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Neuroprotective effects of *Tetrapleura tetraptera* fruit extract on hippocampal histological features in pentylenetetrazol-induced kindling in Wistar rats

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Abstract

Seizures are a hallmark of epilepsy and often lead to cognitive impairment. However, most antiepileptic medications have limited efficacy in this context and are associated with negative consequences with prolonged use. *Tetrapleura tetraptera*, an antioxidant-rich plant known for its anticonvulsant properties, prompted this study to examine its role in hippocampal protection after pentylenetetrazol (PTZ)-induced kindling. Phytochemical screening and median lethal dose assessments of the *T. tetraptera* fruit extract (TFE) were conducted. Forty-nine male Wistar rats (150 – 200 g, *n*=7 per group) were assigned as follows: Control, TFE (500 mg/kg), PTZ alone (40 mg/kg), and PTZ (40 mg/kg) following sodium valproate (200 mg/kg) or TFE at low (250 mg/kg), intermediate (500 mg/kg), and high (1,000 mg/kg) doses over 21 alternate days. PTZ was administered intraperitoneally, while all other treatments were given orally. On day 22, spontaneous alternation behavior (SAB) was assessed, followed by euthanasia and histological analyses. Phytochemical analysis of TFE identified phenols, alkaloids, and flavonoids as key constituents, and its oral median lethal dose was greater than 5,000 mg/kg. Pre-treatment with TFE showed suppressed seizures with quantal protection ($p<0.05$) compared to the PTZ group. While SAB was not significantly different, hippocampal Nissl expression was mildly affected, and neuron-specific enolase (NSE)-positive cells and glial fibrillary acidic protein (GFAP)-positive cells significantly decreased ($p<0.05$) compared with the control group. Therefore, oral administration of TFE is safe in Wistar rats with protection against PTZ-induced seizure and mortality. While short-term memory remained unaffected, the hippocampal Nissl substance, NSE, and GFAP were preserved, particularly in the low and intermediate TFE dose groups.

Keywords: Hippocampus; Pentylenetetrazol; *Tetrapleura tetraptera*; Sodium valproate; Immunohistochemistry; Nissl substance

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1. Introduction

Seizures are a major characteristic of epilepsy, occurring recurrently due to an imbalance in excitatory glutamate and inhibitory gamma-aminobutyric acid (GABA) neurotransmitters.[1,2] This disorder could be inherited or acquired from conditions,

such as hippocampus sclerosis, trauma, intracranial infections, and exposure to toxic agents, among others.[3,4] The incidence of epilepsy is reported as 50/100,000 in developed countries and twice as high in developing countries.[5,6] Anxiety, depression, and low self-esteem are often associated with this disorder.[7,8]

Epilepsy is more common in adults and frequently leads to cognitive impairment, particularly affecting the hippocampus, which is crucial for memory retention.[9,10] This memory function of the hippocampus can be studied through spontaneous alternation behavior in a T- or Y-maze, which helps identify impairments.[11] Cognitive impairment associated with epilepsy may have deleterious effects, and the majority of antiepileptic medications exhibit limited efficacy in this regard.[12,13] Individuals with epilepsy also face a higher risk of death than the general population.[14,15] Histological changes in the hippocampus are involved in epilepsy, with extensive pyramidal cell loss in the cornu ammonis 1 and 3 (CA1 and 3) regions.[9,10] Evaluation of hippocampal changes in epilepsy can include histological analysis of Nissl substance and the regulation of neuronal and astrocytic markers, such as neuron-specific enolase (NSE) and glial fibrillary acidic protein (GFAP), among other immunohistochemical or biochemical methods.[16-18]

Most antiepileptic drugs act by blocking sodium or calcium channels, reducing the release of excitatory glutamate and increasing inhibitory GABA levels.[19,20] However, GABA can also have pro-convulsive effects, either directly or indirectly.[19] Epilepsy treatment is often long-term, sometimes extending throughout a patient's life. Aside from this limitation, the use of these drugs is also associated with various adverse reactions.[21,22] Valproic acid, a carboxylic acid antiepileptic drug, or its sodium salt, sodium valproate, has a long history of efficacy in seizure management,[23-25] and serves as a standard antiepileptic drug control in this study.

Plants have been reported to offer medicinal and nutritive constituents. One such plant, *Tetrapleura tetraptera* Taub., belonging to the *Fabaceae* family,[26] is used in the management of various diseases and ailments in Nigeria and other African countries.[27-31] In addition, some local ethnic groups have reported using the plant for epilepsy management.[27,32]

T. tetraptera is known for several medicinal properties, including antimalarial, antibacterial, anti-inflammatory, antioxidant, hypoglycemic, central nervous system depressant, hypothermic, and antiepileptic effects in experimental animals.[31,33-42] The plant also exhibits anticonvulsant, analgesic, and hypothermic properties.[27,28] These properties make *T. tetraptera* a prospective treatment option for epilepsy and its associated neuronal deficits. Establishing the optimal dosage of *T. tetraptera* extract is crucial to maximize its efficacy in epilepsy protection while minimizing potential side effects. However, it is essential to assess the potential toxicity of the extract, which can be done through a median lethal dose test to prevent adverse outcomes upon usage.[43]

In experimental models, epilepsy is often induced in rodents using chemicals, such as pentylenetetrazol (PTZ), with the resulting seizure activity scored according to the Racine scale.[44,45] This research investigated the effects of *T. tetraptera* plant extract on PTZ-induced kindling and its potential neuroprotective effects on the hippocampus in a rat model.

2. Material and methods

2.1. Animal handling

Forty-nine male Wistar rats, 9 – 10 weeks old and weighing 150 – 200 g, were utilized in this study. The rats were obtained and housed in the animal facility of the Faculty of Basic Medical Sciences at the university. Each rat was cared for and handled following the guidelines for the use of laboratory animals set forth by the United States National Institute of Health.[46] This research was approved by The Faculty of Basic Medical Sciences Research and Ethical Committee (approval reference: UU_FBMSREC_2022_001).

The rats were maintained throughout the experiment at a room temperature of 25 – 28°C with a 12:12 h natural light/dark cycle. They were given *ad libitum* access to a standard pelletized rat diet (Grand Cereals, Nigeria) and drinking water. The rats were divided into seven groups (n=7), designated as control and test groups.

2.2. Preparation of *T. tetraptera* extract

Fresh, mature fruits of the *T. tetraptera* Taub., commonly known as Uyayak by the Ibibio ethnic group of Nigeria, were obtained from a local market in Calabar, Nigeria. A taxonomist identified the fruit, which was assigned specimen number UUPH/A32(f) and deposited at the Pharmacognosy Herbarium of the Faculty of Pharmacy, University of Uyo, Nigeria. The fruits were chopped into pieces after the seeds were removed. The fruits were first washed and air-dried before being pulverized into a smooth paste. A total of 1,342 g of the paste was macerated with 80% ethanol for 72 h, filtered through Whatman No. 1 filter paper. The resulting filtrate was concentrated at 45°C in a water bath and subsequently stored at 4°C until further use.

2.3. Preparation of PTZ and sodium valproate solutions

All drugs were prepared daily. PTZ (Sigma-Aldrich, Germany) was dissolved in distilled water, and 40 mg/kg

body weight was administered intraperitoneally to the rats.[45] Sodium valproate (Sanofi-Aventis, United Kingdom) tablets (200 mg) were dissolved in distilled water, and a dose of 200 mg/kg body weight was administered orally.

2.4. Phytochemical screening of *T. tetraptera* fruit

Qualitative and quantitative analyses of the phytochemicals present in the ground *T. tetraptera* fruits were conducted following standard methods.[47]

2.5. Determination of median lethal dose of *T. tetraptera*

The ethical approval for the lethal dose testing on mice was also granted by the Faculty of Basic Medical Sciences Research and Ethical Committee. To assess the toxicity of the *T. tetraptera* fruit extract, an oral median lethal dose was carried out on 25 CD-1 mice, following the Organization for Economic Cooperation guidance document. The up-and-down procedure was used to determine the median lethal dose.[43] Briefly, the 25 CD-1 mice were assigned to five groups (n = 5). After overnight fasting, the mice were administered the extract, with water provided ad libitum. Two hours after administration, the mice were allowed access to food.[43] In Group 1, *T. tetraptera* was administered at 1,000 mg/kg, and the mice were observed for signs of toxicity. After 48 h, with no deaths recorded, the procedure was repeated for the second through fifth groups at doses of 2,000, 3,000, 4,000, and 5,000 mg/kg, respectively. As no signs of toxicity or death were recorded during the experiment, the dosing procedure was discontinued, and the lethal dose was estimated.

2.6. Administration of *T. tetraptera*, sodium valproate, and PTZ

The *T. tetraptera* fruit extract (2 g) was dissolved in 20 mL distilled water. Calculated doses of 250 (5%), 500 (10%), and 1,000 (20%) mg/kg of the median lethal dosage (LD_{50}) of *T. tetraptera* fruit extract were administered. In addition, 200 mg/kg sodium valproate was administered orally, 1 h before the PTZ intraperitoneal injections, which were given on alternate days (every 48 h) until day 21. The administration schedule is shown in Table 1.

2.7. Kindling induction and determination of seizure scores

To induce kindling, 40 mg/kg PTZ was administered intraperitoneally every 48 h. Kindling was achieved on alternate day 21. Following the administration of PTZ, seizure scores, and mortality rates were observed and recorded for up to 30 min. The seizure stage and accompanying behaviors were noted using the following Racine scale: Stage 0 – No response; stage 1 – Ear and facial twitching; stage 2 – Myoclonic jerks; stage 3 – Myoclonic jerks with rearing; stage 4 – Forelimb clonus; stage 5 – Seizures with rearing, rolling over to the side, and generalized clonic-tonic seizures; stage 6 – Death. The rats were monitored for 24 h after the last administration to check for mortality. The percentage of quantal protection against seizures and death was calculated. Quantal protection was determined as the number of live rats per group divided by the total number of rats in the group.[45]

2.8. Spontaneous alternation behavior test

On day 22, a spontaneous alternation behavior test was conducted in a T-maze. Briefly, the rats were acclimated in the test room for one h before the test commenced. The T-shaped maze consisted of two short, goal arms (left and right) and one long, start arm. Gates were placed at the junctions of the arms and were opened when the rat was placed in the start arm. The rats were allowed to choose between entering either of the short arms.

Each animal underwent five successive trials, each lasting 60 s. When all four paws of the rat entered one of the short arms, the gate was closed, and an arm entry was recorded before the rat was returned to the holding cage. If a rat did not enter a goal arm within the allotted time, the trial was scored as blank, and the rat was returned to the holding cage. After each trial, the T-maze arena was cleaned with 70% ethanol. The frequency with which the rats alternated between the short arms and the total trial duration were recorded. The percentage alternation was then calculated.[11,48]

2.9. Termination of the experiment

Ketamine hydrochloride (Rotex Medica, Germany) was administered intraperitoneally as an anesthetic agent at a dose of 50 mg/kg. The thoracic cavity of each rat was opened, and a cannula was inserted into the left ventricle of the heart for perfusion fixation using 10% buffered formalin. The entire brain was removed and post-fixed in 10% buffered formalin for 48 h, followed by processing for paraffin wax sectioning for histological and immunohistochemical analysis.

2.10. Nissl substance and immunohistochemistry protocols

Briefly, the left dorsal hippocampal brain region of the rats was routinely dehydrated using a graded series of ethanol, cleared in xylene, and embedded in paraffin wax. Serial sections (10 μm thick) were mounted on slides and were rehydrated for both Nissl substance staining and immunohistochemistry. For the Nissl substance study, the sections were stained with 1% cresyl violet, supplemented with a few drops of acetic acid, for 10 min.

Table 1. Schedule of groupings and administration protocol

Groups (*n*=7)	Drugs	Dosages (mg/kg)	Duration (alternate days)
Group 1	Control, distilled water	5 mL/kg	11
Group 2	*Tetrapleura tetraptera*	500 mg/kg	11
Group 3	PTZ	40 mg/kg	11
Group 4	Sodium valproate pre-treatment then PTZ	200 mg/kg then 40 mg/kg	11
Group 5	Low-dose *Tetrapleura tetraptera* pre-treatment then PTZ	250 mg/kg then 40 mg/kg	11
Group 6	Intermediate-dose *Tetrapleura tetraptera* pre-treatment then PTZ	500 mg/kg then 40 mg/kg	11
Group 7	High-dose *Tetrapleura tetraptera* pre-treatment then PTZ	1,000 mg/kg then 40 mg/kg	11

Notes: Pentylenetetrazol was administered intraperitoneally, while *Tetrapleura tetraptera* fruit extract and sodium valproate were administered orally. All administrations were carried out every alternate day for 21 days (a total of 11 alternate days).
Abbreviation: PTZ: Pentylenetetrazol.

The excess stain was then washed off with distilled water. For the immunohistochemical study, antigen retrieval was performed in a citrate buffer solution (pH 6.0) in a microwave oven for 5 min, followed by protein blocking with 3% hydrogen peroxide for 10 min. Sections were then pre-incubated in 2% normal goat serum for 30 min and incubated with either a monoclonal mouse anti-NSE (22C9, 1:100, Leica Biosystems, United States) or monoclonal mouse anti-GFAP (NCL-L-GFAP-GA5, 1:100, Leica Biosystems, United States) for 2 h. Afterward, sections were incubated for 1 h with goat anti-mouse secondary antibody (1:100). The reaction was detected using the avidin-biotin complex with diaminobenzidine as the chromogen. Finally, sections were counterstained with Harris hematoxylin.

All stained sections were dehydrated in increasing concentrations of alcohol, cleared in xylene, and cover-slipped with dibutylphthalate polystyrene xylene. Processed slides were examined under a light microscope, and photomicrographs were captured using a computer-assisted digital microscope camera.

2.11. Hippocampus cell counts

The populations of Nissl substance-stained Cresyl violet and NSE- and GFAP-immunopositive cells in the hippocampus were quantified manually using ImageJ® software (United States of America). Images of the three layers of the hippocampal cornu ammonis 3 (CA3) regions were acquired from three brain sections per animal, with three animals per group. Each image was mapped randomly using the ImageJ® gridlines as a guide. Manually counting was conducted using a tool to select Nissl, NSE, or GFAP-stained cells located on the upper and right borders of each mapped area.

2.12. Statistical analyses

One-way and repeated measures analyses of variance were employed to compare the calculated means and percentages. Subsequently, Tukey's or Bonferroni's *post-hoc* tests were performed using GraphPad Prism software (version 5.0; United States of America) to determine statistical significance at $p \leq 0.05$. All results are presented as mean ± standard error of the mean or standard deviation.

3. Results

3.1. Phytochemical analysis of *T. tetraptera*

Qualitative screening of *T. tetraptera* fruit extract revealed the presence of phenols, flavonoids, alkaloids, saponins, tannins, terpenoids, steroids, phytate, anthraquinones, oxalates, cardiac glycosides, and hydrogen cyanide. The quantitative analysis of *T. tetraptera* fruit extract showed that phenols (7.80 g/100 g) had the highest concentration, followed by alkaloids (6.20 g/100 g), flavonoids (5.30 g/100 g), saponins (3.40 g/100 g), tannins (1.20 g/100 g), and phytate (0.40 g/100 g). Oxalates, cardiac glycosides, and hydrogen cyanide were present in trace amounts.

3.2. Oral median lethal dose

Mice that received oral administration of *T. tetraptera* fruit extract at doses ranging from 1,000 mg/kg to 5,000 mg/kg showed no signs of death or toxicity. Hence, the median lethal dose of *T. tetraptera* ethanol fruit extract was determined to be greater than 5,000 mg/kg.

3.3. Antiseizure activity of *T. tetraptera*

Repeated administration of PTZ at a sub-convulsive dose of 40 mg/kg for 11 alternate days resulted in a steady increase in seizure scores, leading to generalized clonic-

tonic seizures. A repeated measure one-way analysis of variance revealed a significant difference (F = 20.78, p<0.001). Bonferroni's *post-hoc* test further indicated that the seizure scores increased significantly from days 1 to 11 (alternate days) (Table 2).

A one-way analysis of the variance of the seizure scores between groups on an alternate day 11 revealed a significant difference (F = 8.52, p<0.001). The seizure scores in the PTZ group were significantly higher (p<0.05) compared to the group pre-treated with 250 mg/kg and 500 mg/kg of *T. tetraptera*. However, no significant difference was observed between the 1,000 mg/kg *T. tetraptera* and sodium valproate groups. In addition, the group pre-treated with sodium valproate exhibited significantly higher (p<0.05) seizure scores compared to the 500 mg/kg *T. tetraptera* group (Table 3).

3.4. Quantal protection action of *T. tetraptera*

The percentage quantal protection for the groups pre-treated with sodium valproate (85.71%), low-dose *T. tetraptera* (71.42%), or intermediate-dose *T. tetraptera* (85.71%) was higher compared to the PTZ group (57.14%). However, there was no significant difference between the PTZ group and the high-dose *T. tetraptera*-pre-treated group (57.14%) (Table 4).

Consistently, the mortality rates in the groups pre-treated with sodium valproate (14.29%), low-dose *T. tetraptera* (28.58%), and intermediate-dose *T. tetraptera* (42.86%) were lower than those in the PTZ group (57.14%), which did not differ from the high-dose *T. tetraptera*-pretreated group (42.86%) (Table 4).

3.5. Spontaneous alternation behavior

A repeated measures analysis of variance and Tukey's *post-hoc* tests revealed no significant difference (p = 0.1475, F = 1.751) in spontaneous alternation behavior between the test groups: *T. tetraptera* alone, PTZ alone, pre-treated sodium valproate, and pre-treated *T. tetraptera* at low, intermediate, and high doses (Figure 1).

3.6. Cresyl violet-stained cells

In the control group, the CA3 region of the hippocampus exhibited distinct and intensely stained Nissl substance across all three layers: molecular, pyramidal, and polymorphic (Figure 2A). Similarly, the CA3 region of the hippocampus in the *T. tetraptera* fruit extract group showed distinct and intense Nissl staining in these layers, resembling the staining pattern of the control group (Figure 2B).

The PTZ group exhibited less prominent neuronal nuclei and a slightly weaker Nissl substance staining intensity in the CA3 layers of the hippocampus compared

Table 2. Daily seizure scores of the experimental groups after pentylenetetrazol administration

Alternate days	1 (n=35)	2 (n=35)	3 (n=35)	4 (n=35)	5 (n=35)	6 (n=35)	7 (n=35)	8 (n=35)	9 (n=35)	10 (n=35)	11 (n=35)
Seizure scores	0.0±0.0	1.03±1.98	1.37±2.00*	2.49±2.24***	0.5±0.78*	2.03±2.38***	2.83±2.20***	3.20±2.44***	3.31±2.75***	3.29±2.80***	3.14±2.79***

Notes: Repeated measures analysis of variance and Bonferroni's *post-hoc* tests were performed. Values are expressed as mean±standard deviation. F=20.78, p<0.001. *Indicates a significant difference from day 1 at P<0.05. ***Represents a significant difference from day 1 at p<0.001.

Table 3. Seizure score differences on alternate day 11 between experimental groups after pentylenetetrazol administration

Groups	PTZ (*n*=7)	PTZ+SV (*n*=7)	PTZ+TT (LD) (*n*=7)	PTZ+TT (ID) (*n*=7)	PTZ+TT (HD) (*n*=7)
Seizure scores	4.29±1.60	3.50±1.76	1.20±0.84**	1.00±0.63***[b]	3.25±0.96

Notes: One-way analysis of variance and Bonferroni's *post-hoc* tests were performed. Values are expressed as mean±standard deviation. F=8.52, p<0.001. **Indicates a significant difference from the PTZ group at p<0.01. ***Represents a significant difference from the PTZ group at p<0.001. [b]Significantly different from the PTZ+SV group at p<0.05.
Abbreviations: HD: High dose; ID: Intermediate dose; LD: Low dose; PTZ: Pentylenetetrazol; SV: Sodium valproate; TT: *Tetrapleura tetraptera*.

Table 4. Quantal protection and mortality rate of the experimental groups after pentylenetetrazol administration

Groups	Quantal protection	Percentage protection (%)	Mortality rate	Percentage mortality (%)
PTZ (*n*=7)	4/7	57.14	3/7	42.86
PTZ+SV (*n*=7)	6/7	85.71	1/7	14.29
PTZ+TT (LD) (*n*=7)	5/7	71.42	2/7	28.58
PTZ+TT (ID) (*n*=7)	6/7	85.71	1/7	14.29
PTZ+TT (HD) (*n*=7)	4/7	57.14	3/7	42.86

Abbreviations: HD: High dose; ID: Intermediate dose; LD: Low dose; PTZ: Pentylenetetrazol; SV: Sodium valproate; TT: *Tetrapleura tetraptera*.

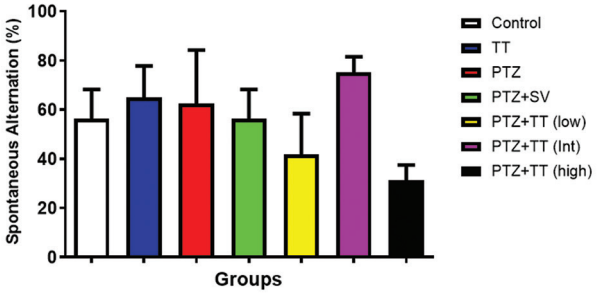


Figure 1. Spontaneous alternation behavior test. A repeated measure analysis of variance and Tukey's *post-hoc* tests. No significant differences were observed among the groups (F=1.751; p=0.1475). Notes: Values are expressed as mean ± standard error of the mean. Sample size per group: Control=5; TT=5; PTZ=4; PTZ+SV=4; PTZ+TT (low)= 4; PTZ+TT (int)=5; PTZ +TT (high)=4.
Abbreviations: PTZ: Pentylenetetrazol; SV: Sodium valproate; TT: *Tetrapleura tetraptera*.

to the control group (Figure 2C). The hippocampal CA3 layers in the groups pre-treated with sodium valproate or high-dose *T. tetraptera* showed no apparent Nissl substance staining intensity compared to the control group (Figure 2D and G). Similarly, the CA3 region in the low-dose *T. tetraptera*-pretreated group showed no apparent difference in Nissl substance staining intensity compared to the control group (Figure 2E). The CA3 region of the intermediate-dose *T. tetraptera*-pretreated group also showed no apparent difference in Nissl substance staining intensity compared to the control group (Figure 2F).

A repeated measure analysis of variance and a Tukey's *post-hoc* test revealed no significant difference (p>0.05) in the number of cresyl violet-stained pyramidal cells between the test groups and the control group, or among the test groups. However, the number of other cresyl violet-stained cell types in the sodium valproate and intermediate-dose *T. tetraptera*-pretreated groups was significantly lower (p<0.05) compared to the control group (Figure 3).

3.7. Neuron-specific enolase immunoreactivity

Expression of NSE was observed in the hippocampal CA3 region of the control group (Figure 4A). The hippocampal CA3 regions of the *T. tetraptera*-alone and the low-dose *T. tetraptera*-pretreated groups showed increased NSE expression intensity throughout the layers compared to the control group (Figure 4B and E). The CA3 region of the PTZ group also exhibited increased NSE expression intensity throughout the layers compared to the control group (Figure 4C). In contrast, the CA3 regions of the sodium valproate and the intermediate- and high-dose *T. tetraptera*-pretreated groups showed reduced NSE expression intensity in some neurons of the layers compared to the control group (Figure 4D, 4F, and 4G).

A repeated measures analysis of variance and *post-hoc* tests revealed that the number of NSE-positive pyramidal cells was significantly higher (p<0.05) in the *T. tetraptera* group, but significantly lower (p<0.05) in the PTZ group and the intermediate- and high-dose *T. tetraptera*-pre-treatment groups compared to the control group. There were significantly fewer (p<0.05) NSE-positive pyramidal cells in the PTZ group compared to the *T. tetraptera*-alone and sodium valproate-pretreated groups, with no significant differences (p>0.05) when compared to the low, intermediate, and high-dose *T. tetraptera* groups (Figure 5).

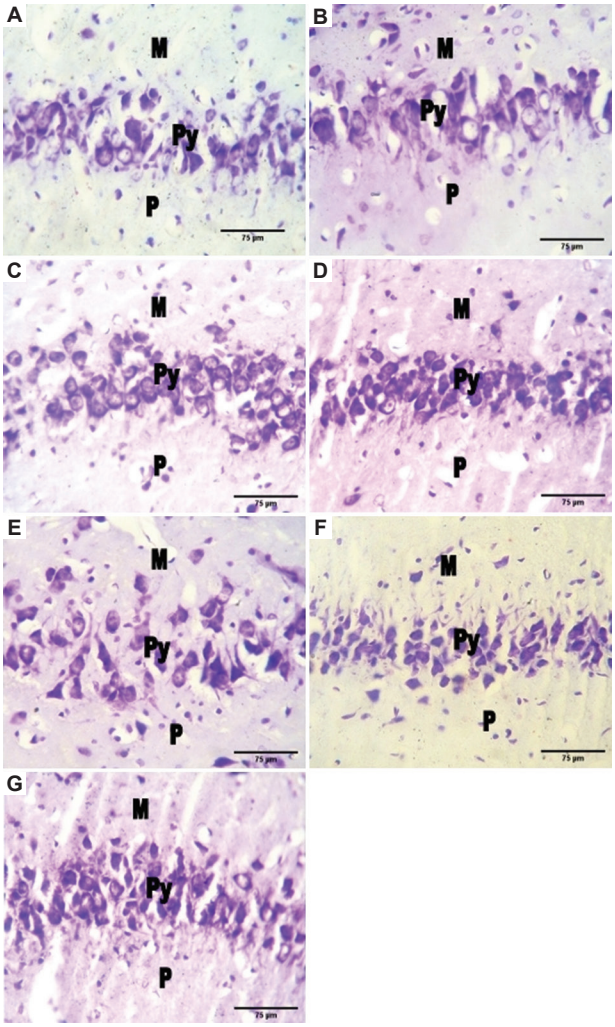


Figure 2. Sections of the hippocampal cornu ammonis 3 region stained with cresyl fast violet (scale bars: 75 µm; magnification power: ×400). (A) Control group showing deeply stained Nissl substance. (B) *Tetrapleura tetraptera* group with strongly stained Nissl substance. (C) Pentylenetetrazol group showing the reduced staining intensity of Nissl substance. (D) Sodium valproate pre-treatment group with moderately stained Nissl substance. (E) Low-dose *T. tetraptera*-pretreatment group showing less intense Nissl substance staining. (F) Intermediate-dose *T. tetraptera*-pretreatment group with intensely stained Nissl substance. (G) High-dose *T. tetraptera*-pretreatment group showing moderately stained Nissl substance.
Abbreviations: M: Molecular; P: Polymorphic layers; Py: Pyramidal layer.

Other NSE-positive cell types were significantly fewer (p<0.05) in the test groups compared to the control group. However, the intermediate-dose *T. tetraptera* group had significantly (p<0.05) more NSE-positive cells than the PTZ group (Figure 5).

3.8. Glial fibrillary acidic protein immunoreactivity

Glial fibrillary acidic protein was expressed in the hippocampal CA3 regions of the control and *T. tetraptera* groups (Figure 6A and B). The PTZ group exhibited slightly reduced GFAP expression in the hippocampal CA3 region compared to the control group (Figure 6C). In contrast, the hippocampal CA3 regions of the groups pre-treated with sodium valproate, as well as the low, intermediate, and high doses of *T. tetraptera*, showed less GFAP expression compared to the control group (Figure 6D-G).

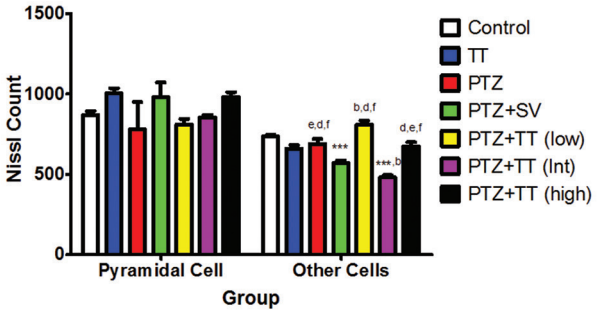


Figure 3. Nissl-stained cell count of the cornu ammonis 3 region of the hippocampus. A repeated measures analysis of variance and *post-hoc* tests were performed. Values are expressed as mean±standard error of the mean. Notes: ***Indicates significant difference from the control group at p<0.001. [b,d,e,f]Significantly different from TT, PTZ+SV, PTZ+TT (low), and PTZ+TT (int), respectively, at p<0.05. Sample size per group: Control=3; TT=3; PTZ=3; PTZ+SV=3; PTZ+TT (low)=3; PTZ+TT (int)=3; PTZ+TT (high)=3.
Abbreviations: PTZ: pentylenetetrazol; SV: Sodium valproate; TT: *Tetrapleura tetraptera*.

A repeated measures analysis of variance and *post-hoc* tests revealed that the number of GFAP-labeled cells was significantly lower (p<0.05) in the *T. tetraptera*-alone group, as well as in the sodium valproate, intermediate-dose, and high-dose *T. tetraptera*-pretreatment groups, compared to the control group. However, there was no significant difference (p>0.05) in the GFAP-labeled cells between the PTZ group and the low-dose *T. tetraptera*-pretreated group, in contrast to the control group. The number of GFAP-labeled cells was significantly higher (p<0.05) in the PTZ group compared to the sodium valproate and the intermediate- and high-dose *T. tetraptera*-pretreated groups (Figure 7).

4. Discussion

The study investigated the effects of *T. tetraptera* fruit extract on spontaneous alternation behavior, Nissl substance staining, and NSE and GFAP immunoreactivity in PTZ-induced epileptic Wistar rats. Phytochemical screening of *T. tetraptera* fruit extract revealed high concentrations of phenols, alkaloids, saponins, tannins, flavonoids, terpenoids, and steroids, while cardiac glycosides and anthraquinones showed weak positive reactions. Plants, due to the presence of these phytochemicals, play an active role in medicine and

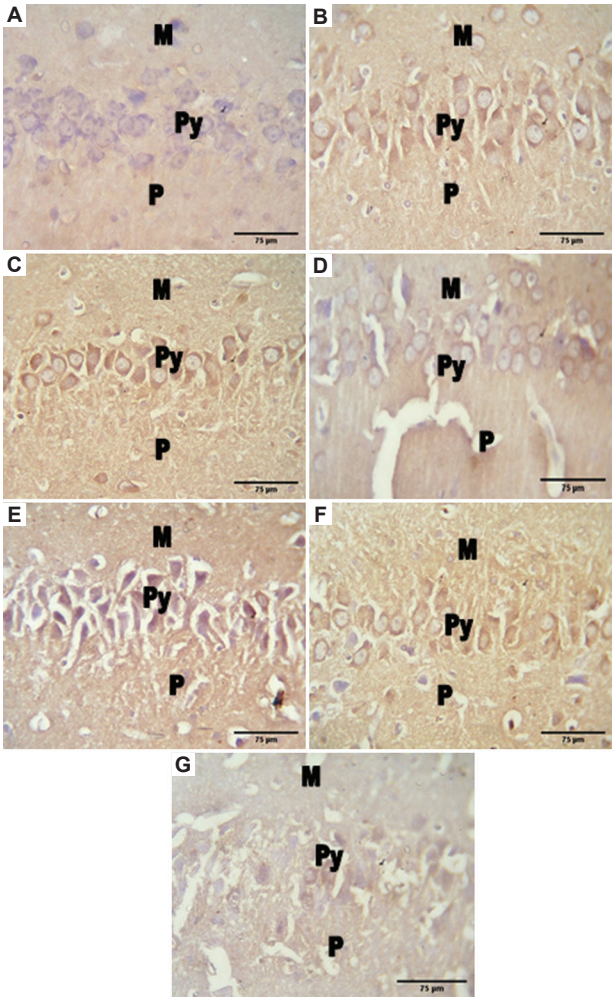


Figure 4. Sections of the hippocampal cornu ammonis 3 with neuron-specific enolase (NSE)-positive cells (brown staining; scale bars: 75 µm; magnification power: ×400). (A) Control group with NSE-positive cells. (B) *Tetrapleura tetraptera* group showing increased intensity of NSE expression. (C) Pentylenetetrazol group showing increased intensity of NSE expression. (D) Sodium valproate-pretreated group showing reduced intensity of NSE expression. (E) Low-dose *T. tetraptera*-pretreatment group showing slightly increased NSE expression. (F and G) Intermediate- and high-dose *T. tetraptera*-pretreatment groups showing reduced NSE expression intensity.
Abbreviations: M: Molecular; P: Polymorphic layers; Py: Pyramidal layer.

nutrition, offering antioxidant properties that help protect cells from oxidative stress, among other benefits.[49-51] The combination of these phytochemicals contributes to the diverse range of beneficial physiological activities associated with *T. tetraptera*.[28,31,52] The present results corroborate previous findings[52,53] that reported the presence of similar phytochemical profiles in both the aqueous and ethanol extracts of *T. tetraptera*.

The safety of *T. tetraptera* was assessed through acute toxicity testing. The result indicated an oral LD_{50} of

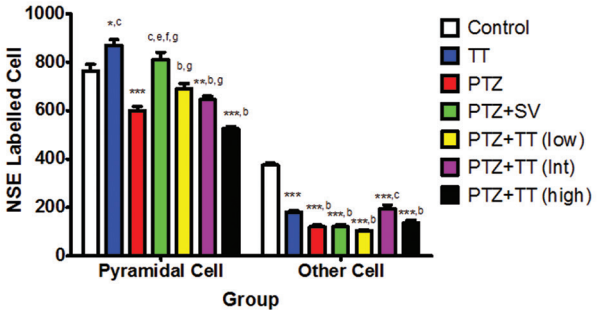


Figure 5. Neuron-specific enolase-positive cell count in the hippocampal cornu ammonis 3 region of the experimental groups. A repeated measures analysis of variance and Tukey's *post-hoc* tests were performed. Values are expressed as mean±standard error of the mean. Notes: Sample size of per group: Control=3; TT=3; PTZ=3; PTZ+SV=3; PTZ+TT (low)=3; PTZ+TT (int)=3; PTZ+TT (high)=3. *,**, and ***Indicate significant differences from the control at $p<0.05$, $p<0.01$, and $p<0.001$, respectively. [b,c,e,f,g]Significantly different from TT, PTZ, PTZ+TT (low), PTZ+TT (int), and PTZ+TT (high), respectively, at $p<0.05$.
Abbreviations: PTZ: pentylenetetrazol; SV: Sodium valproate; TT: *Tetrapleura tetraptera*.

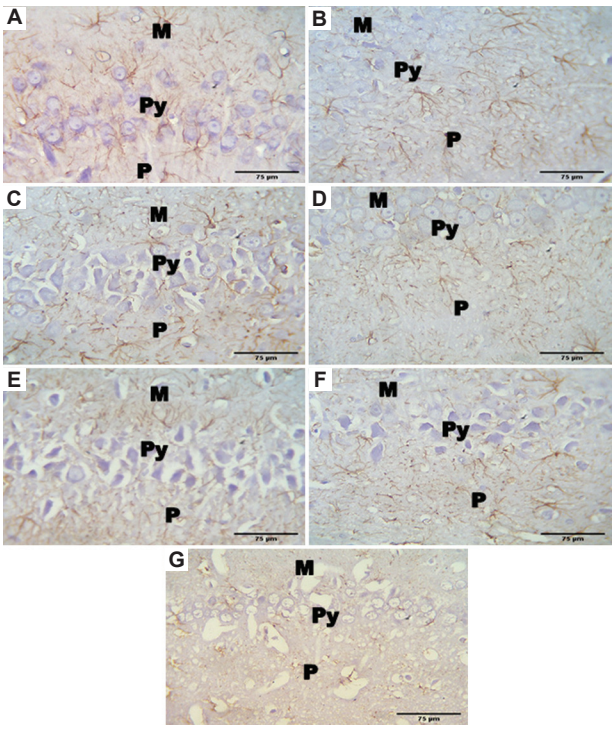


Figure 6. Sections of the hippocampal cornu ammonis 3 showing glial fibrillary acidic protein (GFAP)-positive cells (brown staining; scale bar: 75 µm; magnification power: ×400). (**A** and B) Control and *Tetrapleura tetraptera* groups showing expression of GFAP. (C) Pentylenetetrazol group showing decreased expression of GFAP. (D and E) Sodium valproate and low-dose *T. tetraptera*-pretreated groups showing decreased expression of GFAP. (F and G) Intermediate- and high-dose *T. tetraptera*-pretreated groups showing decreased expression of GFAP.
Abbreviations: M: Molecular; P: Polymorphic layers; Py: Pyramidal layer.

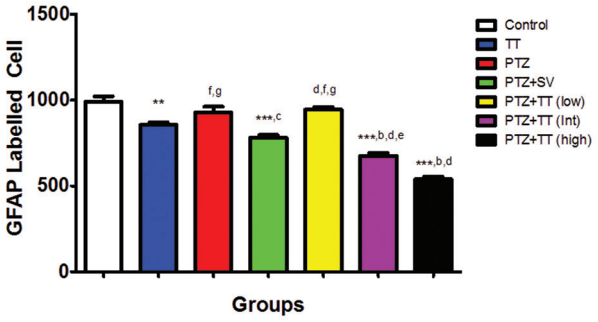


Figure 7. Glial fibrillary acidic protein (GFAP)-labeled cell count of the hippocampal cornu ammonis 3 region of the experimental groups. A repeated measures analysis of variance and Tukey's *post-hoc* tests were performed. Values are expressed as mean±standard error of mean. Notes: Sample size per group: Control=3; TT=3; PTZ=3; PTZ+SV=3; PTZ+TT (low)=3; PTZ+TT (int)=3; PTZ+TT (high)=3. ** and ***indicate significant differences from the control at $p<0.01$ and $p<0.001$, respectively. [b,c,d,e,f]Significantly different from TT, PTZ, PTZ+SV, PTZ+TT (low), PTZ+TT (int), and PTZ+TT (high), respectively, at $p<0.05$.
Abbreviations: PTZ: pentylenetetrazol; SV: Sodium valproate; TT: *Tetrapleura tetraptera.*

over 5,000 mg/kg in mice, supporting the safety of the extract, as this concentration is typically the end-point of LD_{50} determination.[54] These findings align with those of a previous study,[55] which reported an LD_{50} of over 10,000 mg/kg body weight in a mouse model.

Kindling is a recognized experimental model for human epilepsy, induced through sub-convulsive doses of chemicals or electrical stimulation.[56,57] PTZ, a chemical convulsant, exerts its effects by inhibiting GABA activity at the $GABA_A$ receptor, thus mimicking epilepsy.[58] In the present study, PTZ administration resulted in increasing seizure scores and kindling, which progressively intensified with each administration. Increased seizure scores, as assessed using the Racine scale, denote the worsening seizure severity, consistent with previous reports.[45,57] Sodium valproate, a standard antiepileptic drug, failed to suppress seizures in this study, as the seizure scores in this group did not differ significantly from those in the PTZ group. This result contrasts with previous studies that reported antiseizure effects of sodium valproate at doses of 200 and 300 mg/kg,[44] which were also used in the present study. While sodium valproate is known to attenuate PTZ-induced seizures,[59] this contrasting result may be due to a spike in glycine levels, as sodium valproate also interferes with glycine synthesis, potentially leading to increased seizure frequency.[60]

T. tetraptera attenuated PTZ-induced kindling, with significant reductions ($p<0.05$) in seizure scores observed in the low-, intermediate-, and high-dose *T. tetraptera*-pretreated groups compared to the PTZ group. These findings suggest that the administered doses of *T. tetraptera* possess anticonvulsant activity, potentially reducing the severity of PTZ-induced seizures, a result consistent with a previous report.[36] Although the exact mechanism underlying *T. tetraptera*'s anticonvulsant effect remains to be elucidated, it is likely mediated through GABAergic transmission. This hypothesis is supported by a prior study indicating that alkaloids and flavonoids,[61] which are also constituents of *T. tetraptera*, enhance GABA transmission.

Pentylenetetrazol administration resulted in mortality, with the PTZ group exhibiting a lower percentage of quantal protection, suggesting toxicity, as previously reported.[62] The groups pre-treated with sodium valproate, intermediate doses of *T. tetraptera*, and intermediate doses of *T. tetraptera* demonstrated greater protection and lower mortality compared to the PTZ group, supporting previous findings.[36] Sodium valproate, an established antiepileptic drug (54), exhibited expected antiseizure activity, whereas *T. tetraptera's* effect may be attributed to its rich phytochemical constituents, which have been shown to be safe in the present study. Notably, the high-dose *T. tetraptera*-pretreatment group showed no significant difference in quantal protection or mortality when compared to the PTZ group, suggesting that high doses of *T. tetraptera* may be unnecessary or potentially harmful, either exacerbating or having no effect on PTZ toxicity.

The spontaneous alternation behavior test is commonly used to assess short-term memory and identify memory impairments associated with various disease conditions.[11,48] The present results revealed no significant difference ($p>0.05$) between the test and control groups, suggesting that the treatment regimens did not influence spontaneous alternation. This lack of effect may be attributed to the treatment's inadequacy in influencing spatial brain functions. Given that spontaneous alternation is related to learning and memory,[48] this study found no impairment in these functions. Chemical kindling models, including those using PTZ, have previously reported cognitive, learning, and memory impairments.[63] The discrepancy between these findings and the present study could be due to the animal model used. Furthermore, the current results contrast with another study in which PTZ kindling resulted in learning and memory deficits in mice,[57] which may be attributed to the differences in behavioral paradigms tested.

Nissl substance, present in all neurons, is a rough endoplasmic reticulum component essential for protein synthesis[64] and frequently disintegrates during chromatolysis.[16] PTZ also affects this macromolecule.[9,56] In the current study, the CA3 hippocampal region of the

PTZ group demonstrated disintegrated and dispersed Nissl substance, which appeared slightly less stained, indicating PTZ toxicity. This observation aligns with a previous report.[56] The cellular population in the Nissl-stained pyramidal and other cell types was not significantly different in the PTZ group compared to the control, suggesting that the cellular population was not adversely affected, and no immediate loss of protein synthesis function occurred.

On the other hand, the *T. tetraptera*-alone group demonstrated high Nissl stain intensity in most pyramidal neurons, while no apparent difference was observed in the sodium valproate, pre-treated group or in the low-, intermediate-, and high-dose *T. tetraptera* groups. These observations indicate that Nissl substance distribution was not adversely affected. A previous report suggests that sodium valproate provides neuroprotection,[65] which may explain the current results in this drug-pre-treated group. The findings in the *T. tetraptera*-pre-treatment groups may be attributed to its rich phytochemical composition, which is known to offer neuroprotective benefits.[66,67] The cellular population in the Nissl-stained pyramidal and other cell types was not significantly different in these test groups compared to the control, further indicating that the cellular populations remained unaffected.

Neurons can also be identified immunohistochemically by their expression of, or immunoreactivity to, anti-NSE. This anti-NSE binds to NSE, a cytoplasmic glycolytic enzyme, and its reactivity reflects the metabolic state of the neurons. Increased NSE expression suggests high metabolic activity.[17] In the present study, there was increased intensity of NSE expression in the hippocampus of the PTZ group, with the population of NSE-positive cells significantly lower compared to the control. This observation indicates increased neuronal activity, potentially accompanied by injury to the hippocampal neurons. In response to injury, NSE expression and activity are markedly upregulated,[68] and neurotoxic agents have been associated with increased NSE expression.[69] Increased NSE levels may result from dysfunction in neuronal metabolic activity and synaptic connections, which may have been induced by PTZ.

Decreased intensity of NSE expression was observed in the hippocampus of the sodium valproate, intermediate, and high-dose *T. tetraptera*-pretreated groups compared to the control group. Although the number of NSE-positive pyramidal cells was not significantly different in the sodium valproate pre-treated group, it was significantly lower in the intermediate- and high-dose *T. tetraptera*-pretreated groups compared to the control. These results indicate an increase in neuronal metabolic activity in these groups, which may not be harmful. Sodium valproate, known for its neuroprotective properties,[65] may explain the intensity of NSE expression observed in this group. *T. tetraptera* has also been reported to have neuroprotective effects,[36] likely due to its rich phytochemicals, as demonstrated in the present study. *T. tetraptera* may exert its effect by influencing ionotropic GABA receptors and inhibiting acetylcholinesterase, thereby increasing GABA levels while antagonizing N-methyl-D-aspartate.[66,67,70] This neuroprotective effect of *T. tetraptera* corroborates findings from another study,[71] which reported similar effects of *Moringa oleifera*, a plant containing comparable phytochemicals, on this glycolytic enzyme.

In the low-dose *T. tetraptera* group, there was increased intensity of NSE expression, although the number of NSE-positive pyramidal cells did not significantly differ from the control. This observation suggests increased neuronal activity in this group. Given that the NSE-positive cell population was not significantly altered, it may indicate a non-adverse effect of the *T. tetraptera* pre-treatment.

Astrocytes are glial cells found in the hippocampus, as well as in other regions of the brain. They can be identified immunohistochemically through the expression of GFAP, an intermediate filament protein that provides mechanical strength to astrocytes.[18] Increased GFAP expression indicates astrocyte activation. In the present study, the PTZ group showed a slight decrease in GFAP expression in the CA3 region of the hippocampus compared to the control, which may indicate neurotoxicity, although the difference in population was not statistically significant. Following injury and astrogliosis, GFAP is typically expressed at much higher levels,[72] which contrasts with the findings in the present study. Decreased GFAP expression is also commonly reported in neurodegenerative or other detrimental conditions,[73] which may have been the case in the present study.

The CA3 regions of the hippocampus in the *T. tetraptera* group showed a similar GFAP expression to the control, although with a significantly smaller population. This observation suggests little to no adverse effect from the plant, as previously reported in the study. The CA3 regions of the hippocampus in the sodium valproate, and low, intermediate, and high doses of *T. tetraptera*-pretreatment groups exhibited decreased GFAP expression, with a significantly smaller ($p<0.05$) population. These findings may indicate a protective effect, as these pre-treated substances are known to offer neuroprotection. In this context, decreased GFAP expression could be due to either cytoskeletal destabilization or a loss of GFAP antigenicity.[74]

The hippocampus, particularly the CA3 region, plays a key role in cognitive processes such as learning and memory and is highly susceptible to trauma. Any alteration in the

normal regulation of its cellular and structural components can adversely affect or alter its functions,[10] as observed with the PTZ treatment. In contrast, the functions and structure of the hippocampus appear to be protected with *T. tetraptera* pre-treatment in the present study, with the mechanism of action likely involving the elevation of the GABAergic pathway.

In summary, PTZ-induced kindling in rats, possibly through GABAergic inhibition and glutamatergic stimulation, led to mortality from excitotoxicity, Nissl substance dispersal, NSE upregulation, and GFAP downregulation in the hippocampal CA3. Pre-treatment with *T. tetraptera* doses may have modulated both the GABAergic and glutamatergic pathways to suppress kindling, resulting in high quantal protection and minimal effects on Nissl substance, NSE, and GFAP.

5. Conclusion

This study demonstrated that PTZ-induced seizures may result in rat mortality, along with histological and immunohistochemical alterations in the hippocampal CA3 region. Oral administration of *T. tetraptera* fruit extract, a safe and phytochemical-rich plant, provided protection against PTZ-induced seizures and mortality in Wistar rats. In addition, the extract preserved hippocampal Nissl substance, NSE, and GFAP expression. The low (250 mg/kg) and intermediate (500 mg/kg) doses of *T. tetraptera* fruit extract offered the greatest protection, even in comparison with sodium valproate. While further research is needed to clarify its mechanism of action, this plant extract may hold promise for seizure management.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Clementina F. Iniodu, Moses B. Ekong
Formal analysis: Moses B. Ekong
Investigation: Clementina F. Iniodu
Methodology: Clementina F. Iniodu, Moses B. Ekong
Supervision: Gabriel J. Ekandem, Moses B. Ekong
Writing – original draft: Clementina F. Iniodu, Moses B. Ekong
Writing – review & editing: All authors

Ethics approval and consent to participate

This study involved the use of animals. Ethical approval for the research was obtained from the Faculty of Basic Medical Sciences Research and Ethical Committee, with approval number: UU_FBMSREC_2022_001.

Consent for publication

Not applicable.

Availability of data

Not applicable.

Further disclosure

Part of this set of findings has been presented in a conference and has been published as an abstract.

References

1. Sumadewi KT, Harkitasari S, Tjandra DC. Biomolecular mechanisms of epileptic seizures and epilepsy: A review. *Acta Epileptologica*. 2023;5(1):28.

   doi: 10.1186/s42494-023-00137-0

2. WHO. *Epilepsy*. World Health Organization; 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/epilepsy [Last accessed on 2023 Dec 06].

3. Balestrini S, Arzimanoglou A, Blümcke I, *et al*. The aetiologies of epilepsy. *Epileptic Disord*. 2021;23(1):1-16.

   doi: 10.1684/epd.2021.1255

4. Deme S. A study of correlation of CT scan brain and EEG in epilepsy. *IAIM*. 2016;3(10):55-61.

5. Beghi E, Giussani G, Nichols E, *et al*. Global, regional, and national burden of epilepsy, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol*. 2019;18(4):357-375.

   doi: 10.1016/S1474-4422(18)30454-X

6. Camfield P, Camfield C. Incidence, prevalence and aetiology of seizures and epilepsy in children. *Epileptic Disord*. 2015;17(2):117-123.

   doi: 10.1684/epd.2015.0736

7. Alkoblan FI, Alsoadan MM, Alhajri AA, *et al*. Social anxiety, social support, and quality of life in patients with epilepsy at a tertiary care hospital in Saudi Arabia. *Cureus*. 2023;15:e45447.

   doi: 10.7759/cureus.45447

8. Temple J, Fisher P, Davies C, Millar C, Gemma Cherry M. Psychosocial factors associated with anxiety and depression in adolescents with epilepsy: A systematic review. *Epilepsy Behav*. 2023;149:109522.

   doi: 10.1016/j.yebeh.2023.109522

9. Ekong MB, Bassey OO, Ebeh DI, *et al*. *Rauvolfia vomitoria* phenol extract relieves pentylenetetrazol-induced seizures in Swiss mice and protects some temporal lobe structures. *Acta Epileptol*. 2024;6(1):35.

   doi: 10.1186/s42494-024-00183-2

10. Sendrowski K, Sobaniec W. Hippocampus, hippocampal sclerosis and epilepsy. *Pharmacol Rep.* 2013;65(3):555-565.

    doi: 10.1016/S1734-1140(13)71033-8

11. D'Isa R, Comi G, Leocani L. Apparatus design and behavioural testing protocol for the evaluation of spatial working memory in mice through the spontaneous alternation T-maze. *Sci Rep.* 2021;11(1):21177.

    doi: 10.1038/s41598-021-00402-7

12. Novak A, Vizjak K, Rakusa M. Cognitive impairment in people with epilepsy. *J Clin Med*. 2022;11(1):267.

    doi: 10.3390/jcm11010267

13. Sayed NM, Aldin MTK, Ali SE, Hendi AE. Cognitive functions and epilepsy-related characteristics in patients with generalized tonic-clonic epilepsy: A cross-sectional study. *Middle East Curr Psychiatry*. 2023;30(1):15.

    doi: 10.1186/s43045-023-00293-6

14. Devinsky O, Spruill T, Thurman D, Friedman D. Recognizing and preventing epilepsy-related mortality: A call for action. *Neurology*. 2016;86(8):779-786.

    doi: 10.1212/WNL.0000000000002253

15. Trinka E, Rainer LJ, Granbichler CA, Zimmermann G, Leitinger M. Mortality, and life expectancy in Epilepsy and Status epilepticus-current trends and future aspects. *Front Epidemiol*. 2023;3:1081757.

    doi: 10.3389/fepid.2023.1081757

16. Moon LDF. Chromatolysis: Do injured axons regenerate poorly when ribonucleases attack rough endoplasmic reticulum, ribosomes and RNA? *Dev Neurobiol*. 2018;78(10):1011-1024.

    doi: 10.1002/dneu.22625

17. Rahmy TR, Hassouna IA. Immunohistochemical investigation of neuronal injury in cerebral cortex of cobra-envenomed rats. *J Venom Anim Toxins Incl Trop Dis*. 2004;10(1):53-76.

    doi: 10.1590/S1678-91992004000100005

18. Brenner M. Role of GFAP in CNS injuries. *Neurosci Lett*. 2014;565:7-13.

    doi: 10.1016/j.neulet.2014.01.055

19. Perucca E, Bialer M, White HS. New GABA-targeting therapies for the treatment of seizures and epilepsy: I. Role of GABA as a modulator of seizure activity and recently approved medications acting on the GABA System. *CNS Drugs*. 2023;37(9):755-779.

    doi: 10.1007/s40263-023-01027-2

20. Sills GJ, Rogawski MA. Mechanisms of action of currently used antiseizure drugs. *Neuropharmacology*. 2020;168:107966.

    doi: 10.1016/j.neuropharm.2020.107966

21. Chen B, Choi H, Hirsch LJ, *et al*. Psychiatric and behavioral side effects of antiepileptic drugs in adults with epilepsy. *Epilepsy Behav*. 2017;76:24-31.

    doi: 10.1016/j.yebeh.2017.08.039

22. Perucca P, Gilliam FG. Adverse effects of antiepileptic drugs. *Lancet Neurol*. 2012;11(9):792-802.

    doi: 10.1016/S1474-4422(12)70153-9

23. *National Center for Biotechnology. Valproic Acid*; 2024. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Valproic-Acid [Last accessed on 2024 Dec 24].

24. Tomson T, Battino D, Perucca E. Valproic acid after five decades of use in epilepsy: Time to reconsider the indications of a time-honoured drug. *Lancet Neurol*. 2016;15(2):210-218.

    doi: 10.1016/S1474-4422(15)00314-2

25. DeVane CL. Pharmacokinetics, drug interactions, and tolerability of valproate. *Psychopharmacol Bull*. 2003;37(Suppl 2):25-42.

26. Ojewole JA, Adewunmi CO. Anti-inflammatory and hypoglycaemic effects of *Tetrapleura tetraptera* (Taub) [*Fabaceae*] fruit aqueous extract in rats. *J Ethnopharmacol*. 2004;95(2-3):177-182.

    doi: 10.1016/j.jep.2004.06.026

27. Aderibigbe AO, Iwalewa EO, Adesina SK, Adebanjo AO, Ukponmwan OE. Anticonvulsant, analgesic and hypothermic effects of aridanin isolated from *Tetrapleura tetrapetra* fruit in mice. *J Biol Sci*. 2007;7(8):1520-1524.

    doi: 10.3923/jbs.2007.1520.1524

28. Adesina S, Iwalewa E, Johnny I. *Tetrapleura tetraptera* taub- ethnopharmacology, chemistry, medicinal and nutritional values- a review. *Br J Pharm Res*. 2016;12(3):1-22.

    doi: 10.9734/BJPR/2016/26554

29. Kemigisha E, Owusu EO, Elusiyan CA, Omujal F, Tweheyo M, Bosu PP. *Tetrapleura tetraptera* in Ghana, Nigeria and Uganda: Households uses and local market. *Forests Trees Livelihoods*. 2018;27(4):243-256.

    doi: 10.1080/14728028.2018.1498027

30. Mensah RQ, Adusei S, Azupio S, Kwakye R. Nutritive value, biological properties, health benefits and applications of *Tetrapleura tetraptera*: An updated comprehensive review. *Heliyon*. 2024;10(6):e27834.

    doi: 10.1016/j.heliyon.2024.e27834

31. Wahab OM. Ethnomedicinal antiepileptic plants used in parts of Oyo and Osun States, Nigeria. *Bot Res Int*.

2015;8(4):77-81.

doi: 10.5829/idosi.bri.2015.8.4.12823

32. Ojewole JAO. Analgesic and anticonvulsant properties of *Tetrapleura tetraptera* (Taub) (*Fabaceae*) fruit aqueous extract in mice. *Phytother Res*. 2005;19(12):1023-1029.

doi: 10.1002/ptr.1779

33. Adusei S, Otchere JK, Oteng P, Mensah RQ, Tei-Mensah E. Phytochemical analysis, antioxidant and metal chelating capacity of *Tetrapleura tetraptera*. *Heliyon*. 2019;5(11):e02762.

doi: 10.1016/j.heliyon.2019.e02762

34. Aladesanmi AJ. *Tetrapleura tetraptera*: Molluscicidal activity and chemical constituents. *Afr J Tradit Complement Alternat Med*. 2007;4(1):23-36.

35. Ekong FM, Ekanem AU, Aquaisua AN, Ekong MB. *Tetrapleura tetraptera* fruit extract protects mice heart morphology from chronic dietary-salt intake. *SciBase Hum Nutr Food Sci*. 2024;1(1):1004.

36. Ekong MB, Iniodu C, Essien IO, Edem S. *Tetrapleura tetraptera* (Schumach.) Taub. fruit extract improves cognitive behaviour and some brain areas of pentylenetetrazol-kindling rats. *Nig J Neurosci*. 2021;12(1):29-39.

doi: 10.47081/njn2021.12.1/004

37. Joel JS, Sheena OE, Martins OE, Onyemauche NSC, Emmanuel AA. Comparative antioxidant capacity of aqueous and ethanol fruit extracts of *Tetrapleura tetraptera*. *J Biol Sci*. 2017;17(4):185-193.

doi: 10.3923/jbs.2017.185.193

38. Koma O, Olawumi O, Godwin EU, Theophilus O. Phytochemical screening, *in-vitro* antimicrobial activity and antioxidant characteristics of *Tetrapleura tetraptera* extracts. *Eur J Med Plants*. 2016;17(2):1-10.

doi: 10.9734/EJMP/2016/29585

39. Lekana-Douki JB, Oyegue Liabagui SL, Bongui JB, Zatra R, Lebibi J, Toure-Ndouo FS. *In vitro* antiplasmodial activity of crude extracts of *Tetrapleura tetraptera* and *Copaifera religiosa*. *BMC Res Notes*. 2011;4(1):506.

doi: 10.1186/1756-0500-4-506

40. Odesanmi OS, Lawal RA, Ojokuku SA. Effects of ethanolic extract of *Tetrapleura tetraptera* fruit on serum lipid profile and kidney function in male Dutch-white rabbits. *Nig Q J Hosp Med*. 2011;21(4):299-302.

41. Ozaslan M, Karagoz ID, Lawal RA, *et al*. Cytotoxic and anti-proliferative activities of the *Tetrapleura tetraptera* fruit extract on Ehrlich ascites tumor cells. *Int J Pharmacol*. 2016;12:655-662.

42. Ekong MB, Bassey OO, Pessu NA, *et al*. *Tetrapleura tetraptera Fruit Extracts Ameliorate Pentylenetetrazol-Induced Seizures as Well as Ensuing Cognitive Deficit and Oxidative Stress*; 2024. [Research Square Preprint].

doi: 10.21203/rs.3.rs-3382014/v1

43. Bruce R. An up-and-down procedure for acute toxicity testing. *Fundam Appl Toxicol*. 1985;5(1):151-157.

doi: 10.1016/0272-0590(85)90059-4

44. Ngoupaye GT, Adassi MB, Foutsop AF, Yassi FB, Ngo Bum E. Pentylenetetrazole kindling-induced epilepsy rat models: Insight on the severity state, a comparative study. *IBRO Neurosci Rep*. 2022;13:164-176.

doi: 10.1016/j.ibneur.2022.08.003

45. Dhir A. Pentylenetetrazol (PTZ) kindling model of epilepsy. *Curr Protoc Neurosci*. 2012;9:Unit9.37.

doi: 10.1002/0471142301.ns0937s58

46. National Research Council. *Guide for the Care and Use of Laboratory Animals*. 8th ed. United States: National Academies Press; 2011. p. 12910.

doi: 10.17226/12910

47. Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London: Chapman and Hall; 1998.

48. Deacon RMJ, Rawlins JNP. T-maze alternation in the rodent. *Nat Protoc*. 2006;1(1):7-12.

doi: 10.1038/nprot.2006.2

49. Cho KS, Lim YR, Lee K, Lee J, Lee JH, Lee IS. Terpenes from forests and human health. *Toxicol Res*. 2017;33(2):97-106.

doi: 10.5487/TR.2017.33.2.097

50. Nimse SB, Pal D. Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Adv*. 2015;5(35):27986-28006.

doi: 10.1039/C4RA13315C

51. Vermerris W, Nicholson R. Phenolic compounds and their effects on human health. In: *Phenolic Compound Biochemistry*. Berlin: Springer Netherlands; 2006. p. 235-255.

doi: 10.1007/978-1-4020-5164-7_7

52. Ebana R, Edet U, Ekanemesang U, Ikon G, Etok C, Edet A. Antimicrobial activity, phytochemical screening and nutrient analysis of *Tetrapleura tetraptera* and *Piper guineense*. *Asian J Med Health*. 2016;1(3):1-8.

doi: 10.9734/AJMAH/2016/29362

53. Godfrey NE. Proximate and phytochemical composition of the pulp of *Tetrapleura tetraptera* fruits consumed in Abakaliki, Nigeria. *Int J Eng Res Technol*. 2015;4:1286-1294.

doi: 10.17577/IJERTV4IS060622

54. OECD. *Guidance Document on Acute Oral Toxicity Testing*. OECD; 2002.

doi: 10.1787/9789264078413-en

55. Sylvester E, Emmanuel U, Essien N, Olajumoke A. Acute

toxicity study and ascertaination of wound healing effect of the acetone fraction of *Tetrapleura tetraptera* fruit in excision wound model. *J Adv Med Pharm Sci*. 2015;3(3):112-121.

doi: 10.9734/JAMPS/2015/15776

56. Aldawsari HM, Eid BG, Neamatallah T, Zaitone SA, Badr JM. Anticonvulsant and neuroprotective activities of *Phragmanthera austroarabica* extract in pentylenetetrazole-kindled mice. *Evid Based Complement Alternat Med*. 2017;2017(1):5148219.

doi: 10.1155/2017/5148219

57. Liang KG, Mu RZ, Liu Y, Jiang D, Jia TT, Huang YJ. Increased serum S100B levels in patients with epilepsy: A systematic review and meta-analysis study. *Front Neurosci*. 2019;13:456.

doi: 10.3389/fnins.2019.00456

58. Hansen SL, Sperling BB, Sánchez C. Anticonvulsant and antiepileptogenic effects of GABAA receptor ligands in pentylenetetrazole-kindled mice. *Prog Neuropsychopharmacol Biol Psychiatry*. 2004;28(1):105-113.

doi: 10.1016/j.pnpbp.2003.09.026

59. Zhu Y, Zhang S, Shen M, *et al.* Anticonvulsant effects of dingxian pill in pentylenetetrazol-kindled rats. *Evid Based Complement Alternat Med*. 2019;2019:4534167.

doi: 10.1155/2019/4534167

60. Dhamija R, Gavrilova RH, Wirrell EC. Valproate-induced worsening of seizures: Clue to underlying diagnosis. *J Child Neurol*. 2011;26(10):1319-1321.

doi: 10.1177/0883073811402204

61. Singh P, Singh D, Goel RK. Phytoflavonoids: Antiepileptics for the future. *Int J Pharm Pharm Sci*. 2014;6(8):51-66.

62. Li B, Wang L, Sun Z, *et al.* The anticonvulsant effects of SR 57227 on pentylenetetrazole-induced seizure in mice. *PLoS One*. 2014;9(4):e93158.

doi: 10.1371/journal.pone.0093158

63. Esmaeilpour K, Sheibani V, Shabani M, Mirnajafi-Zadeh J. Effect of low frequency electrical stimulation on seizure-induced short- and long-term impairments in learning and memory in rats. *Physiol Behav*. 2017;168:112-121.

doi: 10.1016/j.physbeh.2016.11.001

64. Brady S, Colman DR, Brophy P. Subcellular organization of the nervous system. In: *From Molecules to Networks*. Netherlands: Elsevier; 2014. p. 23-52.

doi: 10.1016/B978-0-12-397179-1.00002-6

65. Harrison IF, Crum WR, Vernon AC, Dexter DT. Neurorestoration induced by the HDAC inhibitor sodium valproate in the lactacystin model of Parkinson's is associated with histone acetylation and up-regulation of neurotrophic factors. *Br J Pharmacol*. 2015;172(16):4200-4215.

doi: 10.1111/bph.13208

66. Hussain G, Rasul A, Anwar H, *et al.* Role of plant derived alkaloids and their mechanism in neurodegenerative disorders. *Int J Biol Sci*. 2018;14(3):341-357.

doi: 10.7150/ijbs.23247

67. Garlapati PK, Raghavan AK, Shivanna N. Phytochemicals having neuroprotective properties from dietary sources and medicinal herbs. *Phcog J*. 2014;7(1):1-17.

doi: 10.5530/pj.2015.7.1

68. Haque A, Polcyn R, Matzelle D, Banik NL. New insights into the role of neuron-specific enolase in neuro-inflammation, neurodegeneration, and neuroprotection. *Brain Sci*. 2018;8(2):33.

doi: 10.3390/brainsci8020033

69. Ekong MB, Peter AI, Edagha IA, Ekpene UU, Friday DA. *Rauwolfia vomitoria* inhibits olfaction and modifies olfactory bulb cells. *Brain Res Bull*. 2016;124:206-213.

doi: 10.1016/j.brainresbull.2016.05.008

70. Cortes N, Posada-Duque RA, Alvarez R, *et al.* Neuroprotective activity and acetylcholinesterase inhibition of five *Amaryllidaceae* species: A comparative study. *Life Sci*. 2015;122:42-50.

doi: 10.1016/j.lfs.2014.12.011

71. Ekong MB, Ekpo MM, Akpanyung EO, Nwaokonko DU. Neuroprotective effect of *Moringa oleifera* leaf extract on aluminium-induced temporal cortical degeneration. *Metab Brain Dis*. 2017;32(5):1437-1447.

doi: 10.1007/s11011-017-0011-7

72. Gleichman AJ, Carmichael ST. Astrocytic therapies for neuronal repair in stroke. *Neurosci Lett*. 2014;565:47-52.

doi: 10.1016/j.neulet.2013.10.055

73. Bondan EF, Martins MDFM, Viani FC. Decreased astrocytic GFAP expression in streptozotocin-induced diabetes after gliotoxic lesion in the rat brainstem. *Arq Bras Endocrinol Metab*. 2013;57(6):431-436.

doi: 10.1590/S0004-27302013000600004

74. Liu C, Li Y, Lein PJ, Ford BD. Spatiotemporal patterns of GFAP upregulation in rat brain following acute intoxication with diisopropylfluorophosphate (DFP). *Curr Neurobiol*. 2012;3(2):90-97.