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Tetrapleura tetraptera (Schumach.) Taub. Fruit Extract Improves Cognitive Behaviour and Some Brain Areas of Pentylenetetrazol-Kindling Rats

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ABSTRACT

Epilepsy is a neuronal disorder that arises from imbalances of some neurotransmitters and manifests as recurrent seizure and cognitive impairment. Most antiepileptic drugs are either expensive, not effective or are associated with adverse effects warranting efficacious alternatives. This study, therefore, investigated the activity of *Tetrapleura tetraptera* (Schumach.) Taub fruit extract (TTE) on the behaviour and some brain areas of pentylenetetrazol (PTZ)-kindling rats. Thirty-five male Wistar rats (150-200 g) were assigned into five groups (1-5, n=7): Control (distilled water); TTE (500 mg/kg); PTZ (40 mg/kg); PTZ (40 mg/kg) pre-treated with either sodium valproate (SV, 200 mg/kg) or TTE (500 mg/kg). All treatments were oral, except for the PTZ (intraperitoneally), and carried out 48 hourly, until kindling was also fully achieved (21 days). Subsequently, there was a beam walking behavioural test, deep anaesthesia and animals' sacrifice, while whole brains were processed for histology. The results showed that seizure was induced with higher mortality in the PTZ group, and was suppressed with higher quantal protection in the PTZ groups pre-treated with either TTE or SV. There was no difference ($p>0.05$) in beam walk slips and latency. Simultaneously, the PTZ group showed some degenerative cellular changes in the hippocampus and temporal cortex, with significantly ($p<0.05$) higher cellular density, except in the cerebellum. These cellular changes were either minimal or not apparent in the PTZ groups pre-treated with either TTE or SV compared with the control group. In conclusion, TTE protected against PTZ - induced seizures and brain histopathology of rats, with results similar to the standard anticonvulsant drug, SV.

Key words: *Epilepsy; Pentylenetetrazol; Tetrapleura tetraptera; Beam walking; Brain histology; Sodium valproate*

INTRODUCTION

Epileptic seizures are characterized by recurrent and unpredicted interruptions of normal brain functions due to abnormal electrical discharge of neurons, resulting from imbalances of inhibitory GABAergic and excitatory glutaminergic neurotransmitters (Fisher et al. 2005; Deme 2016). This disorder is more common among males and in the first two decades of life, with over 50 million epidemiology and incidences of 2.4 million people per year (Samokhina and Samokhin 2018). The aetiology of epilepsy is unknown, and its prevalence is further compounded by the failure of available treatments due to unpleasant side effects, unpredictable pharmacological actions or high cost (Ogunrin et al. 2005; Puig-Lagunes et al. 2016).

Epilepsy is reportedly predisposed by changes in such brain areas as the hippocampus, temporal cortex, and cerebellum (Bonilha et al. 2010; Schmahmann 2019; Marcián et al. 2020; Streng and

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Krook-Magnuson 2020). These changes include degeneration and loss of neurons, hyperplasia of interneurons, glia reactions and atrophy (Saniya et al. 2017; Samokhina and Samokhin 2018; Marcián et al. 2020; Streng and Krook-Magnuson 2020), manifesting as seizure, cognitive decline or unconsciousness (Zhu et al. 2004; Dhir 2012; Saniya et al. 2017).

Medicinal plant therapies have become popular in treating disease conditions (Ekong et al. 2017; Nduohosewo and Ekong 2020). *Tetrapleura tetraptera* (Schumach.) Taub. (*T. tetraptera*), a nutritive and medicinal plant used in the management of some diseases and neurological disorders in Nigeria, has also been reported in the management of epilepsy (Akintola et al. 2015; Wahab 2015; Adesina et al. 2016). Experimental data also supports the anticonvulsant role of whole *T. tetraptera* fruit extract or its metabolites (Ojewole 2005; Aderibigbe et al. 2007; Adesina et al. 2016). Other activities of *T. tetraptera* include anti-inflammatory, antioxidant, hypoglycaemic, hypolipidaemic, hypokalaemic and central nervous system depressant, among others (Atawodi et al. 2011; Lekana-Douki et al. 2011; Wahab 2015; Adesina et al. 2016; Erukainure et al. 2017).

Tetrapleura tetraptera, widely used in Nigeria for food seasoning, pomades and soap (Akintola et al. 2015), besides treating diverse health issues, belongs to the family, Fabaceae (Ojewole and Adewunmi 2004). The plant, commonly called Aridan, is also known as Prekese (Twi of Ghana), and Aidan and Uyayak (Yoruba and Ibibio respectively, of Nigeria), and is found in the lowland forest of many tropical African countries, including Nigeria. It fruits are long (22–27 cm), green when tender and dark red-brown when fully ripe, with characteristic fragrance and pungent aromatic odour (Nwoba 2015).

The fruit of *T. tetraptera* is rich in calcium, phosphorous, potassium, zinc, iron, sodium and vitamins, with phytochemicals including tannins, phenolic compounds, saponins, alkaloids, steroids, flavonoids, phlobatannins and terpenoids (Ojewole 2005; Erukainure et al. 2017). The reported changes in brain microstructures underlying epilepsy and the role of *T. tetraptera* fruit extract in mitigating convulsion, motivated this investigation on the effect of *T. tetraptera* fruit extract on behaviour and some brain microstructures of rats following pentylenetetra-zol kindling.

MATERIALS AND METHODS

Animal Handling

Thirty-five male Wistar rats, nine to ten weeks old, weighing between 120 - 180 g, were obtained from the Animal House of the Faculty of Basic Medical Sciences, University of Uyo, and acclimatized for two weeks. The animals were housed in spacious wooden cages to enhance free movement and good ventilation. Wood shavings were used as bedding, while the cages were cleaned every day. The room temperature of 25-28 °C and 12:12 h light and dark cycle were maintained throughout the duration of the experiment, and allowed a standard rat pelletized diet (Grand Cereals, Nigeria) and water *ad libitum*. Approval for the study was granted by the Faculty of Basic Medical Sciences Ethical Committee, University of Uyo. All recommendations and protocols involving handling and care of the animals by the National Research Council of the United States of America were strictly adhered to (National Research Council 2011).

Collection, Authentication and Preparation of Extract of *T. tetraptera*

Fresh fruits of *T. tetraptera* were obtained from a local market in Calabar Metropolis, Nigeria. The plant was identified by the Principal curator at the Pharmacognosy Herbarium in the Faculty of Pharmacy of the University of Uyo, where it was also authenticated, with specimen voucher number UUPH/A32(f) deposited. The fruits of *T. tetraptera* were cut into pieces, washed, air-dried and pulverized into a fine powder with an electric blender. The dried sample (1,342 g) was weighed and macerated in ethanol for 72 h. It was filtered, and the filtrate concentrated to dryness in a water bath at 45 ℃ and stored in the refrigerator at 4 ℃.

Preparation of Drugs and *T. tetraptera* Fruit Extract

Pentylenetetrazol (Sigma-Aldrich, USA) solution for induction of convulsion was prepared in normal saline and administered intraperitoneally at a sub-convulsive dose of 40 mg/kg body weight of the rats. This was administered every two days (for eleven alternate days) to induce kindling as recommended by Dhir (2012). Sodium valproate pack (Sanofi-Aventis, United Kingdom) was the standard anticonvulsant used. A tablet (200 mg) of sodium valproate was dissolved in 5 mL of distilled water (40 mg/mL), and an equivalent of 200 mg/kg body weight dose for each rat calculated. Two grams of *T. tetraptera* fruit extract was dissolved in 20 mL of distilled water (100 mg/mL), and 500 mg/kg equivalent for each rat was calculated. The *T. tetraptera* and sodium valproate were administered orally an hour before the pentylenetetrazol injections, which lasted till day twenty one. All administered chemicals/extracts were prepared just before use.

Animal Grouping and Treatment

The thirty-five Wistar rats were divided into five groups (n=7): Control and test groups (1-5). Group 1, the control group received distilled water (0.2 mL/kg), while the test groups 2-5, received respectively, *T. tetraptera* (500 mg/kg, 10% of the median lethal dose that was determined previously), pentylenetetrazol (40 mg/kg, Ueno et al. 2020), pentylenetetrazol pre-

treated with sodium valproate (200 mg/kg, therapeutic dose, and then 40 mg/kg pentylenetetrazol), pentylenetetrazol pre-treated with *T. tetraptera* (500 mg/kg and then 40 mg/kg pentylenetetrazol) for eleven alternate days (every two days, making 21 days).

Pentylenetetrazol Induced Kindling and Determination of Seizure Scores

Kindling was induced by repetitive intraperitoneal injections of pentylenetetrazol (40 mg/kg) every 48 h, which was fully achieved on day 21 (alternate day 11) with eleven injections. For groups 4 and 5, sodium valproate and *T. tetraptera*, respectively, were administered an hour before the pentylenetetrazol injections. Immediately after each pentylenetetrazol injection, seizure scores and mortality rates were observed and recorded within 30 min. The seizure and stages of activity were measured using the Racine scale as follows: Stage 0 (no response), stage 1 (ear and facial twitching), stage 2 (myoclonic jerks without rearing); stage 3 (myoclonic jerks and rearing), stage 4 (forelimb clonus), stage 5 (seizures characterized by rearing, turning over into side position, generalized clonic-tonic seizures) and stage 6 (death). The rats were also monitored for 24 h after the last treatments to check for mortality. Quantal protection (number of animals alive in each group divided by the total number of animals in the group) and the percentage quantal protection and mortality were calculated from the data obtained (Dhir 2012; Erkeç and Arihan 2015).

Beam Walking Behavioural Test

On day 22 of the experiment, the beam walking behaviour for assessing locomotor activity and grip strength in rats was carried out. A metre (100 cm) long narrow wooden beam of 2 cm diameter suspended from its end at an elevation of 30 cm was used. The animals were brought into the test room an hour before the test. Each rat was gently placed at the centre of the beam, facing one of the ends, and allowed to walk to the end of the beam. The rats were allowed 60 s for each trial, and a total of four trials with a 10 s interval. The frequency of hind foot slips from the beam was recorded.

Termination of the Experiment

The animals were weighed prior to drug/extract administrations and every five days thereafter to determine body weight changes. The animals were anaesthetized using ketamine hydrochloride (50 mg/kg body weight, Rotex Medica, Germany) intraperitoneally. Each animal's thoracic cavity was opened up, and a cannula connected to the heart's left ventricle for perfusion and fixation with 10% buffered formalin after normal saline flushing. After perfusion-fixation, the whole brain was then excised and post-fixed in 10% buffered formalin for 48 h and subsequently processed for histological studies using haematoxylin and eosin (H & E) staining method as described in Suvarna et al. (2019).

Cell Count and Microscopy

Tissue sections were viewed under the light microscope (Zeiss Primo Star, Germany), and photomicrographs were obtained using the microscope camera-linked to a computer. The cell population was estimated manually using ImageJ® software (version 1.52p). Briefly, digital images of the hippocampi, frontal and cerebella cortices were obtained for each section and randomly mapped with the ImageJ® gridlines. Counting of cell nuclei was done manually, considering the nuclei on the upper and right borders of the mapped areas.

Statistical Analysis

Repeated (beam walking test) and one-way (other data) analysis of variance were used to compare the means. Thereafter Tukey's post-hoc test was carried out, both using Graphpad Prism 5 software (version 5.01) to find the level of significance at $p \leq 0.05$. The results were expressed as mean ± standard error of mean (SEM).

RESULTS

Rats Body Weight

By day 6 of the experiment, weight loss was evident in the experimental groups. These body weights were regained subsequently in the experimental groups (Control: 9.21%, *T. tetraptera*: 8.35%, pentylenetetra-

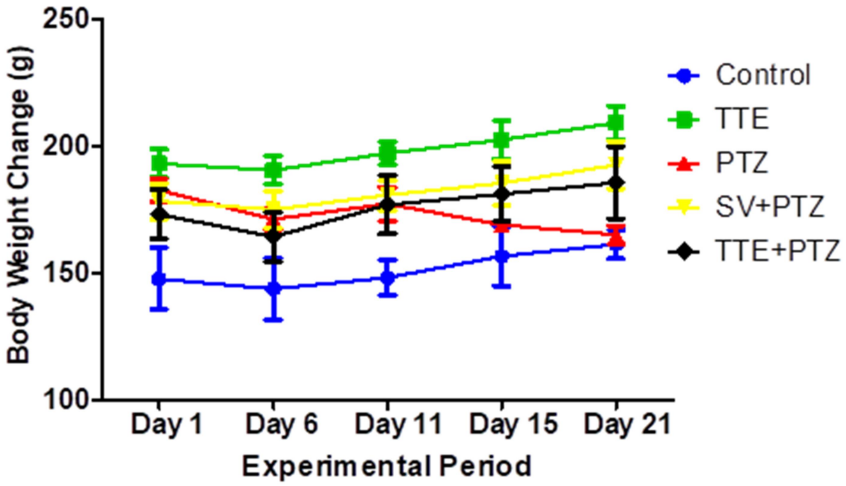

Fig. 1: Body weight changes. PTZ (pentylenetetrazol), SV (sodium valproate), TTE (*T. tetraptera*)

zol pre-treated with sodium valproate: 7.97%, and pentylenetetrazol pre-treated with *T. tetraptera*: 7.16%), except the group administered pentylenetetrazol alone (-9.64%), where the body weights were on a continuous decline (Fig. 1).

Anti-Seizure and Quantal Protection Activities of *T. tetraptera*

There was reduced seizure score in the pentylenetetrazol group pre-treated with *T. tetraptera*, which was significant ($p < 0.05$) on alternate day 11 compared with the group administered pentylenetetrazol alone. The seizure score was also reduced in the pentylenetetrazol group pre-treated with sodium valproate, although not significantly ($p > 0.05$) compared with the group administered pentylenetetrazol alone (Fig. 2a).

There was higher quantal protection for the pentylenetetrazol groups pre-treated with either *T. tetraptera* (six alive) or sodium valproate (six alive) compared with the group administered group administered pentylenetetrazol alone (four alive). Invariably, there was lower mortality in the pentylenetetrazol groups pre-treated with either *T. tetraptera* (one death) or sodium valproate (one death) compared with the group administered pentylenetetrazol alone (three deaths) (Fig. 2b).

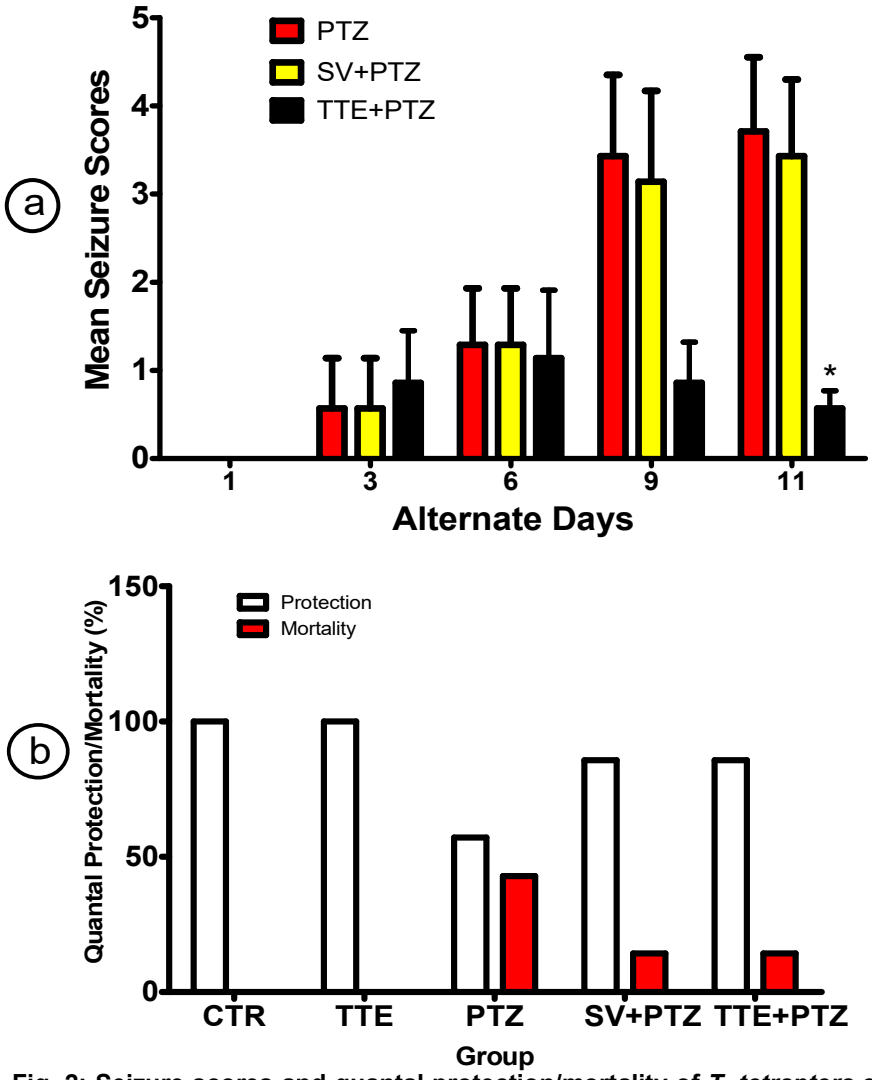

Fig. 2: Seizure scores and quantal protection/mortality of *T. tetraptera* on pentylenetetrazol-induced seizure. * = significantly different from PTZ group at $p < 0.05$. Mean ± standard error of mean. PTZ (pentylenetetrazol), SV (sodium valproate), TTE (*T. tetraptera*). The control and *T. tetraptera* groups administered distilled water and *T. tetraptera* respectively, did not show any seizure and hence, were not represented.

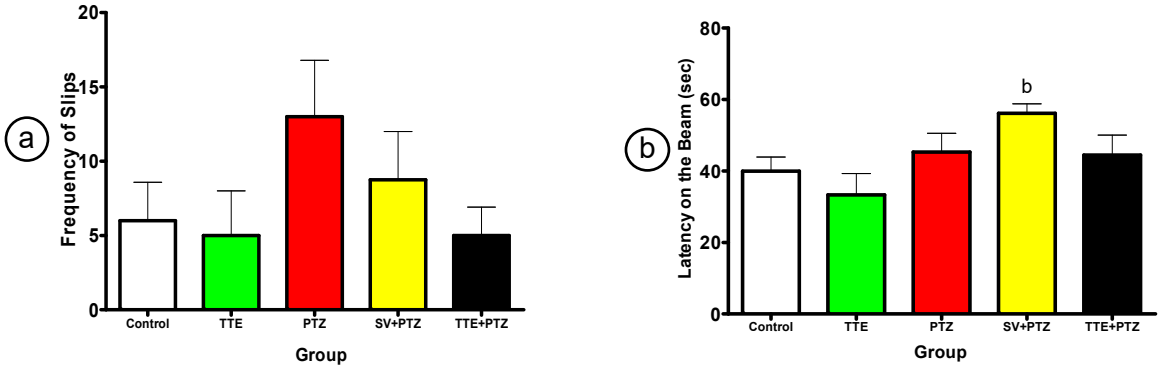

Fig. 3: Beam walking behaviour test. a. Frequency of slips was not significantly different. b. Latency on the beam walking. b significantly ($p > 0.05$) higher compared with the TTE group. PTZ (pentylenetetrazol), SV (sodium valproate), TTE (*T. tetraptera*). n=4

Beam Walking Behaviour Test

The frequency of hind feet slips was not significantly different ($p > 0.05$) among the experimental groups, although this was higher in the group administered pentylenetetrazol alone. There was no difference ($p > 0.05$) of the latency on the beam between the test groups and the control. However, there was significantly ($p < 0.05$) higher latency in the pentylenetetrazol group pre-treated with sodium valproate compared with the group administered *T. tetraptera* alone, while there was no latency difference ($p > 0.05$) among the other test groups (Fig. 3).

Histological Observations

Hippocampus

The histological presentations of the different hippocampal regions appeared similar to the CA3, which also served as a representative of the entire hippocampus proper. The control group showed normal histological features in the three cortical layers; outer molecular, middle pyramidal and inner polymorphic: The molecular layer consisted of sparsely populated neuronal and glial cells. The pyramidal layer consisted of a dense population of large pyramidal-shaped neurons, with neuroglia distributed within. The polymorphic layer also contained sparsely populated neurons and neuroglia (Fig. 4a).

The cortical layers; molecular, pyramidal and polymorphic, were also present in the test groups' hippocampi. In the group administered *T. tetraptera* alone, there were few pyramidal cells showing karyorrhexis. In the group administered pentylenetetrazol alone, some of the pyramidal cells showed either pyknosis or karyorrhexis. The pentylenetetrazol group pre-treated with sodium valproate showed pyknosis of some pyramidal cells. In contrast, the pentylenetetrazol group pre-treated with *T. tetraptera* showed few pyramidal cells with karyorrhexis, all compared with the control group (Fig. 4b-e).

The hippocampal cell density was significantly ($p < 0.05$) higher in the groups administered either pentylenetetrazol alone or pentylenetetrazol pre-treated with *T. tetraptera* compared with the control, and groups administered either *T. tetraptera* alone or pentylenetetrazol pre-treated with sodium valproate. However, there was no difference ($p > 0.05$) in cell density amongst the rest of the groups (Fig, 5).

Temporal Cortex

The outer three cortical layers, molecular, external granular and external pyramidal of the control group's temporal cortex, were studied. The molecular layer contained mainly neuronal processes and sparsely distributed cells. The granular contained a dense population of granular neurons with other smaller

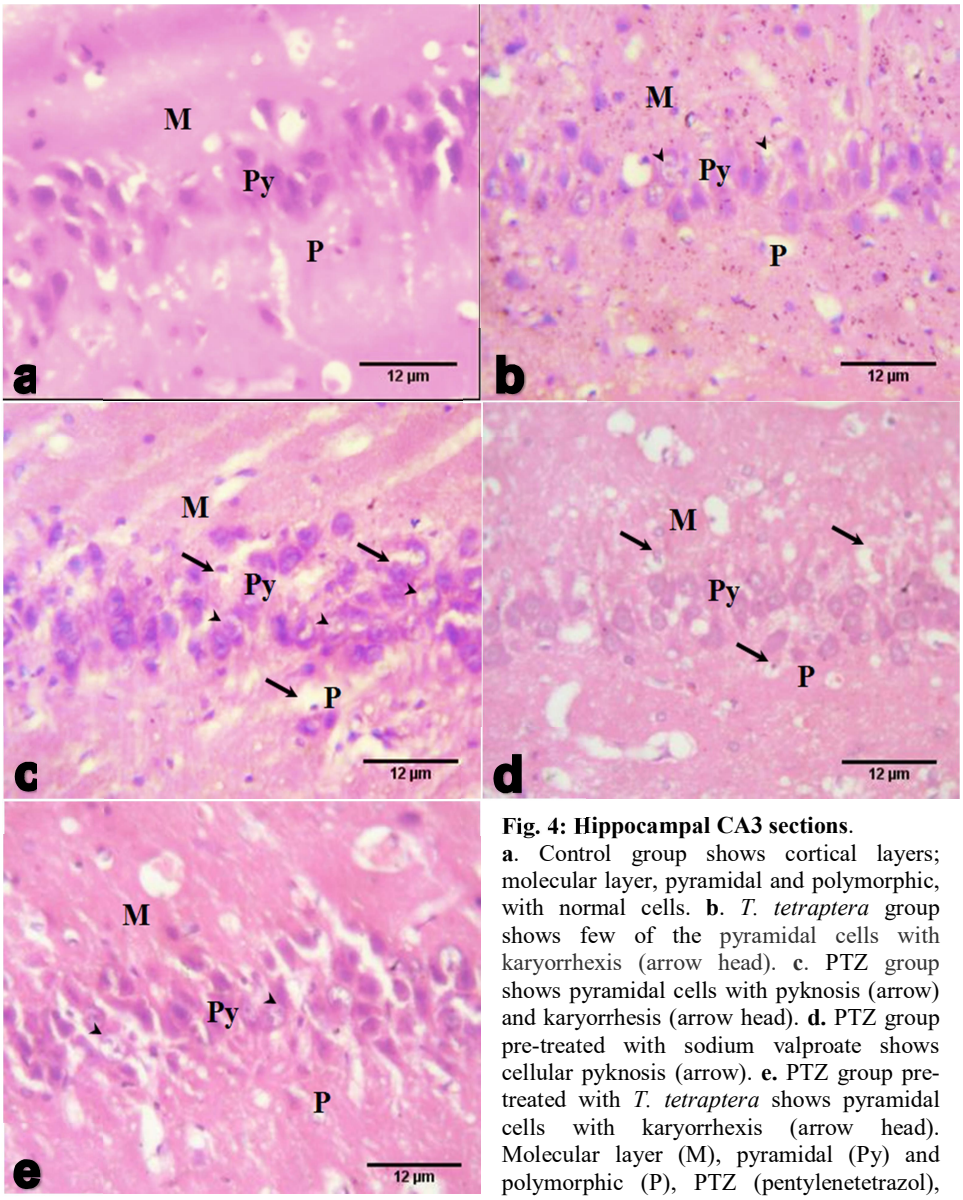

Fig. 4: Hippocampal CA3 sections.
a. Control group shows cortical layers; molecular layer, pyramidal and polymorphic, with normal cells. **b**. *T. tetraptera* group shows few of the pyramidal cells with karyorrhexis (arrow head). **c**. PTZ group shows pyramidal cells with pyknosis (arrow) and karyorrhesis (arrow head). **d.** PTZ group pre-treated with sodium valproate shows cellular pyknosis (arrow). **e.** PTZ group pre-treated with *T. tetraptera* shows pyramidal cells with karyorrhexis (arrow head). Molecular layer (M), pyramidal (Py) and polymorphic (P), PTZ (pentylenetetrazol), H&E ×400

cells distributed within. The pyramidal layer contained a less dense population of pyramidal-shaped neurons with other small cells distributed within (Fig. 6a).
The temporal cortical layers; molecular, granular and pyramidal, were also studied in the test groups. The temporal cortex of the group administered *T. tetraptera* alone showed cellular hyperplasia, with karyorrhexis in some of the cells. In the group administered pentylenetetrazol alone, there was cellular hyperplasia, with wide areas of karyorrhectic cells. In the group administered pentylenetetrazol pre-treated with sodium valproate, there was cellular hyperplasia, with some karyorrhexis in some of the cells, while the group administered pentylenetetrazol pre-treated with *T. tetraptera* showed apparently normal, but hyperplasia of cells, all compared with the control group (Fig. 6b-e).
The temporal cortical cell density was significantly ($p < 0.05$) higher in the test groups compared with the control. The cell density was also significantly ($p < 0.05$) higher in the group administered pentylenetetrazol alone compared with the groups administered either pentylenetetrazol pre-treated with *T. tetraptera* or sodium valproate. However, there was no difference ($p > 0.05$) in cell density amongst the rest of the groups (Fig. 5).

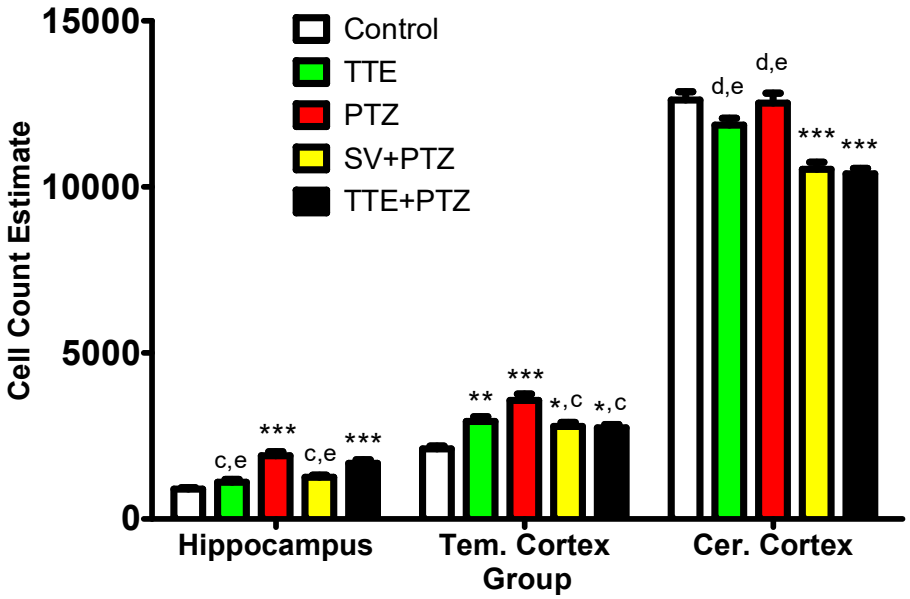

Fig. 5: Total cell counts of the experimental groups. Values are expressed as Mean ± SEM (standard error of mean).*, **, *** Significantly different from the control at $p < 0.05$, 0.01, 0.001, respectively; c,d,e Significantly different from PTZ, PTZ+SV and PTZ+TTE, respectively, at $p < 0.05$; PTZ (pentylenetetrazol); SV (sodium valproate); TTE (*T. tetraptera*) (n=4).

Cerebella Cortex
The control group showed the outermost molecular, middle Purkinje and the innermost granular layers. The molecular layer contained mostly nerve processes and sparsely populated cells. The Purkinje layer contained a single layer of Purk-inje neurons with other smaller cells distributed within. The inner granular layer contained a dense population of granular cells and sparsely distributed glomeruli within (Fig. 7a).
The cerebella cort-ical layers; mole-cular, Purkinje and granular, were also present in the test groups. The group administered *T. tetraptera* alone showed slight shrinkage of the Purkinje neuronal bodies, which was in addition to the shrinkage of the granular neurons in the group administered pentylenetetrazol alone. The group administered pentylenetetrazol pre-treated with sodium valproate showed apparently normal appearance, while the group administered pentylenetetrazol pre-treated with *T. tetraptera* showed slight cellular shrinkages, all compared with the control group (Fig. 7b-e).
Cellular population of the cerebella cortex were significantly ($p < 0.05$) lower in the groups administered either pentylenetetrazol pre-treated with *T. tetraptera* or sodium valproate compared with the control, and groups administered *T. tetraptera* or pentylenetetrazol alone. However, there was no difference ($p > 0.05$) in cell density amongst the rest of the groups (Fig. 5).

DISCUSSION

The present study investigated *T. tetraptera* fruit extract's actions on pentylenetetrazol-kindling Wistar rat model on body weight, beam walking behavioural test, and the histology of some brain areas. There was an increase in the test groups' body weight, except that of the pentylenetetrazol group, which decreased compared with the control group. The loss in body weight suggests that pentylenetetrazol may have raised the animals' metabolic activities, resulting in the use-up of available energy and more adipose tissues in such animals. This is because animals' growth depends on their metabolic processes (Galgani and Ravussin 2008).
The gain in body weights in the other test groups may be due to normal metabolism or reduced catabolic/increased anabolic activities. *T. tetraptera* is reported to have high nutritional value, which provides readily available energy to the body (Nwoba,

2015). These energy reserves may reduce the use of the body reserves, and may be a reason for the body weight gain by the animals in the pentylenetetrazol group pre-treated with *T. tetraptera* of the present study. Sodium valproate is reported to increase body weight (Kanemura et al. 2012; Bai et al. 2018), which may have been a reason for the animals' body weight in the pentylenetetrazol group pre-treated with sodium valproate of the present study.

Most rats were fully kindled by the eleventh pentylenetetrazol administration, which is similar to a previous report of full kindling within the same period (Erkeç and Arihan 2015). There were significant ($p < 0.05$) decreased seizure scores, as well as mortality, with equally increased quantal protection in the pentylenetetrazol groups pre-treated with either *T. tetraptera* or sodium valproate, compared to the pentylene\tetrazol alone group. These may indicate a neuroprotective action of *T. tetraptera* to suppress the severity of pentylenetetrazol-kindled seizures. Pentylenetetrazol exerts it convulsion effects by inhibiting the activity of GABA at the GABAA receptor. The enhancement of GABA neurotransmission atenuate convulsions (Kukuia et al. 2016), indicating that *T. tetraptera* may have acted through this pathway to improve GABA transmission (Ogunrin et al. 2015). The present result supports Paramdeep et al. (2014), who reported that alkaloids and flavonoids, which are present in *T. tetraptera* enhanced GABA transmission by stimulating the GABA receptors and voltage-gated ion channels (Schachter et al. 2015). Sodium valproate in the other hand is reported to act through the enhancement of GABA transmission (Cook and Besalem-Owen 2011), and may be the mechanism of action in the present study. Pentylenetetrazol kindling also increases the binding, density and concentrations of glutamate receptors (Ekonomou and Angelatou 1999), which *T. tetraptera* may have also acted.

In the beam walking behaviour, the frequency of hind feet slips and latency on the beam was not different ($p > 0.05$), although higher in the pentylenetetrazol alone group compared with the control group. This may indicate some neurocognitive changes, even though subtle. There was also no difference ($p > 0.05$) in these behavioural parameters in the pentylenetetrazol group pre-treated with *T. tetraptera*, an indication of non-effect. However, these results in the pentylenetetrazol group pre-treated with *T.*

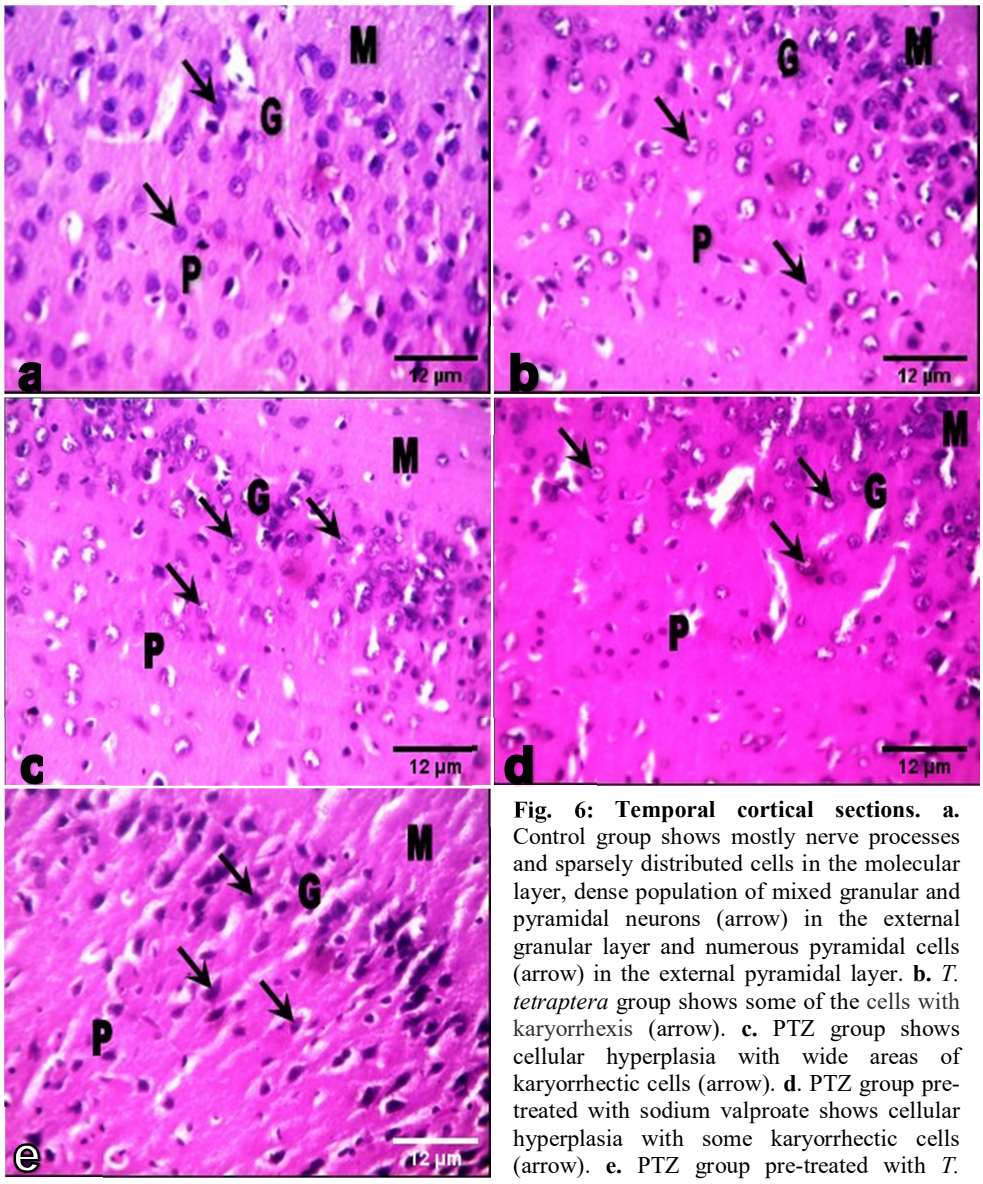

Fig. 6: Temporal cortical sections. a. Control group shows mostly nerve processes and sparsely distributed cells in the molecular layer, dense population of mixed granular and pyramidal neurons (arrow) in the external granular layer and numerous pyramidal cells (arrow) in the external pyramidal layer. **b.** *T. tetraptera* group shows some of the cells with karyorrhexis (arrow). **c.** PTZ group shows cellular hyperplasia with wide areas of karyorrhectic cells (arrow). **d**. PTZ group pre-treated with sodium valproate shows cellular hyperplasia with some karyorrhectic cells (arrow). **e.** PTZ group pre-treated with *T. tetraptera* shows normal (arrow), but hyperplasia of cells. M (molecular), G (external granular), P (external pyramidal), PTZ (pentylenetetrazol), H&E, ×400

tetraptera were similar to the control, suggesting a subtle neuroprotection by *T. tetraptera* against pentylenetetrazol. The pentylenetetrazol group pre-treated with sodium valproate also showed similar hind feet slips results, indicating its neuroprotection role. Foot slips frequency from the beam measures motor coordination and grip strength (Goldstein and Davis, 1990; Carter et al. 2001). These are mainly modulated in the cerebellum (Kiernan and Rajakumar 2014), indicating that this brain area may not have been affected.

The hippocampus histology showed pyknosis and karyorrhexis of the pyramidal cells of the pentylenetetrazol group with significantly ($p < 0.05$) higher cell density, indicating neurotoxicity. Pyknosis and karyorrhexis are degenerative cellular changes occurring due to apoptosis (Lehman-Mckeeman et al. 2013), which in the central nervous system are usually preceded by cellular hyperplasia due to invading microglia and astrogliosis (Röhl et al. 2007). The present result is consistent with Erkeç and Arihan (2015) and Ueno et al. (2020), who reported morphological changes in neurons, with extensive pyramidal cell loss in the CA1 and CA3 regions of the hippocampus due to pentylenetetrazol- kindling. The pentylenenetetrazol group pre-treated with *T. tetraptera* showed less cellular degeneration, with significant ($p < 0.05$) higher cell density, indicating a protective action in the hippocampus. *T. tetraptera* is reported to have a neuroprotective action against convulsive agents (Ishola et al. 2015; Iniodu et al 2019), which in the present study may not have been optimum, probably due to insufficient dosage. The pentylenetetrazol group pre-treated with sodium valproate also showed milder cellular degeneration, with no difference in cell density, indicating its neuroprotective role in the hippocampus.

The temporal cortex of the group administered pentylenetetrazol alone showed hyperplasia, wide areas of karyorrhectic cells with significantly ($p < 0.05$) higher cell density, which is similar to the observations in the hippocampus of the present study. This result which indicates neurotoxicity is reported in pentylenetetrazol kindling (Erkeç and Arihan 2015; Mishra et al. 2018; Ueno et al. 2020), The temporal cortex of the pentylenetetrazol group pre-treated with *T. tetraptera* showed normal cellular appearance, with significantly ($p < 0.05$) higher cell density, indicating the protective action in the temporal cortex. This is similar to its action in the hippocampus of the

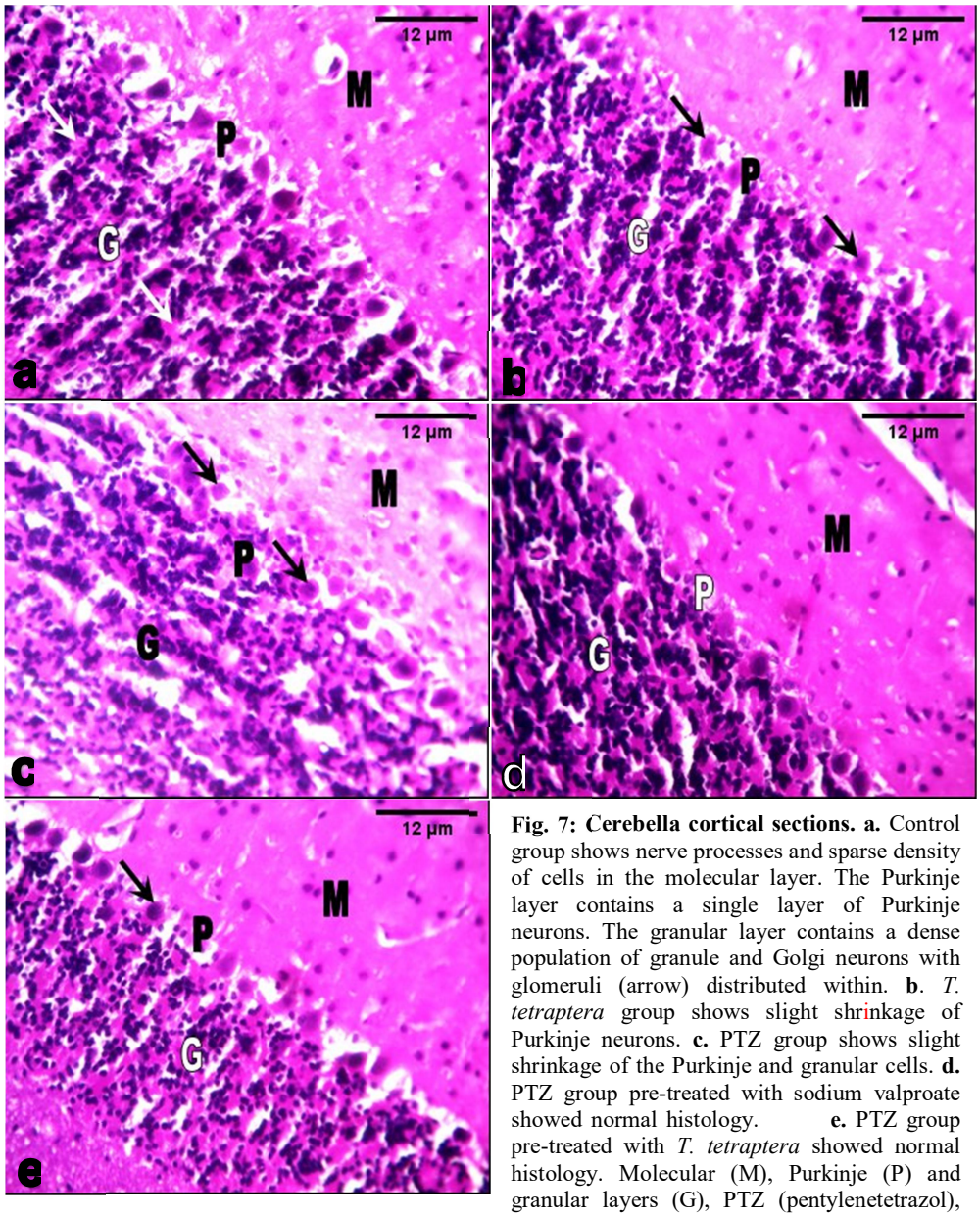

Fig. 7: Cerebella cortical sections. a. Control group shows nerve processes and sparse density of cells in the molecular layer. The Purkinje layer contains a single layer of Purkinje neurons. The granular layer contains a dense population of granule and Golgi neurons with glomeruli (arrow) distributed within. **b**. *T. tetraptera* group shows slight shrinkage of Purkinje neurons. **c.** PTZ group shows slight shrinkage of the Purkinje and granular cells. **d.** PTZ group pre-treated with sodium valproate showed normal histology. **e.** PTZ group pre-treated with *T. tetraptera* showed normal histology. Molecular (M), Purkinje (P) and granular layers (G), PTZ (pentylenetetrazol), H&E, ×400

present study. The pentylenetetrazol group pre-treated with sodium valproate also showed mild cellular changes, indicating its neuroprotective role in the temporal cortex.

The cerebella of the group administered pentylenetetrazol alone showed slight shrinkage of the Purkinje and granular cells with no difference in cell density, indicating mild neurotoxicity. Pavlova et al. (2004) reported that caspase-3, involved in apoptosis, does not cause cerebella degeneration when activated in pentylenetetrazol-kindling. This inability to cause degeneration may be a reason for the mild effect in the cerebella histology of the present study. The pentylenetetrazol group pre-treated with *T. tetraptera* showed normal histology and significantly ($p < 0.05$) lower cell density, indicating trauma, which *T. tetraptera* may have protected against. This is in agreement with Iniodu et al. (2019), who reported a protective action of *T. tetraptera* on the cerebellum. The pentylenetetrazol group pre-treated with sodium valproate also showed normal histology with significantly ($p < 0.05$) lower cell density.

The cerebellum modulates motor coordination (Kiernan and Rajakumar 2014), besides other functional roles (Schmahmann 2019). These functions are influenced by the hippocampus and cerebral cortex (Kiernan and Rajakumar 2014; Watson et al. 2019), indicating that cerebella dysfunction may also result from a dysfunction of these other brain areas. Pentylenetetrazol kindling increases glutamate concentrations (Dhir, 2012). High brain glutamate concentration is reported with excitoxicity and brain degeneration (Lewerenz and Maher et al. 2015). These may also be an underlying aetiology of the degenerative changes in cells of the hippocampus, temporal and cerebella cortices in the present study. Ishola et al. (2015) reported that the neuroprotective ability of *T. tetraptera* arises from it antioxidant properties, which is consistent with the report of alkaloids and flavonoids activities (Paramdeep et al. 2014), also present in *T. tetraptera*. Sodium valproate is reported in neurotoxicity, where it also acts through oxidative stress resulting in thinning of white matter and total brain volume reduction (Pardoe et al. 2013; Chaudhary and Parvez 2018). These may be a reason for the cellular changes observed in the brain areas of the pentylenetetrazol group pre-treated with sodium valproate of the present study.

In summary, pentylenetetrazol activated seizure possibly by inhibiting the activity of GABA at the GABAA receptor, reducing GABAergic transmission, while increasing the binding, density and concentrations of glutamate receptors, and thereby increasing glutamate transmission. These activities may have resulted in metabolic rate increase, leading to body weight loss, and neuronal damage/ degeneration, especially of the hippocampus and temporal cortex among other brain areas. *T. tetraptera* fruit extract or their metabolites antagonized pentylenetetrazol activities possibly at the GABAergic and glutamate receptors level similar to sodium valproate, resulting in the prevention of seizure, maintenance of body weight and neuroprotection of the animals.

Conclusion

Pentylenetetrazol kindling induced reduction in body weight and non-significant behaviour changes, as well as degenerative cellular changes. *T. tetraptera* fruit extract administered prior to pentylenetetrazol mitigated the convulsive actions and protected the animals by improving body weight, hind grip strength and cellular degeneration. The protective actions of *T. tetraptera* in the present study appeared similar to the standard anticonvulsant drug, sodium valproate.

Conflict of Interest

None declared.

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