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Original Research Article

Glyphaea brevis (Spreng) Monachino (Tiliaceae) leaf fractions protect against pentylenetetrazole (PTZ)-induced convulsion in mice

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Abstract

Purpose: *To investigate the anticonvulsant effect of Glyphaea brevis leaf fractions using a pentylenetetrazole (PTZ)-induced seizure model in mice.*
Methods: *Glyphaea brevis leaf methanol extract was partitioned using n-hexane and hydro-methanol (8:2). Seizure was assessed in terms of onset of myoclonic and tonic-clonic seizure, duration of seizure and frequency of seizure. The concentrations of glutathione of catalase, glutathione peroxidase, superoxide dismutase and malondialdehyde in the brain of the mice were also determined.*
Results: *The administration of the different fractions of Glyphaea brevis leaf prior to induction of seizure with PTZ prolonged the latency of convulsion in mice as well as a significant ($p < 0.05$) decline in the intensity of convulsion was observed. In relation to the untreated mice, there was a rise in the levels of glutathione peroxidase, superoxide dismutase, and catalase in the brain, but a decrease in malondialdehyde levels.*
Conclusion: *The study results show that the fractions of Glyphaea brevis leaf have anticonvulsive properties, proving that the plant's use in folklore medicine for treating convulsion.*

Keywords: *Glyphaea brevis, Pentylenetetrazole, Seizure, Diazepam, Antioxidant enzymes*

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INTRODUCTION

Seizure is a serious neurological disorder and the most common disabling chronic illness of the central nervous system affecting about 50 million people globally [1]. However, seizure is normally attributed to persistent and constantly evolving neurological conditions that damage the neuron. An example of such a condition is an imbalance between the concentrations of excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmitters within the central nervous system (CNS). This has been the most common development mechanism for epilepsy [2]. Although the functioning of GABAergic system could be influenced by other health conditions, it is the most inhibitory system in the CNS [3]. Hence, a deficit in gamma-aminobutyric acid (GABA) concentration could lead to health changes in the CNS that could trigger convulsive episodes.

Normally, epileptic seizures reduce the effect of antioxidants in the brain, resulting in an increase in free radicals. This exacerbates oxidative stress [4], a condition in which free radicals are abundant and glutaminergic receptors in the CNS are overexpressed and activated [5]. These free radicals cause epilepsy, brain edema, and lipid peroxidation, including coma and possibly death. The brain could easily be damaged because of high levels of oxidative metabolism and low antioxidant defenses, affecting cell structure, and causing damage [2]. This imbalance increases the risk of harm to the brain. Free radical and scavengers and antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), defend biological systems from the damaging effects activated species [4]. The plant, *Glyphaea brevis* (Spreng) Monachino (Tiliaceae) can be found in rocky savannas, forest regrowth, swamps, fallow land, and forest galleries. The leaves are also used for dyspepsia, ulcers, antimicrobial, anti-inflammatory, and antioxidant properties [6]. Even though this plant has been used in the treatment convulsion in traditional medicine among some Africans, there is a paucity of studies to validate this claim. Thus, the research was carried out to assess the anticonvulsant properties of n-hexane and hydro-methanol fractions of *Glyphaea brevis* leaves in pentylenetetrazole-induced seizures in mice.

EXPERIMENTAL

Collection of plant material

The fresh leaves of *Glyphaea brevis* were harvested in December 2019 from Ozom Mgbagbu Owa, in Ezeagu Local Government Area, Enugu State of Nigeria and validated by a taxonomist, Mr. A Ozioko of the Bioresources Conservation and Development Center, Nsukka, Enugu State, Nigeria.

Preparation of plant material and extraction

The leaves of *Glyphaea brevis* leaves were first air dried at room temperature of 25 ^{o}C and then carefully crushed into powder using a miller. The pulverized sample (1200 g) was macerated inside methanol (6 litres) with thorough shaking at regular intervals for 72 hours at temperature of 25 ^{o}C. The mixture is then passed through muslin cloth before being filtered with Whatman filter paper No 1. The filtrate was concentrated at 45 °C under reduced pressure using a rotary evaporator, and the concentrated extract was accurately measured and stored at 4 °C in refrigerator for further use.

Phytochemical analysis of *Glyphaea brevis* leaves

The phytochemical analyses of the methanol extract of *Glyphaea brevis* leaves were performed using the methods of Trease and Evans [7], Harbourne [8], Soni and Sosa [9].

Solvent-solvent partitioning

The methanol extract of *Glyphaea brevis* was fractionated using solvent-solvent partitioning. The extract was partitioned using 20 % methanol (v/v) and n hexane. The plant extract (20 g) was poured into a separating funnel after been immersed in the hydro-methanol solution (200 ml) at the ratio of 8:2. Then, n hexane (250 mL) was introduced and shaken vigorously. The mixture was allowed to settle, giving rise to two layers. The different fractions of hydro-methanol and n hexane were collected separately. The process was repeated until no observable colour change was observed.

Animals

The experimental animals used for this study were forty (40) adult male albino mice, aged 8 to 12 weeks old. The mice were procured from the animal house of Zoology and Environmental Biology Department, University of Nigeria, Nsukka (UNN). They were placed in a clean, well-ventilated cages with unrestricted access to standard mice feed (Pecco Foods, Enugu, Nigeria) and water *ad libitum* and allowed to acclimate for 14 days.

Experimental design

A total of forty (40) adult albino mice were utilized for this research. The mice were sheltered in metal cages with access to unlimited feed and water supply. As they were acclimatizing to the laboratory environment 14 days prior to the start of the experiment *ad libitum*. The mice were divided into eight (8) groups of 5 animals each, acclimatized to the laboratory 14 days prior to the experiments. The experimental protocol was carried out according to the guidelines established by the European Union on Animal Care (CCE Council 86/609) [10]. Group 1 served as the untreated; group 2 was given 5 mg/kg b.w. diazepam; Groups 3 - 5 received graded doses of 100, 300 and 600 mg/kg of hydro-methanol fractions of *Glyphaea brevis* while groups 6 - 8 received graded doses of n hexane fraction of *Glyphaea brevis* (100, 300 and 600 mg/kg) respectively. The oral administration of the standard drug, diazepam, and graded doses of the different fractions of *Glyphaea brevis* leaf

lasted for two weeks. Approval for the study was granted by the Ethics and Biosafety Committee of Faculty of Biological Sciences (UNN/FBS/EC/1020).

Induction of seizure using pentylenetetrazole

The mice were kept in a wooden 60 × 60-cm square field surrounded by a 40 cm high wall. On the final day of the experiment, 30 minutes after the administering diazepam and the graded doses of the different fractions of *Glyphaea brevis* leaf, single (60 mg/kg) pentylentetrazole (PTZ) doses were used to induce convulsive seizures which were assessed based on the onset of seizure, clonic-tonic seizure onset, number of jerks, frequency of seizures, duration of seizure and mortality.

In addition, the cerebral cortex of the mice was collected, homogenized, and used in determining the concentrations of antioxidant enzymes (GPx, CAT and SOD) and malondialdehyde. Superoxide dismutase activity was evaluated using Fridovich [11] method while the catalase activity was evaluated using the procedure outlined by Aebi [12]. The activity of glutathione peroxidase was then evaluated as reported in the article by Paglia and Valentine [13], even as malondialdehyde concentration evaluated using the method explained by Wallin [14] as highlighted by Randox kit (Randox Laboratories, USA).

Statistical analysis

The data was analyzed and expressed as mean standard deviation, with statistical significance determined using one-way analysis of variance (ANOVA). Version 16 of Statistical Package for Social Sciences (SPSS) was used to conduct the analysis. Data obtained from the test groups were compared to the untreated group and at $P < 0.05$, the discrepancies were considered significant.

RESULTS

Phytochemical profile of *Glyphaea brevis*

Alkaloids, flavonoids, phenolics, terpenoids, saponins, glycosides and tannins were detected in *Glyphaea brevis* leaf extract during phytochemical analyses (Table 1).

Table 1: Phytochemical profile of *Glyphaea brevis* leaves extract

Phytochemical	Abundance
Alkaloids	+
Phenolic	+
Flavonoids	+
Saponins	+
Tannins	+
Glycosides	+
Terpenpoids	+

+ = Detected

Anticonvulsant effect of *Glyphaea brevis*

The onset of myoclonic seizure in the mice that received the graded doses of different fractions of *Glyphaea brevis* leaves were prolonged. Myoclonic jerks were not detected in the rats that received the standard drug, diazepam. Tonic-clonic seizures were not detected in the rats that received the standard drug, diazepam. However, in mice given graded doses of different fractions of *Glyphaea brevis* leaves, a significant ($p < 0.05$) increase in the time it took for tonic-clonic seizures to begin was observed in a dose related manner. The frequency of seizure decreased significantly ($p < 0.05$) as the doses of *Glyphaea brevis* leaf fraction were increased. Significant ($p < 0.05$) increase in the frequency and duration of seizure were found in the experimental rats that did not receive any treatment. The mice that were given the standard drug, diazepam did not have any seizures. However, the duration of seizures was reduced significantly ($p < 0.05$) and dose-dependently in mice given graded of different fractions of *Glyphaea brevis* leaves (Table 2).

Table 2: Anticonvulsant effect of *Glyphaea brevis* leaf fractions (mean ± SD, n = 5)

Group	Onset of myoclonic jerks (min)	Onset of tonic-clonic seizure (min)	Frequency of seizure	Duration of seizure (min)	Protection (%)
Untreated	1.37±0.29^{a}	2.76±0.86^{a}	5.25±0.96^{g}	3.58±0.70^{g}	-
5 mg/kg diazepam	-	-	-	--	100
100 mg/kg HMF	10.85±1.62^{b}	13.00±1.41^{b}	3.50±0.58^{e}	2.39±0.04^{f}	-
300 mg/kg HMF	19.50±3.11^{d}	21.95±2.25^{d}	2.75±0.50^{d}	1.28±0.14^{c}	25
600 mg/kg HMF	26.00±2.86^{e}	28.75±2.21^{f}	1.15±0.20^{a}	1.16±0.02^{a}	75
100 mg/kg HF	12.00±2.38^{c}	15.75±3.10^{c}	3.75±0.50^{f}	2.24±0.06^{e}	-
300 mg/kg HF	19.50±3.11^{d}	23.25±1.71^{e}	2.25±0.50^{c}	1.35±0.10^{d}	50
600 mg/kg HF	31.08±2.72^{f}	36.00±3.86^{g}	1.25±0.12^{b}	1.24±0.04^{b}	50

n = 5. HMF = hyrdo-methanol fraction; HF = n-hexane fraction. Mean values having different superscript letters down the columns differ significantly at $p < 0.05$

Table 3: Effect of *Glyphaea brevis* leaves fractions on enzymatic antioxidant and malondialdehyde levels (mean ± SD, n = 5)

Group	Catalase (U/mg)	Superoxide dismutase (U/mg)	Glutathione peroxidase (U/mg)	Malondialdehyde (mg/ml)
Normal Control	6.35 ± 0.29^{c}	10.28 ± 0.34^{cd}	16.03 ± 0.58^{f}	1.35 ±0.05^{a}
Untreated	4.90 ± 1.05^{b}	8.13 ± 0.08^{b}	8.98 ± 0.51^{a}	5.32 ±0.06^{h}
Diazepam	8.13 ± 0.31^{d}	10.53 ±0.28^{d}	13.78 ± 0.30^{e}	1.73±0.02^{b}
100 mg/kg b.w HMF	5.21 ± 0.59^{a}	9.55 ± 0.81^{cd}	10.68 ± 0.52^{cd}	2.79±0.01^{e}
300 mg/kg b.w HMF	6.62 ± 0.31^{c}	6.98 ± 0.22^{e}	11.21 ± 0.38^{f}	2.39±0.01^{d}
600 mg/kg b.w HMF	6.90 ± 0.50^{c}	7.24 ± 0.24^{f}	12.33 ± 0.58^{f}	1.96±0.02^{c}
100 mg/kg b.w HF	3.89 ± 0.39^{b}	9.76 ± 0.68^{c}	11.55 ± 0.27^{b}	3.91 ±0.01^{f}
300 mg/kg b.w HF	6.60 ± 0.33^{c}	11.46 ± 0.71^{a}	16.43 ± 1.09^{bc}	2.75 ±0.03^{e}
600 mg/kg b.w HF	7.15 ± 0.19^{c}	12.49 ± 0.91^{a}	16.79 ± 0.34^{d}	2.31 ±0.03^{d}

b.w = body weight; HMF = methanol fraction; HF = n-hexane fraction. D Mean values having different superscript letters down the columns differ significantly at $p < 0.05$

Effect of *Glyphaea brevis* leaves fractions on antioxidant enzymes and malondialdehyde of experimental mice

Significant ($p < 0.05$) reductions in CAT, SOD and GPx activities, as well as an increased malondialdehyde concentration were observed in the brain of mice that were induced but not treated. On the other hand, improved antioxidant enzyme activities were observed in the groups that received diazepam and different doses of the plant fractions (Table 3).

DISCUSSION

The phytoconstituent screening of the *Glyphaea brevis* leaf extract showed the presence of alkaloids, flavonoids, terpenoids, saponins, tannins and glycosides. The findings are consistent with Mubo and Ogunlakin [15] findings. Several studies have shown that flavonoids possess antiepileptic activity which has been attributed to the modulated $GABA_A$-Cl-channel complex [16]. Terpenoids, alkaloids and tannins have also been reported to possess anticonvulsive activities modulating neuronal activity and suppressing epileptiform activity [17].

The administration of subconvulsive dose of pentylenetetrazole, a $GABA_A$ receptor blocker causes a progressive increase in convulsion which ultimately leads to seizure. Pentylenetetrazole is a tetrazole derivative which noncompetitively inhibit the gamma-aminobutyric acid (GABA)(A) receptor complex. However, the results from this study demonstrate that *Glyphaea brevis* n hexane and hydro-methanol leaf fractions possessed anticonvulsant activity, possibly by protecting the gamma-aminobutyric acid receptor from PTZ inhibition. The administration of graded doses of the hydro-methanol and n hexane fractions of *Glyphaea brevis* leaves significantly delayed the initiation of myoclonic jerks, and associated tonic-clonic seizure respectively compared to the untreated group. The various fractions of *Glyphaea brevis* leaves also reduced the frequency and duration of seizures respectively in the mice which received graded doses of the leaf fractions. The anticonvulsant effect seen in the mice which received graded *Glyphaea brevis* leaves fractions doses might be due to the phytochemical content of the separate fractions, possibly suppressing pentylenetetrazole (PTZ) activity, while also inhibiting receptors of N-Methyl-D aspartate (NMDA). This can therefore enhance the GABA activity that regulates voltage-gated and synaptic conductance of neuronal excitation leading to convulsion.

Antioxidant enzymes play major roles in cellular defense of the brain against oxidative damage and can reduce the potential risk of some neurological disorders via the inhibition of lipid peroxidation [4]. The antioxidant enzymes have the capacity to counteract ROS, representing the first defence line against any oxidative injury. SOD, on the other hand, catalyzes superoxide radical dismutation, liberating H_2O_2, which is then removed by CAT activity. Free radicals have been suggested as a potential cause of neuronal changes in neurodegeneration that results in behavioural deficits [4]. The brain is especially, vulnerable to oxidative stress due to its high concentration of polyunsaturated fatty acids (which easily undergo lipid peroxidation) and increased oxygen consumption [18]. In addition, the brain contains a lot of iron which can trigger the Fenton reaction, which produces free radicals. This study showed that the administration of graded doses of hydro-methanol and n-hexane fractions of *Glyphaea brevis* produced significant rise in the actions of CAT, SOD and GPx in the brain of mice treated with the fractions, indicating that the plant has neuroprotective effects, assisting the brain to relieve oxidative stress which is a major cause of seizure. Accumulated superoxide radical can

either initiate lipid peroxidation directly or indirectly through the product of its metabolism if it is not scavenged by the antioxidant. The significant and dose-dependent decrease in MDA concentration in the brain of mice that received the fractions may be attributed to inhibition and suppression of lipid peroxidation by the antioxidant compounds in the fractions. It could be said that the *Glyphaea brevis* fractions attenuated PTZ-induced seizures of the mice by enhanced interference with GABAergic neurotransmission as well as scavenging the free radicals generated in the mice brain by PTZ induction.

CONCLUSION

The findings of this research suggest that the fractions of *Glyphaea brevis* leaf have anticonvulsant properties, providing some support for plant's traditional use in treating convulsions.

DECLARATIONS

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Conflict of interest

This work had no conflict of interest associated with it.

Contribution of authors

We declare that this work was completed entirely by the named authors in this article, and the authors will bear all liabilities to claims relating to the article content. Olivia U Njoku conceived the study concept and designed it. Martins O Ogugofor collected and prepared the plant sample. Olivia U Njoku and Martins O Ogugofor collected and analysed the data. Martins O Ogugofor and Olivia U. Njoku wrote the entire manuscript. The study manuscript was also read and accepted for academic publication by the authors.

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