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Antiepileptic Property of *Tetraponera aethiops* Venom in Pilocarpine-picrotoxin Model of Temporal Lobe Epilepsy

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Abstract

Aims: The present is undertaken to evaluate the antiepileptic properties of Tetraponera aethiops venom in mice pilocarpine-picrotoxin model of temporal lobe epilepsy and to investigate the mechanisms of action.

Methodology: A total of seven groups of six mice were prepared. One hour before the induction of status epilepticus through intraperitoneal injection of pilocarpine (360 mg/kg), all groups with the exception of group one and two received the different doses (0.1, 0.25, 0.05 and 0.1 mg/kg) of the Tetraponera aethiops venom while group one and two received distilled water. Twenty-four hours later the anticonvulsant effects of the crude venom were evaluated after the intraperitoneal injection of a sub-convulsive dose of picrotoxin. The animals received their various treatments for seven days. One hour after the last treatment on day seventh day, they were sacrificed and brain of each mouse was dissected and hippocampi isolated. The levels of malondialdehyde, reduced glutathione, gamma aminobutyric acid and the activity of gamma aminobutyric acid transaminase were determined in the homogenates of hippocampus. Results: Tetraponera aethiops venom showed a significant increase in the latency time of tonic-clonic seizures ($P<0.001$) in mice, as well as a reduction in the number ($P<0.001$) and duration ($P<0.001$) of seizures. A significant effect was seen as the crude venom could reverse the alteration of the oxidative stress makers and GABAergic neurotransmission. Conclusion: Findings from this study showed that Tetraponera aethiops venom ameliorated pilocarpine-picrotoxin-induced temporal lobe epilepsy through enhancement of antioxidant defence mechanism and amelioration of GABAergic transmission.

Keywords: *Tetraponera aethiops*; Venom; Antiepileptic; Temporal lobe epilepsy; Antioxidant; GABAergic

1. Introduction

Epilepsy is a chronic brain disorder characterized by abnormal electrical activity of cerebral neurons. It affects about 1% of the world population [1, 2], and it is the most widespread neurological disorder in Africa, affecting an estimated 25 million people [3]. Temporal lobe epilepsy, the most prevalent form of epilepsy, is often linked to behavioural alterations, such as anxiety, depression, and cognitive impairments. It is established that during human temporal lobe epilepsy, recurrent seizures are correlated with decrease in brain GABA concentration, increase in the activity of GABA-transaminase, and decrease in L-glutamate decarboxylase activity within cerebrospinal fluid and hippocampal tissue [4, 5]. Among other biochemical processes involved in the refractoriness of kindling and kindled seizures in patients with temporal lobe epilepsy, generation of reactive oxygen species is considered as one of the leading causes of recurrent and spontaneous seizures [6, 7]. In fact, recurrent seizures result in overproduction of mitochondrial superoxide radicals, which alter protein function, permeability of membrane, and expression of genes in the central

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nervous system. In spite of newly introduced antiepileptic drugs, about one-third of patients with temporal lobe epilepsy are pharmacoresistant [8-10]. Indeed, existing antiepileptic drugs are merely symptomatic and do not prevent underlying natural history of epilepsy or associated comorbidities. Current antiepileptic drugs used against temporal lobe epilepsy neither provides a cure nor prevent relapse. In addition, antiepileptic drugs cannot stop the neurodegeneration neither reverse apoptosis and necrosis of neurons. Therefore, it is urgent to develop disease-modifying therapies with curative effects.

Natural products, sources of new pharmacological substances, have large chemical diversity and architectural complexity. Toxins obtained from insects' venom has indicated neuroprotective properties in animal models of brain disorders. Venom from the tropical ant, *Pseudomyrmex triplarinus,* has demonstrated anti-inflammatory properties in rodent carrageenin model of oedema [11]. A partially purified extract prepared from the insects' venom from South American tree ant *Pseudomyrmex sp.* was tested in a double-blind, controlled study of patients with rheumatoid arthritis. Venom treated patients demonstrated an improvement in global efficacy and a decrease in the number of tended painful joints and swollen joints [12]. Previous studies have demonstrated the anticonvulsant effects of toxins isolated from invertebrate venoms. The peptide fraction (350 μg/animal) isolated from the venom of the wasp *Polybiapaulista* protected 60% of the rats against generalized tonic-clonic seizures induced by pentylenetetrazol [13]. Recently, studies have shown that the denatured venom of the ant *Dinoponera quadriceps* protects mice against bicuculline-induced convulsions and death [14, 15]. Recent study showed that melittin (MEL) which is an active compound of bee venom protected 90% of MEL-treated bicuculline-induced seizure rats [16]. Furthermore, parawixin 11 (isolated from the venom of the spider *Parawixia bistriata*) protected animals against seizures induced by pentylenetetrazol, picrotoxin, pilocarpine, and kainic acid [17]. *Tetraponera aethiops* is an arboreal plant-ant involved in an obligatory mutualistic association with the myrmecophyte Barteria fistulosa (Passifloraceae), myrmecophytes being plants sheltering colonies of specialized "plant-ant" species in hollow structures called domatia [18, 19]. Previous study revealed that the *Tetraponera aethiops* venom peptidome contains twelve venom peptides, among which nine were identified through the combination of transcriptomic and proteomic data [20]. *Tetraponera aethiops* venom peptides, which are either linear or dimeric, possess substantial sequence identities with myrmeciitoxins previously described as both pain-inducing and insecticidal. It has been reported that the aqueous extract of *Tetraponera aethiops* venom is used orally for the treatment of epilepsy, inflammation, pain, anxiety and memory impairment, by traditional healers form the tropical region in Africa. However, very little research has been done on biological activity of *Tetraponera aethiops* venom. Taken together, these findings demonstrate that the tropical ants (*Tetraponera aethiops*) venom is a potential source of new anticonvulsants molecules. Therefore, we hypothesised that *Tetraponera aethiops* crude venom could antagonise *status epilepticus*, epileptogenesis, epileptic seizures and anxiety behaviour in a pharmaco-resistant model of epilepsy. The overall objective of this research was to evaluate the antiepileptic and anxiolytic properties of *Tetraponera aethiops* crude venom in pilocarpine-picrotoxin model of temporal lobe epilepsy, and to investigate the effects of its effects on some parameters of oxidative and nitrosative stresses, and GABAergic transmissions.

2. Material and methods

2.1. *Tetraponera aethiops* collection and authentication

Live specimens of *Tetraponera aethiops* were collected in Ekonjo (coordinates 4° 03′ 53′′ N and 9° 09′ 52′′ E, altitude: 540 M), Fako Division, in the South West region of Cameroon. These ants used for this study has been classified under the subfamily *Pseudomyrmecinae*. The colonies were immediately placed into plastic boxes whose walls were coated with Fluon® to prevent the workers from climbing out.

2.2. Preparation of the crude extracts of *Tetraponera aethiops* venom

Venom was obtained from two thousand ants by dissection of their abdomens. After each ant had been killed, the sting and venom glands were pulled from the abdomen with a forceps under the low magnification of a dissecting microscope. The venom of *Tetraponera aethiops* was obtained by holding the isolated abdomen of the ant by the petiole and collecting the venom issuing from the everted sting in microcapillaries or in the depression of a microscope slide. The venom was collected with sterile saline (4°C) after a number of glands had been compressed, and the liquid was filtered with a porous paper filter to separate pieces of gland. The pooled venom was filtered through a 0.45 ft sterile filter unit (Nalgene, Rochester, US), and stored in small aliquots in glass tubes at -70°C. They were then centrifuged for 5 min at 14,400 rpm, 4°C, to pellet empty reservoirs and membranes, and finally lyophilized to obtain a crude extract of *Tetraponera aethiops*. To determine the dry venom weight per ant, the venom from 100 ants was pooled, dried and weighed (15.03 mg) using a microscale. To evaluate the biological activity of the crude venom of *Tetraponera aethiops* a series of sequential dilutions with distilled water were performed, and several concentrations where obtained. The extract was prepared 30 minutes to 1 hour before its administration to mice, and was administered orally (*per os* (*p.o.*))

1 hour before the pharmacological test, at a volume of 10 mL/kg of body weight. The doses were obtained from the main dose used by the traditional practitioner and the concentration (initial concentration; Ci = mass/volume) was obtained from calculation, knowing that the volume of administration is 10 mL/kg.

The initial concentration was prepared by introduction of 25 mg of crude extract in 250 mL distilled water, and the corresponding concentration was Ci = 1 mg/10 mL = 0.1 mg/mL. The three other concentrations were prepared by dilution of the initial concentration at ½, ¼, and 1/10, respectively. The respective concentrations were 0.1 mg/kg, 0.25 mg/kg, 0.5 mg/kg and 1 mg/kg. The doses were calculated following the formula: Dose = Ci × volume of administration; with: volume of administration = 10 mL/kg (Ci: initial concentration; Dose i = Ci × volume of administration; Dose 1 = 0.1 mg × 10 mL = 1 mg/kg: Dose 2 = 0.05 mg × 10 mL = 0.5 mg/kg (or Dose i/2); Dose 3 = 0.025 mg × 10 mL = 0.25 mg/kg (or Dose i/4); Dose 4 = 0.01 mg × 10 mL = 0.1 mg/kg (or Dose i/10).

In fact, the following concentrations were used: 0.01, 0.025, 0.5 and 0.1 mg/mL respectively for the doses of 0.1, 0.25, 0.05 and 0.1 mg/kg. Oral administration of the venom extract was performed using a non-flexible gavage needle with round end, fixed at the extremity of a 1 mL syringe with respect to the volume of administration 10 mL/kg.

2.3. Chemicals and reagents

In this study, we used sodium valproate (depakine Chrono®) from SANOFI, and diazepam (valium Roche®) from ROCHE as positive control drugs respectively for antiepileptic test, and anxiolytic test. Methyl scopolamine, reduced glutathione, thiobarbituric acid, n-butanol, pyridine, sodium dodecyl sulphate, 5′5-dithiobis (2-nitrobenzoic acid), pilocarpine, trichloroacetic acid, Griess reagents and all the other reagents used for the evaluation of oxidative stress markers (MDA GSH) and the brain γ-aminobutyric acid content estimation are obtained from Sigma, St. Louis, MO, USA.

2.4. Experimental animals and ethical consideration

In this study we used 48 adult male mice (*Mus musculus* Swiss, weighing 20–25 g). Animals were house in standard cages under ambient conditions, with unlimited access to food and water. Each animal was used only once. All experiments were performed according to the Guide for the Care and Use of Laboratory Animal published by the United States National Institutes of Health (NIH publication No. 85-23, revised 1996). All efforts were made to minimize animal suffering and reduce the number of animals used.

2.5. Pharmacological testing

2.5.1. Acute pilocarpine-induced status epilepticus test

Swiss mice Mus musculus were used for the acute pilocarpine-induced *status epilepticus*. They were randomly grouped in different groups of six mice each and administered the following treatment: group 1 (normal control group) and 2 (negative control group) received orally 10 mL/kg of distilled water; groups 3 - 6 received, orally different doses of *Tetraponera aethiops* crude venom (test groups; 0.1, 0.25, 0.05 and 0.1 mg/kg); and group 7, received sodium valproate (positive control group 1; 300 mg/kg). Forty minutes after the first administration of the different treatments to mice, they were injected intraperitoneal with N-methylscopolamine at a dose of 1 mg/kg to lower the peripheral effects of pilocarpine. Twenty minutes after the injection of N-methyl-scopolamine, *Status epilepticus* was induced in each mouse by acute intraperitoneal injection of 360 mg/kg pilocarpine [21, 22]. Mice from the normal control group named group 1 have not received N-methylscopolamine and pilocarpine, however they received saline. Immediately after the intraperitoneal injection of pilocarpine, each animal was returned to its cage, and their behaviour were observed for a 6-hour duration. Severity and duration of acute epileptic seizures were categorised based on the Racine scale [23]. Hypoactivity followed by behavioural changes (orofacial modifications, twitching of vibrissae, yawning, salivation, eye blinking and toni-clonic seizures) were observed in the studied animals. During the experiment, animals were video-monitored for the appearance of *status epilepticus*. The severity of seizures was assessed using the Racine scale: stage 0: no response; stage 1: hyperactivity and clonus of vibrissae; stage 2: shaking of the head and myoclonic jerks; stage 3: unilateral clonus of the forelimbs; stage 4: rearing and bilateral clonus of the forelimbs; stage 5: tonic-clonic seizures with loss of the righting reflex. Animals were selected for further studies (day-second) based on the development of stage 5 attack of the Racine scale for two consecutive periods [24].

2.5.2. Anticonvulsant properties of Tetraponera aethiops crude venom during the acute phase of temporal lobe epilepsy induced by picrotoxin in pilocarpine-treated mice

The test was carried out according to the method previously described with slight modifications [25]. Picrotoxin was used at a dose of 1 mg/kg to induce *excitus* and convulsions in pilocarpine-treated mice 24 hours after the first injection of pilocarpine. This procedure was done to facilitate the occurrence of neuropathology in mice. Briefly, animals were

treated for the second day, or twenty-three hours after the injection of pilocarpine, with distilled water for group 1 and 2, the respective doses of the venom for group 3 – 6, and sodium valproate for group 7, respectively. One hour later, a sub-convulsive dose of picrotoxin (1 mg/kg) was injected intraperitoneally to mice (groups 2 to 7), except group 1 that was injected intraperitoneally with saline. Each animal was observed immediately for a period of 30 min, and the incidence of seizures (the latency time to first tonic-clonic seizure, number and duration of tonic-clonic seizures and the number and duration clonic seizures) were noted [22, 25]. Tonic-clonic seizures involve both tonic (a sudden stiffness or tension in the muscles of the arms, legs or trunk) and clonic (twitching or hock-like jerks of a muscle or a group of muscles) phases of muscle activity. The latency of tonic-clonic seizures was used to determine the seizure score. This score was calculated according to the following formula: Score = 1- negative control group latency/test group or positive control group latency [26].

2.5.3. Biochemical analysis

Evaluation of brain GABA concentration and determination of GABA-transaminase activity.

An hour later, animals were sacrificed and their hippocampus were sampled, weighted and used for the biochemical analyses. Ninhydrin reacts with succinic semialdehyde acid to form a complex, the absorbance at 512 nm wavelength of which is proportional to the amount of GABA. The amount of GABA in the homogenates was assessed by the colorimetric technique [27]. The amount of GABA in the homogenate was determined using GABA standards (50, 100, 150, 200, 250, 300, 350, and 400 µg), each mixed with 1.5 mg of glutamate dissolved in 2 mL of TCA (10%). The concentration of GABA was expressed in µg/g of tissue [28].

The coloration of the complex generated by succinic semialdehyde acid and 3-methyl-2-benzothia-zolone-2-hydrazone in the presence of 12% $FeCl_3$ is proportional to the activity of GABA-transaminase (GABA-T) in the homogenate [29]. The amount of semialdehyde succinic acid generated during the incubation was quantified against the blank at 610 nm by a spectrophotometer at 30 and 90 seconds. The GABA-T activity was expressed in µg/min/mg of tissue [30-32].

Quantification of nitrosative and oxidant stress markers

Lipid peroxidation level in the hippocampus homogenates was measured through the thiobarbituric assay [33, 34]. The level of malondialdehyde **(**MDA) expressed in µmol/g of tissue was calculated using Beer Lambert's formula and the extinction coefficient of 1.6×10^5 M/cm.

Glutathione was measured using the Ellman Method [35]. GSH was reacted with 5, 5′-dithio-bis-2-nitrobenzoic acid (Ellman's reagent) generating a yellow chromophore which was measured at 412 nm using a UV spectrophotometer and the concentration of glutathione was expressed in mol/g of tissue.

2.6. Statistical analysis

Eight group of animals were used in this study (seven groups for the anticonvulsant test, and one group of animals was added during the behavioural testing). Results were expressed as means ± standard error of mean (SEM), for six animals per group; statistical differences between controls and treated groups were tested by a one-way analysis of variance (ANOVA), followed by Dunnet multiple comparison test. The differences were considered significant at $P<0.05$. Statistical analyses were performed using GraphPad prism 9.0.0 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Effects of *Tetraponera aethiops* crude venom on the latency to *status epilepticus*

As shown on Figure 1, results obtained from one way ANOVA demonstrated a significant effect of *Tetraponera aethiops crude venom* oral administration and pilocarpine injection on the latency to status epilepticus [$F(6, 35) = 47.86$, $P < 0.001$]. Dunnett's multiple comparison test demonstrated that *Tetraponera aethiops* crude *venom* significantly increased the latency to status epilepticus from 17.88±1.10 min in the pilocarpine-treated distilled water group (disease group or negative control group) to 33.91± 4.22 min ($P < 0.001$) and 39.46±6.96 min ($P < 0.001$) in the *Tetraponera aethiops crude venom* test groups at the respective doses of 0.5 and 1 mg/kg. Also, latency of status epilepticus increased to 43.65± 8.76 min ($P < 0.001$) with sodium valproate relative to the disease group (Figure 1).

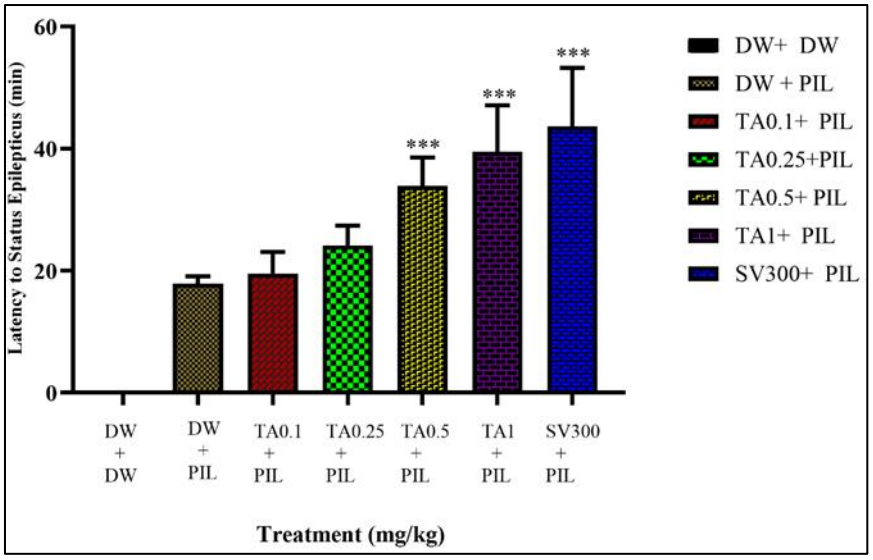

Figure 1 Effects of *Tetraponera aethiops* crude venom on the latency to status epilepticus

Results are expressed as mean ± S.E.M. n= 6 animals. Statistical differences were tested by a one-way ANOVA, followed by Dunnett's multiple comparison test, the differences were considered significant at $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. DW, distilled water; TA, *Tetraponera aethiops* crude venom (0.1 mg/kg); PIL, pilocarpine (360 mg/kg); SV300, sodium valproate (300 mg/kg).

3.2. Effects of *Tetraponera aethiops* crude venom on latency to first tonic-clonic seizure 24 hours after status epilepticus in pilocarpine-picrotoxin treated mice

The oral administration of *Tetraponera aethiops* crude venom and sodium valproate showed a significant increase in the latency to first-tonic seizures 24 hours after status epilepticus in pilocarpine-picrotoxin treated mice. Analysis of one-way ANOVA indicated a significant effect of *Tetraponera aethiops* crude venom oral administration in pilocarpine-picrotoxin treated mice on the latency to first tonic-clonic seizures [$F(6, 35) = 85.17$, $P < 0.001$]. Dunnett's test demonstrated that *Tetraponera aethiops* crude venom significantly increased latency to first tonic-clonic seizures from 44.67±5.65 sec in the pilocarpine-picrotoxin treated distilled water group (disease group or negative control group) to 202.17± 127.91 sec ($P < 0.001$), 653.16 ± 163.71 sec ($P < 0.001$), and 745.67 ± 131.30 sec ($P < 0.001$) at respective doses of 0.25, 0.5 and 1 mg/kg of *Tetraponera aethiops* crude venom. Similarly, sodium valproate significantly increased the latency to first tonic-clonic seizure by 997.67 ± 141.79 sec ($P < 0.001$) when compared to the disease group (Figure 2).

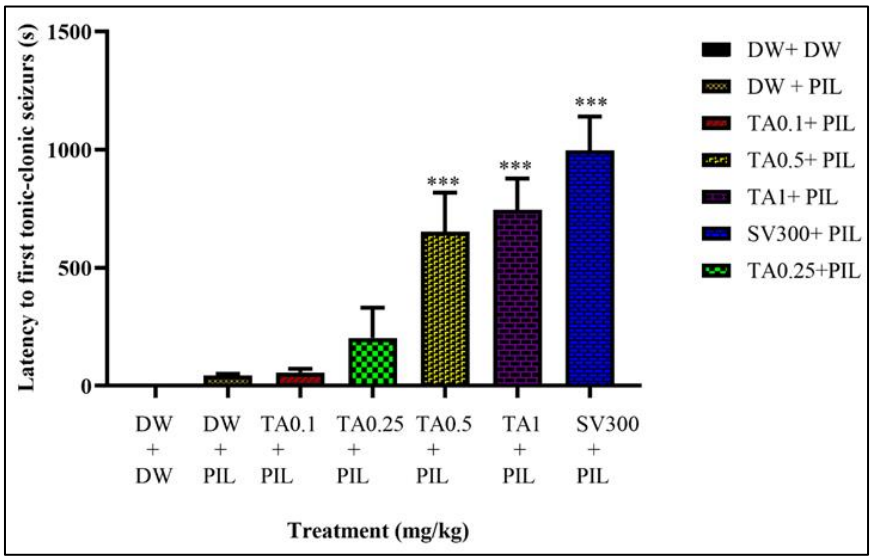

Figure 2 Effects of *Tetraponera aethiops crude* venom on the latency to first tonic-clonic seizures in pilocarpine-picrotoxin treated mice

Results are expressed as mean ± S.E.M. n= 6 animals. Statistical differences were tested by a one-way ANOVA, followed by Dunnett's multiple comparison test, the differences were considered significant at *P<0.05, **P<0.01, ***P<0.001. DW, distilled water; TA0.1, *Tetraponera aethiops* crude venom (0.1 mg/kg); PIL, pilocarpine (360 mg/kg); SV300, sodium valproate (300 mg/kg).

3.3. Effects of *Tetraponera aethiops* crude venom on the number and duration of tonic- clonic seizures; number and duration of clonic seizures 24 hours after *status epilepticus* in pilocarpine-picrotoxin-treated mice

As demonstrated on Figure 3a, one way ANOVA showed a significant effect of the oral administration of *Tetraponera aethiops* crude venom in pilocarpine-picrotoxin treated mice on the duration of clonic seizures [F (6, 35) = 131.5, P < 0.001]. Dunnett multiple comparison test indicated that *Tetraponera aethiops* crude venom significantly decreased the duration of clonic seizures from 57.33 ± 6.74 sec in the disease group to 46.67± 6.86 sec (P < 0.01), 16.50 ± 5.09 sec (P < 0.001) and 14.33 ± 3.01 sec (P < 0.001) in the *Tetraponera aethiops* crude venom test groups administered with the respective doses of 0.25, 0.5 and 1 mg/kg. Also, the duration of clonic seizure decreased to 8.33 ± 2.06 sec (P < 0.001) with sodium valproate compared to the disease group (Figure 3a).

One way ANOVA also indicated a significant effect of the oral administration of *Tetraponera aethiops* crude venom in pilocarpine-picrotoxin treated mice on the number of clonic seizures [F (6, 35) = 49.83, P < 0.001]. Dunnett's test indicated that *Tetraponera aethiops* crude venom significantly decreased the number of clonic seizures from 37.67 ± 9.50 in the disease group to 26.83 ± 1.17 (P < 0.01), 10.33 ± 1.03 (P < 0.001) and 8.67 ± 1.03 (P < 0.001) in the *Tetraponera aethiops* crude venom treated groups administered at the doses 0.25, 0.5 and 1 mg/kg respectively. Interestingly, the number of clonic seizures decreased to 6.33 ± 2.07 sec (P < 0.001) with sodium valproate administration when compared with the disease group (Figure 3b).

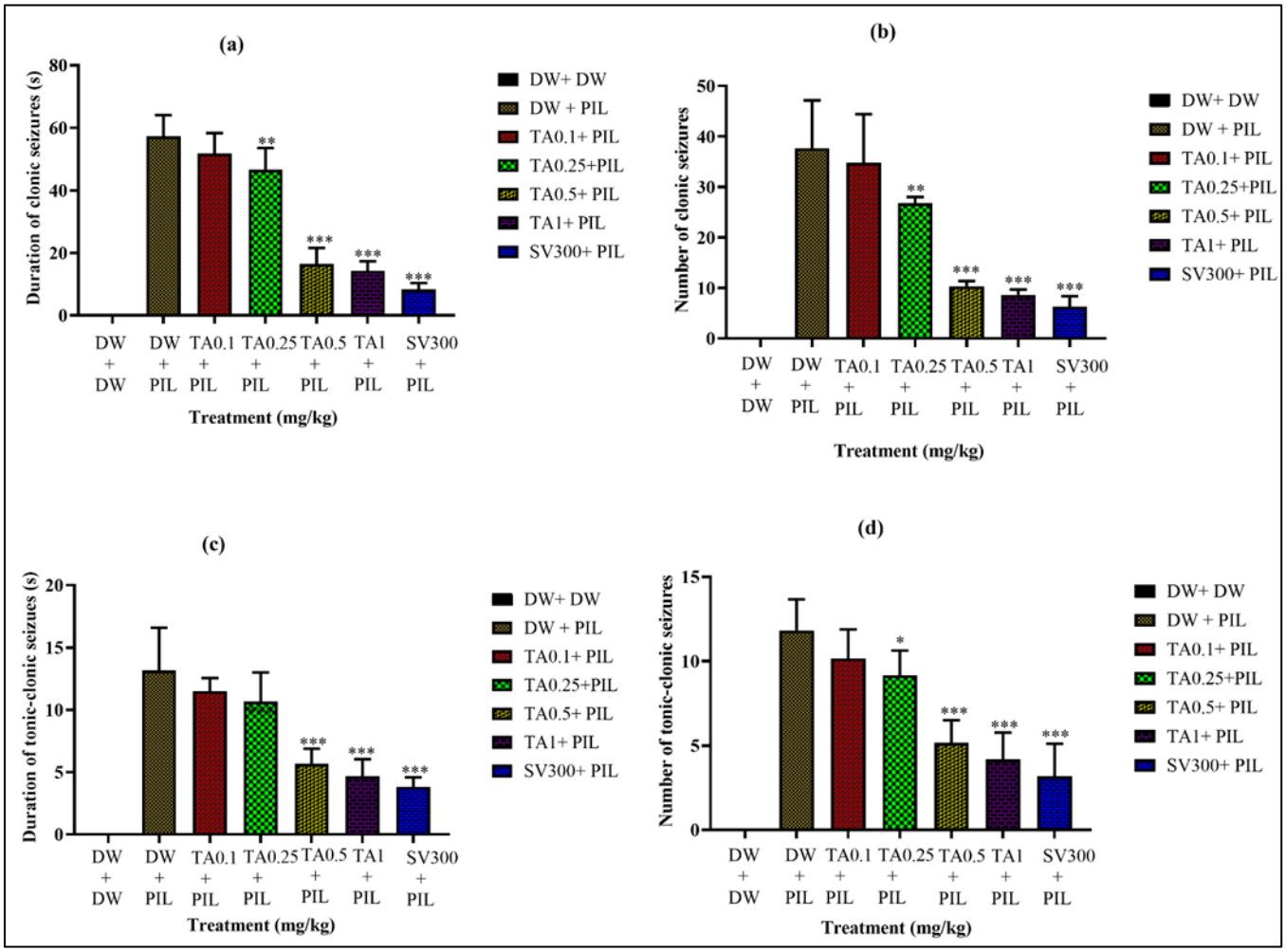

Figure 3 Effects of *Tetraponera aethiops* venom on the number and duration of tonic- clonic seizures; number and duration of clonic seizures 24 hours after status epilepticus in pilocarpine-picrotoxin-treated mice

Results are expressed as mean ± S.E.M. n= 6 animals. Statistical differences were tested by a one-way ANOVA, followed by Dunnett's multiple comparison test, the differences were considered significant at *P<0.05, **P<0.01, ***P<0.001. DW, distilled water; TA0.1, *Tetraponera aethiops* crude venom (0.1 mg/kg); PIL, pilocarpine; SV300, sodium valproate (300 mg/kg).

One way ANOVA revealed significant effect of *Tetraponera aethiops* crude venom oral administration in pilocarpine-picrotoxin treated mice on the duration of tonic-clonic seizures [F (6, 35) = 43.43, P < 0.001]. Dunnett multiple comparison test demonstrated that *Tetraponera aethiops* crude venom significantly decreased the duration of tonic-clonic seizures from 13.17 ± 3.43 sec in the disease group to 5.67 ± 1.21 sec (P < 0.001), and 4.67 ± 1.36 sec (P < 0.001) in the *Tetraponera aethiops* crude venom treated groups at the respective doses of 0.5 and 1 mg/kg. Likewise, the duration of tonic-clonic seizure decreased to 3.83 ± 0.75 sec (P < 0.001) with sodium valproate oral administration when compared to the disease group (Figure 3c).

One way ANOVA demonstrated a significant effect of *Tetraponera aethiops* crude venom oral administration in pilocarpine-picrotoxin treated mice on the number of tonic-clonic seizures [F (6, 35) = 68.27, P < 0.001]. Dunnett's test demonstrated that *Tetraponera aethiops* crude venom significantly decreased the number of tonic-clonic seizures latency from 11.83 ± 1.83 in the disease group to 9.17 ± 1.47 (P < 0.05), 5.17 ± 1.33 (P < 0.001) and 4.17 ± 1.60 (P < 0.001) in the *Tetraponera aethiops* treatment groups at the respective doses of 0.25, 0.5 and 1 mg/kg. Similarly, the number of tonic-clonic seizure decreased to 3.17 ± 1.94 (P < 0.001) with sodium valproate as compared to the disease group (Figure 3d).

3.4. Effects of *Tetraponera aethiops* venom crude on the score of seizures

The resultant effect of *Tetraponera aethiops* crude venom showed a significant increase in seizure score likewise the sodium valproate treated animals. One way ANOVA demonstrated a significant effect of *Tetraponera aethiops* crude venom oral administration in pilocarpine-picrotoxin treated mice on the score of seizures [F (6, 35) = 5.619, P < 0.001]. Dunnett's multiple comparison test demonstrated that *Tetraponera aethiops* crude venom significantly increased the seizure score from zero (0) in the disease group to 0.78 ± 0.05 (P < 0.05), 0.93 ± 0.03 (P < 0.01) and 0.94 ± 0.02 (P < 0.01) in the *Tetraponera aethiops* crude venom test groups at the respective doses of 0.25, 0.5 and 1 mg/kg. Also, score of seizures increased to 0.95 ± 0.01 (P < 0.01) with sodium valproate when compared to the disease group (Figure 4).

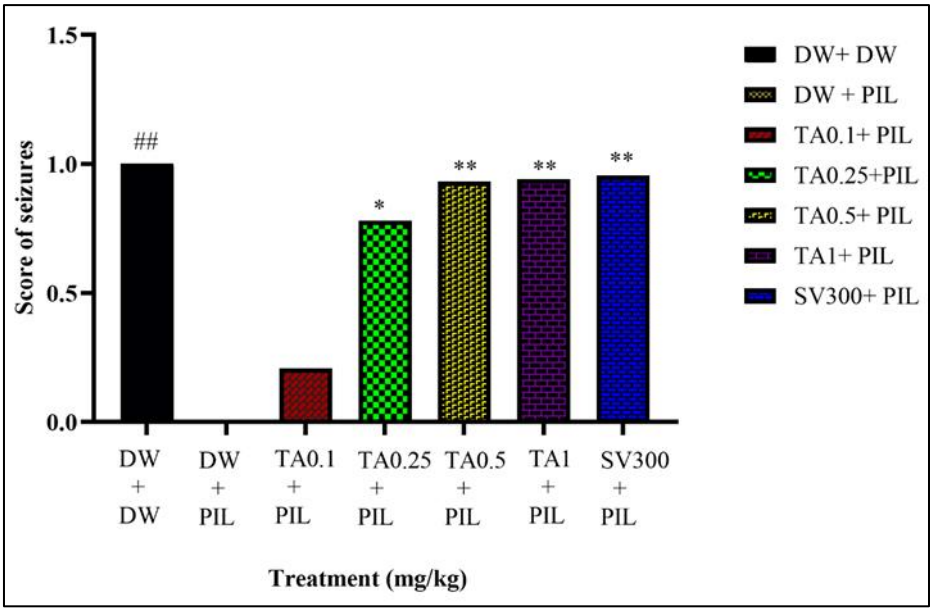

Figure 4 Effects of *Tetraponera aethiops* crude venom on the score of seizures **in pilocarpine-picrotoxin treated mice**

Results are expressed as mean ± S.E.M. n= 6 animals. Statistical differences were tested by a one-way ANOVA, followed by Dunnett's multiple comparison test, the differences were considered significant at *P<0.05, **P<0.01, ***P<0.001. DW, distilled water; TA0.1, *Tetraponera aethiops* crude venom (0.1 mg/kg); PIL, pilocarpine; SV300, sodium valproate (300 mg/kg).

3.5. Effects of *Tetraponera aethiops* crude venom on oxidative stress markers

One way ANOVA showed a significant effect of oral *Tetraponera aethiops crude venom* administration among pilocarpine-picrotoxin treated mice in MDA levels [F (7, 40) = 329.3, *P* < 0.001] (Fig. 4a). Dunnett's test demonstrated that there was a significant increase in MDA level in the hippocampus of the disease group (384.33± 22.89) as compared to the distilled water control treated mice (normal control group) (124.67± 10.89, P < 0.001**)**. Consequently, pre-treatment of animals with *Tetraponera aethiops venom* decrease the level of MDA to 335.67 ± 12.89 nmol/g (P < 0.01), 226.5 ± 7.83 nmol/g (P < 0.001), and 147.67 ± 9.00 nmol/g (P < 0.001) at the doses of 0.25, 0.5 and 1mg/kg respectively (Fig. 4a). Similarly, Sodium valproate and diazepam significantly decreased the level of MDA up to 143.17 ± 6.22 nmol/g (P < 0.001) and 126.50 ± 6.67 nmol/g (P < 0.001) when compared to the disease group (Fig. 4a).

One way ANOVA illustrated a significant effect of oral administration of *Tetraponera aethiops* crude venom among pilocarpine-picrotoxin treated mice in the level of GSH [F (7, 40) = 23.59, P < 0.001] (Fig. 5a). Dunnett's test indicated that there was a significant decrease in the GSH level in the hippocampus of the disease group (125.17 ± 6.17 mol/g) as compared to distilled water control treated mice (210.17 ± 5.17 mol/g, P < 0.001). Meanwhile, the pre-treatment of animals with *Tetraponera aethiops crude* venom increased the GSH level to 162.50 ± 11.833 mol/g (P < 0.01), and 184.33 ± 6.22 mol/g (P < 0.001) at the doses of 0.5 and 1mg/kg respectively (Fig. 5a). Likewise, Sodium valproate and diazepam significantly increased GSH level to 192.83 ± 12.11 mol/g (P < 0.001) and 208.50 ± 23.67 mol/g (P < 0.001) when compared to the disease group (Fig. 5a).

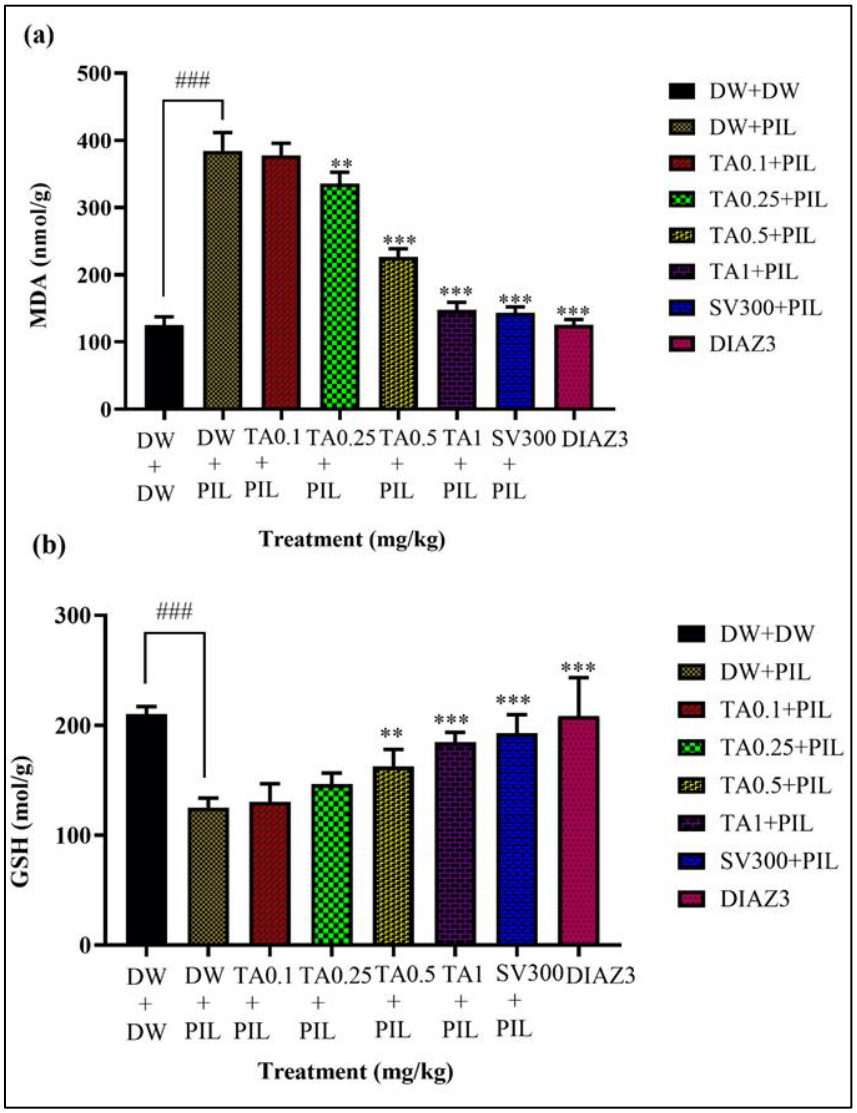

Figure 5 Effects of *Tetraponera aethiops* crude venom on levels of malondialdehyde and glutathione in pilocarpine-picrotoxin treated mice

Results are expressed as mean ± S.E.M. n= 6 animals. Statistical differences were tested by a one-way ANOVA, followed by Dunnett's multiple comparison test, the differences were considered at ##P < 0.01, ###P < 0.001 significantly different compared with the normal group. P < 0.05, ** P < 0.01, *** P < 0.001 significantly different compared with the distilled water-treated pilocarpine-picrotoxin mice. DW, distilled water; TA0.1, *Tetraponera aethiops* crude venom (0.1 mg/kg); PIL, pilocarpine; SV300, sodium valproate (300 mg/kg), DIAZ, diazepam (3 mg/kg).

3.6. Effects of *Tetraponera aethiops* crude venom on the level of gamma-aminobutyric acid and the activity of gamma-aminobutyric acid transaminase in the hippocampus of pilocarpine-picrotoxin-treated mice

Oral administration of *Tetraponera aethiops* crude venom alternated the GABAergic effects caused by pilocarpine-picrotoxin administration. One way analysis of variance showed a significant effect of *Tetraponera aethiops crude* venom oral administration on GABA concentration [F (7, 40) = 39.63, P < 0.001] in mice brain hippocampus. Dunnett's test indicated that there was a significant reduction in GABA concentration in the hippocampus in the disease group (214.67± 4.00 µg/min/mg) in comparison to the distilled water treated group (374.67± 14.56, p < 0.001 µg/min/mg). Meanwhile, the pre-treatment of mice with *Tetraponera aethiops crude* venom increase GABA level to 269.33± 13.00 µg/min/mg (P < 0.001), 334.50 ± 11.83 µg/min/mg (P < 0.001), and 355.67± 13.67 µg/min/mg (P < 0.001) at the doses of 0.25, 0.5 and 1mg/kg respectively (Fig. 6a). However, Sodium valproate and diazepam also significantly increased GABA level to 374.33 ± 7.83 µg/min/mg (P < 0.001) and 387.83 ± 6.94 µg/min/mg (P < 0.001) when compared to the disease group (Fig. 6a).

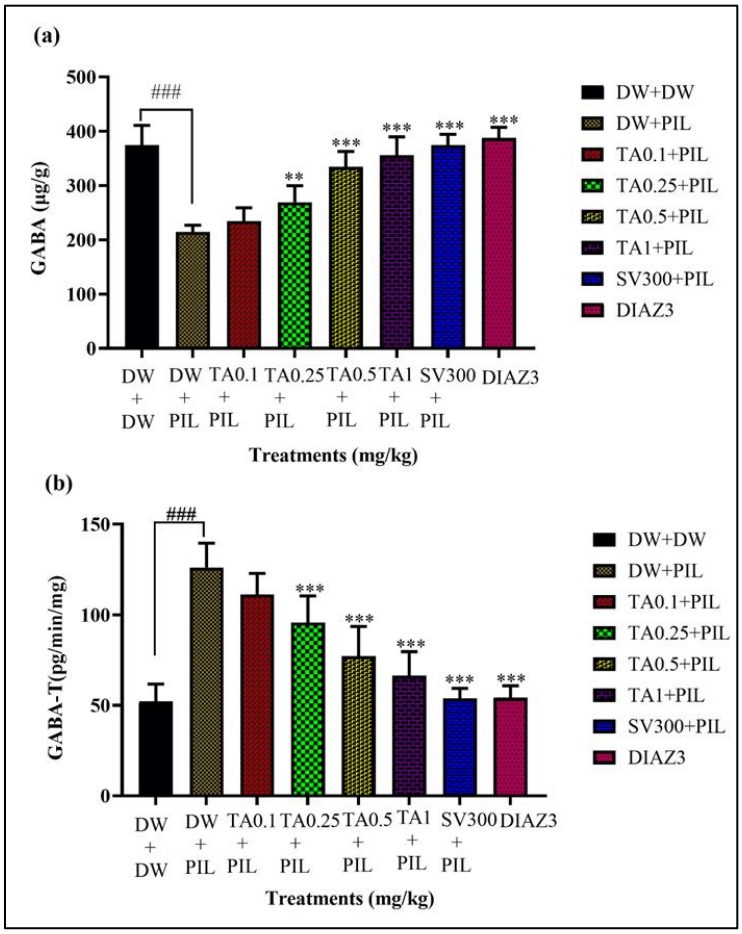

Figure 6 Effects of *Tetraponera aethiops* crude venom on the level of gamma-aminobutyric acid and the activity of gamma-aminobutyric acid transaminase in the hippocampus **of pilocarpine-picrotoxin treated mice**

Results are expressed as mean ± S.E.M. n= 6 animals. Statistical differences were tested by a one-way ANOVA, followed by Dunnett's multiple comparison test, the differences were considered at ##P < 0.01, ###P < 0.001 significantly different compared with the normal group. ** P < 0.01, *** P < 0.001 significantly different compared with the distilled water-treated pilocarpine-picrotoxin mice. DW, distilled water; TA0.1, *Tetraponera aethiops* crude venom (0.1 mg/kg); PIL, pilocarpine; SV300, sodium valproate (300 mg/kg), DIAZ, diazepam (3 mg/kg).

One way ANOVA illustrated a significant effect of oral administration of *Tetraponera aethiops crude* venom on the concentration of GABA-T [F (7, 40) = 34.07, P < 0.001] in the hippocampus. Dunnett's test indicated that there was a significant increase in GABA-T concentration in the hippocampus in the disease group (126.17 ± 11.11 pg/min/mg) as compared to the distilled water treated control group (52.16 ± 7.78, p < 0.001 pg/min/mg). Consequently, the pre-treatment of animals with *Tetraponera aethiops crude* venom decreased GABA-T concentration to 95.67 ± 11.56 pg/min/mg (P < 0.001), 77.17 ± 13.78 pg/min/mg (P < 0.001), and 66.33± 10.78 pg/min/mg (P < 0.001) at doses of 0.25, 0.5 and 1mg/kg respectively (Fig. 6b). Also, sodium valproate and diazepam significantly decreased GABA-T level to 53.83 ± 3.89 µg/min/mg (P < 0.001) and 54.17 ± 5.22µg/min/mg (P < 0.001) in relation to the disease group (Fig. 6b).

4. Discussion

Administration of pilocarpine, an experimental seizures inducer, induced convulsions in mice according to the Racine scale [24]. The pilocarpine model was developed by [36], and by [37]. The administration of pilocarpine induced status epilepticus accompanied with neuronal damage and death of hippocampus cells. Pilocarpine activate M1 muscarinic receptors, leading to the activation of phospholipase C, which in turn produces diacylglycerol and inositol triphosphate, resulting in alterations in calcium and potassium, leading to enhanced excitability [38]. High concentrations of Ca^{2+} trigger the release of glutamate leading to status epilepticus [39]. *Tetraponera aethiops* crude venom administered to mice at different doses though was not able to stop the development of seizures, but it delayed the onset of epileptic status induced by pilocarpine-picrotoxin [22, 25]. One hour after the administration of pilocarpine, *Tetraponera aethiops* crude venom delayed the onset of *staus epilepticus*. During the second day of study *Tetraponera aethiops* crude venom increased the latency to tonic-clonic seizures and the score of seizures; and decreased the number and duration of tonic-clonic seizures, in pilocarpine-picrotoxin challenged mice. These effects are similar to the effects of sodium valproate (which is an antiepileptic reference substance); and show that *Tetraponera aethiops* crude venom extracts possesses antiepileptogenic and anticonvulsant properties.

Furthermore, after the animals were sacrifice, with the brain collected and hippocampus isolated and homogenate prepared for the evaluation of oxidative stress parameters. It was revealed that *Tetraponera aethiops* crude venom acted as an antioxidant there by reducing the level of malondialdehyde whilst increasing the level of glutathione. Malondialdehyde is a marker of lipid oxidation. It is known that malondialdehyde is one of the end products of the oxidation of polyunsaturated fatty acids. Therefore, a high level of malondialdehyde is an indicator of oxidative stress and cellular damage [40-42]. Similarly, a low or very high level of glutathione is the manifestation of oxidative stress because glutathione by reducing hydrogen peroxide prevents any deleterious effect such as lipid peroxidation [43]. The observed decrease in malondialdehyde and increase in glutathione justify an antioxidant activity of *Tetraponera aethiops* crude venom.

More so, the level Gamma aminobutyric acid (GABA) which is the major inhibitory neurotransmitter of the central nervous system was determined. GABA is synthesized at the pre-synaptic neuron by decarboxylation of glutamate, by glutamate decarboxylase [44]. GABAergic inhibition can be pre-synaptic (release of GABA from the GABAergic nerve terminal into pre-synaptic nerve terminals causing a reduction of neurotransmitter release) or post-synaptic (caused by the interaction of GABA with specific postsynaptic receptors). GABA released from GABAergic nerve terminals binds to two distinct types of GABA receptors $GABA_A$ ionotropic and $GABA_B$ metabotropic receptors to produce neuronal inhibition. Inhibitory synapse causes an inflow of Cl^- or outflow of K^+ making the synaptic membrane hyperpolarized, decreasing the possibility of an axon discharge. The hippocampus or ammon's horn is one of the most vulnerable areas of TLE to develop cell loss after seizures [38]. The histological pattern of hippocampal sclerosis in TLE patients is characterised by the loss of pyramidal cells in the prosubicular and CA1 field of the hippocampus [45]. Some studies show that the majority of abnormal observation during TLE in hippocampus lead to structural and functional reorganization of their neuronal network [46], implicating a selective loss of some GABAergic interneuron types and changes in many $GABA_A$ receptors sub-units. Receptors in the CA1 region (sub unit α2 and α5) with reduced inhibition expression are also found in mice model of pilocarpine induced epilepsy [47]. This deficit may limit the capacity of CA1 pyramidal stratum neurons to control an excessive excitatory stimulation occurring during epilepsy induction [48]. The administration of pilocarpine-picrotoxin led to the reduction of inhibition in the hippocampus. There was an observable increase in GABA level and a decrease in GABA-T activity by the *Tetraponera aethiops* crude venom suggesting the interaction of the crude venom with the GABAergic neurotransmission, in the anticonvulsant effects of this venom. Sodium valproate has anticonvulsant effects on seizure and is able to increase the brain GABA concentration through the blockage GABA reuptake; the inhibition of GABA transaminase; the activation of GABA decarboxylase and the enhancement of GABA release at the axon terminals [37]. These results suggest that *Tetraponera aethiops* crude venom is able to restore and maintain the balance between neuronal excitation and inhibition and thus have anticonvulsant and anxiolytic activities.

5. Conclusion

From this study, it can be concluded that *Tetraponera aethiops* crude venom possesses potent antiepileptogenic effects; significantly increased *status epilepticus* latency, the number and duration of tonic-clonic seizures, improves the score of seizures and reduces the progression of temporal lobe epilepsy induced by pilocarpine-picrotoxin in mice. The antiepileptic potential of *Tetraponera aethiops* crude venom is expressed through mechanisms that involve the antioxidant properties by decreasing the lipid peroxidation, and augmenting endogenous antioxidant in the hippocampus of pilocarpine-picrotoxin treated mice. It also decreases in GABA-T activities and increases in GABA concentration indicate the involvement of GABAergic modulation by *Tetraponera aethiops* crude venom in the antiepileptic properties.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Statement of ethical approval

All experiments were performed according to the Guide for the Care and Use of Laboratory Animal published by the United States National Institutes of Health (NIH publication No. 85-23, revised 1996) and the University of Buea, Cameroon (Permit number: UB-IACUC- N°16/2022).

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