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ANTICONVULSANT SCREENING OF CHLOROFORM-SOLUBLE FRACTION OF METHANOLIC STEM-BARK EXTRACT OF *LONCHOCARPUSSERICEUS*, POIR (PAPILIONACEAE)

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ABSTRACT
Lonchocarpus sericeus is used in African traditional medicine in the management of epilepsy. Previous study showed that the crude methanol extract possess significant anticonvulsant activity. In this study, the chloroform soluble portion of the methanol extract of the stem bark of Lonchocarpus sericeus was screened for anticonvulsant activity using maximal electroshock** test **in chicks, 4-aminopyridine andpentylenetetrazols induced seizures in mice. The fraction (ISO mgkg^{1}) protected 50% of the animals against pentylenetetrazole induced seizure in mice** whereas sodium **valproate, a standard anti-epileptic drug, offered 100% protection. In 4-aminopyridine induced seizure model, the fraction at doses of 75 and 300 mgkg^{1} protected 33.3% of the animals against mortality, but phenobarbitone (20mgkg^{1}) protected 100%. There was no activity against maximal electroshock convulsion. Phytochemical screening of the fraction revealed the presence of phenolic compounds, flavonoids, triterpenoids and steroids. The fraction was found to have an intraperitoneal LD_{50} of 1131.3 mgkg^{1}. The results showed that the fraction contains compounds with some anticonvulsant activity worthy of further investigations.

Keywords: Lonchocarpus sericeus, anticonvulsant, pentylenetetrazole, aminopyridine, epilepsy.

INTRODUCTION

Epilepsy is a disorder of brain function characterized by the periodic and unpredictable occurrence of seizures. These seizures are transient signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain (McNamara, 2006). Incidence of epilepsy in developed countries is approximately 50 per 100,000 while that of developing countries is 100 per 100,000 (WHO, 2006). The ideal anti-seizure drugs would suppress all seizures without causing any unwanted effects. Unfortunately, the drugs used currently not only fail to control seizure activity in some patients, but frequently cause unwanted effects that range in severity from minimal impairment of the Central Nervous System (CNS) to death from aplastic anemia or hepatic failure (McNamara, 2006). As majority of anti-epilepsy drugs are consumed life long, it is necessary to investigate for an agent that is highly efficacious as well as safe in terms of drug related toxicity.

Medicinal plants used for the therapy of epilepsy in traditional medicine have been shown to possess promising anticonvulsant activities in animal models of anticonvulsant screening. Plants such as *Nigella sativa,* (Hosseinzadeh *et al.*, 2004), *Glycyrrhiza glabra* (Ambawade *et al.*, 2002) and *Drosera burmannii* (Gauthaman *et al.*, 2009) were reported to have anticonvulsant activity. Musa *et al.,* (2006) reported the anticonvulsant activity of the crude methanol extract of stem-bark of *Lonchocarpus sericeus*.

In an attempt to isolate the bioactive principle responsible for this activity, the chloroform soluble fraction of the methanol extract was evaluated for anticonvulsant potential.

MATERIALS AND METHODS

Collection and Identification of the Plant material

The stem bark of *Lonchocarpus sericeus* was collected from Kongo Campus of Ahmadu Bello University, Zaria-Nigeria in the month of June, 2010 after identification of the tree on the field using description in the literature (Burkill, 1995). The identity of the stem bark was confirmed and authenticated by Mal. Musa Mohammed of the Herbarium section, Department of Biological Sciences, Ahmadu Bello University Zaria by comparing with existing specimen of voucher number 1085.

Extraction and Fractionation

The stem bark was washed, air dried under shade, powdered manually and referred to as plant material. A weighed quantity (2000g) of the plant material was extracted with 70% methanol in a soxhlet extraction apparatus. The extract was concentrated *in vacuo* to yield 100g of brown mass subsequently referred to as methanol extract.

The methanol extract was suspended in water and partitioned successively with n-hexane, chloroform, ethyl acetate and n-butanol to obtain hexane fraction (HXF), chloroform fraction (CF), ethyl acetate fraction (EAF) and n-butanol fraction (BuF) respectively. CF was concentrated *in vacuo* affording 10 g of a yellowish brown residue.

Experimental Animals

Locally bred adult Swiss albino mice of either sex weighing 20 ± 2 g were obtained from Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria-Nigeria. Day old Ranger cockerels (30 ± 5 g) were obtained from Zarm farms Kwara State, Nigeria. The mice, maintained on standard rodent feed and water *ad libitum,* were housed in polypropylene cages at room temperature throughout the study. All experimental protocols were in accordance with the guidelines for the care and use of experimental animals as accepted internationally and in compliance with Ahmadu Bello University, Zaria-Nigeria Research Policy. The experiments were conducted in a quiet laboratory between the hours of 900 h to 1600 h.

Drugs/Chemicals and Equipment

Methanol, n -hexane, chloroform, ethyl acetate and n-butanol (Sigma Co. USA), normal saline (Dana, Nigeria), 4-Aminopyridine (Sigma Co. USA), pentylenetetrazole (Sigma, UK), phenobarbitone (Lab. Renaudin, France), phenytoin (Sigma, UK) and valproic acid (Sanofi synthelabo)

Preliminary Phytochemical Screening

The chloroform fraction was tested for the presence of alkaloids, flavonoids, phenolic compounds, cardiac glycosides, triterpenes and steroids using standard protocols (Trease and Evans, 2002).

Acute Toxicity Study

The method previously described by Lorke (1983) was adopted. All mice were injected intraperitoneally (i.p). Briefly, the study was divided into two phases. In the first phase, nine mice of either sex were divided into three groups of three mice each. Group I received 10 $mgkg^{-1}$ extract while groups II and III received 100 $mgkg^{-1}$ and 1000 $mgkg^{-1}$ respectively. The animals were observed for signs and symptoms of toxicity including mortality for twenty-four hours after treatment. In the second phase, four mice of either sex were divided into four groups of one mouse each. The first mouse received extract at a dose of 200 $mgkg^{-1}$ while the second, third and fourth received the extract at doses of 400 $mgkg^{-1}$, 800 $mgkg^{-1}$ and 1600 $mgkg^{-1}$ respectively. The mice were also observed for twenty four hours. The final LD_{50} was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose *i.e* the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

Pentylenetetrazole (PTZ) Induced Seizure in mice

The method previously described by Swinyard (1989) was employed. Thirty mice were randomly divided into five groups of six mice each. Group one (negative control group) was given 10 $mlkg^{-1}$ i.p of normal saline. Groups two, three and four were given 75 $mgkg^{-1}$, 150 $mgkg^{-1}$ and 300$mgkg^{-1}$ i.p of CF respectively. Group five (positive control) was given 200 $mgkg^{-1}$i.p valproic acid. Thirty minutes post-treatment, 100 $mgkg^{-1}$ of freshly prepared solution of s.c PTZ (CD_{97}) was administered to each mouse. The mice were observed for thirty minutes for presence or absence of threshold seizures (i.e an episode of clonic spasm of at least five seconds duration).

4-Aminopyridine (4-AP) Induced Seizure Test

The method previously described by Yamaguchi and Rogawski (1992) was adopted. Thirty albino mice were randomly divided into five groups of six mice each. Group one (negative control) was given 10 $mlkg^{-1}$ of normal saline. Groups two, three and four were given graded doses of the extract. Group five was administered 20 $mgkg^{-1}$ phenobarbitone to serve as positive control. After thirty minutes, 15 $mgkg^{-1}$ of freshly prepared 4-AP was administered subcutaneously to each mouse. Ability of the extract to protect the mice from lethality within a thirty minutes observation period was considered an indication of anticonvulsant activity.

Maximal Electroshock Seizure Test (MEST) in Chicks

The method of Swinyard and Kupferberg (1985) was employed. Day old ranger cockerels were randomly divided into five groups of ten chicks each. The first group received normal saline (10 $mlkg^{-1}$) i.p, second, third and fourth groups were administered graded doses of the extract and the fifth group was administered 20 $mgkg^{-1}$ of phenytoin. Thirty minutes later, maximal electroshock was administered to induce seizure in the chicks using Ugobasile electroconvulsive machine (Model 7801) with corneal electrodes placed on the upper eyelids of the chicks. The shock duration, frequency and pulse width were set and maintained at 0.8 s, 100 pulses/sec and 0.6 ms respectively. A current of 90 mA which produced tonic seizures in 80% of the control chicks, was used throughout the study. An episode of tonic extension of the hind limbs was regarded as full convulsion, while lack of tonic extension of hind limbs was regarded as protection. In unprotected animals, the recovery time was recorded.

Statistical Analysis

The protective index in each study was recorded as percentage protection. The onset of seizure and recovery time after seizure was expressed as mean ± S.E.M. The mean values of treated groups were compared with that of the control group using SPSS Software version 13. Differences were considered significant at $p<0.05$ or lower.

RESULTS

Phytochemical Screening

Preliminary phytochemical screening of CF revealed the presence of phenolic compounds, flavonoids, steroids and triterpenoids (Table 1).

Table 1: Result of Preliminary phytochemical screening

Constituent	Test	Observation	Inference
1. Steroids and triterpenes	a. Liebermann burchards test	Brownish-red ring	+ ve
	b. Salkwoski test	Reddish brown colour at interphase	+ ve
2. Flavonoids	a. Shinodas test	Red colouration	+ ve
	b. Ferric chloride test	Green colouration	+ ve
3. Saponins	Froth test	No froth	- ve
4. Phenolic compound	a. Lead subacetate test	Yellow colouration	+ve
	b. Ferric chloride test	Greenish black colouration	+ve

Key: + ve present, - ve absent

Acute Toxicity Study

The median lethal dose (LD_{50}) of the CF in mice was estimated to be 1131.3mgkg^{-1} body weight (Table 2).

Table 2: Result of Acute Toxicity Studies

First phase			
Group	**Number of animals**	**Dose (mgkg^{-1})**	**Death observed**
I	3	10	0/3
II	3	100	0/3
III	3	1000	2/3
Second phase			
Group	**Number of animals**	**Dose (mgkg^{-1})**	**Death observed**
I	1	200	0/1
II	1	400	0/1
III	1	800	0/1
IV	1	1600	1/1

Therefore, $LD_{50} = \sqrt{(1600 \times 800)}$

$= 1131.3$ mgkg^{-1}

Anticonvulsant studies

In the Subcutaneous pentylenetetrazole model, all the control animals exhibited threshold seizures and loss of righting reflex, with tonic forelimbs extension. CF offered maximum protection against threshold seizure with 50% protection at a dose of 150 mgkg^{-1} (Table 3).

Table 3: Effect of CF against Pentylenetetrazole-induced seizure in mice

Treatment (mgkg^{-1})	Percentage protection	Mean Onset of Seizure in Unprotected animals (min)	Percentage protection against Mortality
Normal saline	-	6.33 ± 1.2	13.33
CF 75	16.7	5.6 ± 1.75	50.00
CF 150	50.00	8 ± 0.58	83.33
CF 300	33.33	5.8 ± 1.39	67.67
VPA (200)	100.00	-	100.00

Protection against seizure and mortality expressed as percentages; Mean onset of seizure expressed as mean ± SEM; CF: Chloroform soluble fraction; VPA: Valproic acid; n=6

CF was unable to offer protection (quantal protection) against seizure induced by 4-Aminopyridine at all the tested doses. Similarly, there was no significant difference in the latency to death for the extract treated groups as compared to normal saline groups (Table 4).

Table 4: Effect of CF against subcutaneous 4-Aminopyridine-induced seizure in mice

Treatment ($mgkg^{-1}$)	Percentage protection	Mean Onset of Seizure in Unprotected animals (min)	Percentage protection against Mortality
Normal saline	13.33	15.8 ± 1.2	33.33
CF 75	23.33	22.5 ± 2.9	33.33
CF 150	13.33	17.4 ± 1.44	13.33
CF 300	26.67	19.75 ± 3.66	33.33
PBT (20)	100	-	100

Protection against seizure and mortality expressed as percentages; Mean onset of seizure expressed as mean ± SEM; CF: Chloroform soluble fraction; PBT: Phenobarbitone; n=6

CF did not offer significant protection against maximal electroshock seizure in the chick at all the doses tested. There was also no significant decrease in the mean recovery time in the unprotected animals (Table 5).

Table 5: Effect of CF on Maximal Electroshock seizure in chicks

Treatment ($mgkg^{-1}$)	Percentage protection	Mean Recovery time in Unprotected animals (min)
Normal saline	0	4.0 ± 0.68
CF 75	0	5.2 ± 0.57
CF 150	0	7.2 ± 0.65
CF 300	0	6.1 ± 0.78
Phenytoin (20)	70	5.67 ± 1.86

Protection against seizure expressed as percentages; Mean recovery time expressed as mean ± SEM; CF: Chloroform soluble fraction; n=10

DISCUSSION

The results obtained from this study suggest that CF contains some biologically active compounds that possess some anticonvulsant activity. The intraperitoneal median lethal dose of CF found to be 1131.3 $mgkg^{-1}$ in mice suggests that it is relatively toxic (Matsumura, 1985). However, it is relatively safe at the doses employed in the studies.

Anticonvulsant activity in subcutaneous PTZ test identifies compounds that can raise the seizure threshold in the brain (White *et al.*, 1998). One of the mechanisms by which such drugs act is the suppression of T-type calcium currents in thalamic neurons (Rho and Sanker, 1999). Similarly, agents such as the barbiturates and benzodiazepines, which enhance GABAergic transmission, are known to be protective against PTZ-induced seizure. The ability of CF to protect the animals against PTZ-induced seizure suggest that CF contains compounds useful in petit mal epilepsy since all the antiepileptic drugs that are effective in the treatment of petit mal epilepsy can suppress various seizure patterns induced by pentylenetetrazole (Loscher *et al.*, 1991). Maximal electroshock seizure can be prevented by sodium channel blockers such as phenytoin, valproate, felbamate and lamotrigine (Browning, 1992; Sayyah *et al.*, 2004) or agents that block glutamatergic neurotransmission mediated by N-methyl-D-Aspartate (NMDA) receptors (Subramaniam *et al.*, 1995). The absence of anticonvulsant activity in MEST suggests that CF may not be useful in the treatment of generalized tonic clonic and partial seizures.

K^+ channels play a significant role in controlling all aspect of neuronal excitability (Wickenden, 2002). Sodium channel blockers, such as phenytoin which prevent seizure spread effectively antagonize seizures induced by K^+ channel blocker such as 4-Aminopyridine while those with specific actions on other cellular targets may be weak or inactive, presumably because they are unable to attenuate the spread of intense (non-NMDA receptor mediated) excitation evoked by 4-Aminopyridine (Yamaguchi and Rogawski, 1992). The inability of the fractions to produce significant activities against 4-AP induced seizure suggests that they may likely not be interacting with K^+ channel in producing their anticonvulsant activities. Triterpenes, steroids, and flavonoids have been reported to possess anticonvulsant activity in some experimental seizure models such as PTZ (Katsure *et al.*, 2002; Chauhan *et al.*, 1988). These constituents may be responsible, either singly or in combination for the anticonvulsant potential of the chloroform fraction of *Lonchocarpus sericeus*.

CONCLUSION

This study has shown that CF may have anticonvulsant activity against petit mal epilepsy. The mechanism for anticonvulsant activity is not clear but further studies to establish the mechanism of anticonvulsant action is in progress in our laboratory.

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