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Assessment of Anticonvulsant Activity of Ethanol Extract of *Piper guineense* Leaf on Experimental-Induced Convulsions in Wistar Rats

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Abstract

Epilepsy is a disorder of central nervous system that affects a significant number of persons in developed and underdeveloped countries of the world. *Piper guineense* is among the biodiversity employed in South Eastern Nigeria tradomedicinal practice in the management of epilepsy in humans. Because the neuropharmacological activities of *Piper guineense* have not yet been widely and scientifically elucidated, this study was designed to assess the anticonvulsant activity of ethanol extract of *Piper guineense* leaf on experimental-induced convulsions in wistar rats. This was done by extracting the plant material and monitoring the anticonvulsant activity of the plant extract at increasing doses (100, 200, 400 mg/kg; p.o.) on animal models of epilepsy such as Maximal Electrical Shock (MES), Pentylenetetrazole (PTZ) and Picrotoxin induced convulsions. The results obtained indicate that: (i) the extract (400 mg/kg) significantly ($p<0.05$) decreased the duration of tonic hind limb extension and also reduced mortality to 60% in MES-induced convulsion (ii) on PTZ-induced convulsion, the phenobarbitone (20 mg/kg; i.p.) and extract (400 mg/kg) respectively reduced the mortality to 30% and 40%, but when used concurrently, mortality was reduced to 10%. (ii) On picrotoxin-induced convulsion, the extract exhibited significant ($p<0.05$) dose-dependent delay in onset of seizure, increased seizure latency and reduced mortality. This work therefore, justifies the tradomedicinal use and also substantiates the existing reports on *Piper guineense* leaf as anticonvulsant agent.

Keywords: *Piper guineense* leaf; Ethanol extract; Anticonvulsant; Mortality; Seizure latency

Introduction

Epilepsy is a chronic neurological disorder of global health concern. Its etiology is primarily due to imbalance between excitatory and inhibitory neurotransmission that results to abnormality in synchronized firing of group of neurons in the brain [1,2]. Epilepsy is characterized by recurrent distortion of the nervous system that leads to excessive discharge from cerebral neurons [3]. Epilepsy contributes about one percent of the total global burden of diseases, with prevalence rate of about two percent [4]. Based on the possible causes, epilepsy can be classified into two: (i) genetic epilepsy, resulting from genetic predisposition of the brain to produce seizure (ii) acquired epilepsy, resulting from acute trauma or insult that can cause alterations of the molecular, cellular and physiological properties of the brain that can provide origin to seizures [5]. Other possible seizure initiators which include altered permeability of neuronal membrane, diminished inhibitory regulation of neurons and neurotransmitter imbalance, have been reported [2,6].

All currently available anticonvulsant drugs are synthetic compounds [7] and are associated with chronic toxicity (teratogenic effects), high cost, poor compliance and approxima -tely 30% of patients continue to have seizure relapse with antiepileptic therapy [8-10]. Despite successful development of various antiepileptic drugs in recent decade, the search for new therapies with better efficacy, less toxicity and tolerability remains an important goal [11].

There is a growing interest in the traditional systems of medicine in developing countries. About 80% of world population rely on traditional medicines or folk remedies for their health care needs [12,13]. Plants have been reported as a cheap and rich source of new agents with potential therapeutic effects [14].

Piper guineense, a flora of tropical regions of Central and West Africa, belongs to the family of *Piperaceae*. Its leaf is an edible vegetable in Nigeria and proximate analysis reveals that the plant contains crude proteins, fats, carbohydrates, vitamins and minerals [15]. *Piper guineense* has broad applications in traditional medicine in the treatment of diseases. Some of the reported ethnobotanical uses of *Piper guineense* include: Anticonvulsant activity [16,17], sedative and anxiolytic activities [18], antibacterial activity [19,20]. Study on the ethanol leaf of the plant shows the presence of alkaloid (piperine) as one of the antiulcer principles [21].

This study, therefore, was designed to assess the anticonvulsant activity of ethanol extract of *Piper guineense* leaf on experimental-induced convulsions in wistar rats, with a view to justify, or otherwise, its tradomedicinal claim and/or to substantiate, or otherwise, the existing published reports as an agent with anticonvulsant activity.

Materials and Methods

Identification of plant material

Matured fresh leaves of the plant were identified as *Piper guineense* leaves and assigned a voucher specimen number, UPH/P/251, in the Laboratory of Plant Science and Biotechnology, University of Port Harcourt, Nigeria.

Animal ethics approval

This study was conducted under the animal ethics approval (Reference Number: MAU/SREC/A/22), granted by Senate Research and Ethics Committee of Madonna University, Nigeria. Guideline for care and handling of animals were strictly followed as prescribed by [22].

Extraction of plant material

About 2.5 kg of air-dried leaves of *Piper guineense* were pulverized to pass through sieve #20. About 300 g of the pulverized plant material was macerated in 1.5 liter 80% ethanol for 72 hours with 6 hourly agitation. The resulting solution was filtered through Whatmann No.1 filter paper. The marc was re-macerated and re-filtered. The obtained filtrates were pooled together and concentrated in a flask using rotary evaporator operated at 40-45°C. Yield was calculated by subtracting the initial weight of empty flask from final weight of the flask containing the solid residue. The difference in weight was taken as the weight of the extracted residue. The extraction and concentration processes were repeated severally (*i.e.,* five times) until sufficient quantity of solid crude extract was obtained. The solid extract was stored for subsequent use in an air-tight container and then labeled as *Piper guineense* Extract (PGE).

Phytochemical analysis

The obtained PGE was subjected to phytochemical tests by using methods proposed by [23], to detect the presence or absence of various phytoconstituents.

Acute oral toxicity test

In a procedure proposed by [24] where low, medium and high doses would be selected for treatment, PGE was orally administered at increasing doses, up to 4000 mg/kg, to different groups of rats, consisting of five rats per group. The rats were initially monitored continuously for 4 hours for behavioral and physiological changes. Subsequently, the rats were monitored for signs of toxicity and lethality at interval of 4 hours for 12 hours and then once daily for 14 days. With no obvious signs of toxicity and lethality, $1/40^{th}$ (2.5%: Low dose), $1/20^{th}$ (5%: Medium dose) and $1/10^{th}$ (10%: High dose) of the maximum test dose (4000 mg/kg) would be considered as doses to be employed in the present investigation.

Experimental design for assessing anticonvulsant activity

Three sets of sixty adult Wistar rats (160 g-180 g) were adequately fed and allowed free access to water. The three sets of rats were used in different seizure models: Maximal Electrical Shock (MES), Pentylenetetrazole (PTZ) and Picrotoxin. The rats in each set were randomized into six groups (n=10) and each group was treated daily as follows for 7 days:

- Group 1 (negative control) received 10 ml/kg 3% v/v Tween 80
- Group 2 (positive control) received 20 mg/kg Phenobarbitone
- Group 3 received 100 mg/kg PGE
- Group 4 received 200 mg/kg PGE
- Group 5 received 400 mg/kg PGE
- Group 6 received 400 mg/kg PGE+20 mg/kg Phenobarbitone

The vehicle (3% v/v Tween 80) and PGE were administered per oral (p.o.) while the standard drug (Phenobarbitone) was administered by intraperitoneal (i.p.) routes. About 30 minutes after respective treatment on the 7th day, convulsions were induced using MES [25], PTZ (65 mg/kg; i.p.) [26] and picrotoxin (4 mg/kg; i.p.) [27]. Following induction of seizure by MES, the rats were monitored (time in seconds) for various phases convulsion (flexion, tonic hind limb extension, clonus, stupor and recovery), number of rats that convulsed and mortality, while in PTZ and picrotoxin-induced seizures, the rats were not only monitored for number showing convulsion and mortality, but also monitored for latency of clonic and tonic seizures [28].

Statistical analysis

Obtained data are presented in tables as ± Standard Error of Mean (SEM) of n=10 and was evaluated by one-way Analysis of Variance (ANOVA), followed by Duncan's Post-Hoc multiple comparison test using SPSS version 24. Probability value of less than 0.05 ($p<0.05$) was considered significant.

Results

Yield

The quantitative yield (11.36 g) of PGE obtained in the first extraction was low when compared to the amount (300 g) of macerated plant material. Five rounds of extraction were performed and a total of 55.68 ± 2.19 g of solid extract was obtained.

Phytochemistry

Phytochemical analysis of crude PGE presented in **Table 1** reveals the presence of all the tested phytoconstituents: Phenols, flavonoids, alkaloids, carotenoids, saponins, tannins, glycosides and terpenoids.

Table 1: Phytochemical analysis of ethanol extract of *Piper guineense* leaf.

Test	Observation
Phenols	+
Flavonoids	+
Alkaloids	+
Carotenoids	+
Saponins	+
Tannins	+
Glycosides	+
Terpenoids	+
Note: + = Present	

Acute oral toxicity test

This test reveals that at limit/maximum test dose of 4000 mg/kg PGE, there were no behavioral/physiological changes and no signs of toxicity and lethality within 4 hours, 12 hours and 14 days of observation in rats. With no obvious signs of toxicity and lethality, 100, 200 and 400 mg/kg were considered in this investigation to represent low, medium and high doses respectively.

Anticonvulsant activity

As shown in **Table 2,** induction of seizure in rats with MES produced flexion, tonic hind limb extension, stupor, recovery and 100%mortality in the vehicle-treated group (3% v/v Tween 80). Pretreatment with phenobarbitone (20 mg/kg, i.p.) completely abolished the extension phase of seizure and reduced the mortality to 30%. Pretreatment with PGE (400 mg/kg, p.o.) not only produced significant ($p<0.05$) decrease in duration of tonic hind limb extension, but also reduced mortality to 60%.

Table 2: Effect of PGE and phenobarbitone on mes-induced convulsions in rats.

Group	Treatment (per kg b.w)	Time (second) of various phase of convulsion					No of animals convulsed/ No used	%Mortality
		Flexion	Extension	Clonus	Stupor	Recovery		
1	10 ml 3% v/v Tween 80	10.22 ± 0.60	11.16 ± 0.81	11.91 ± 0.80	48.40 ± 1.03	81.60 ± 2.14	10/10	100
2	20 mg Phenobarbitone	4.14 ± 0.18[a]	-	11.43 ± 0.56	20.15 ± 0.77[a,b]	49.44 ± 1.71[a,b]	3/10	30
3	100 mg PGE	6.72 ± 1.04[a]	9.32 ± 0.60	10.07 ± 0.22[a]	22.63 ± 0.84[a,b]	70.33 ± 1.71[a]	10/10	100
4	200 mg PGE	5.50 ± 0.21[b]	8.40 ± 1.18	9.65 ± 0.90[a]	23.70 ± 1.11[a,b]	64.24 ± 2.02[b]	10/10	100
5	400 mg PGE	4.37 ± 1.12[a]	7.82 ± 0.41[a,b]	9.13 ± 0.25[a]	25.16 ± 0.31[a,b]	55.17 ± 1.05[a,b]	6/10	60
6	400 mg PGE +20 mg Phenobarbitone	7.15 ± 0.63[a,b]	7.15 ± 0.63[a]	8.62 ± 0.51[a,b]	25.85 ± 1.01[a,b]	42.34 ± 0.95[a,b]	2/10	20
Note: Values represent mean ± SEM (n=10), [a]$p<0.05$, [b]$p<0.01$ compared to negative control (Group 1).								

Induction of seizure in rats with PTZ (65 mg/kg, i.p.) precipitated clonic-tonic convulsions and 100% mortality in the vehicle-treated group (3% v/v Tween 80). Pretreatment with Phenobarbitone (20 mg/kg, i.p.) and PGE (400 mg/kg, p.o.) respectively reduced the mortality to 30% and 40%. Further reduction of mortality to 10% was observed when Phenobarbitone (20 mg/kg, i.p.) and PGE (400 mg/kg, p.o.) were used concurrently as shown in **Table 3.**

Table 3: Effect of PGE and phenobarbitone on PTZ–induced convulsion in rats.

Group	Treatment dose	Latency of clonic convulsion (mins)	Latency of tonic convulsion (mins)	No of animals convulsed/No used	%Mortality
1	10 ml 3% v/v Tween 80	3.16 ± 1.42	4.41 ± 1.05	10/10	100
2	20 mg Phenobarbitone	10.08 ± 0.85^{a,b}	15.33 ± 1.61^{a,b}	3/10	30
3	100 mg PGE	5.51 ± 1.07^{b}	7.04 ± 1.11^{a}	10/10	100
4	200 mg PGE	7.36 ± 0.62^{a,b}	10.28 ± 0.88^{a,b}	8/10	80
5	400 mg PGE	8.75 ± 1.04^{a}	12.45 ± 1.25^{a,b}	4/10	40
6	400 mg PGE+20 mg Phenobarbitone	13.10 ± 1.25^{a,b}	16.22 ± 0.78^{a,b}	1/10	10
Note: Values represent mean ± SEM (n=10), $^{a}p<0.05$, $^{b}p<0.01$ compared to negative control (Group 1).					

From the result in **Table 4,** induction of seizure with picrotoxin (4 mg/kg, i.p.) produced tonic-clonic convulsion after 4.08 ± 1.13 minutes in the group treated with vehicle (3% v/v Tween 80). Pretreatment with PGE produced significant ($p<0.05$) dose-dependent delay in onset of seizure, increase in seizure latency and reduction in mortality.

Table 4: Effect of PGE and phenobarbitone on picrotoxin–induced convulsion in rats.

Group	Treatment (per kg b.w)	Latency of clonic convulsion (mins)	Latency of tonic convulsion (mins)	No of animals convulsed/No used	%Mortality
1	10 ml 3% v/v Tween 80	4.08 ± 1.13	5.53 ± 0.94	10/10	100
2	20 mg Phenobarbitone	12.42 ± 1.51^{a,b}	16.74 ± 1.40^{a,b}	5/10	50
3	100 mg PGE	6.73 ± 2.01^{a}	8.35 ± 1.31^{a}	9/10	90
4	200 mg PGE	9.11 ± 1.32^{a,b}	11.87 ± 1.28^{a,b}	8/10	80
5	400 mg PGE	10.65 ± 1.17^{a,b}	13.16 ± 1.15^{a,b}	6/10	60
6	400 mg PGE + 20 mg Phenobarbitone	14.44 ± 0.83^{a,b}	18.35 ± 1.27^{a,b}	3/10	30
Note: Values represent mean ± SEM (n=10), $^{a}p<0.05$, $^{b}p<0.01$ compared to negative control (Group 1).					

Discussion

The quantitative yield of PGE in this study was low and this finding conforms to our earlier report that biologically active compounds are usually present in plants in low amounts [20].

Plants which occur in nature and contain abundant phytoconstituents, have been documented as promising source for development of new therapeutic agents [29]. Some phytoconstituents reported to have neuropharmacological activity include glycosides [30] and alkaloids [31]. Flavonoids have been reported as potential alternative medicine for epilepsy [32] and can act as benzodiazepine-like molecules that modulate central nervous system GABA-mediated chloride conductance in animal models of seizure. Saponins and terpenoids have been reported to effectively attenuate MES and PTZ-induced seizures in animals [33,34].

The result of phytochemical analysis **(Table 1)** in this study suggests that anticonvulsant activity exhibited by *Piper guineense* leaf may be associated with the presence of glycosides, alkaloids, flavonoids, tannins and terpenoids.

Report has shown that the aim of determining safety of medicinal plants is to identify the nature of possible adverse effects and to reveal the concentrations at which the effects occur [35]. Plants have been reported to have advantages of toxicity considerations based on their long term use [36]. *Piper guineense* is a common edible plant in South Eastern Nigeria. This study did not record any signs of toxicity nor lethality up to the limit/maximum test dose of 4000 mg/kg PGE within the periods of observation in rats.

Maximal Electric Shock (MES) is a model that can induce generalized tonic-clonic convulsion and can be used to identify agents that possess the ability to prevent spread of seizure when the brain's neuronal circuits are maximally activated [37]. Many drugs that increase brain content of Gamma Amino Butyric Acid (GABA) have been reported to exhibit anticonvulsant activity against MES-induced seizures [26,38]. The extensor phase of MES-induced convulsion is selectively inhibited by drugs effective in generalized tonic-clonic seizure [26]. Phenobarbitone is effective against electrically-induced convulsions in rats and mice and it acts by two mechanisms: Enhancing the activation GABAA receptors and inhibiting excitatory synaptic responses, thus, affecting the duration and intensity of artificially-induced seizures [39]. Judging from the result presented in **Table 2**, PGE at 400 mg/kg decreased mortality and the duration of hind limb tonic extension and clonic convulsions induced by maximal electrical shock. The extract at 400 mg/kg concurrently administered with 100 mg/kg phenobarbitone may have exhibited a synergistic reduction in seizure duration, evidenced by reduction of mortality to 20% and therefore, protected against MES-induced seizure. Similar protective effect against MES-induced seizure has been reported on the same plant [16].

Although Pentylenetetrazole (PTZ)-induced seizure is most widely used animal model, the mechanism of action is not fully comprehended. At molecular level, it is believed that PTZ may elicit seizures by noncompetitive inhibition of the activity of GABA at GABAA receptors [40-42]. GABA is an inhibitory neurotransmitter that plays a role in the pathogenesis of seizures. Seizure is thought to occur and attenuated when GABAergic neurotransmission in the brain is respectively inhibited and enhanced [26,43-46]. Standard antiseizure drugs such as phenobarbitone, is believed to produce its effect by enhancing GABA-mediated inhibition in the brain [39,47]. In the present study **(Table 3),** PGE produced antiseizure activity that can be significantly similar to phenobarbitone on PTZ-induced seizure and hence, may suggest that anticonvulsant activity of the plant extract is by enhancing GABA-mediated inhibition. Similar findings have been reported on other plants with anticonvulsant activity [26,47,48].

Picrotoxin has been reported to elicit seizure by blocking the action of GABA on GABAA receptor-linked chloride ion channel, which when open, usually allows enhanced conductance of chloride ion into the brain cells, resulting from activation of GABAA receptor by GABA [46,49]. From the result of the study in **Table 4**, Phenobarbitone and PGE individually and in combination, significantly ($p<0.05$) delayed the latency of picrotoxin-induced seizure, hence, may suggest that PGE enhanced GABA neurotransmission probably by opening the chloride channels associated with GABAA receptors. Similar finding has been reported on another plant that delays the latency of Picrotoxin-induced seizure [47].

Conclusion

This study concludes that *Piper guineense* leaf possesses anticonvulsant activity, hence, justifies its tradomedicinal use as anticonvulsant agent and also substantiates the existing reports. However, further studies on *Piper guineense* leaf are required to isolate and characterize the bioactive principle(s) responsible for anticonvulsant activity.

Conflict of Interest

There is no conflict of interest among the authors.

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