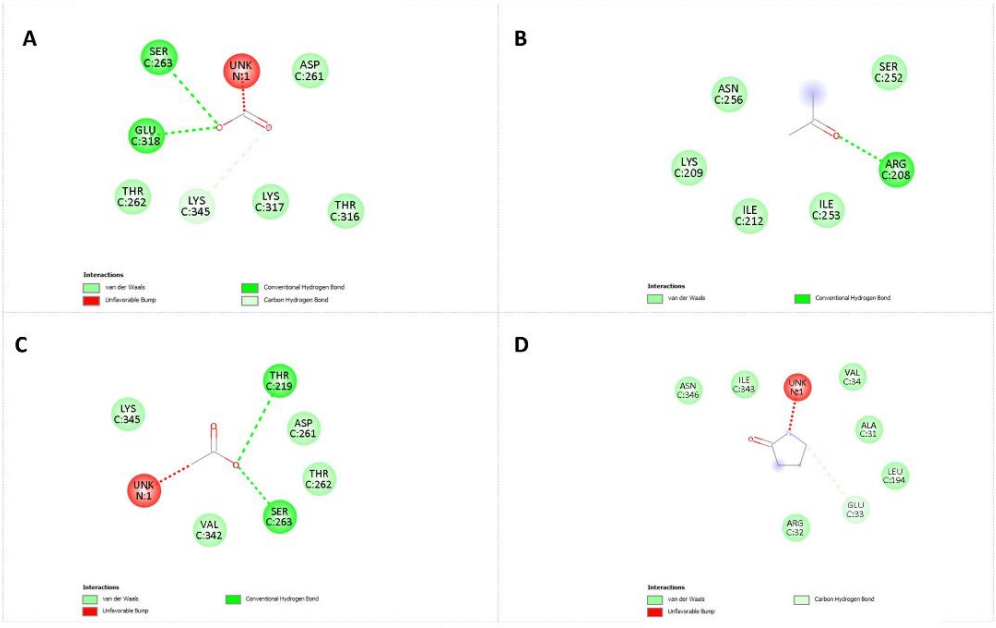

Figure 4. Bond Interactions in Molecular Docking of FFAR2 receptor
Bond interaction of FFAR2 with: (A) Acetoacetate, (B) Acetone, (C) beta-hydroxybutyrate, (D) Levetiracetam

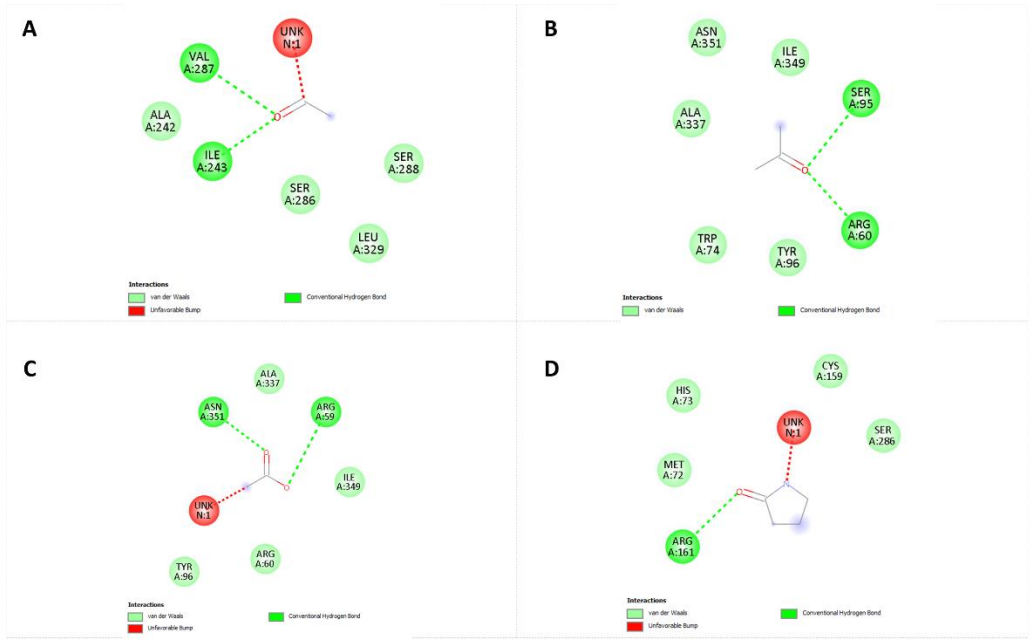

Figure 5. Bonding Interactions in Molecular Docking of FFAR3 receptor. Bond interaction of FFAR2 with: (A) Acetoacetate, (B) Acetone, (C) beta-hydroxybutyrate, (D) Levetiracetam

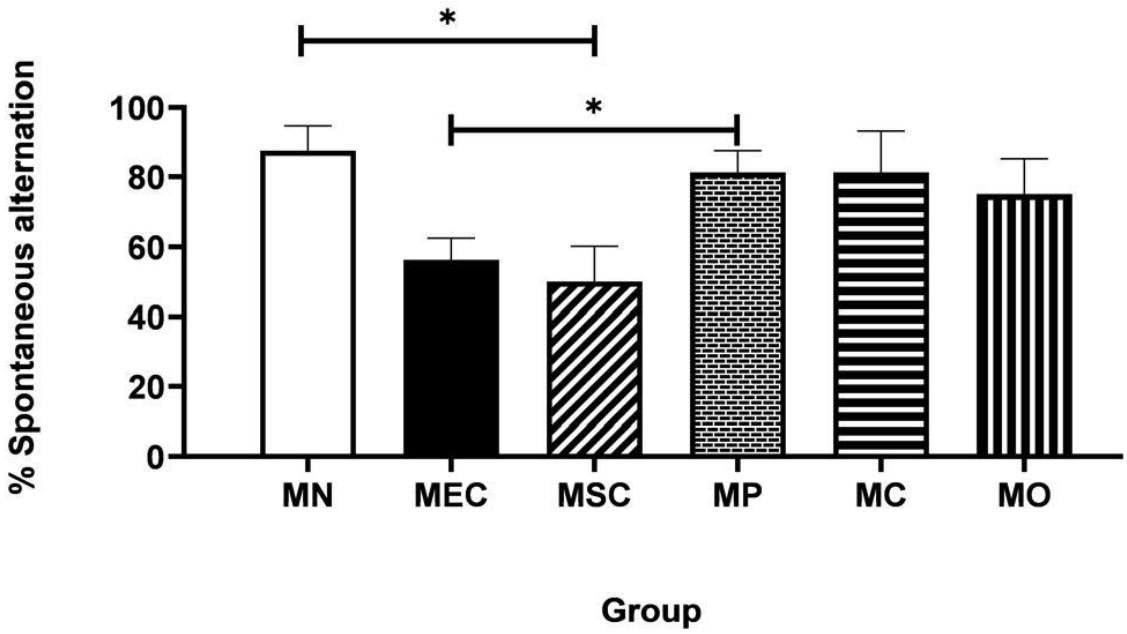

Figure 6: Effect of PTZ-kindled on short-term memory using Y-maze
MN = Normal; MEC = Experimental Control; MSC = Standard Control; MP = Palm Kernel Oil; MC = Castor Oil; MO = Olive Oil. n=5, One-way ANOVA, *P<0.05

The Effect of different kindling models on body weight: The mean body weight analysis shows no significant interactions between variables, F (10, 54) = 0.9974, p = 0.4573. The row factor showed a highly significant difference across the groups, F (2, 54) = 14.60, p = 0.0001. Although the day 1 row factor had no significant difference (p=0.993) (Figure 7).

The row factor for day 5 showed the MSC had a notable rise in comparison to MP (p= 0.0424). The row factor for day 10 showed MP significantly decreased compared to MN (p=0.0141), MO significantly decreased compared to MN (p=0.0329), MP showed a notable decrease compared to MEC (p=0.0135), and MO showed a significant decrease in comparison to MEC. There is also a notable decrease in MP compared to MSC (p=0.0031), MC had a noticeable decrease compared with MSC (p=0.00315), and there was also a significant decrease in MO compared with MSC (p=0.0080) (Figure 7).

Effect of kindling models on Brain Weight: No statistical change was observed across the groups; [F (5,18) =1.259; P=0.2855] (Figure 8).

Effect of kindling models on brain-body weight ratio: Brain-body weight ratio shows no significant difference across groups; [F (5,18) = 1.121; P=0.3846] (Figure 9).

Morphological Analysis

Effect of kindling models on neuronal number in CA3 Pyramidal Cell Layer: Effect of PTZ kindling modeling of seizure on neuron number in the pyramidal cell layer of CA3 showed a significant reduction in CA3 neuronal count in the MEC group compared to the MN [F (5,18) =3.417; P=0.0373]. However, the MSC group and MP group showed an increase compared to MEC, MC, and MO but not at a significant level (Figure 10).

Effect of kindling models on DNA fragmentation: The effect of PTZ kindling modeling of seizure on hippocampus CA3 DNA fragmentation, using the Feulgen reaction, shows that the MEC group had a significant high mean value of DNA fragmented optical density compared to MN [F(5,18) = 81.59; P = 0.0001], MP [F(5,18) = 81.59; P = 0.0001], MC [F(5,18) = 81.59; P = 0.0064], and MO [F(5,18) = 81.59; P = 0.0064]. It also shows that the MN group had the lowest mean value of DNA fragmented optical density compared to MEC [F (5,18) = 81.59; P = 0.0001], MSC [F (5,18) = 81.59; P = 0.0001], and MP [F (5,18) = 81.59; P = 0.0005]. MC [F (5,18) = 81.59; P = 0.0001], MO [F (5,18) = 81.59; P = 0.0001]. The MP group also shows a decreased mean value of DNA fragmented optical density compared to MSC [F (5,18) = 81.59; P = 0.0001], MC [F (5,18) = 81.59; P = 0.0001], and MO [F (5,18) = 81.59; P = 0.0001] (Figure 11).

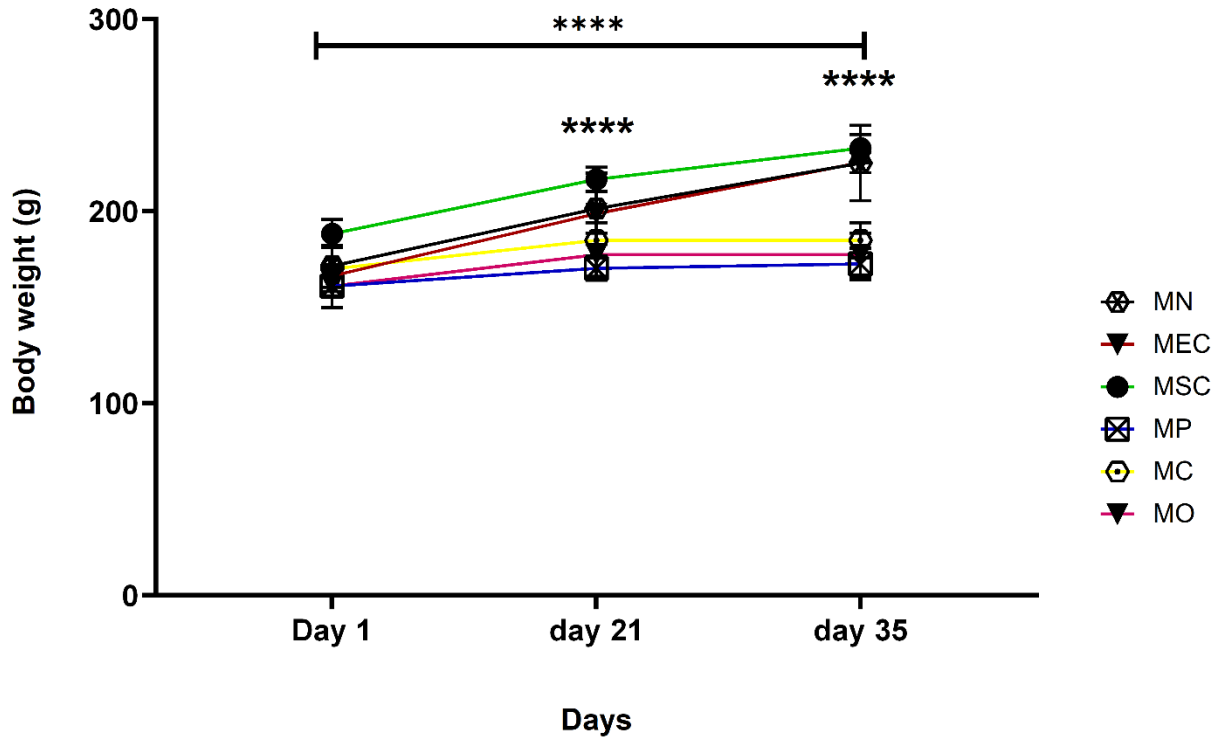

Figure 7: Effect of PTZ-kindled on Body Weight
MN = Normal; MEC = Experimental Control; MSC = Standard Control; MP = Palm Kernel Oil; MC = Castor Oil; MO = Olive Oil. n=5, Two-way ANOVA, ****P<0.0001.

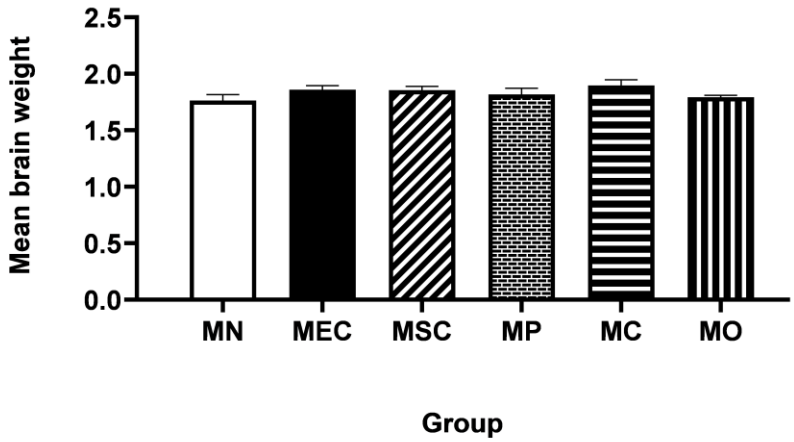

Figure 8: Effect of PTZ-kindled on brain Weight
MN = Normal; MEC = Experimental Control; MSC = Standard Control; MP = Palm Kernel Oil; MC = Castor Oil; MO = Olive Oil. n=5, One-way ANOVA, P<0.05.

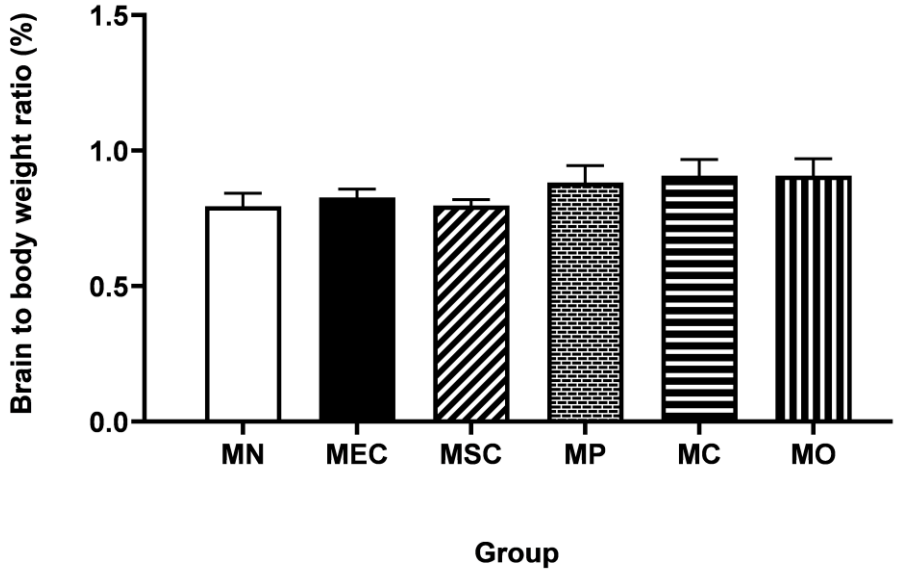

Figure 9: Effect of PTZ-kindled on brain-Body Weight Ratio
MN = Normal; MEC = Experimental Control; MSC = Standard Control; MP = Palm Kernel Oil; MC = Castor Oil; MO = Olive Oil. n=5, One-way ANOVA, P<0.05.

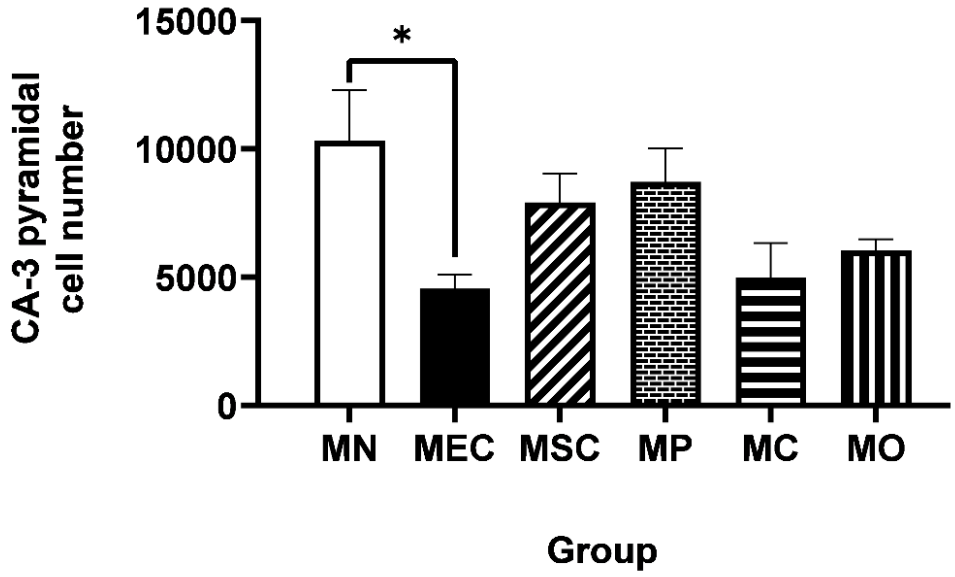

Figure 10: Effect of PTZ-kindled showing One-way ANOVA analysis of number of neurons in CA3 Pyramidal Cell Layer
MN = Normal; MEC = Experimental Control; MSC = Standard Control; MP = Palm Kernel Oil; MC = Castor Oil; MO = Olive Oil. n=5, One-way ANOVA, *P<0.05.

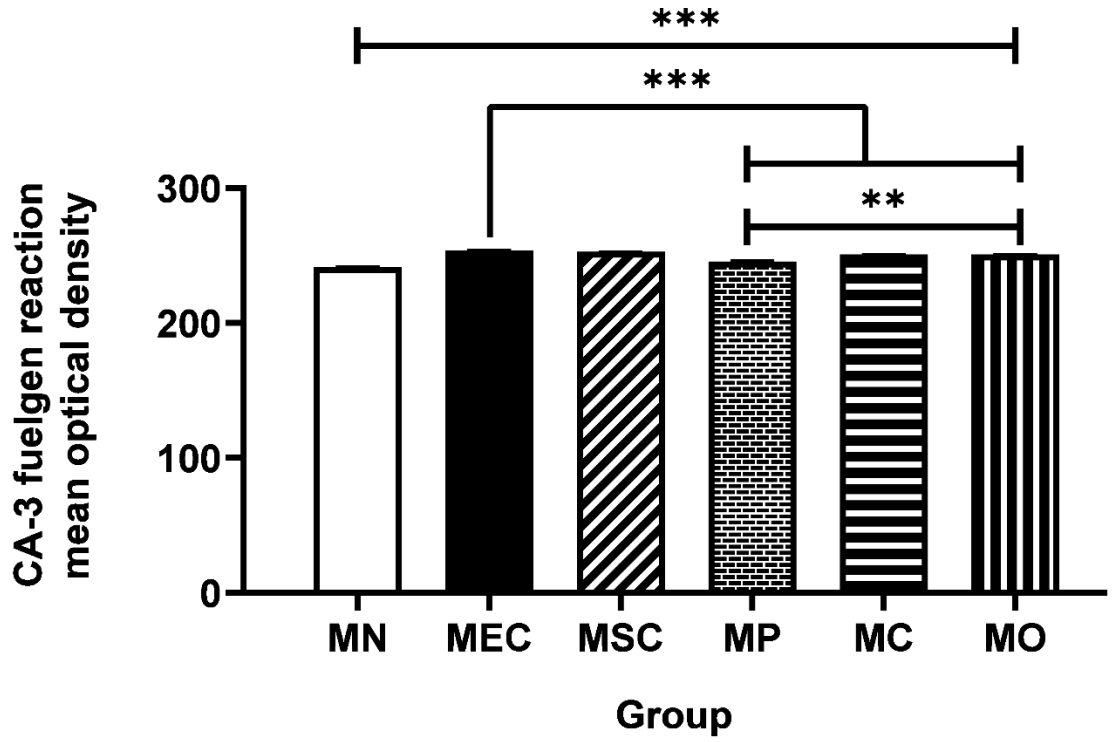

Figure 11: Effect of PTZ-kindled on DNA fragmentation of CA-3 Feulgen reaction (mean optical density)
MN = Normal; MEC = Experimental Control; MSC = Standard Control; MP = Palm Kernel Oil; MC = Castor Oil; MO = Olive Oil. n=5, One-way ANOVA, **P<0.005, ***P<0.001

DISCUSSION

This study determined the therapeutic effects of different ketogenic diet (KD) formulations, which comprise palm kernel oil, castor oil, and olive oil, compared with the standard anti-epileptic drug (levetiracetam) on the PTZ model of seizure in Wistar rats' hippocampus CA3 alteration. With the focus on molecular docking studies (ketone bodies, levetiracetam, and PTZ binding affinity to ligands), physical observation (seizure score), and morphological (number of neurons and DNA fragmentation) analysis, collectively, these provide insights into the neurotherapeutic potential of ketone bodies under epileptic conditions.

The *in-silico* comparison of binding affinities between ketone bodies and standard anti-seizure medications helps uncover the molecular processes via which the ketogenic diet (KD) operates, particularly in individuals suffering from drug-resistant epilepsy (Kumar *et al.,* 2022; Domańska *et al.,* 2025; Na *et al.,* 2025). While the KD is an effective treatment for intractable epilepsy, its exact mechanisms of action remained a long-standing question. Therefore, comparing the binding profiles of ketone bodies (like beta-hydroxybutyrate or BHB, acetoacetate, and acetone) to known drug targets helps identify potential direct interactions with seizure-related receptors and ion channels. BHB has been shown to bind to and act as an antagonist for the G-protein-coupled receptor FFAR3 (Oteng and Liu, 2023; Pali *et al.,* 2025) This interaction is significant because FFAR3 mediates sympathetic nervous system activity; its inhibition by BHB is thought to reduce sympathetic outflow (Nagliya *et al.,* 2024). While acetoacetate and acetone have been demonstrated to provide protection against seizures induced by GABAA receptor antagonists (Nagliya *et al.,* 2024). In general, Ketone bodies directly inhibit specific channels and receptors, including the direct inhibition of AMPA receptors, reducing excitatory neurotransmission, enhancement of GABAergic and adenosinergic inhibitory effects, thereby activating ATP-sensitive potassium channels (Kadowaki *et al.,* 2017; Simeone *et al.,* 2017). Primarily studies have linked BHB to FFAR3, other studies suggest that short-chain fatty acids (SCFAs), which share structural similarities with ketone bodies, influence the CNS by binding to both FFAR2 (GPR43) and FFAR3 against epilepsy (García-

Rodríguez and Giménez-Cassina, 2021; Lymperopoulos *et al.,* 2022).

In this study, the standard drug exhibited the strongest binding affinity across all receptor systems evaluated (FFAR2 and FFAR3), however this binding affinity is of weak van der Waals force, that is the strong binding affinity observed between the FFAR2 and FFAR3 can be displaced, therefore questioning the standard drug's ability to completely treat epilepsy. The moderate and consistent affinities demonstrated by acetoacetate and β-hydroxybutyrate is fortified with strong hydrogen bond. This suggests that ketone bodies (acetoacetate and β-hydroxybutyrate) may serve as a regulator of neuronal and inflammatory processes, possibly contributing to seizure suppression and neuroprotection observed in vivo (Simeone *et al*., 2018; Viggiano *et al*., 2016). Also, the synergistic effect of the three ketone bodies might provide a more robust anti-seizure suppressor than the standard drugs, as observed in this study.

Following PTZ administration, seizure responses varied across the groups; the normal control rats (MN) showed no seizure activity and scored zero (0) throughout the experiment. While PTZ-treated rats developed seizure symptoms such as tonic-clonic convulsions, confirming a successful kindling seizure model score of five (5) towards the end of the experiment. However, rats treated with ketogenic diets (MP, MO, and MC) and levetiracetam (MSC) demonstrated reduced seizure severity and improved behavioral stability, indicating the anticonvulsant potential of the intervention. These findings align with previous reports that ketogenic diets enhance mitochondrial energy metabolism, reducing reactive oxygen species and advocating for the use of ketone bodies as alternatives energy substrates (Masino and Rho, 2019; Sondhi *et al.,* 2020; Kapoor *et al.,* 2021). The amelioration of fully kindled state observed with the ketogenic diets and standard anti-seizure medication groups, confirmed anti-seizure activity of ketogenic diets and standard anti-seizure medication in protecting against PTZ to strongly sensitized the hippocampus CA3 neurons, may result in decreased levels of excitability, specifically in the hippocampus CA3, It is acknowledged to possess high excitability levels.

KDs improved memory (as measured in the Y-maze) in pentylenetetrazol (PTZ)-kindled rats by reverse cognitive impairment, by showing a strong ability to normalize behavioral alterations, indicating an improvement in cognitive/behavioral state. The spontaneous alternation percentage obtained from the Y-maze showed that the control group (MN) exhibited the highest spontaneous switching indicating optimal spatial working memory. The PTZ control (MEC) and standard control (MSC) showed reductions in spontaneous alternations indicating impaired memory function. Ketogenic diet treatment with palm kernel oil (MP) showed significant improvement in spontaneous alternation suggesting enhanced cognitive performance. However, the castor oil (MC) and olive oil (MO) also showed enhanced cognitive performance. This aligns with reports showing that ketogenic fatty acids can improve or stabilize memory function in epilepsy models (Dahlin *et al.,* 2022; Shabbir *et al.,* 2025). To strengthen behavioral interpretation, additional memory paradigms such as Morris Water Maze or Barnes Maze can be done.

As expected, Body weight analysis indicated that rats on ketogenic diets experienced reductions in body weight, consistent with enhanced fat metabolism and reduced carbohydrate availability characteristic of ketosis (Newman and Verdin, 2017; Shabbir *et al.,* 2025). The non-differences in organ weight and non-significant differences in brain-to-body weight ratios suggest that the ketogenic diets were well tolerated and did not cause abnormal development to the brain or atrophy, aligning with previous studies on dietary fat tolerance in rodents (McDonald and Cervenka, 2018; McDonald and Cervenka, 2020).

A decrease in neuronal cell number is often due to neuronal loss from excitotoxicity or apoptosis leading to impaired synaptic transmission and hippocampal atrophy as seen in conditions like Alzheimer's disease and epilepsy. Conversely, an increase in hippocampus CA3 cell number in ketogenic diets groups may occur through neurogenesis, which enhances cognitive recovery and reactive gliosis (Dyńka *et al.,* 2022). In this study, a significant neuronal loss was evident in the PTZ control group, confirming seizure-induced neurodegeneration in the CA3 hippocampus, as earlier reported (Wilcox and Vezzani, 2014; Vezzani and Bartfai, 2019). However, KD-treated groups, particularly the MP group, showed improved neuronal survival and higher neuronal counts approximating those of the MN. This demonstrates KD's neuroprotective and restorative potential, likely through its ability to reduce oxidative stress, inhibit apoptosis, and enhance mitochondrial efficiency (Kim *et al.,* 2015; Ildarabadi *et al.,* 2024; Parveen *et al.,* 2025).

Feulgen reaction, as a quantitative marker of DNA stability and integrity, it shows the decreased optical density indicating normal viable, proliferating nuclei,

while increased optical density indicates DNA fragmentation representing advanced necrosis common in neurodegeneration (Madabhushi *et al.,* 2014). The MEC group showed significantly elevated mean optical density (MOD), indicating increased DNA fragmentation, a marker of epigenetic stress and neuronal injury (Blumcke *et al.,* 2017). All KD-treated groups exhibited significantly reduced MOD values, suggesting that KD mitigated DNA damage and epigenetic dysregulation. This action can be linked to β-hydroxybutyrate, a principal ketone body, which functions as an endogenous histone deacetylase (HDAC) inhibitor, promoting neurotherapeutic gene expression (Achanta and Rae, 2017; Wood *et al.,* 2019; Kim *et al.,* 2022). Therefore, the observed reduction in MOD reflects KD's epigenetic modulation that supports cellular repair in damaged hippocampal neurons. This finding in this study can be further confirmed using a TUNEL and comet assay to quantify the amount of DNA damage by PTZ and repair due to standard drugs (levetiracetam), and ketogenic diets (palm kernel oil, castor oil, and olive oil).

CONCLUSION

This study revealed that ketone molecules like β-hydroxybutyrate and acetoacetate interact moderately with strong bonding to key neuronal and inflammatory receptors (FFAR2/3), supporting their potential role in amelioration maybe via neurochemical stability and enhancing hippocampal neurogenesis and inhibiting DNA fragmentation. This study concludes that ketogenic diet formulations based on palm kernel, castor, and olive oils exhibit neurotherapeutic efficacy in ameliorating PTZ-induced hippocampus CA3 alterations in Wistar rats.

Conflict of Interests

The authors have no relevant financial or non-financial interests to disclose.

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