Niger. J. Physiol. Sci. 39 (December 2024): 295 – 304
www.njps.physiologicalsociety.com

Niger. J. Physiol. Sci.

Full length Research Article

Effect of *Musa paradisiaca* Stem Juice on Acute Status Epilepticus, Hippocampal Histology and Behaviour in Rats with Pentylenetetrazole-Induced Seizures

Bassey I.F.[a], Eru E.M.*[a], Udoaffah G.U.[a], Nnenna W.A.[a], Ajaba M.O.[b], Anani S.E.[a], Umoh N.M.[a], Ekanem A.E.[a], Okpabi F.[b], Ushie A.[c]

[a]*Department of Anatomical Sciences, University of Calabar, Calabar, Cross River State, Nigeria.*
[b]*Department of Science Laboratory Technology, University of Calabar, Calabar, Cross River State, Nigeria.*
[c]*Faculty of Clinical Sciences, University of Port Harcourt, Rivers State, Nigeria.*

Summary: Status epilepticus, histology, and neurobehavioral parameters of pentylenetetrazole-induced epileptic wistar rats were being investigated following administration of Musa paradisiaca (MP) stem juice. A seizure was induced sixty minutes after the administration of graded doses of Musa paradisiaca stem juice and diazepam, an anticonvulsant drug. A neurobehavioral test was carried out after pentylenetetrazole (PTZ) induction. The animals were arbitrarily given five groups, namely: A, B, C, D, and E. Group A animals functioned as the normal control, given rat pellets and distilled water. Group B served as the PTZ control, and the animals were administered 65mg/kg body weight of PTZ intraperitoneally. Group C served as the PTZ + Diazepam (DZP) treated group, and the animals were administered orally 4 mg/kg body weight of diazepam 60 minutes before administration of PTZ intraperitoneally. Group D and E served as the PTZ + MP [Low dose and High dose] pre-treated groups, given 2500 mg/kg and 5000 mg/kg body weight of MP stem juice, respectively, for 7 days orally before administration of PTZ intraperitoneally and 1 hour post treatment with MP stem juice. Significant antiepileptic effects on status epilepticus were observed in MP stem juice (2500mg/kg and 5000mg/kg) and the Diazepam group of animals. These antiepileptic effects were clearly seen in the delay recorded in latency of seizure and latency of tonic-clonic seizure, a reduced frequency of myoclonic jerks and a 100% mortality index rate recorded in the MP stem juice pre-treated animals. Histological sections of the hippocampus pre-treated with Musa paradisiaca stem juice and diazepam revealed significant differences in the structural integrity when compared with the epileptic control. The novel object recognition task test carried out showed a positive score for long-term memory as attained by animals in Group D and E when compared to Group B animals that had a negative score for long-term memory. In this study, the administration of Musa paradisiaca stem juice limited the extent of status epilepticus, reduced cytoarchitectural damage caused by epilepsy, and further enhanced long-term recognition memory in the MP stem juice pre-treated epileptic rats.

Keywords: *Pentylenetetrazole, Musa paradisiaca, Status epilepticus, Neurobehavioral*

**Authors for correspondence: erumba@unical.edu.ng, Tel: +2347065560843*

Manuscript received- January 2023; Accepted- December 2023

DOI: https://doi.org/10.54548/njps.v39i2.16

INTRODUCTION

The brain is considered a master organ due to its ability to regulate the activity of the entire human system. It is composed of a number of subcomponents that are responsible for organizing reflexes and other behavioural responses, as well as for cognition, learning, memory, planning, and the execution of voluntary movements (singh, 2004). Under the medial temporal lobe of the cerebrum, one on each side of the brain, lies the hippocampus. The hippocampus, along with its adjacent temporal and parietal lobe structures, constitutes the hippocampal formation (Guyton and Hall, 2000). The hippocampal formation is involved in the creation of new historical and truthful memories, and it also serves as a "memory gateway" through which new memories must pass before being permanently stored in the brain Serra *et al.,* 2020). Anterograde amnesia will result from damage to the hippocampus, which is the inability to form and retain new memories. According to Anand and Dhikav (Anand and Dhikav, 2012), the hippocampus is a common focus site in epilepsy and may be harmed by chronic seizures. The brain's high complexity makes it susceptible to damage. According to a 2015 report by the National Institute of Neurological Disorders and Stroke, difficult births, poor nutrition, and exposure to infectious diseases and toxins can cause or aggravate neuropsychiatric disorders. Hundreds of millions of people worldwide are affected by neurological disorders. Every year, more than 6 million people die as a result of a

stroke, with low- and middle-income countries accounting for more than 80% of these deaths according to a review by Leone *et al.,* (2021). In this review, about 60 million people have epilepsy worldwide.

Epilepsy is derived from the Greek word "epilepsia," which is composed of two words: "epi" meaning "upon" and "lepsi" meaning seizures (Harper, 2001). This neurologic disorder disrupts the transmission of electrical signals within the brain and it is marked by a persistent propensity to generate epileptic seizures, as well as neurobiological, cognitive, psychological, and social consequences of this condition (Fische *et al.*, 2005). There is a difference between seizures and epilepsy [also known as a "seizure disorder"]. A seizure is a temporary incidence of indications caused by an abnormal increase or synchronous neuronal activity in the brain (Bazil *et al*., 2019). All human cerebral cortices are capable of producing seizures if sufficiently stimulated. According to research, approximately 10% of individuals will have one at some point in their lives (Epileptic Foundation, 2010). The term "epilepsy" refers to a condition in which a person has an abnormally elevated likelihood to have seizures, such that they are susceptible to having them on a regular basis during normal life (World Health Organization, 2019). Using electroencephalograms, which detect abnormal neuronal signals in the brain, the origin of seizures can be determined (Fischer *et al.,* 2014). These seizures occur occasionally or on regular basis, are most prevalent in adults, and have been hypothesized to cause mental issues, such as high rates of depression and anxiety. Therefore, epilepsy has a profound impact on the entire central nervous system. Stress, sleep deprivation or fatigue, insufficient food intake, alcohol or drug abuse, and failure to take prescribed anticonvulsant medications may increase the risk of seizures in susceptible individuals (Diaz-Negrillo, 2013). Epilepsy is considered extremely dangerous, especially if the condition status epilepticus (SE) develops. Status epilepticus is defined as epileptic activity that persists for 30 minutes or longer. According to the International League against Epilepsy Task Force on Classification of Status Epilepticus, SE conceptually refers to "a condition resulting either from the failure of the mechanisms responsible for seizure termination or from the initiation of mechanisms which lead to abnormally prolonged seizures" (Trinka *et al.,* .i2013). Seizures can manifest as either prolonged or recurrent attacks without periods of recovery in between. This condition was estimated to affect over 100,000 Americans annually and result in approximately 50,000 deaths (World Health Organization, 2009. 31% to 43% of patients with SE do not respond to standard medications (Mayer *et al.* , , 2002) Without immediate care, this condition can result in permanent brain damage or death. In an early foundational work on primate models, Meldrum and Horton (1973) and Meldrum *et al*., (1973) described associated neuronal loss in SE. Even in the absence of hypoxia, acidosis, hypoglycaemia, and other confounding factors, programmed necrosis and apoptosis may mediate cell death according to (Niquet *et al.,* . 2003)

In mice, rats, cats, and primates, pentylenetetrazole (PTZ), a tetrazol derivative, induces convulsions. It does so apparently by blocking GABA-mediated inhibition through an action at the GABA receptor (Olsen , 2001; Kliouеva *et al.,* 2001). For research into brain excitability and the development of antiepileptic medicines, PTZ injection is a popular behavioural technique (Loscher, 2002; Pitkanen *et al*., 2015). PTZ is a model drug for generalized seizures, according to the behavioral and electroencephalogram (EEG) manifestations of rodent seizures brought on by the drug. When used to cause recurring seizures, PTZ has been used as a model for generalized tonic-clonic seizures. PTZ can be given subcutaneously, intravenously, or intraperitoneally. The amount and method of administration impact the latency to seizure onset and behavioural symptoms; typically, a single systemic administration is sufficient to elicit seizures. PTZ can result in status epilepticus when given in large doses (Holmes and Ben-Ani, 1998). Young rats can also be repeatedly given PTZ in the kindling model of epilepsy (Erkeç and Arihan, 2015; Aiyeloja and Bello, 2006).

The plantain, *Musa paradisiaca*, belongs to the Musaceae family and is an herbaceous plant that can grow up to 9 meters long. It has a strong pseudo-tree-like pseudo-stem and a crown of large, elongated, oval, dark-green leaves with a prominent midrib that can measure up to 365 cm in length and 61 cm in width. More than 70 million people in sub-Saharan Africa rely on it as a staple meal, and it is widely produced in the tropics.

It is known as Ogede (Yoruba), Abrika (Igbo), Okamu-ayaba (Hausa), and Ukọm (Efik /Ibibio) in Nigeria (Ajaba, 2016; Onyenekwe *et al.,* 2013). According to Onyenekwe *et al*., (2012), plantain juice (pseudo stem) contains alkaloids, saponins, flavonoids, glycosides, anthraquinones, phlobatannins, and tannins. It has been demonstrated that flavonoids prevent the prompting, promotion, and progression of tumors due to their anti-oxidant properties (Citraro *et al.,* 2016). Oxidative stress may be involved in the initiation and progression of epileptogenesis as a result of excessive free-radical production. A recent report by Citraro *et al*., (2016) on the anticonvulsant effect of flavonoid-rich extract from *Citrus sinensis* (sweet orange) in certain experimental models of epilepsy provides additional support for the neurorehabilitative effect of this active ingredient (flavonoid). These phytochemicals provide *Musa paradisiaca's* juice its synergistic therapeutic qualities, which explains why it is used to treat a wide range of illnesses (Fischer *et al.,* 2019; Kim *et al.,* 2012; Agbo *et al.,* 2015; Agbo *et al.,* 2019; University of Florida Herbarium, 2022)

In light of the foregoing and other studies on the anti-oxidative effects of flavonoid-rich extracts, this study assessed the antiepileptic activity, learning, and memory effects of *Musa paradisiaca* stem juice in a PTZ-induced model of epilepsy

MATERIALS AND METHODS

Experimental animals: Thirty male albino wistar rats weighing between 110-160g were obtained from the animal house of the Department of Biochemistry, University of Calabar, Calabar. The animals were allowed one week of acclimatization and housed in cages under room temperature (25±20C), relative humidity (50±5%) and a 12 hour light/dark cycle in the animal house of the Faculty of Basic Medical Sciences. During the whole experiment, the animals could eat rat food and drink tap water whenever they wanted.

Sample collection and preparation: Fresh plantain pseudo stems used for this experiment were obtained from the plantation of Mrs. Kinini Frank in Ikot Nkebre, 8 miles from Calabar Municipality, Cross River State. The stems were authenticated at the Department of Botany, University of Calabar, Nigeria and the voucher specimen was placed in the Department's herbarium with voucher number HERB/BOT/UCC/ 145. Plantain stems were cleaned of dirt by washing. To extract the juice, they were then cut into slices and crushed with a mortar and pestle. At least 600ml of the juice were extracted and stored for further use right away at 4^{o}C day and night. This method is according to University of Florida Herbarium (UFH) (2022)

Acute toxicity test: Oral acute toxicity of *Musa paradisiaca* juice was determined in rats as described by Lorke (1983). The maximum tolerated dose in the current study was determined to be larger than 5000 mg/kg after it was determined that *Musa paradisiaca* juice has no drug-related toxicity.

Chemicals used: Both the chemicals (PTZ) and the drugs (Diazepam) were obtained commercially from Bez Pharmacy, Calabar, Cross River State, and were of analytical grade. Thus, the chemicals are ultra-pure and are ideal for providing consistent results.

Drug preparation: PTZ was freshly prepared to the desired concentration of 13mg/ml (195mg of PTZ dissolved in 15ml of normal saline). The drug will be given at a dose of 65mg/kg bodyweight by taking volumes of the stock corresponding to the dose for each rat.

Experimental design: The design consisted of 30 male Wistar rats divided into 5 groups (A-E) of 6 animals each. The groups were a normal control, an epileptic, and the 3 epileptic treated groups. In this study, diazepam was used as a reference drug to check anti-convulsing activity in PTZ induced epilepsy. The doses used were based on the LD50 determined for the juice, which was greater than 5000mg/kg. Group A animals served as the normal control and were administered rat pellets and distilled water for 14 days. Group B animals that served as the epileptic control received 65mg/kg body weight of pentylenetetrazole once. Group C animals were treated with 4mg/kg body weight of Diazepam before and after epilepsy induction. Group D was the *Musa paradisiaca* juice group and received 2500mg/kg body weight of *Musa paradisiaca* stem juice daily for 7 days, while group E animals received 5000mg/kg body weight of *Musa paradisiaca* stem juice daily for 7 days.

The drugs (PTZ and Diazepam) and extracts were administered by intraperitoneal (I.P) and oral routes, respectively. Seven (7) days of pre-treatment with the juice of MP, one-hour pre/post treatment with diazepam, and a one-hour post treatment with 65mg/kg of PTZ, were given to the animals, and the animals were observed over a period of thirty minutes after. During this period of observation, the onset of seizures (time it takes for seizures to begin), latency to seizure, frequency of seizures, and mortality rate were noted. From the data obtained, quantal protection (number of animals alive in each group divided by the total number of animals in the group), percentage protection (percentage of animals alive) and percentage mortality (percentage of animals dead) were calculated.

Ethical approval: Approval was obtained for all experimental procedures from the Faculty Animal Research Ethics Committee of the Faculty of Allied Medical Sciences, University of Calabar, Calabar, Cross River State, Nigeria with an Approval number: *086ANA2321*

Collection of tissue samples for analysis: At the conclusion of the 14-day experiment, the animals were sacrificed and perfused using chloroform vapour and dissected. Their brains were surgically excised, the hippocampus localized and fixed in formal saline preparation for histological analyses.

Histological evaluation: Formal-saline fixed tissue sections of the hippocampus were processed for histopathological examination through careful histological procedures in a specific order that included dehydration, clearing, impregnation, embedding, sectioning, slide preparation, staining, and microscopy. The tissue sections were stained using haematoxylin and eosin (H&E) as described by Bancroft and Stephens (1982). Images were captured with a light microscope at a magnification of X400.

Novel Object recognition task: The object recognition task is conducted in an open field (OF) box (38 x 38 cm), which is also used to assess exploratory behaviour. All animal testing were conducted under diffuse lighting conditions via a 60-Watt white light bulb. A video camera suspended above the centre of the open field records all behaviours for later analysis. Behaviour were scored live using a video camera-based computer tracking system (Lime Light, Actimetrics). The computerized event-recorder program Hindsight for MS-DOS version 1.5 can also be used to record behaviour.

Statistical analysis: Data were presented as mean ±standard error of mean. Data were computed and analyzed using one way ANOVA to compare group means of antiepileptic activity and neurobehavioral test. The data were analyzed with the help of a statistical package, SPSS version 22.0 for Windows, considered significant at $p<0.05$.

RESULTS

Latency of seizure: The latency of seizure recorded for groups C, D and E was 350.86 ± 64.38, 373.00±60.25, and 299.18 ±56.28 sec respectively. These values were significantly higher ($P<0.05$) compared to group B, which recorded 66.69±3.24 (Figure 1).

Latency of tonic-clonic seizure: Treated groups (C, D, and E) exhibited significantly higher values of 589.88±190.76, 618.39±56.66 and 1655.79±126.49 sec, respectively, to delay the initiation of tonic-clonic seizure in the animals as compared to PTZ control group B, which only delayed it for about 132.37±7.47 sec (Figure 2). In group D (Pentylenetetrazole + Musa paradisiaca [High dose]), this effect on how long it took for a tonic-clonic seizure to start was more noticeable.

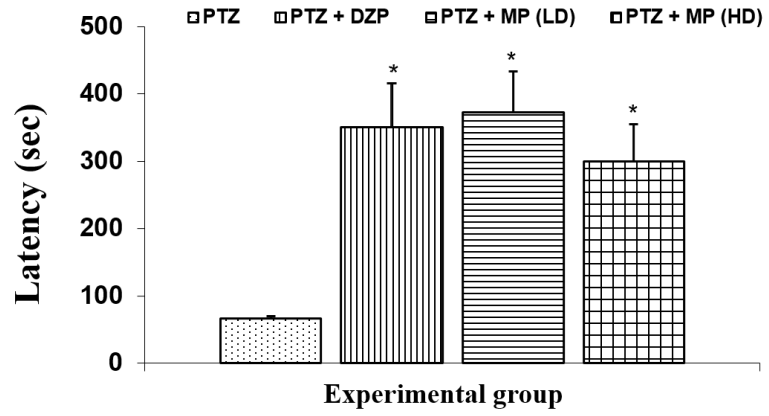

Figure 1:
Comparison of latency of seizure in the different experimental groups.
*Values are expressed as mean±SEM, n = 5. * = p<0.05 vs PTZ*

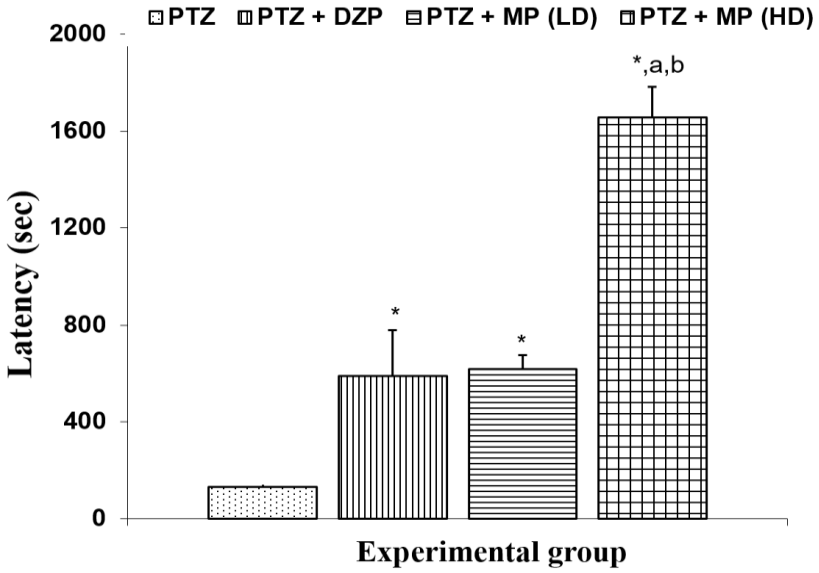

Figure 2:
Comparison of latency of tonic clonic seizure in the different experimental groups.
Values are expressed as mean +SEM, n =5.
** = p<0.05 vs PTZ; a = p<0.05 vs PTZ + DZP*
b = p<0.05 vs PTZ + IM (LD)

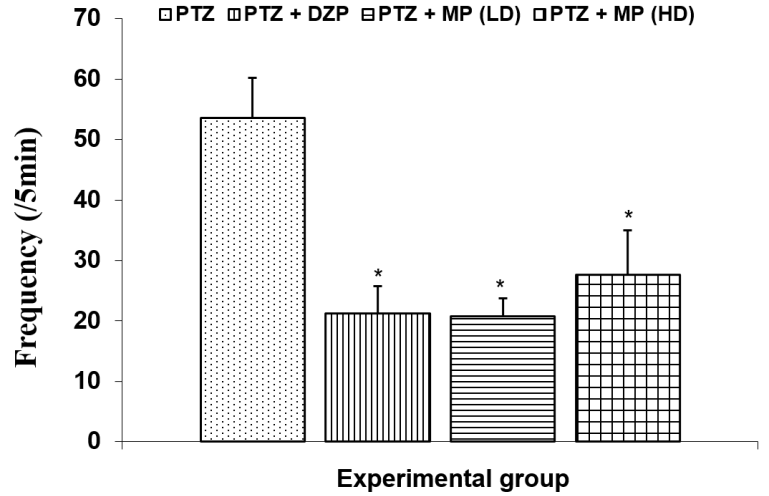

Figure 3:
Comparison of frequency of jerks in the different experimental groups.
Values are expressed as mean +SEM,
n = 5. * = p<0.05 vs PTZ

The Frequency of Myoclonic Body Jerks: The frequency of myoclonic jerks was also observed and the result showed that PTZ control rats (group B) exhibited jerks for 53.60 ± 6.62 minutes in contrast to the treated rats (group C, D and E) with significantly lower duration for their jerks, which were recorded as 21.25 ± 4.49, 20.80 ± 2.94, and 27.60 ± 7.41 minutes. (Figure 3)

Mortality index rate: Seizure phases developed in these animals amongst experimental groups B, C, D, and E were ear and facial twitching, myoclonic body jerks, clonic forelimb convulsions, generalized clonic convulsions, turning onto one side position, and generalized clonic tonic convulsions (or death within 30 minutes). These seizure phases will sequentially result in mortality cases recorded amongst the experimental population. A 50% mortality index rate was recorded in group B (PTZ), a 16.67% mortality index rate for group C (PTZ + DZP), a 0% mortality index rate and 100% protection rate for group D (PTZ + MP [Low dose]) and group E (PTZ + MP [High dose]) (Table 1).

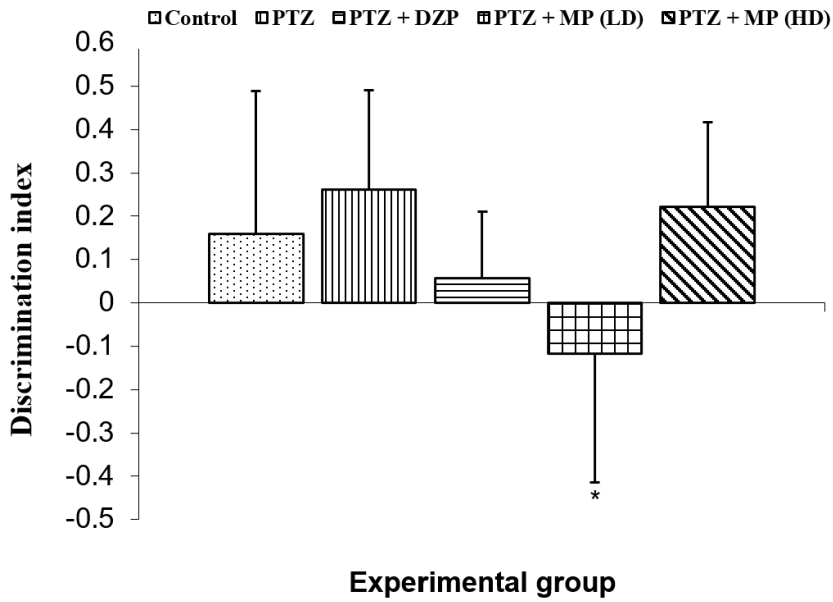

Figure 4:
Comparison of discrimination index for short term memory during Novel object recognition task in the different experimental groups.
Values are expressed as mean +SEM, n =5

.

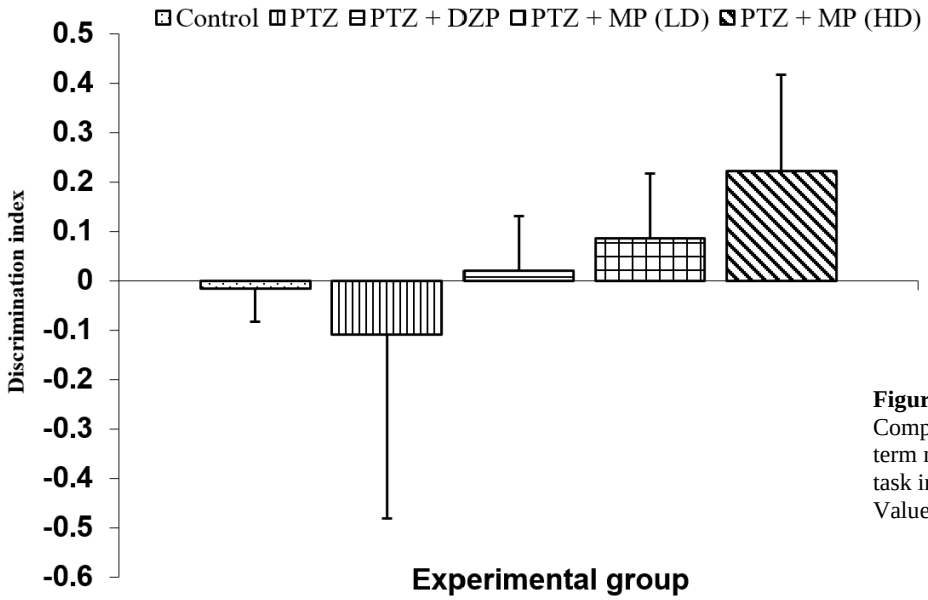

Figure 5:
Comparison of discrimination index for long term memory during Novel object recognition task in the different experimental groups.
Values are expressed as mean +SEM, n =5

Table 1:
Effect of *Musa paradisiaca* stem juice on PTZ-induced seizure

Group	Treatment / Dose	No of rats	Quantal Protection	Percentage Protection %	Percentage Mortality %
A	Normal control (Distilled water)	6	-	-	-
B	PTZ (65mg/kg.b.w)	6	3/6	50	50
C	PTZ + DZP (4mg/kg.b.w)	6	5/6	83.33	16.67
D	PTZ+MP Juice (2500mg/kg.b.w)	6	6/6	100	0.00
E	PTZ+MP Juice (5000mg/kg.b.w)	6	6/6	100	0.00

Histological findings: The hippocampus of group B (PTZ control) animals induced with epilepsy and not treated showed a distorted architecture, a reduction in neuronal cell population, pyknosis, and chromatolysis (Plate 1B). In the *Musa paradisiaca* treated groups (Plate 1D and 1E), there was a marked reduction in cellular damage, which also had similar features to group C (Plate 1C). Group E (Plate 1E) showed realignment of cellular layers, with the molecular layer, polymorphic cell layers, and pyramidal layer having densely populated neuronal cells with prominent nuclei, scanty glia cells, and blood vessels. Sections from the normal control group showed the hippocampus consisting of an outer molecular layer and an intermediate pyramidal layer. The pyramidal cell layer consists of densely populated and closely packed pyramidal cells of various sizes with prominent nuclei. Interspersed in these two layers were microglia cells (Plate 1A). This is in contrast to animals that were administered 65mg/kg body weight of PTZ only.

Sections from this group showed a partially distorted architecture with indistinct layers that appeared to consist of sparsely populated neuronal cell bodies with an obvious shrinkage of the cell body, pyknosis, and intense chromatolysis. The pyramidal cell layer appears scanty and degenerated pyramidal cells are seen (Plate 1B). Observations made on the treated groups began with animals that were given 4 mg/kg body weight of diazepam before and after epileptogenesis. The sections showed the hippocampus with distinct cellular layers. The pyramidal cell layer is clearly visible and appears to consist of a scanty population of pyramidal cells with prominent oval to ovoid nuclei. Intervening blood vessels were also seen between the molecular and pyramidal cell layers (Plate 3). Similar changes were also noted in an animal group administered 2500mg/kg body weight of *Musa paradisiaca stem* juice extract. The section of the hippocampus showed realignment of the different cell layers, which are now distinct. The pyramidal cell layer appeared filled with neuronal cells which had round to ovoid nuclei. There is also the presence of microglia cells (Plate 4). Sections of the hippocampus in animals treated with 4000 mg/kg body weight of *Musa paradisiaca stem juice* showed distinct layers having densely populated neuronal cells with prominent nuclei. They were microglia cells and intervening blood vessels between molecular and pyramidal layers (Plate 5). These changes can be compared to those in the normal control group of animals.

DISCUSSION

In this study, the juice (2500mg/kg and 5000mg/kg b.w) from the stem of *Musa paradisiaca* exhibited significant antiepileptic activities in the PTZ-induced model of epilepsy. This was observable as the juice showed a significant effect on the recorded seizure parameters, analyzed histological sections, and neurobehavioural assessment. Seizure parameters recorded were latency of seizure, latency of tonic clonic seizure, frequency of myoclonic jerks, and mortality rate analysis.

Goldberg and Coulter (2013) alludes that latency of seizure is the time between the start of epilepsy (epileptogenesis) and the first seizure. Tonic-clonic seizures, on the other hand, are distinguished by clonic or myoclonic movements that progress to tonic muscle extension of the limb and trunk muscles followed by clonus, and they can result in significant morbidity and mortality, according to Hammond (2016).The juice from *Musa paradisiaca* stem recorded a significant delay in the action of PTZ in the treated groups as compared to the PTZ control rats. This effect was evidenced by a significant delay in the latency of seizure and tonic clonic seizure among the juice treated groups D and E, as compared to the PTZ control group (Figure 1 and 2). These results are in similitude with reports from Reddy *et al*., (2018) on the effect of *Musa sapientum* stem extract on acute and chronic experimental models of epilepsy.

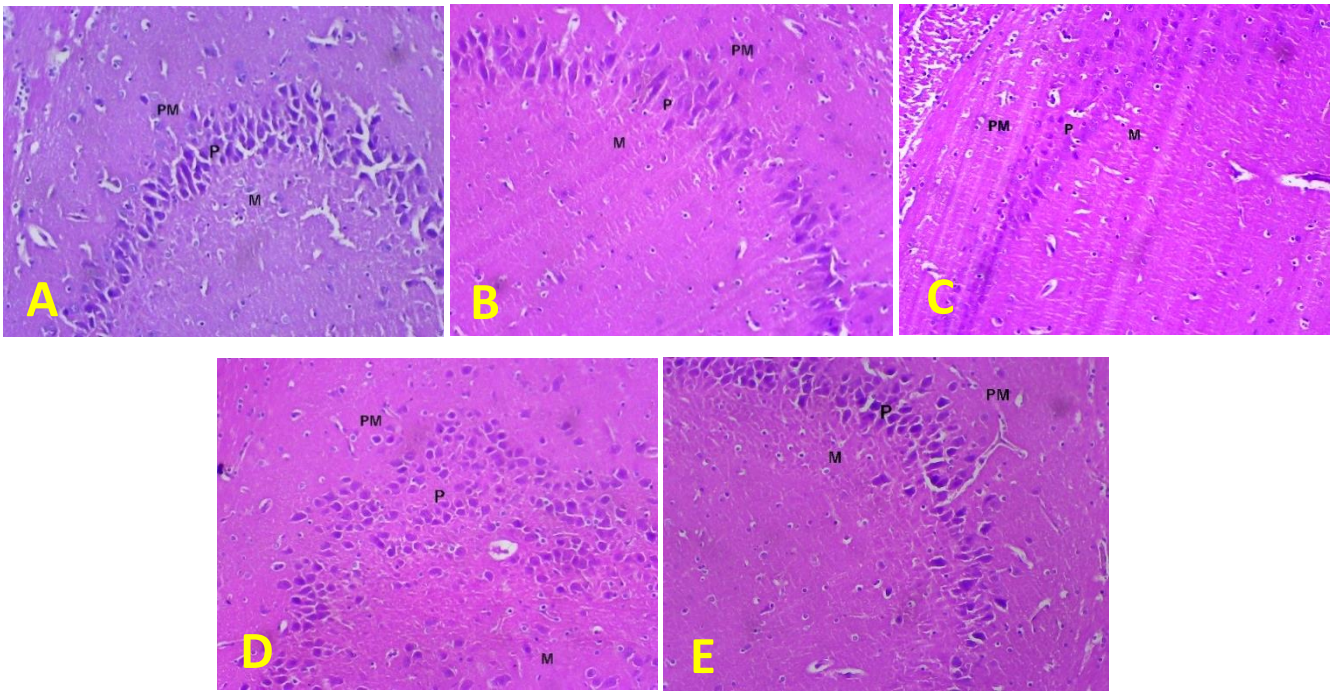

Plate 1 (A-E)

1A: Section of hippocampus from group A (normal control) showing the molecular layer (M), polymorphic cell layer (PM) and pyramidal layer (P) with densely populated and closely packed pyramidal neurons (P).

1B: Section of hippocampus from group B (PTZ control) showing indistinct and partially distorted cellular architecture; the molecular layer (M), polymorphic cell layer (PM) and pyramidal layer (P) with sparsely distributed pyramidal neurons (P).

1C: Section of hippocampus from group C (PTZ + DZP) showing distinct cellular layers; showing molecular layer (M), polymorphic cell layer (PM) and pyramidal layer (P). The pyramidal cell layer is clearly visible and appeared to consist of scanty population of pyramidal cells.

1D: Section of hippocampus from group D (PTZ + MP [LD]) showed realignment of the different cell layers which are now distinct; the molecular layer (M), polymorphic cell layer (PM) and pyramidal layer (P) with numerous pyramidal neurons.

1E: Section of hippocampus from group D (PTZ + MP [HD]) showed distinct layers; the molecular layer (M), polymorphic cell layer (PM) and pyramidal layer (P) having densely populated neuronal cells with prominent nuclei.

In line with this research, there was a significant increase in the latency to the onset of myoclonic jerks and the duration of clonic convulsions following PTZ administration.

According to Shibasaki and Hallet (2005), myoclonus is a movement disorder that presents itself with sudden, brief, shock-like jerks. Kojovic *et al*., (2011) reviewed that myoclonus may be classified as physiological, essential, epileptic, symptomatic, or psychogenic based on aetiology. In the present study, as expected, the PTZ control group of animals initially produced myoclonic jerks, which subsequently became sustained and led to a generalized tonic-clonic seizure. In addition, it was observed that the frequency of myoclonic jerks in the treated groups (C, D, and E) decreased much more rapidly than in the PTZ control rats (group B), which exhibited a higher frequency of jerks

The MP stem juice also offered 100% quantal protection to the treated animals in groups D and E, thereby significantly reducing seizure severity posed by the chemo-convulsant drug (PTZ) without any record of mortality in this group. This is in contrast to the PTZ-induced group of animals, where half of the population died due to seizure severity (Table 1). In line with this result, Massey *et al., (*2014) reported that severity of seizures leads to sudden unexplained death in epileptic patients (SUDEP) whose mechanisms remain unknown, with both cardiac and pulmonary derangement having been hypothesized. Stachowicz *et al*., (2008) also emphasized that seizure severity leads to a convulsive syndrome, which is a multi-causal phenomenon characterized by the decrease or absence of neuronal inhibition usually mediated by Gamma amino butyric acid. His reports still follow that PTZ antagonizes GABAergic receptors, leading to a decrease in GABAergic tone, which is associated with an increase in neuronal excitability that favours the hyper synchronization of neuronal electrical activity and the appearance of convulsive episodes. This will in turn trigger numerous pathologies which will result in increased mortality. The MP stem juice administered to the treated animals acted to reduce this seizure severity by neutralizing PTZ levels in the blood of these treated animals, thereby minimizing its dose-dependent action on GABAergic receptors and avoiding the death that would have ensued. The neutralizing effect of MP stem juice has also been reported by Borges *et al.,* (2005).
Positive responses of extract-treated animals during this phase of seizure evaluation may be attributable to the capacity of MP stem juice to neutralize specific chemical components. Furthermore, these aforementioned antiepileptic activities of the MP stem juice may be due to the presence of phytochemicals in the juice of the *Musa paradisiaca* plant, as well documented by Akpuaka and Ezem (2011) and Akpabio *et al*., (2012)]. In particular, Ostróżka-Cieślik (2002) reported that antioxidants are essential to protect the human organism. This will support our findings because in this study, the high concentrations of flavonoids in the MP stem juice administered to the animals in group D (PTZ + MP) had the greatest impact, blocking the toxic effects of PTZ on neuronal excitability and preventing convulsion-induced crises.

Epilepsy is typically associated with prominent cell loss and altered cytoarchitecture of the brain, specifically the hippocampus, according to research (Arellano *et al.,* 2004). Additionally, numerous deformities, such as loss of plasticity, are commonly observed in affected neurons. The extent of metabolic workup is governed by the type of seizure and syndrome (Pearl, 2004). An imbalance between generated reactive metabolites, also known as reactive oxygen species (ROS), and the body's antioxidant system is known to cause oxidative stress. However, if the imbalance persists, it can cause cell damage and eventually disease. Seizures and seizure-induced neuronal damage are facilitated in part by free radical damage, which has been identified as a key factor (Shin *et al*., 2011: Frantseva *et al*., 2000). In sequence to this finding, in the current study, the hippocampus of PTZ control animals induced with epilepsy and not treated showed a distorted architecture, a reduction in neuronal cell population, pyknosis, and chromatolysis. This corresponds to reports from Sutula *et al*., (2002) that prolonged seizure characteristics which may progress to tonic-clonic convulsive seizures are more likely to lead to neuronal loss. On the other hand, Olajuyigbe and Afolayan (2011) demonstrated in their study that phenols and flavonoids, which are excellent antioxidants resident in MP stem juice, would greatly mop up free circulating radicals that may result in neuronal damage, and thus in the *Musa paradisiaca* treated groups (D and E), there was a marked reduction in cellular damage, which also had similar features to group C (PTZ + DZP). Broadbent *et al*., (2010) reported that the hippocampus is important for object recognition memory, and if there are lesions on this structure, moderate and reliable anterograde memory impairment will occur. While Eru *et al*.,(2022; 2021a; 2021b; 2020a, 2020b) linked hippocampal cells death with learning and memory enhancement in cognitive dysfunction rats induced with scopolamine hydrobromide post treatment with *Telfairia occidentalis* seeds and *Talinium triangulare* leaves aqueous extract, Anani *et al.,* (2020) and Udoh *et al.,* (2020) reported the neuroprotective effect of *Averrhoa carambola* aqueous fruit extract on the hippocampus and *Cytrus esculentus* reversed radiation-induced damage testicles in rats respectively . In light of these reports, the anatomico-functional relationship of the hippocampus was meticulously examined in this study. The novel object recognition test was used to conduct a neurobehavioral test for learning and memory. According to Lueptow (2017), the novel object recognition task is an efficient and adaptable method for studying learning and memory in rats and is appropriate for the detection of neuropsychological changes following pharmacological, biological, or genetic manipulations. Oliveira *et al.,* [(2010) added that during a NOR task, memory is consolidated and spatial or contextual characteristics of objects can be relocated in different parts of the brain. The discrimination index is the most common memory measurement for the novel object task (Denninger *et al.,* 2018). A comparison of the discrimination index for short-term memory revealed that treated animals in the MP [LD] group had a negative score, which was significantly different from the positive scores of other groups (Figure 4). During this phase of the experiment, animals in group E performed better than those in the normal control and PTZ + DZP groups. The final analysis for long-term memory in the discrimination phase revealed a positive score for the MP [LD] and MP [HD] treated groups, in contrast to the PTZ control group, which displayed a negative score (Figure 5), indicating a decline in long-term recognition

memory due to structural hippocampal damage caused by epileptic seizures, as recorded in the histological analysis (Plate 2). These findings are consistent with previous research by Osim *et al*., (2017), which demonstrated that long-term consumption of unripe plantain improved learning and memory in an experimental mouse model. Throughout this experiment, diazepam had a positive effect on the animals, indicating that it has no negative effect on learning and memory.

In conclusion, these results indicate that MP stem juice exhibited significant antiepileptic activity in reducing the detrimental effects of status epilepticus and the associated structural deficit. In addition, MP stem juice was associated with positive behavioural parameters that are indicative of an enhanced long-term recognition memory.

Acknowledgements

The following people are acknowledged for their contributions towards the success of this work: Grace F. Bassey, Bassey E. Kanu, Collins Igbaji, Joseph Jacob and Cynthia Echendu

REFERENCES

Adamson, S.S. and Ganiyu, O. (2012). Aqueous Extracts from Unripe Plantain (Musa paradisiaca) Products Inhibit Key Enzymes Linked with Type 2 Diabetes and Hypertension in vitro. Jordan J. Biol. Sci.. 5(4): 239-246.

Agbo, B. E., Nta, A. I. and Ajaba, M. O. (2019). Bio-pesticidal Properties of Neem (Azadirachta indica). Adv. Trends in Agric, Sci.,. Book Publisher International, 1:10-26. DOI:10.9734/bpi/atias/v1

Agbo, B. E., Nta, A. I. and Ajaba, M. O..(2015). A Review on the Use of Neem (Azadirachta indica) as a Bio-pesticide, J. Biopestic Environ., 2 (1):58-65

Ajaba, M. O., Brooks, A. A. and Emanghe, U. E. (2016). Antimicrobial potency of neem (Azadirachta indica) leaf extracts on pathogenic bacteria isolated from wound infection. Int. J. Sci. Eng. Res. 7(1): 1501-1507.

Akpabio, U. D., Udiong, D.S. and Akpakpan, A. E. (2012). The Physicochemical characteristics of plantain (Musa paradisiaca) and banana (Musa sapientum) pseudo stem wastes. Adv. Nat. Appl. Sci. 6 (2): 172.

Akpuaka, M. U. and Ezem, S. N. (2011). Preliminary photochemical screening of some Nigerian dermatological plants. J. Basic Phys. Res. 2 (1): 5.

Anani, S. E., Eru, E. M., Okon, D., Uruakpa, K. C., Ugbem, T. I., Igiri, A. O. (2020). The Effect of Averrhoa Carambola (Star Fruit) Aqueous Fruit Extract on the Hippocampal Astrocyte Expression following Diazepam-Induced Neurotoxicity in Wistar Rats. Trop J Nat Prod Res. 4 (12):1170-1173.

Arellano, J. I., Muñoz, A., Ballesteros-Yáñez, I., Sola, R. G. and DeFelipe, J. (2004). Histopathology and reorganization of chandelier cells in the human epileptic sclerotic hippocampus, Brain. 127 (1): 64.

Bancroft, J. and Stevens, A. (1982). Theory and Practice of Histological Techniques. (2nd ed). New York, Churchill Livingston. 131-135.

Bazil, C. W., Morrell, M. J. and Pedley, T. A. (2005). Epilepsy. In: Rowland LP. (11th ed) Merritt's Neurology. Philadelphia; pp. 1008.

Borges, M. H., Alves, D. L. F., Raslan, D. S., Pilo-Veloso, D., Rodrigues, V. M., Homsi-Brandeburgo, M. I., De Lima, M. E. (2005). Neutralizing properties of Musa paradisiaca L. (Musaceae) juice on phospholipase A2, myotoxic, hemorrhagic and lethal activities of Crotalidae venoms. Jour. Ethnopharmacol., 98 (1-2): 21-9.

Broadbent, N. J., Gaskin, S., Squire, L. R. and Clark, R. E. (2010). Object recognition memory and the rodent hippocampus. Learn Mem., 17:11

Citraro, R., Navarra, M., Leo, A., Donato-Di-Paola, E., Santangelo, E., Lippiello, P., Aiello, R., Russo, E. and De Sarro, G. (2016). The Anticonvulsant Activity of a Flavonoid-Rich Extract from Orange Juice Involves both NMDA and GABA-Benzodiazepine Receptor Complexes. Mol. (Basel, Switzerland), 21 (9); 1261.

Denninger, J. K., Smith, B. M. and Kirby, E. D. (2018). Novel Object Recognition and Object Location Behavioral Testing in Mice on a Budget. J Vis Exp., (141), e58593, 1-10.

Díaz-Negrillo, A. (2013). Influence of sleep and sleep deprivation on ictal and interictalepileptiform activity. Epilepsy Res Treat., 2013: 1-7, doi:10.1155/2013/492524.

Epilepsy Foundation (2010). About epilepsy and seizures. Available at: www.epilepsyfoundation.org/about/statistics.cfm.

Erkeç, O. E. and Arihan, O. (2015). Pentylenetetrazole Kiindling Epilepsy Model. Epilepsia; 21(1):12.

Eru, E. M., Anani, S. E., Paulinus, S. O., Mesembe, O. E., Uruakpa, K. C., Umoh, N. M., Oku, M. E., Akpaso, M. I., Igiri, A. O. (2022). Scopolamine-Induced Alzheimer's disease in Wistar Rats; Aqueous Talinum Triangulare Potency on the Hippocampal Nissl Bodies and Long-Term Learning and Memory. Trop J Nat Prod Res. 6 (1):117-122.

Eru, E. M., Paulinus, S. O., Udo-Affah, G. U., Uruakpa, K. C., Oku, M. E., Anani, S. E., Umoh, N., Akpaso, M. I. and Igiri, A. O. (2021b). Hippocampal Astrogliotic Reduction in Scopolamine Hydrobromide-Induced Alzheimer's Cognitive Dysfunction Wistar Rats Following Administration of Aqueous Extract of Telfairia occidentalis (Hook F.) Seeds. Niger. J. Physiol. Sci. 36: 241 - 244 www.njps.physiologicalsociety.com

Eru, E. M., Paulinus, S. O., Igiri, A. O. and Akpaso, M. I. (2020a). Enhanced Effect of Aqueous Extract of Telfaria occidentalis Seed on the Microstructure of the Hippocampus of Scopolamine Hydrobromide-Induced Cognitive Dysfunction Rats. Asian. J. Res. Rep. Neurol, 3 (1): 5-10.

Eru, E. M., Paulinus, S. O., Udo-Affah, G. U., Uruakpa, K. C., Oku, M. E., Edet, U. I. and Igiri, A. O. (2020b). Neurobehavioral Enhancement in Scopolamine Hydrobromide-Induced Alzheimer Type Cognitive Dysfunction in Rats Following Administration of Aqueous Seed Extract of Telfairia occidentalis (Hook.f.) Cucurbitaceae. Trop J Nat Prod Res. 4(7):282-285.

Eru, E. M., Udo-Affah, G. U., Paulinus, S. O., Uruakpa, K. C., Oku, M. E., Anani, S. E., Blessed, N. C., Nyiam, T., Umoh, N. M. and Igiri, A. O. (2021a). Restorative Potentials of Aqueous Telfairia occidentalis Seeds extract on the Hippocampal Nissl Granules and Short Term Memory in Scopolamine Hydrobromide-Induced

Alzheimer's Type Cognitive Dysfunction Rats. Trop. J. Nat. Prod. Res. 5(1):182-187.

Fischer, V. A., Bassey, I. F., and Fischer, C. E. (2019). Methanolic Leaf Extract of Vernonia amygdalina on the Testes of Streptozotocin Induced Diabetic Wistar Rats. J Pharm Biol Sci., 14(3):84-88

Fisher, R. S., Scharfman, H. E. and DeCurtis, M. (2014). How can we identify ictal and interictal abnormal activity? Adv Exp Med Biol., 813:3-23.

Fisher, R. S., van Emde Boas, W., Blume, W., Elger, C., Genton, P., Lee, P. and Engel, J. Jr. (2005). Epileptic seizures and epilepsy: Definitions proposed by the International League against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia., 46 (4): 470 - 472.

Frantseva, M. V., Velazquez, J. L., Hwang, P. A. and Carlen, P. L. (2000). Free radical production correlates with cell death in an in vitro model of epilepsy. Eur J Neurosci., 12 (4):1431- 1439.

Goldberg, E. M. and Coulter, D. A. (2013). Mechanisms of epileptogenesis: A convergence on neural circuit dysfunction. Nat. Rev. Neurosci., 14:349

Guyton, A. C. and Hall, J. E. (2000). Textbook of Medical Physiology. (10th ed.) London: W. B. Saunders Company, pp. 198-216

Hammond, N. (2016). Tonic–Clonic Seizures☆. Reference Module in Biomedical Sciences. doi:10.1016/b978-0-12-801238-3.995

Harper, D. O. (2001). Meaning of epilepsy. Brit Med Jour, 2: 321.

Holmes, G. and Ben-Ari, Y. (1998). Seizures in the developing brain: Perhaps not so benign after all. Neuron., 21: 1234.

Kim, J. H, Kang, J. W., Kim, M. S., Bak, Y., Park, Y.S., Jung, K. Y., Lim, Y. H. and Yoon, D. Y. (2012). The apoptotic effects of the flavonoid N101-2 in Human cervical cancers. Toxicol In vitro; 26 (1): 73

Klioueva, I. A., Van Luijtelaar, E. L., Chepurnova, N. E. and Chepurnov, S. A. (2001). PTZ-induced seizures in rats: Effects of age and strain. Physiol. Behav., 72: 426.

Kojovic, M., Cordivari, C. and Bhatia, K. (2011). Myoclonic disorders: a practical approach for diagnosis and treatment. Ther Adv neurol Disord, 4 (1), 47–62.

Leone, M., Ciccacci, F., Orlando, S., Petrolati, S., Guidotti, G., Majid, N. A, Tolno, V. T., Sagno, J., Thole, D., Corsi, F. M., Bartolo, M. and Marazzi, M. C. (2021). Pandemics and Burden of Stroke and Epilepsy in Sub-Saharan Africa: Experience from a Longstanding Health Programme. Int. J. Environ. Res. Public Health, 18 (5), 2766.

Lorke, D. G. (1983). A new approach to practical acute toxicity testing. Arch Toxicol., 54:287.

Loscher, W. (2002). Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. Epilepsy Res., 50:123.

Lueptow, L. M. (2017). Novel Object Recognition Test for the Investigation of Learning and Memory in Mice. J V Exp., 126: 55718.

Massey, C. A., Sowers, L. P., Dlouhy, B. J. and Richerson, G. B. (2014). Mechanism of sudden unexpected death in epilepsy: the pathway to prevention. Nat Rev Neurol., 5: 282

Mayer, S. A., Claassen, J., Lokin, J., Mendelsohn, F., Dennis, L. J., and Fitzsimmons, B. F. 2002. Refractory status epilepticus: frequency, risk factors, and impact on outcome. Arch Neurol., 59(2), 205. doi:10.1001/archneur.59.2.205

Meldrum, B. S. and Horton, R. W. (1973). Physiology of Status Epilepticus in Primates. Arch Neurol., 28(1), 1–9. doi:10.1001/archneur.1973.00490190019

Meldrum, B. S. (1973). Systemic Factors and Epileptic Brain Damage. Arch Neurol., 29(2), 82. doi:10.1001/archneur.1973.0049026

National Institute of Neurological Disorders and Stroke. (2015). "Epilepsy: Hope through Research". "Retrieved from" https://www.ninds.nih.gov/

Niquet. J., Baldwin, R. A., Allen, S. G., Fujikawa, D. G. and Wasterlain, C. G. (2003). Hypoxic neuronal necrosis: Protein synthesis-independent activation of a cell death program. Proc Natl Acad Sci U S A, 100 (5), 2825–2830. doi:10.1073/pnas.0530113100

Olajuyigbe, O. O. and Afolayan, A. J. (2011). Phytochemical Assesment and Antioxidant Activities of Alcoholic and Aqueous Extracts of Acacia mearnsii De Wild. Int J Mol , 7(8): 856-861

Oliveira, A. M. M., Hawk, J. D., Abel, T. and Havekes, R. (2010). Post-training reversible inactivation of the hippocampus enhances novel object recognition memory. Learn Mem.17: 160

Olsen, R. W. (1981). GABA-benzodiazepine-barbiturate receptor interactions. J Neurochem., 37(1): 1-13.

Onyenekwe, P. C., Okereke, O. E. and Owolewa, S. O. (2013). Phytochemical screening and effect of Musa paradisiaca stem extrude on rat haematological parameters. Curr Res J Biol Sci., 5 (1): 26-29.

Osim, E., Peter, E., Ofem, O. and Okon, U. (2017). Chronic Consumption of Plantain (Musa paradisiaca) Diet Increases Learning and Memory in Mice. Pharm Biol., 7(2): 64-68.

Pearl, P. L. (2009). New treatment paradigms in neonatal metabolic epilepsies. J Inherit Metab Dis 32(2): 204-213.

Pitkänen, A., Lukasiuk, K., Dudek, F. E. and Staley, K. J. (2015). Epileptogenesis. Cold Spring Harb. Perspect Med 5(10): 1-17.

Ramanjaneyulu, R. and Ticku, M. K. (1984). Interactions of pentamethylenetetrazole and tetrazole analogues with the picrotoxinin site of the benzodiazepine-GABA receptor-ionophore complex. Eur. J. Pharmacol., 98: 345.

Reddy, A. J., Dubey, A. K., Handu, S. S., Sharma, P., Mediratta, P. K., Ahmed, Q. M. and Jain, S. (2018). Anticonvulsant and Antioxidant Effects of Musa sapientum Stem Extract on Acute and Chronic Experimental Models of Epilepsy. Pharmacogn Res., 10(1): 49–54.

Serra, L., Bozzali, M., Fadda, L., De Simone, M. S., Bruschini, M., Perri, R., Caltagirone, C. and Carlesimo, G. A. (2020). The role of hippocampus in the retrieval of autobiographical memories in patients with amnestic Mild Cognitive Impairment due to Alzheimer's disease. J Neuropsychol., 14(1): 46-68.

Shibasaki, H., and Hallett, M. (2005). Electrophysiological studies of myoclonus. Muscle & Nerve., 31(2): 157–174. doi:10.1002/mus.20234

Shields, W. D. (2006). Infantile spasms: little seizures, BIG consequences. Epilepsy curr., 6(3):63-69.

Shin, E. J., Jeong, J. H., Chung, Y. H., Kim, W. K., Ko, K. H., Bach, J. H., Hong, J. S., Yoneda, Y. and Kim, H. C. (2011). Role of oxidative stress in epileptic seizures. Neurochem. Int; 59(2):122-137.

Singh, I. (2004). Introduction to Human neuroanatomy. (6th ed.). New Delhi: Jaypee Brothers Medical Publishers. pp. 25.

Stachowicz, K., Branski, P., Klak, K., Van der Putten, H. and Cryan, J. F. et al. (2008). Selective activation of metabotropic G-protein-coupled glutamate 7 receptor elicits anxiolytic-like effects in mice by modulating GABAergic neurotransmission. Behave. Pharmacol; 19(5-6): 597-603.

Sutula, T., Hagen, J. and Pitkanen, A. (2002). Do seizures damage the brain? (2002). Curr Opin Neurol., 16(2): 189-195.

Trinka, E., Cock, H., Hesdorffer, D., Rossetti, A. O., Scheffer, I. E., and Shinnar, S., Shorvon, S. And Lowenstein, D. H. (2015). A definition and classification of status epilepticus: report of the ILAE Task Force on Classification of Status Epilepticus. Epilepsia. 56(10):1515–1523. doi:10.1111/epi.13121

Udoh, B. E., Erim, A. E., Paulinus, S. O., Eru, E. M, Ukpong, E. V., Efanga, I., Archibong, B. E., Egong, A. E., Udo, E. E., Egbe, N. O. (2020). Effects of Cyperus esculentus (Tiger Nut) Extract on the Irradiated Testes of Wistar Rats. Trop J Nat Prod Res. 4(11):966-969.

University of Florida Herbarium, UFH, (2022). Preparation of Plant Specimens for Deposit as Herbarium vouchers. Retrieved July 12, 2022 from https://www.floridamuseum.ufl.edu/herbarium/methods/vouchers/

World Health Organization. (2001). Epilepsy: Aetiology, epidemiology and prognosis. Fact sheet No.165. Retrieved 15-06-2009.

World Health Organization. (2019)s. Epilepsy: Signs and symptoms, rates of disease, causes, treatment, prevention, social and economic impacts, human rights and WHO response. Fact sheet; pp. 51.