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Studies on the Anticonvulsant Activity of Extract and Fractions from Zapoteca Portoricensis (Jacq) HM. Hernández.

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Abstract: In Nigerian traditional medicinal practices, Zapoteca portoricensis (Jacq) HM. Hernández roots are used as anti-diarrhoea, antispasmodic as well as treating convulsive disorders. The present study aimed to evaluate the anticonvulsant activity of the root extract and fractions of Zapotecca portoricensis in mice.
Three different *in vivo* test models were used: pentylenetetrazole (PTZ), picrotoxin (PTX) and strychnine (STR) induced-convulsions in mice. Mice were allotted to five (5) groups; group I served as the seizure control, groups II-IV served as the test groups that received *Z. portoricensis* extract and fractions (100, 200 and 400 mg/kg, p.o.), respectively while group V served as the standard control.

The root extract and fractions of *Zapoteca portoricensis* dose-dependently exhibited significant anticonvulsant activity and high percentage protection against mortality compared with the control. In the PTZ and STR-induced convulsions, EF and MF at 400 mg/kg dose, showed the highest significant (p<0.001) delay in the time of onset of myoclonic spasms (MS) and tonic-clonic phase (TCP), respectively while ME (400 mg/kg) showed highest significant (p<0.001) delay against PTX-induced MS and TCP.

The results indicate that *Zapoteca portoricensis* root possesses significant anticonvulsant activity, thus a potential source of effective drug against convulsive disorders.

Keywords: Zapoteca portoricensis, Anticonvulsant, Pentylenetetrazole, Picrotoxin, Strychnine.

1 Introduction

Epilepsy accounts for around 2 % of the most common abnormality of the brain, making it second to stroke [1]. Globally, it is the third most common neurological disorder affecting approximately 3% of the population. It is characterized by recurrent seizures and usually episodes of unconsciousness and/or amnesia due to decreased resistance of excitatory neurons to fire and downregulation of inhibitory neurons [2]. There are approximately 50 million epileptics worldwide and 80% of them are from developing countries. The incidence in developed countries is approximately 50 per 100,000 while that of developing country is between 7 and 14 per 1000 [3]. Among the common pathologies that give rise to epilepsy are brain tumours, meningitis, infections of the CNS, metabolic if the aetiology cannot be identified or "symptomatic" if the aetiology is secondary to an identifiable condition. Many therapeutic approaches have been utilized in the management, control, and/or treatment of epileptics [5]. However, about 30% of epileptic cases are refractory to current antiepileptic drugs (AEDs). The refractoriness, dose-related toxicity, high cost and unavailability of standard AEDs call for alternative therapeutic options.

In recent years, the search for newer agents with better efficacy and tolerability from natural sources has been the major goal in epilepsy research [6]. In Africa alone, several herbal medicinal products are in widespread use in treating epileptics due to their easy availability and affordability, when compared with standard AEDs. *Zapoteca portoricensis* (Jacq) HM. Hernández belongs to the family Fabaceae. It is endemic in West Africa, West Indies and the Atlantic Coast of America. In South-Eastern Nigeria, It is popularly called "Elugelu" and is used to treat tonsillitis, spasmodic, gastrointestinal disorders as well as convulsive disorders [7]. It has shown antimicrobial [8], antiulcer [7] and anti-

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inflammatory [9] properties. However, the anticonvulsant activity has not been reported. The present study aims at the anticonvulsant activity to scientifically ascertain the traditional use in the treatment of convulsive disorders.

2 Materials and Methods

2.1 Drugs and Chemicals

Pentylenetetrazole (PTZ) (Sigma, Germany), picrotoxin (PTX) (Sigma, Germany); strychnine (STR) (Sigma, Germany) and diazepam (Valium) 5 mg/ml injection (F-Hoffman-La Roche, Switzerland). Solvents used for extraction and fractionation are methanol, n-hexane and ethyl acetate (analytical grades).

2.2 Animals

Adult albino mice weighing between 20-30 g were used for this study. The animals were provided with standard laboratory pellets and water *ad libitum*. All animal experiments were conducted according to International Guidelines for Care and Use of Laboratory Animals (Pub. No. 85 -23, revised 1985) and in strict compliance to the ethical principles, rules and regulations of the institution on the use of laboratory animals for experiments (NHREC/05/01/2008B).

2.3 Collection and Preparation of Plant Material

Fresh roots of *Z. portoricensis* were collected from Orba in Nsukka LGA, Enugu State, Nigeria. Botanical identification was confirmed by Mr A.O. Ozioko, International Centre for Ethnomedicine and Drug Development (Inter-CEDD), Nsukka, Nigeria. The voucher specimen was deposited at the herbarium with the number InterCEDD/16043. The roots were air-dried and powdered to a coarse size using a milling machine (Lab mill, serial no. 4745, Christy and Norris Ltd, England). The powdered root (3 kg) was extracted with about 10 L of methanol by maceration process. The resulting filtrate was concentrated using a rotary vacuum to obtain the methanol extract (ME; 160 g; 5.33% w/w). The ME (150 g) was subjected to fractionation in a silica gel (70–230 nm mesh, Merck Germany) column using n-hexane, ethyl acetate and methanol solvents. The fractions were concentrated to obtain the n-hexane fraction (HF; 7.54 g; 5.02% w/w), ethyl acetate fraction (EF; 3.34 g; 2.22% w/w) and methanol fraction (MF; 46.25 g; 30.80% w/w).

2.4 Phytochemical Screening

Qualitative phytochemical screening was performed to identify secondary metabolites present in the methanol extract (ME) as well as the n-hexane, ethyl acetate and methanol fractions using standard procedures [10,11].

2.5 Anticonvulsant activity

2.5.1 Pentylenetetrazole(PTZ)-induced Convulsions.

Five (5) groups of mice (n=5) were used in this model; group I served as the PTZ-induced seizure control (received 10 ml/kg of distilled water, p.o.), groups II-IV received ME, HF, EF and MF (100, 200 and 400 mg/kg, p.o.), respectively while group V served as the standard (received 3 mg/kg of diazepam, i.p.). Thirty minutes later, convulsions were induced by the administration of pentylenetetrazole (PTZ, 60 mg/kg, i.p). Animals were observed for the time of onset of myoclonic spasms and tonic-clonic phases of seizures. Protection (%) of mice against seizure-induced deaths was also recorded in each group. Animals devoid of seizure/ convulsion without subsequent death during the 60 min observation period were considered protected [12,13].

2.5.2 Picrotoxin (PTX)-induced Convulsion

Five (5) groups of mice (n=5) were used in this model; group I (PTX-induced seizure control) received 10 ml/kg of distilled water, p.o.), groups II–V received the extract/fractions/standard drug as described above. Treatments were done 30 min before picrotoxin (PTX, 4 mg/kg, i.p.) administration. Animals were observed for 60 min after administration of PTX for the time of onset of myoclonic spasms, tonic-clonic spasms and death. Animals that showed neither of these signs was considered protected [14,15]. Protection (%) of mice against seizure-induced deaths was recorded in each group.

2.5.3 Strychnine (STC)-induced Convulsion

Five (5) groups of mice (n=5) were used in this model; STC-induced seizure control (group I) received 10 ml/kg of distilled water, p.o.). Mice in groups II–V received the extract/fractions/ standard drug as described above. Treatments were done 30 min before strychnine (STC, 2 mg/kg, i.p.) administration. Animals were observed for 60 min after administration of STC for myoclonic spasms, tonic-clonic seizures and time to death. Animals that showed neither of these signs was considered protected *[14,15]*. Protection (%) of mice against seizure-induced deaths was recorded in each group.

2.6 Statistical Analysis

The data obtained were analysed using the One-Way Analysis of Variance (ANOVA) followed by Post Hoc Dunnett's test. Results were expressed as mean ± SD (n=5).

Differences between mean values were considered significant at 0.1%, 1% and 5% level of significance (i.e. $p<0.001$, $p<0.01$, $p<0.05$).

3 Results

3.1Phytochemical Constituents of the Extract and Fractions of Z. Portoricensis

The result showed that ME contains carbohydrates, saponins, steroids, reducing sugar, flavonoids, terpenoids, glycosides, fats and oil, alkaloids and resins. HF tested positive for carbohydrates, saponins, steroids, flavonoids, terpenoids, fats and oil, alkaloids and resins. EF gave positive reactions for carbohydrates, saponins, steroids, terpenoids, glycosides, fats and oil, alkaloids and resins while MF tested positive for carbohydrates, saponins, steroids, terpenoids, glycosides, alkaloids and resins (Table 1).

(60.00 ± 0.00) of onset of MS and TCP respectively with 100% protection (Table 3).

3.2 Effect of Extract and Fractions of Z. Portoricensis on PTZ-Induced Convulsion

The result showed that EF at all doses (100, 200, 400 mg/kg), HF (400 mg/kg) and MF (400 mg/kg) significantly ($p<0.001$; $p<0.01$) delayed the onset of MS whereas in mice that received ME, no significant ($p>0.001$; $p>0.01$; P>0.05) effect was observed in the time of onset of MS compared with the control group. In the TCP, there was significant ($p<0.001$; $p<0.01$) delay in ME and HF (400 mg/kg); EF and MF (200 and 400 mg/kg), respectively compared with the control. However, EF (200 and 400 mg/kg) and ME (400 mg/kg) offered the highest percentage protection (100%) in mice similar to diazepam (3 mg/kg *i.p.*) with 100% protection. This was followed by ME (100, 200 mg/kg) with 80% protection compared with the control (0%) against PTZ-induced lethality (Table 2).

Table 1: Phytochemical constituents of the extract and fractions of *Z. portoricensis.*

Constituents	ME	HF	EF	MF
Carbohydrates	+	+	+	+
Reducing sugar	+	-	-	-
Glycosides	+	-	+	+
Flavonoids	+	+	-	-
Saponins	+	+	+	+
Alkaloids	+	+	+	+
Terpenoids	+	+	+	+
Tannins	-	-	-	-
Resins	+	+	+	+
Steroids	+	+	+	+
Fats and oil	+	+	+	-
Acidic compounds	-	-	-	-

= Present; - = Absent

3.3 Effect of Extract and Fractions of Z. Portoricensis on PTX-induced Convulsion

The result showed that ME (200 and 400 mg/kg), HF (400 mg/kg) and EF (100, 200, 400 mg/kg) significantly ($p<0.00$; $p<0.01$; $p<0.05$) delayed the time of onset of MS when compared with the control group. MF had no significant ($p>0.001$; $p>0.01$; $p>0.05$) effects on MS. The TCP was found to be significantly ($p<0.001$; $p<0.05$) delayed in ME at all doses (100, 200, 400 mg/kg), MF and HF (400 mg/kg) and EF (200 and 400 mg/kg) compared with the control. ME (200 and 400 mg/kg) offered the highest percentage protection (100%) in treated mice followed by HF and MF with 80% protection at 400 mg/kg, respectively. Diazepam exhibited the highest delay in time

3.4 Effect of Extract and Fractions of Z. Portoricensis on STR-Induced Convulsion

The onset of MS was significantly ($p<0.001$; $p<0.01$; $p<0.05$) delayed by ME and EF at all doses (100, 200, 400); HF and MF (200 and 400 mg/kg), respectively when compared with the control. Similarly, ME, EF and MF at all doses (100, 200 and 400) and HF (200, 400 mg/kg) significantly ($p<0.001$; $p<0.01$; $p<0.05$) delayed strychnine-induced TCP compared with the control. MF and ME at 400 mg/kg respectively, offered the highest percentage protection of 80 % against STR-induced convulsion. The diazepam treated group did not show any signs of convulsions, and thus shows percentage protection of 100% (Table 4).

Table 2: Effect of extract and fractions of *Z. portoricensis* on PTZ-induced convulsion.

Treatment	Dose (mg/kg)	Onset of seizure (min)		% protection
		Myoclonic spasms (MS)	Tonic-clonic phase (TCP)	
Control	10 ml/kg	0.67 ± 0.02	0.81 ± 0.18	0
Diazepam	3	60.00 ± 0.00***	60.00 ± 0.00***	100
ME	100	0.86 ± 0.03	1.08 ± 0.06	80
	200	1.01 ± 0.01	1.16 ± 0.03	80
	400	1.57 ± 0.06	2.07 ± 0.09**	100
HF	100	1.00 ± 0.03	1.21 ± 0.05	60
	200	1.16 ± 0.04	1.45 ± 0.04	100
	400	2.85 ± 0.09**	4.19 ± 0.37***	100
EF	100	5.02 ± 0.81***	7.05 ± 0.77***	40
	200	4.91 ± 1.48***	6.58 ± 1.36***	40
	400	7.58 ± 0.71***	9.40 ± 0.56***	60
MF	100	1.08 ± 0.08	12.97 ± 11.76***	40
	200	1.32 ± 0.02	13.11 ± 11.72***	60
	400	3.53 ± 0.18**	15.69 ± 11.09***	60

Values of onset of seizure time shown are expressed as mean ± SEM; significance ***p<0.001, **p < 0.01, *p <0.05 using ANOVA, post-hoc-Dunnet's test compared with the control, n = 5.

Table 3: Effect of extract and fractions of *Z. portoricensis* on PTX-induced convulsion

Treatment	Dose (mg/kg)	Onset of seizure (min)		% protection
		Myoclonic spasms	Tonic-clonic phase (MS) (TCP)	
Control	10 ml/kg	8.78 ± 0.49	11.32 ± 0.77	0
Diazepam	3	60.00 ± 0.00**	60.00 ± 0.00***	100
ME	100	10.49 ± 0.15	22.79 ± 9.32***	80
	200	12.69 ± 0.18**	24.72 ± 8.84***	100
	400	15.60 ± 0.18***	26.35 ± 8.45***	100
HF	100	8.06 ± 0.29	8.53 ± 0.30	60
	200	10.13 ± 0.22	11.30 ± 0.73	60
	400	16.97 ± 0.22***	17.40 ± 0.44***	80
EF	100	11.46 ± 0.74*	12.81 ± 0.77	40
	200	11.49 ± 0.75*	13.26 ± 0.89*	60
	400	11.87 ± 0.71*	13.43 ± 0.79*	40

MF	100	7.38 ± 0.09	11.32 ± 0.77	40
	200	9.91 ± 0.15	12.03 ± 0.75	60
	400	10.66 ± 0.55	21.09 ± 9.74***	80

Values of onset of seizure time shown are expressed as mean ± SEM; significance *** p<0.001, ** p<0.01, * p<0.05 using ANOVA, post-hoc-Dunnet's test compared with the control, n = 5.

Table 4: Effect of extract and fractions of *Z. portoricensis* on STR-induced convulsion.

Treatment	Dose (mg/kg)	Onset of seizure (min)		% protection
		Myoclonic spasms (MS)	Tonic-clonic phase (TCP)	
Control	10 ml/kg	0.73±0.02	1.21±0.07	0
Diazepam	3	60.00 ± 0.00***	60.00±0.00***	100
ME	100	4.38±0.45*	4.65 ± 0.47*	40
	200	7.63±0.41**	7.92 ± 0.40**	60
	400	9.50±0.39**	9.86 ± 0.41**	80
HF	100	3.32±0.19	3.92 ± 0.37	40
	200	6.59±0.39**	8.53 ± 0.43**	60
	400	8.13±0.18**	19.37 ± 10.17***	60
EF	100	6.24 ± 0.30**	7.14 ± 0.30**	40
	200	7.49 ± 0.49**	8.64 ± 0.58**	40
	400	11.08 ± 0.99***	10.71 ± 1.15***	60
MF	100	3.13 ± 0.40	6.20 ± 1.28**	40
	200	6.11± 0.20**	7.98 ± 0.42**	60
	400	9.69 ± 0.63***	30.61 ± 12.01***	80

Values of onset of seizure time shown are expressed as mean ± SEM; significance *** p<0.001, ** p< 0.01, * p<0.05 using ANOVA, post-hoc-Dunnet's test compared with the control, n = 5.

4 Discussions

In this study, the anticonvulsant activity of *Zapoteca portoricensis* was evaluated against pentylenetetrazole (PTZ), picrotoxin (PTX) and strychnine (STR) induced convulsion models in mice.

The observation after PTZ (60 mg/kg, i.p) administration showed an elicited myoclonic spasm (MS) and tonic-clonic phase (TCP) consistent with many other studies. Evidence has shown that PTZ produces convulsions in experimental animals by inhibiting γ-aminobutyric acid (GABA) pathway in the central nervous system (CNS) via the inhibition of $GABA_A$ receptor-chloride channel complex [16]. This results in a hyper-excitable state of neurons and excessive firing of an action potential, which leads to the development of seizure [17]. The activation of the N-methyl-d-aspartate (NMDA) receptors according to previous report by Kiasalari *et al.* [18] might also be involved in the initiation and propagation of PTZ-induced seizures. The result showed that the methanol extract (ME) and fractions (EF, HF, MF) of *Z. portoricensis* root exhibited varying degrees of anticonvulsant activity in a dose-dependent manner. The 400 mg/kg dose exhibited a maximum delay in the onset of PTZ-induced convulsions when compared to the seizure control group. ME (400 mg/kg) and EF (200 and 400 offered the highest percentage protection (100%). The standard, diazepam exhibited the highest degree of delay in MS and TCP (60.00±0.0) with 100% protection of mice. Diazepam, a positive modulator of $GABA_A$ receptors inhibits seizure frequency and severity

by enhancing the $GABA_A$ receptor-mediated inhibition in the brain [19]. The ability of *Z. portoricencis* root extract and fractions to protect mice against PTZ-induced seizure suggests the presence of constituents with protective activity against PTZ-induced seizure and indicate a promising efficacy against absence seizures. Nisar *et al.* [20] reported that drugs that antagonize PTZ-induced convulsions are generally effective in controlling myoclonic and absence seizures.

The use of picrotoxin-induced seizure as a research model for elucidating the anticonvulsant activity of pharmacological agents has been reported in literature. Picrotoxin, a CNS stimulant and non-competitive antagonist at GABA receptors, was reported to elicit its convulsant effects by blocking the presynaptic inhibition mediated by GABA on $GABA_A$ receptor chloride channels [21]. As presented in Table 3, the methanol root extract and fractions of *Z. portoricencis* demonstrated significant activity against PTX-induced seizure in a dose-dependent manner. The 400 mg/kg appeared to be most effective in delaying the time of onset of MS and TCP. The highest percentage protection of 100 % against PTX-induced seizure deaths was offered by ME (100, 200 mg/kg) followed by 80% for HF and MF at 400 mg/kg dose, respectively. The established suppression and protection against PTX-induced convulsions is suggestive of the enhancement of GABAergic activity [22,23]. A possible mechanism of action may be by enhancing chloride currents through picrotoxin–sensitive chloride channels on the $GABA_A$ receptor complex [24].

Numerous studies have reported the use of strychnine test model for anticonvulsant screening. Strychnine (STR), a potent CNS convulsant, selectively block inhibitory inputs by glycine receptors [25], predominantly at the spinal cord, to induce excitatory responses in the CNS [26]. The result showed a dose-dependent anticonvulsant activity of ME, HF, EF and MF against strychnine-induced convulsions by delaying the time of onset of MS and TCP. MF and HF at 400 mg/kg respectively offered the highest delay in time of onset of MS and TCP and protected 80% and 60%, respectively of the mice against STR-induced seizure deaths. The seizure suppression induced by strychnine indicates an anticonvulsant activity via the glycine inhibitory mechanisms [27].

Furthermore, phytochemical screening of *Z. portoricencis* indicated the presence of saponins, steroids, flavonoids, terpenoids, glycosides and alkaloids. The anticonvulsant activity of triterpenes [28], flavonoids [29], saponins [30,31] and alkaloids [32] have well been documented. Therefore, it is plausible to infer that these constituents may partly be responsible for the observed anticonvulsant effects [33,34].

In conclusion, the extract and fractions of *Z. portoricensis* root exhibited a marked anticonvulsant activity. This gives scientific evidence to the folkloric claims of its use in the management of convulsive disorders. Further research via activity guided isolation is ongoing to characterize the bioactive compound(s) responsible for the anticonvulsant activity.

List of abbreviations:

PTZ: pentylenetetrazole; PTX: picrotoxin; STR: strychnine; MS: myoclonic spasm; TCP: tonic-clonic phase; ANOVA: One-Way Analysis of Variance; AEDs: antiepileptic drugs; WHO: World Health Organization; NMDA: N-methyl-d-aspartate; CNS: central nervous system; GABA: γ-aminobutyric acid;

Competing interests

The authors declare that there is no competing interest regarding the publication of this article.

Authors' contributions:

TCA and BCO designed and supervised all experiments. ULI and FKA performed the experiments. BCO and ULI performed the statistical analysis and wrote the manuscript. TCA made manuscript revisions. All authors read and approved the manuscript.

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