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Tetrapleura tetraptera Fruit Extracts Ameliorate Pentylenetetrazol-Induced Seizures as well as Ensuing Cognitive Deficit and Oxidative Stress

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Abstract

Kindling is an experimental-induced seizure consistent with epilepsy disease, a chronic neurological disorder characterised by spontaneous and repeated seizures. This disease is associated with oxidative stress, and most therapeutic strategies against epilepsy aim at improving the antioxidant defence mechanism in the brain. However, prolonged usage and associated adverse side effects limit antiepileptics, warranting natural antioxidant patronage. The present study investigated the behavioural and antioxidant actions of *Tetrapleura tetraptera* fruit extracts (TT) against pentylenetetrazol (PTZ)-kindling rats. Twenty-five male Wistar rats (150–180 g) were assigned into five groups (1–5, n = 5): Control (normal saline, 5 ml/kg body weight, b.w.), PTZ-only (40 mg/kg/b.w. i.p.), and groups 3–5 administered PTZ (40 mg/kg/b.w. i.p.) after, respectively, receiving oral TT (500 mg/kg/b.w.), TT flavonoid (fTT, 50 mg/kg/b.w.), and sodium valproate (SV, 15 mg/kg/b.w.). All administrations were carried out 48 hourly for 21 days. In the end, buried food, novel object recognition (NOR), Y-maze, elevated plus maze (EPM), and beam walk tests were done, and the rats were sacrificed. Whole brains were processed for antioxidant assays. The results showed a high ($p < 0.05$) seizure score and buried food test latency, preference for the familiar object in the NOR test, aversion to open-arm and reduced grooming in the EPM, reduced beam walk latency, elevated brain malondialdehyde (MDA), and decreased superoxide dismutase (SOD) in the PTZ group. The TT, fTT, and SV suppressed seizure, decreased buried food latency, `preference for the novel object and open-arm, increased grooming, decreased brain MDA, and elevated SOD. In conclusion, TT extracts protected against PTZ-induced cognitive deficits and brain oxidative stress, with results similar to those of the standard anticonvulsant drug, SV.

INTRODUCTION

Epilepsy is one of the most prevalent non-communicable neurological conditions, affecting an estimated 70 million people worldwide, with a high concentration in developing countries (Ba-Diop et al., 2014; Beghi, 2020; WHO, 2023). It arises from abnormal and unhindered neuronal firings with predisposing factors, including genetics, acquired or unknown (Scheffer et al., 2017; Anwar et al., 2020). People suffering from epilepsy have an increased risk of cognitive, behavioural, and psychosocial disorders that can adversely impact their quality of life (Motoki et al., 2021; Rayner et al., 2022).

Most therapeutic strategies against epilepsy are aimed at preventing neuronal excitability, which often results in seizures (Major and Tank, 2004). However, some of the existing antiepileptic drugs are associated with adverse effects (Karimzadeh and Bakrani, 2013), which can exacerbate resistant epilepsy conditions (Löscher and Klein, 2021).

Seizures are associated with decreased antioxidant defence mechanisms in the brain, leading to increased free radicals and oxidative stress induction (Cardenas-Rodriguez et al., 2013; Borowicz-Reutt and Czuczwar, 2020). Alternatives to standard antiseizure drugs abound in phytomedicines with rich antioxidant constituents. These may address some of the adverse concerns by providing the needed

antioxidants alongside antiseizure activities (Aladesanmi, 2007; Odesanmi et al., 2011; Ekong et al., 2021).

The plant *Tetrapleura tetraptera* (*T. tetraptera*) has been reported to have potency against experimentally-induced seizures (Ekong et al., 2021). Apart from its general medicinal and nutritive value, the anti-convulsive potential of *T. tetraptera* is boosted by its antioxidant constituents, such as alkaloids, flavonoids, and phenols (Xi et al., 2008; Lekana-Douki et al., 2011; Erukainure et al., 2014), making it potentially advantageous over conventional antiepileptic drugs. Hence, this study investigated the behavioural and antioxidant effects of *T. tetraptera* fruit extracts against pentylenetetrazol-kindling.

MATERIALS AND METHODS

Mature fruits of *T. tetraptera* were obtained from a local farm in Adim, Biase, Cross River State, Nigeria. This plant was identified and authenticated by taxonomists with specimen number UUPH/A32(f) deposited at the herbarium unit of the Department of Botany and Ecological Studies, University of Uyo, Nigeria. The air-dried fruits were chopped into smaller pieces, their seeds removed, and blended to a paste, which was macerated in 80% ethanol and concentrated to dryness in a water bath at 45 ℃.

Phytochemical Screening/Analysis

The qualitative and quantitative phytochemical analyses of the crude extract of *T. tetraptera* were carried out as described by Harborne (1973), Soforowa (1982), and Trease and Evans (2001). These procedures were done in the laboratory of the Department of Biochemistry, University of Uyo, Nigeria.

Animal Care and Handling

Twenty-five (by power analysis) Wistar rats (150–180 g) were obtained from the Faculty of Basic Medical Sciences Animal House, University of Uyo. The animals were allowed to acclimatise for two weeks, thereafter randomly grouped (groups 1–5, n = 5) and housed in well-ventilated standard animal cages (five rats per cage) at room temperature (25–28°C) and a 12-hour light/dark cycle, with access to a standard chow pellet and water *ad libitum*. Group 1 served as the control and received normal saline (5 mL/kg body weight) as a placebo, while groups 2–5 served as the test (Table 1). The experimental procedure involving animal use and care was conducted following the international guidelines for the care and use of laboratory animals (National Research Council, 2011). Ethical approval for this study was obtained from the Faculty of Basic Medical Sciences Ethical Committee, University of Uyo, with the number: UU/FBMSREC/2022/001.

Table 1
Schedule of treatments of rats in the experimental groups

Group (n = 5)	Treatment	Dosage /route of administration)
1	Normal saline	5 mL/kg (orally)
2	Normal saline and pentylenetetrazol 30 minutes after	5 mL/kg (orally) and 40 mg/kg (intraperitoneally)
3	*T. tetraptera* ethanol extract and pentylenetetrazol 30 minutes after	500 mg/kg (orally) and 40 mg/kg (intraperitoneally)
4	*T. tetraptera* flavonoid extract and pentylenetetrazol 30 minutes after	50 mg/kg (orally) + 40 mg/kg (intraperitoneally)
5	Sodium valproate and Pentylenetetrazol 30 minutes after	15 mg/kg (orally) + 40 mg/kg (intraperitoneally)

Pentylenetetrazol (Batch number: MKCM4261, Sigma-Aldrich Inc., U.S.A.) solution was prepared in normal saline and administered 30 minutes following either *T. teraptera* extracts or sodium valproate (standard anticonvulsant drug, batch number: 677289, Sanofi-Aventis, Riells-Spain) administrations. The pentylenetetrazol solution was administered intraperitoneally, while *T. teraptera* extracts and sodium valproate were administered orally. Administrations were done every 48 hours for up to 21 days.

Pentylenetetrazol-Induced Kindling

To develop a kindling model, each rat received a single dose of 40 mg/kg of pentylenetetrazol every 48 hours. A total of 11 doses of pentylenetetrazol were given to each rat. The seizure in each rat was observed for an hour after each administration (De Sarro et al., 2000). A seizure was classified according to the Racine score (0–5) (Ihara et al., 2016). A rat was kindled when it showed three consecutive seizure scores of 4 or 5 (tonic-clonic seizure with an initial wild run and subsequent loss of righting reflex to tonic-clonic seizure with full extension of fore and hind limbs), after which the pentylenetetrazol administration was discontinued.

Neurobehavioural Study

The behavioural tests were performed between 6:00 AM and 12:00 PM on days 19–22. All the experimental mice were transferred to the test room an hour before the start of each test. Each behavioural test was recorded using a video camera, and the parameters were also scored manually. The behavioural tests were done blind to the treatments.

Buried Food Behavioural Test

On day 22 of the experiment, the buried food behavioural test was carried out. From days 19–21 (three consecutive days), odour familiarisation with a piece of biscuit (Yale Foods Limited, Nigeria) was allowed in the animal cages overnight, and the quantities of biscuit consumed were noted. On day 22, each rat was introduced into a clean experimental cage with bedding for familiarization. After 5 minutes, the rat was transferred into a clean holding cage without bedding. The rat was later re-introduced into the experimental cage with the same biscuit (food stimulus) already hidden 1 cm below the bedding at random corners. The cage lid was replaced, and the rat was allowed 15 minutes. The latency to locate the biscuit was recorded and was considered to have uncovered the biscuit when it started to eat. If the rat could not locate the biscuit within the given time, 900 s was recorded against it. The percentage of rats that located the biscuit per group was calculated (Yang and Crawley, 2009).

Novel Object Recognition Test

On day 22 of the experiment, the novel object recognition test was carried out in an open field consisting of an open field arena with dimensions 100×100×50 cm, made up of plywood. The novel object recognition task was conducted with three objects (A1, A2, and B) of the same height and volume. During habituation on day 20, the animals were allowed to explore an empty arena for five minutes. Twenty-four hours after habituation (day 21), the animals were exposed to the familiar arena with two identical objects (A1 and A2), placed at equidistant, and allowed to explore the arena for ten minutes. Twenty-four hours later (day 22), the animals were allowed to explore the open field in the presence of the familiar (A) and a novel object (B) placed at equidistant, similarly to test memory for ten minutes. The time spent exploring each object was recorded, and the discrimination index was calculated as

$$DI = \frac{T\,(new) - T\,(old)}{T\,(total)}$$

Where DI = discrimination index, T(new) = time with a novel object, T(old) = time with a familiar object, and T(total) = total time with both objects (Lueptow, 2017; Sivakumaran et al., 2018).

Y-Maze Test

On day 21, spontaneous alternation behaviour in the Y-maze was assessed. The apparatus was Y-shaped and consisted of three arms, with one arm serving as the start. The Y-shaped maze was constructed of wood, with each arm 40 cm long, 30 cm high, and 10 cm wide. Arm entry was defined as when the fore and hind paws of the rats were completely within the arm. Spontaneous alternation was defined as rats entering all three arms in the overlapping triplet sets. The percentage of alternation was calculated as {successive triplet sets/(total number of arm entries minus 2)} × 100. The rats were handled at the base of the tail and made to face the starting arm. A timing of eight minutes each was set for each animal. Total arm entry and successive spontaneous alternations were recorded. The apparatus was cleaned with 70% ethanol before testing a new animal to eliminate possible bias due to odours left by the previous animal (Kim et al., 2008; Reza et al., 2009).

Elevated Plus Maze Test

The elevated plus maze test was carried out on day 21. The apparatus for this maze was made of Plexiglas and was in the configuration of a plus sign comprising two open arms (100 × 10 cm) and perpendicular to two closed arms (100 × 10 × 40 cm) with a centre platform (10 × 10 cm) (Kraeuter et al., 2019). A rat was placed in the centre of the maze with its head directed towards the closed arms and allowed to explore for five minutes. The following parameters were scored: open and closed arm durations, rearing, and grooming. After each trial, all arms and the centre area were cleaned with 70% alcohol to control conditions regarding olfactory cues and allowed to dry before the introduction of the next animal.

Beam Walk Behavioural Test

On day 21 of the experiment, the beam walk behaviour was carried out. A metre-long, narrow wooden beam of 2 cm in diametre suspended from its end at an elevation of 30 cm was used. 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 hindfoot slips from the beam was recorded (Ito et al., 2022).

Termination of Experiment

Upon completion of the neurobehavioral tests, the animals were sacrificed after anaesthesia with ketamine hydrochloride (50 mg/kg body weight, Rotex Medica, Germany, Batch number: 20KA03) intraperitoneally. The animals were perfused through the left ventricle of the heart using cold phosphate-buffered saline. The brains were removed and immediately homogenised for biochemical assays.

Biochemical Assay

The brains were homogenised in 0.1M phosphate buffer (pH 7.2). The resulting homogenates were centrifuged at 3,000 g for 15 min, and the supernatants were used for superoxide dismutase (SOD), malondialdehyde (MDA), and glutamate dehydrogenase (GDH) assays (Sun and Zigman, 1978; Katerji et al., 2019).

The SOD activity was determined according to Misra and Fridonich (1977). Briefly, each brain tissue homogenate (10 µL) was added to 970 µL of ethylene diamine tetraacetic acid-sodium carbonate buffer (0.05 M) at a pH of 10.2. Epinephrine (30 mM, 20 µL) was added to start the reaction, and the activity was measured in a spectrophotometer at 480 nm for 4 min.

The MDA level was estimated based on its reaction with thiobarbituric acid-reactive substances. Briefly, a millilitre of the supernatant was precipitated with 2.5 mL of ice-cold trichloroacetic acid and centrifuged at 3,000 g for 10 min. Thiobarbituric acid (0.67%) was added to 2 mL of the supernatant, kept in a boiling water bath for 45 min, and subsequently cooled. The absorbance was read immediately at 532 nm on a spectrophotometer (Fleischer and Packer, 1978).

GDH activity was determined by a coupled enzyme assay in which glutamate is consumed by GDH-generating NADH, which reacts with a probe to generate a colorimetric product. Briefly, brain

homogenates were brought to a final volume of 50 mL with the GDH assay buffer. 100 mL of the GDH master reaction mix was added, mixed, and incubated at 37 ℃, while protected from light. The absorbance at 450 nm was read on a microplate reader and compared with the NADH standard curve (GDH Product Information (2023).

Statistical Analysis

GraphPad Prism 5.0 was used to analyse the data. The data were analysed blind to the treatments using a one-way analysis of variance, followed by a Student Newman-Keuls post hoc test. A P value < 0.05 was considered statistically significant. Normality and variance homogeneity were assessed automatically with the GraphPad Prism software. The normality ranged from 0-100, with 0 being the least value and 100 being the highest value; the Barlett's test shows the test for variance homogeneity. Results were presented as mean ± standard deviation (SD).

RESULTS

T. tetraptera fruit is rich in phytochemicals

The phytochemical screening of *T. tetraptera* fruit was to identify the most abundant and active medicinal constituents. The qualitative screening of *T. tetraptera* showed the presence of alkaloids, flavonoids, saponins, glycosides, polyphenols, terpenoids, tannins, terpenes, and steroids. Amino acids and anthraquinones were also present.

Quantitative analyses showed that phenol (18.3652 mg/100 g) was obtained in the highest quantity from *T. tetraptera.* Flavonoids (13.1650 g) and oxalates (13.8610 g) were also obtained in high quantities. Small quantities of alkaloids (2.6250 g), saponins (3.0651 g), and tannins (3.0870 mg/100 g) were obtained.

T. tetraptera fruit extracts decreased seizure scores

Seizure scores measured the severity of seizures in the rats. The PTZ group showed a high seizure score (5.0) compared with the other test groups administered *T. tetraptera* crude (2.40), flavonoid (3.20), and sodium valproate (2.60), which were significantly less [F(3, 16) = 5.83, P = .007] (Fig. 1).

Neurobehaviour

T. tetraptera fruit extracts enhanced the latency to locate the buried food

The buried food behaviour assessed the olfactory health of the rats. Forty percent of the rats in the PTZ group and 60% in the groups administered *T. tetraptera* crude and flavonoid extracts and sodium valproate located the hidden food, compared with the control of 60%. The latency to locate the hidden food was significantly higher in the PTZ group compared with the control, and the groups administered *T. tetraptera* crude and flavonoid extracts and sodium valproate [F(4, 20) = 7.02, P = .001]. However, there

was no difference in the latency to locate the hidden food between the other test groups and the control (Fig. 2).

T. tetraptera fruit extracts improved novel object recognition/exploration and discriminative index

The novel object recognition behaviour assessed the learning and memory of a novel object over a familiar one. The rats in the PTZ group spent significantly [$F(4, 20) = 5.66$, $P = .003$] more time exploring the familiar object compared to the control, and the groups administered *T. tetraptera* crude and flavonoid extracts and sodium valproate. However, no significant difference was observed in exploration time for the familiar object in these other test groups compared with the control.

The rats in the PTZ group spent a non-significant [$F(4, 20) = 4.27$, $P = .012$] less time exploring the novel object compared to the control and significantly ($p < 0.05$) less time compared to the groups administered *T. tetraptera* crude and flavonoid extracts, but not sodium valproate. However, no significant difference was observed in exploration time for the novel object in these other test groups compared with the control (Fig. 3).

The discrimination indices for the groups were as follows: control (0.3), PTZ (-0.4), *T. tetraptera* crude extracts (0.4), *T. tetraptera* flavonoid extracts (0.4), and sodium valproate (0.3).

T. tetraptera fruit extracts improved spontaneous alternations in the Y-maze

The spontaneous alternation behaviour assessed the short-term working memory of the rats. There was no significant difference between the spontaneous alternations of the PTZ group compared to the control. However, there were significantly higher spontaneous alternations in the groups administered *T. tetraptera* crude and flavonoid extracts, but not the sodium valproate compared to the PTZ group [$F(4, 20) = 5.12$, $P = .005$]. Alternatively, there was no significant difference in spontaneous alternations in these other test groups compared to the control (Fig. 4).

T. tetraptera fruit extracts showed anxiolytic abilities in the elevated plus maze

The elevated plus maze behaviour assessed the anxiety-related state of the rats. There was significantly less time spent in the open arms by the PTZ group compared with the control [$F(4, 20) = 9.32$, $P = .0002$]. The time spent in the open arms by the *T. tetraptera* crude extract and flavonoid, as well as sodium valproate groups, was not different from the control, but was significantly more in the open arms compared with the PTZ group (Fig. 5a).

There was no significant difference in the time spent in the closed arms by the PTZ, *T. tetraptera* crude extract, flavonoid, and sodium valproate groups, compared with the control [$F(4, 20) = 4.29$, $P = .012$]. There was also no significant difference in the time spent in the closed arms in the other test groups, except the *T. tetraptera* crude extract group, which was significantly less compared with the PTZ group (Fig. 5b).

There was no significant difference in the rearing frequency of the PTZ group, but significantly more rearing frequency in the *T. tetraptera* crude extract and flavonoid, as well as sodium valproate groups compared with the control [F(4, 20) = 21.01, P = .0001]. There was no significant difference in rearing frequency in the other test groups compared with the PTZ group (Fig. 5c).

There was a significantly less grooming frequency in the PTZ group, but not significantly different in the *T. tetraptera* crude extract and flavonoid, as well as sodium valproate groups compared with the control [F(4, 20) = 5.40, P = .004]. There were significantly higher grooming frequencies in the other test groups compared with the PTZ group (Fig. 5d).

T. tetraptera fruit extracts did not affect the beam walk behaviour

The beam walk behaviour assessed the ability of the rats to maintain a motor balance. The beam walk latencies of the PTZ and sodium valproate groups were significantly less compared with the control [F(4, 20) = 4.33, P = .011]. The beam walk latencies of the *T. tetraptera* crude extract and flavonoid groups were not different ($p > 0.05$) from the control (Fig. 6).

Biochemical Analysis

Brain Antioxidants and Enzymes

The SOD activity assessed the inert oxidative stress state of the rats. There was a significantly lower SOD level in the PTZ group compared with the control [F(4, 20) = 8.59, P = .0003]. There were also significantly lower SOD levels in the *T. tetraptera* flavonoid and sodium valproate groups but not in the *T. tetraptera* crude extract compared with the control. There were significantly lower SOD levels in the *T. tetraptera* flavonoid and sodium valproate groups, while that of the *T. tetraptera* crude extract group was significantly higher compared with the PTZ group (Fig. 7a).

The MDA level assessed the lipid peroxidation state of the rats. There were significantly higher MDA concentrations in the PTZ group compared with the control [F(4, 20) = 20.60, P = .0001]. Although the MDA concentrations were not significantly different in the *T. tetraptera* crude extract and flavonoid groups, that of the sodium valproate group was significantly less compared with the control. The MDA concentrations of the *T. tetraptera* crude extract and flavonoid, as well as the sodium valproate groups, were significantly lower compared with the PTZ group (Fig. 7b).

The GDH activity assessed the glutamate level regulation of the rats. There was no significant difference in the GDH activity between the test groups compared with the control, as well as among the test groups [F(4, 20) = 0.22, P = .925] (Fig. 7c).

DISCUSSION

The present study investigated the role of *T. tetraptera* fruit extracts on PTZ-kindling Wistar rat model seizure scores, cognitive behavioural tests, and brain biochemical oxidative stress and enzymatic

markers. The results showed that *T. tetraptera* contained antioxidant-rich phytochemicals. Its ethanol and flavonoid extracts suppressed seizures, improved olfaction and memory, and reduced anxiety and brain oxidative stress.

The phytochemical screening of the *T. tetraptera* ethanol fruit extract showed such constituents as flavonoids, phenols, cardiac glycosides, tannins, alkaloids, saponins, steroids, and terpenoids, which is similar to a previous study (Koma et al., 2016). Phytochemicals are beneficial to health and have been reported to have various pharmacological effects, including antioxidant activities (Xi et al., 2008; Erukainure et al., 2014). Flavonoids possess antioxidant activities and inhibit lipid peroxidation, which are essential for overall health and well-being (Heijnen et al., 2002; Boadi et al., 2021). Some flavonoids act as ligands of $GABA_A$, which is a known mitigating pathway for some antiepileptic drugs (Medina et al., 1997; Wasowski and Marder, 2012). Thus, the flavonoid extract of *T. tetraptera* is essential in this study.

The PTZ group showed a high seizure score, indicating the kindled state of the animals. Racine seizure scores of 4–5 are classified as tonic-clonic seizures (Ihara et al., 2016), which the present study showed. PTZ acts by inhibiting the $GABA_A$ receptor and, as such, regulating GABA activity (De Sarro et al., 1999), thereby enhancing glutamate neurotransmitters. PTZ also increases the binding, density, and concentrations of glutamate receptors (Ekonomou and Angelatou 1999), which may be another pathway of action in the present study.

The groups administered *T. tetraptera* ethanol, flavonoid extracts, and sodium valproate before PTZ showed significantly lower seizure scores, indicating their anti-seizure actions. The enhancement of GABA neurotransmission attenuates convulsions (Ogunrin *et al.*, 2015; Kukuia et al., 2016), indicating that *T. tetraptera* extracts may have acted at the $GABA_A$ receptor, where its flavonoid action has been suggested (Wasowski and Marder, 2012). The present result supports Paramdeep et al. (2014) and Ekong et al. (2021), who reported flavonoids enhance GABA transmission and neuroprotection against seizures, respectively. Sodium valproate, on the other hand, acts by enhancing GABA transmission (Cook and Besalem-Owen 2011) and may be the mechanism of action in the present study. Since PTZ kindling also increases the density of glutamate receptors (Ekonomou and Angelatou 1999), *T. tetraptera* extracts may also act through this pathway.

Neurocognitive changes evaluate the state of individuals (Sousa et al., 2020), where epilepsy is reported to compromise (Rayner et al., 2022). Several neurocognitive tests are applied to rodents to assess their brain health state and for drug development (Yang and Crawley, 2009). In the present study, the buried food test assessed the basic olfactory function of smell (Yang and Crawley, 2009; Reza et al., 2009; Lueptow, 2017). Most of the rats in the PTZ group were unable to locate the hidden food, and those that did spent significantly more time, implying altered olfaction. Olfaction is a limbic system function that is very critical for rodents' survival (Rymer, 2020), and epilepsy is implicated in its dysfunction (Khurshid et al., 2019; Motoki et al., 2021).

Most of the rats treated with *T. tetraptera* crude and flavonoid extracts or sodium valproate could locate the hidden food in less time compared with the PTZ-only group. This implies that the treatments may have preserved olfactory functions by suppressing PTZ actions. *T. tetraptera* has a wide range of protective actions in the brain (Ekong et al., 2021) and, as such, protection of the olfactory pathway. Sodium valproate protects the olfactory epithelium (Ogawa et al., 2014) and so may protect the brain areas involved in olfaction, which may have been involved in the present study.

Learning and memory are cognitive functions that aid fundamental processes (Vanderveren et al., 2017). Studying these cognitive functions in rodents includes the use of novel object recognition and spontaneous alternation tests. Although rodents may differ somewhat from humans, the conditions of the novel object recognition test mimic studies that involve human cognition (Lueptow, 2017). In the present study, the discrimination ratio/index, an analogue of recognition memory sensitivity in humans (Sivakumaran et al., 2018), was applied. The discrimination index for the PTZ group was negative, indicating a preference for the familiar object, and ultimately more time was spent on it (Sivakumaran et al., 2018).

Studies indicate that the integrity of the hippocampus and cerebral cortex is critical for the type of recognition memory (Buckmaster et al., 2004; Aggleton et al., 2010; Rajabzadeh et al., 2012), with the perirhinal cortex necessary for short retention time and the hippocampus for long-term object recognition (Reger et al., 2009). The glutamatergic pathway is involved in learning, memory, and cellular plasticity, and the imbalance of this causes spontaneous recurring seizures (Gasiorowska et al., 2021; Limón et al., 2021). These support the cognitive imbalances and seizure state of the PTZ group.

The groups administered *T. tetraptera* crude and flavonoid extracts, and sodium valproate had a positive discrimination index, indicating their preferences for the novel object and, as such, more time spent (Sivakumaran et al., 2018). The hippocampus and cerebral cortex, which are critical for recognition memory (Buckmaster et al., 2004; Aggleton et al., 2010), may have been protected by the interventions with *T. tetraptera* crude and flavonoid extracts and sodium valproate.

Spontaneous alteration is exploited to test spatial working memory in the Y-maze, which is hippocampal-dependent (Kraeuter et al., 2019). Rats with normal working memory remember arms previously visited and demonstrate a preference for less frequented arms (Lalonde, 2002; Kraeuter et al., 2019). The PTZ group showed no significant difference in spontaneous alternations, indicating that this working memory may not have been altered. This result is in line with Lamberty and Klitgaard (2000), who reported the non-alteration of this form of working memory.

There was no significant difference in spontaneous alternations in the test groups administered *T. tetraptera* crude and flavonoid extracts compared to the control. However, there were significantly ($p < 0.05$) higher spontaneous alternations in the groups administered *T. tetraptera* crude and flavonoid extracts but not sodium valproate when compared to the PTZ group. Rats with normal working memory remember arms previously visited and demonstrate a preference for less frequented arms (Lalonde, 2002; Kraeuter et al., 2019). This implies that the extracts enhanced the discrimination of the familiar Y-

maze arms visited previously, making attempts to enter the unfamiliar arm, thus improving spatial working memory (Kraeuter et al., 2019).

The elevated plus maze is used in the study of anxiety-like behaviour in rodents (Walf and Frye, 2007). In the present study, there was aversion for the open arms and less grooming frequency, but not in the closed arms and rearing frequency, by the rats in the PTZ group compared with the control, indicative of anxiety (Walf and Frye, 2007; Estanislau et al., 2019). This result is unsurprising, as PTZ elicits anxiety (Jung et al., 2002).

The time spent in the open arms by the *T. tetraptera* crude, flavonoid extracts, and sodium valproate groups was not different from the control but was significantly more compared with the PTZ group, indicating the anxiolytic effect. There seems to be a link between the facilitation of GABA release and catecholamine inhibition, leading to an anxiolytic effect (Bourin, 2021; Monti and Liebowitz, 2022). This pathway has also been associated with sodium valproate's anxiolytic actions (Bach et al., 2018). *T. tetraptera* crude and flavonoid extracts may have also utilised the same.

Rearing indicates exploration behaviour (Ennaceur, 2014), although it also measures anxiety. This is unreliable depending on whether the animals had maze wall support or not (Ennaceur, 2014; Seibenhener and Wooten, 2015). In the present study, there was no significant difference in the rearing frequency of the PTZ group compared with the control, indicating indifference in exploratory behaviour. There was, however, significantly more rearing frequency in the *T. tetraptera* crude and flavonoid extracts, as well as the sodium valproate groups, compared with the control and PTZ groups, indicating high exploratory behaviour.

The beam walk is a useful test for fine motor coordination and balance. It detects subtle deficits in motor skills and balance (Luong et al., 2011). The beam walk score and latency of the PTZ group were significantly lower compared with the control, indicating motor control impairment. Impairment of motor coordination is reported with PTZ-kindling (Mishra et al., 2021).

The beam walk scores and latency latencies of the *T. tetraptera* crude extract and flavonoid groups were not different from the control, indicating the protective role of these extracts on cerebellar function. Ekong et al. (2021) reported a protective action of *T. tetraptera* crude extract on motor coordination and cerebellar structure, which the present result supports.

Epilepsy is linked to oxidative stress in the brain, which disrupts electrical activities in neurons, leading to seizures (Borowicz-Reutt and Czuczwar, 2020; Lin et al., 2020). MDA level or SOD activity, which are indicators of lipid peroxidation and oxidative stress, respectively, are some of the biochemical parameters in epilepsy evaluation (Deepaare et *al.*, 2008; Shehta et al., 2022). In the present study, there was a significantly higher MDA level in the PTZ group compared to the control, indicating their high lipid peroxidation state.

MDA is a product of lipid peroxidation and has toxic effects on cellular lipid composition. Lipid peroxidation and oxidative stress are pathways by which oxidative stress affects the brain, leading to glutamine synthesis, abnormal accumulation of glutamate, and glutamate receptor activation (Ziylan and Ates, 1989; Oliver et al., 1990; Patel, 1996). Lipid peroxidation may also alter neuronal membrane and myelin sheath functions (Ravera and Panfoli, 2015), leading to neuronal hyper-excitability in seizures. The present result is in agreement with the high MDA level associated with epilepsy (Donmezdil et al., 2016; Keskin Guler et al., 2016).

On the other hand, the MDA levels were not significantly different in the *T. tetraptera* crude extract and flavonoid groups compared with the control, indicating the antioxidant actions of these compounds, which the phytochemical screening and analyses have shown. The MDA concentrations of the *T. tetraptera* crude extract and flavonoid, as well as the sodium valproate groups, were significantly less compared with the PTZ group, supporting the protective antioxidant actions of these compounds.

SOD is an important natural antioxidant in the body, playing a crucial role in the elimination of superoxide anion radicals (Keskin Guler et al., 2016). There was a significantly lower SOD level in the PTZ group compared with the control, indicating their high oxidative stress status. SOD naturally prevents lipid peroxidation and oxidative stress, a cause of excitotoxicity (Patel, 1996; Ighodaro and Akinloye, 2018), as observed in epilepsy.

There was a significantly higher SOD level in the *T. tetraptera* crude extract group compared with the PTZ but not the control, indicating its antioxidant ability. *T. tetraptera* fruit extract is reported to have antioxidant properties (Moukette et al., 2015; Joel et al., 2017). Thus, the incremental SOD activity in the *T. tetraptera* crude extract group is probably through the conversion of superoxide radicals to a less toxic hydrogen peroxide and an associated reduction in the accumulation of superoxide radicals (Lorigados Pedre et al., 2018).

There were significantly lower SOD levels in the *T. tetraptera* flavonoid extract and sodium valproate groups compared with the control, but not in the PTZ groups, indicating the inability of these compounds to prevent oxidative stress. Flavonoids generally possess antioxidant activities; however, this may not have been adequate, suggesting additional components for *T. tetraptera* ethanol extract's antioxidant role. Sodium valproate also possesses antioxidant ability (Beltrán-Sarmiento et al., 2018), which may not have been adequate in the present study.

Glutamate is a neurotransmitter required for neuronal excitability and is implicated in epilepsy excitotoxicity (Pal, 2021). However, its level is modulated by GDH, an enzyme that catalyses the reversible conversion of glutamate to alpha-ketoglutarate and ammonia (Plaitakis et al., 2017). In the present study, there was no significant difference in the GDH activity in the PTZ group compared with the control, indicating normal metabolism of glutamate. PTZ enhances glutamate through $GABA_A$ receptor inhibition (De Sarro et al., 1999), and as such, GDH may not have been involved.

There was no significant difference in GDH activity in groups administered either *T. tetraptera* crude and flavonoid extracts or sodium valproate compared with the control and PTZ groups, indicating normal metabolism of glutamate. These results suggest that the actions of these intervention compounds may not be through the glutamate pathway. Sodium valproate acts through the enhancement of GABAergic transmission (Cook and Besalem-Owen 2011), and since flavonoids possess anticonvulsant properties through GABA enhancement, this pathway has been utilised in the *T. tetraptera* fruit extracts.

Conclusion

PTZ elicited seizure, reduced olfaction, and motor coordination, compromised memory, and stimulated anxiogenic, and oxidative stress. The *T. tetraptera* crude and flavonoid extracts suppressed seizure, improved olfaction, and memory, and reduced anxiety and brain oxidative stress. *T. tetraptera* crude and flavonoid extracts results were similar to the standard anticonvulsant drug, SV, although the crude extract had a better outcome.

Declarations

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Competing Interests

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

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Nelly A. Pessu, Godslove V. Kpobari, Ekereobong I. Okuku, Moses B. Ekong and Okokon O. Bassey. The first draft of the manuscript was written by Okokon O. Bassey and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analysed during the current study are not publicly available as there is no online database for such. However, they are available from the corresponding author on reasonable request.

Ethics Approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Faculty of Basic medical Sciences Research and Ethics Committee of University of Uyo with

reference number: UU/FBMSREC/2022/001.

Consent to Participate

Not applicable

Consent for Publication

Not applicable

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Figures

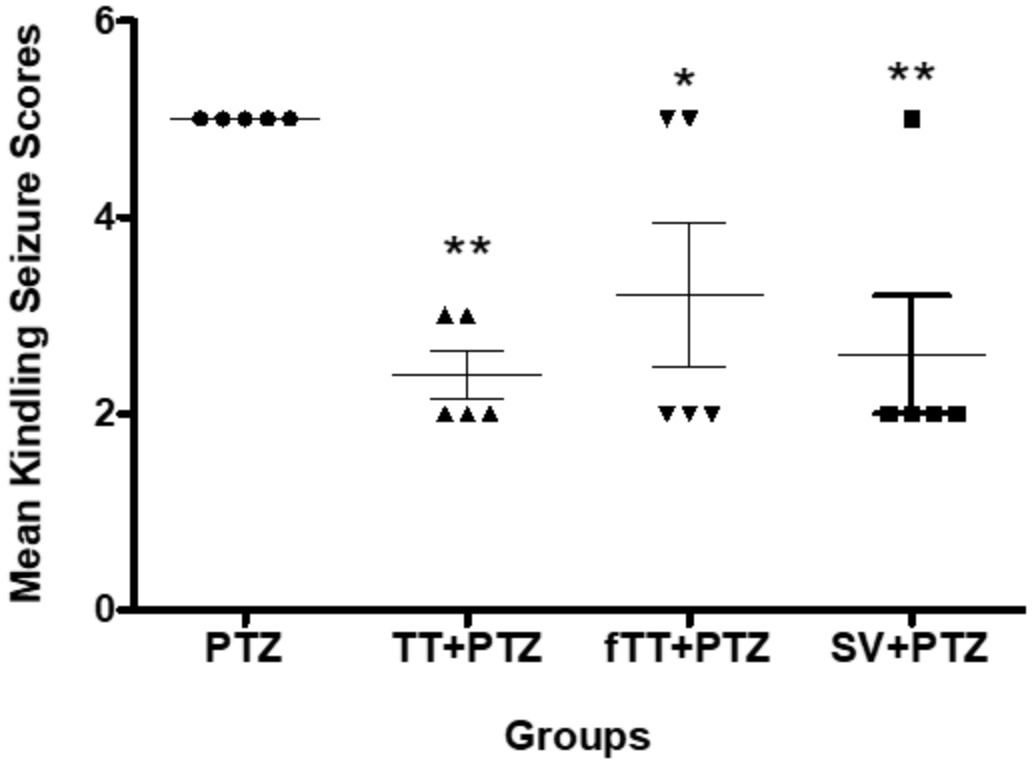


Figure 1

***T. tetraptera* fruit extracts decreased seizure scores.** Data are presented as Mean ± SD; n = 5; ANOVA; [F(3, 16) = 5.83, P =.007]; ** - Significantly difference with the PTZ group at p < 0.01; * - Significantly different with the PTZ group at p < 0.05; PTZ – pentylenetetrazol; TT - *Tetrapleura tetraptera*; fTT - flavonoid extract of *Tetrapleura tetraptera*; SV – sodium valproate

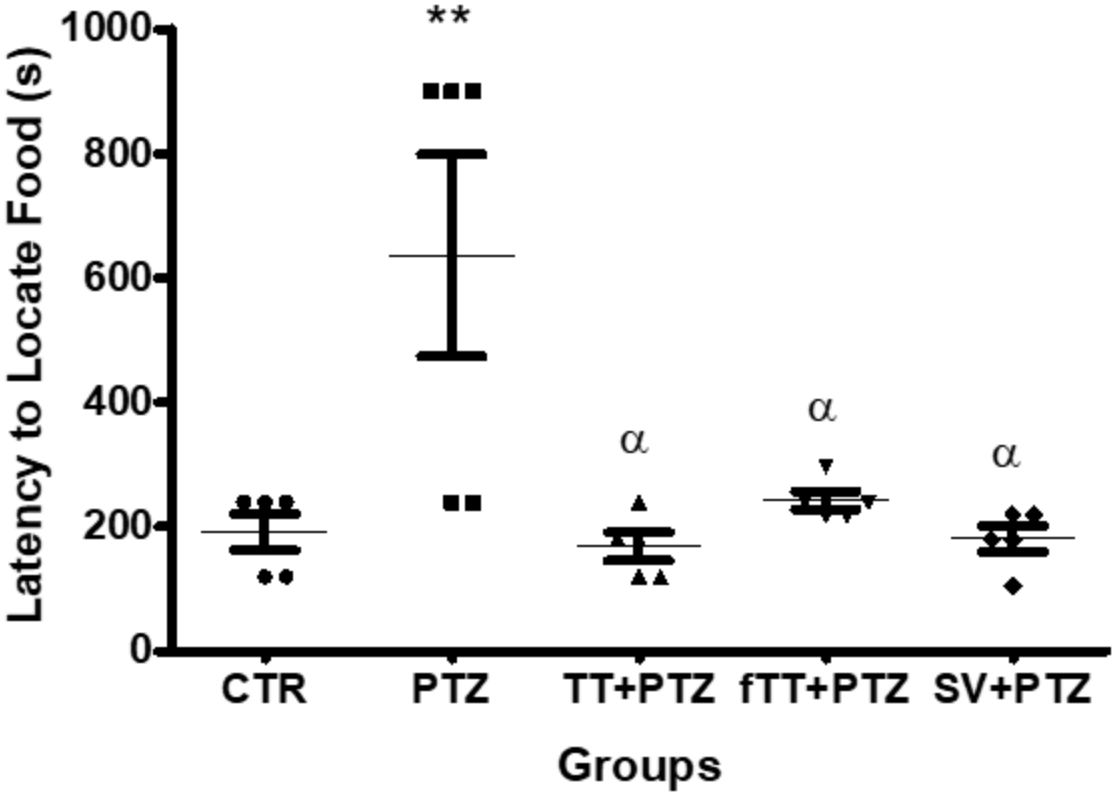


Figure 2

***T. tetraptera* fruit extracts enhanced the latency to locate the buried food.** Data are presented as Mean ± SD; n = 5; ANOVA; [F(4, 20) = 7.02, P =.001]; ** - Significantly different with the control at p < 0.01; α - Significantly different with the PTZ at p < 0.05; CTR – control; PTZ – pentylenetetrazol; TT - *Tetrapleura tetraptera*; fTT - flavonoid extract of *Tetrapleura tetraptera*; SV – sodium valproate

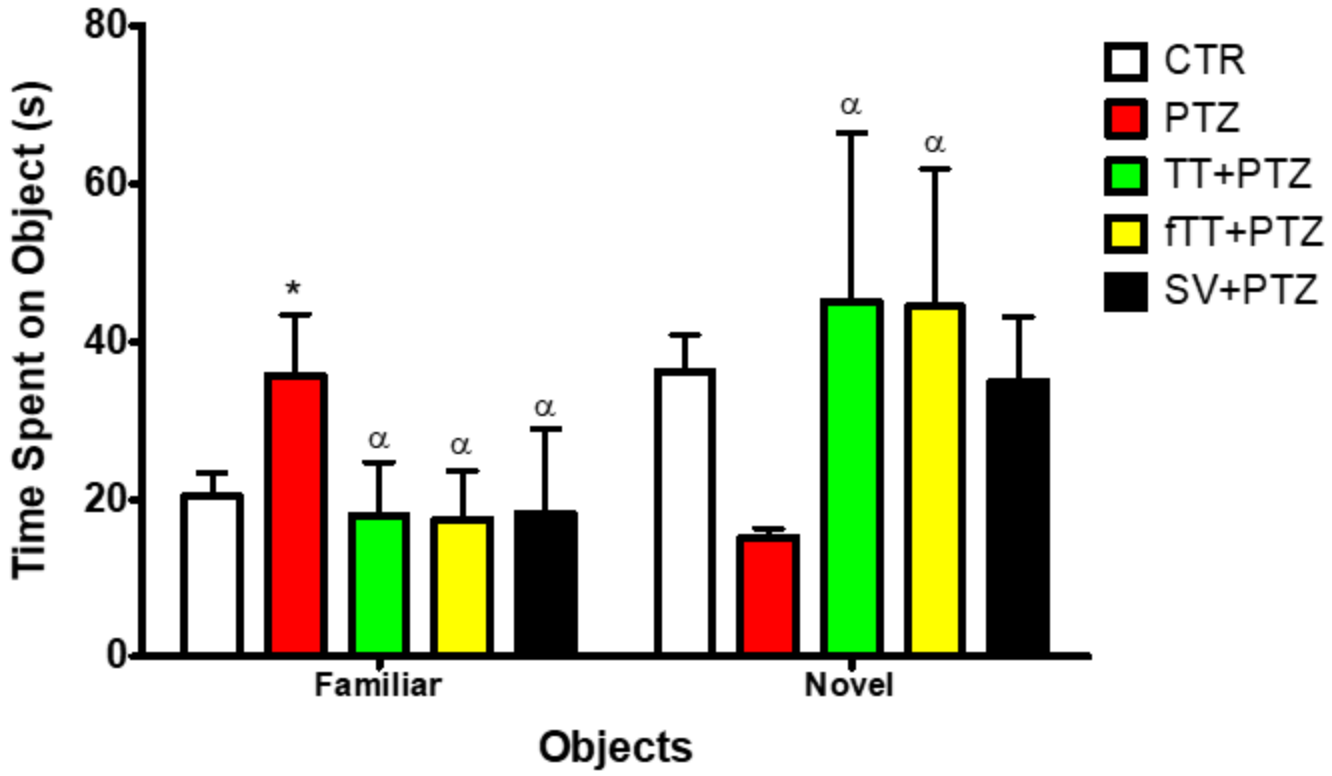


Figure 3

***T. tetraptera* fruit extracts improved novel object recognition/exploration.** Data are presented as Mean ± SD; n = 5; ANOVA; Familiar object [F(4, 20) = 5.66, P =.003]; Novel object [F(4, 20) = 4.27, P =.012]; * - Significantly different with the control at p < 0.05; α - Significantly different with the PTZ group at p < 0.05; CTR – control; PTZ – pentylenetetrazol; TT - *Tetrapleura tetraptera*; fTT - flavonoid extract of *Tetrapleura tetraptera*; SV – sodium valproate

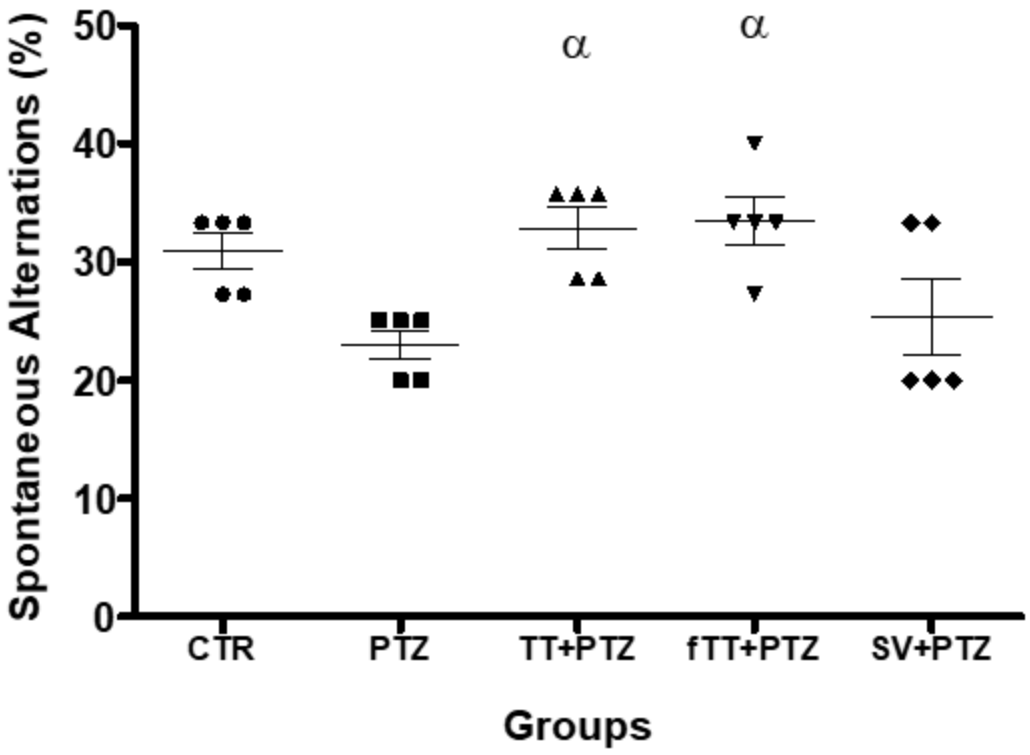


Figure 4

***T. tetraptera* fruit extracts improved spontaneous alternations in the Y-maze.** Data are presented as Mean ± SD; n = 5; ANOVA; [F(4, 20) = 5.12, P =.005]; α - Significantly different with the PTZ at $p < 0.05$; CTR – control; PTZ – pentylenetetrazol; TT - *Tetrapleura tetraptera*; fTT - flavonoid extract of *Tetrapleura tetraptera*; SV – sodium valproate

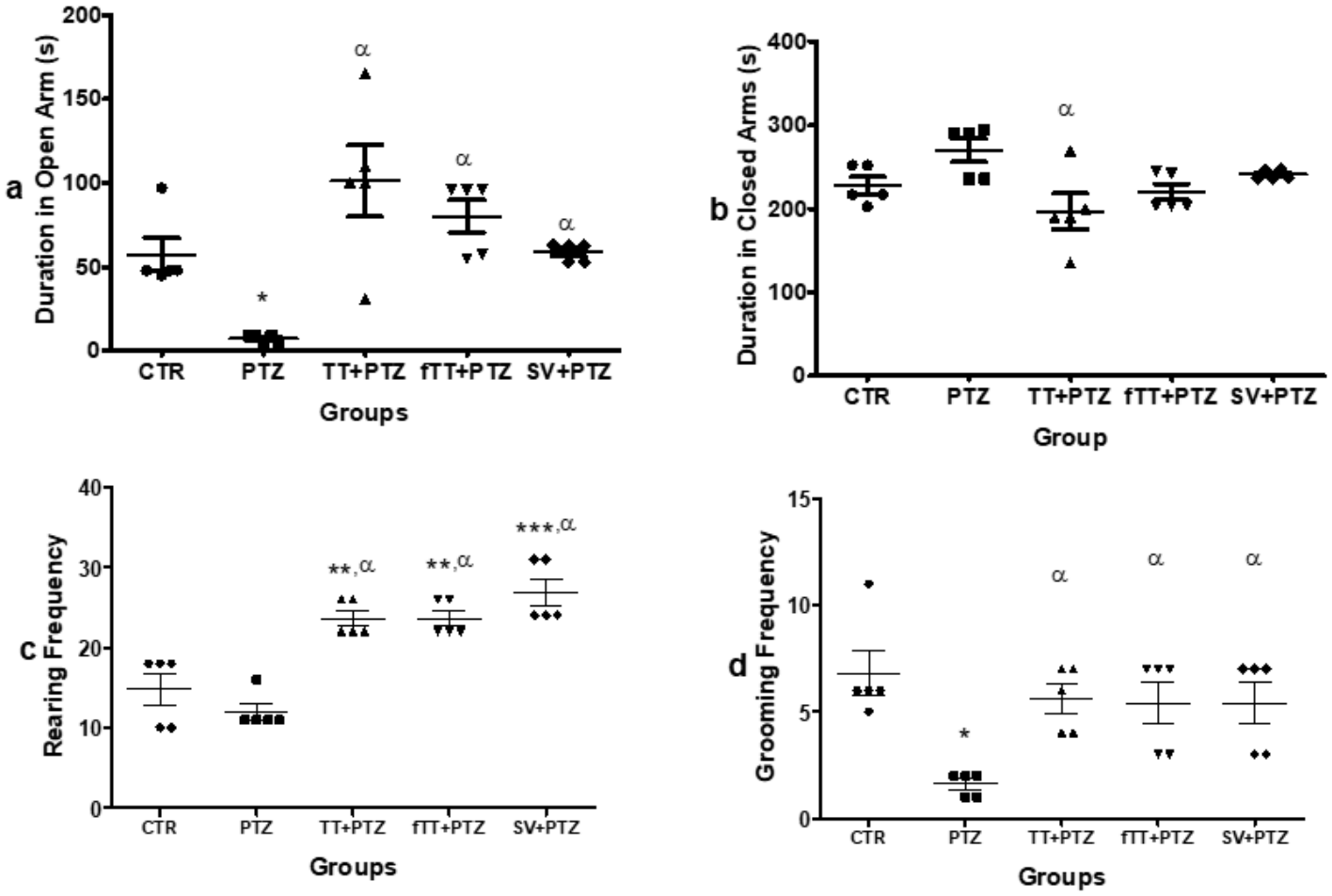


Figure 5

***T. tetraptera* fruit extracts showed anxiolytic abilities in the elevated plus maze. a. Duration in open arm [F(4, 20) = 9.32, P =.0002]; b. Duration in closed arm [F(4, 20) = 4.29, P =.012]; c. Rearing frequency [F(4, 20) = 21.01, P =.0001]; d. Grooming frequency [F(4, 20) = 5.40, P =.004];** Data are presented as Mean ± SD; n = 5; ANOVA; *** - Significantly different with the control at p < 0.001; ** - Significantly different with the control at p < 0.01; * - Significantly different with the control at p < 0.05; α - Significantly different with the PTZ group at p < 0.05; β - Significantly different with the sodium valproate group at p < 0.05; CTR – control; PTZ – pentylenetetrazol; TT - *Tetrapleura tetraptera*; fTT - flavonoid extract of *Tetrapleura tetraptera*; SV – sodium valproate

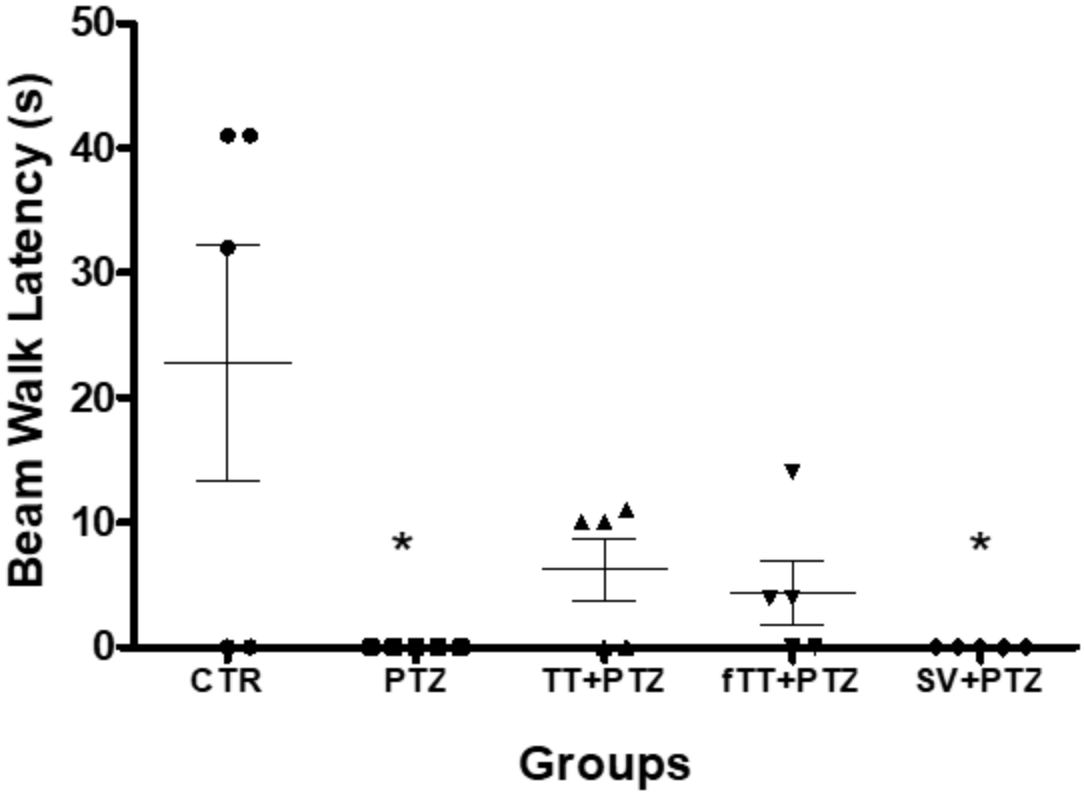


Figure 6

***T. tetraptera* fruit extracts did not affect the beam walk behaviour.** Data are presented as Mean ± SD; n = 5; [F(4, 20) = 4.33, P =.011]; ANOVA; * Significantly different with the control at $p < 0.05$; CTR – control; PTZ – pentylenetetrazol; TT - *Tetrapleura tetraptera*; fTT - flavonoid extract of *Tetrapleura tetraptera*; SV – sodium valproate

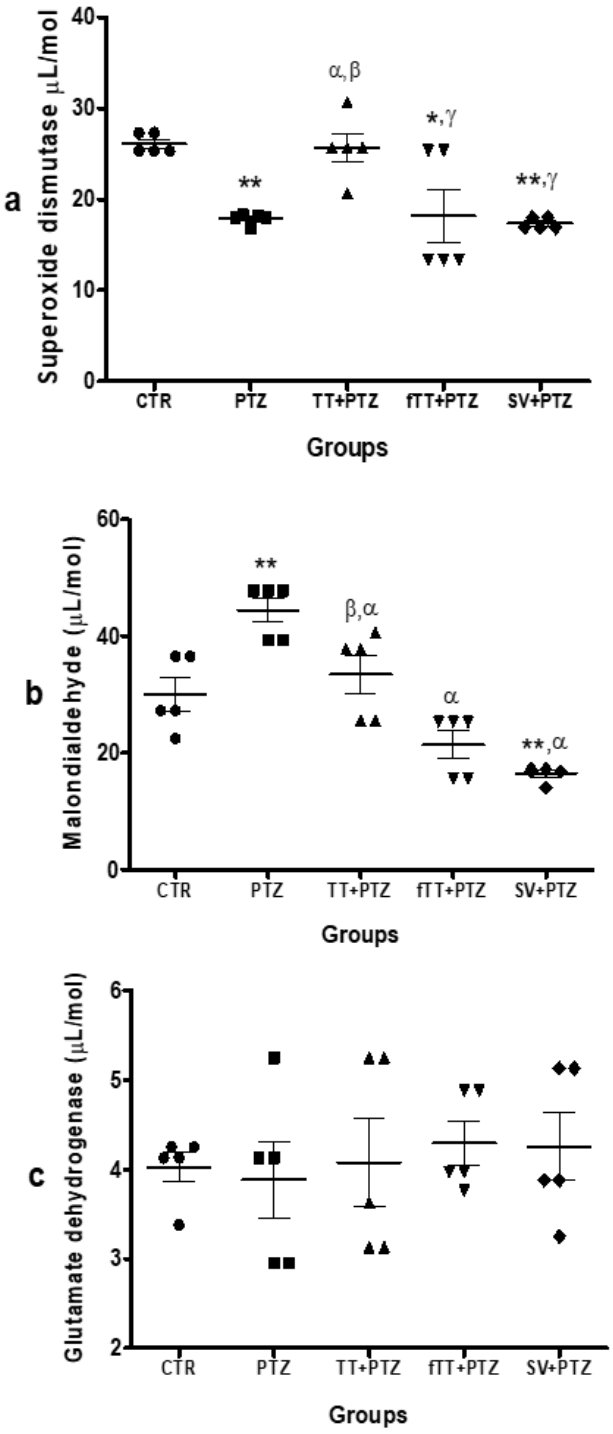


Figure 7

T. tetraptera **fruit extracts improved brain antioxidant concentration and enzyme activities. a. Superoxide dismutase concentration [F(4, 20) = 8.59, P =.0003]; b. Malondialdehyde [F(4, 20) = 20.60, P =.0001]; c. Glutamate dehydrogenase [F(4, 20) = 0.22, P =.925].** Data are presented as Mean ± SD; n = 5; ANOVA; ** - Significantly different with the control at p < 0.01; * - Significantly different with the control at p < 0.05; α - Significantly different with the PTZ group at p < 0.05; β - Significantly different with the sodium valproate

group at $p < 0.05$; CTR – control; PTZ – pentylenetetrazol; TT - *Tetrapleura tetraptera*; fTT - flavonoid extract of *Tetrapleura tetraptera*; SV – sodium valproate