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Anticonvulsant actions of ethanol stem bark extract of *Trichilia roka* (Meliaceae) in mice and chicks

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ABSTRACT

The current antiseizure drugs present with side effects, some of which can lead to discontinuation of epilepsy pharmacotherapy, and a sizeable number of other drugs being less efficacious. Medicinal plants are used for the treatment of epilepsy in Nigeria. The aim of this study is to evaluate the anticonvulsant activity of the stem bark extract of *Trichilia roka* in mice and chicks. Anticonvulsant screening was carried using pentylenetetrazole (PTZ), strychnine (STN) and picrotoxin (PCT) induced seizures while Maximal electroshock (MEST) test in day old chicks. Preliminary phytochemical screening of the extracts was carried out on the extract using conventional protocols. The LD_{50} was determined in mice. The intraperitoneal LD_{50} was calculated to be 118.32 mg/kg in mice. Flavonoids, tannins, alkaloids, saponins, glycosides, anthraquinones and steroids were found to be present. Significant ($p \leq 0.05$) prolongation of the mean onset of seizures was recorded with *Trichilia roka* (15 and 30 mg/kg) compared with normal saline treated groups in PTZ induced seizures. *Trichilia roka* at all the doses tested did not significantly reduce the mean recovery time of seizures induced by MEST. The extract at 7.5 and 15 mg/kg prolonged the latency of convulsion induced by STN. The extract failed to show significant prolongation of the latency of seizures induced by picrotoxin. It can then be inferred, that *Trichilia roka* contained bioactive constituents that are beneficial in petit mal epilepsy and justify their use in Traditional Medicine.

Keywords: Epilepsy, *Trichilia roka*, Maximal electroshock, Pentylenetetrazole, Strychnine and picrotoxin.

INTRODUCTION

Epilepsy is a chronic neurological disorder with a severe morbidity [1]. It was earlier tagged in the ancient days as disease of lightening, characterized by asynchronous, dysrrythmic electrical discharges in the brain [2]. It is estimated that 0.5-1% of world population are affected by this disorder, about 85% of this population are residing in developing countries [3, 4]. In Nigeria, for instance, the prevalence is 37-41 per 100 [5]. Conventional antiseizure drugs like phenobarbitone, phenytoin, sodium valproate, clonazepam among others, have been in use for a quite period of time. Even with the successful usage of these drugs, lack of specificity in exerting their antiseizure effect within the Central Nervous System (CNS), has led to varying side effects observed with these agents. It is therefore worthwhile, to search for ideal antiseizure drugs with high specificity, efficacy and tolerable side effects. Medicinal plants are occupying a strategic position in drug discovery and development process [6]. Herbs and medicinal plants have been in existence for the treatment of plethora of diseases and disorders from time immemorial.

Against this backdrop, natural products with ethnomedical documentation for the treatment of epilepsy are evaluated. *Trichilia roka* (Meliaceae) is a tree that grows 5-12 m high, with straight, spindly bole, crown open, with the leaves at the end of the branches. The plant is well distributed in Sudanese and Guinean Savannahs, especially on rocky soils. It is found in Senegal, Cameroon, Madagascar and Nigeria [7]. It is called "Goron Talaka" by the Hausas of northern Nigeria, meaning "poor man's kola nut" because of its edible fruits that resembles kola nut. Traditionally, it is used to treat: epilepsy, asthma, dysmenorhea and headache [7].

Previous studies on this plant reported antimicrobial activity [8]. Furthermore, antiplasmodial and antioxidant activities in animal models were also evaluated [9]. Its use in hypertension, viral disease and oedema was equally scientifically justified [10].

MATERIALS AND METHODS

Plant material

The stem bark was collected on 8th June, 2013 at Galadimawa village, in Kaduna State, Nigeria. The plant was identified by Taxonomist in the Herbarium section, Department of Biological Sciences,

Ahmadu Bello University, Zaria, Nigeria by comparing with already deposited voucher specimen number 7232.

Preparation of plant material

The stem bark was cleaned, cut into smaller size and air dried at room temperature. The material was then milled into powder using pestle and mortar. Extraction was carried out by cold maceration where 287 g of the powder was soaked in 7 L of 70%v/v ethanol (70% ethanol: 30% water) for 3 days with occasional shaking using glass stirrer. The resultant mixture was filtered using Whiteman filter paper (No.1) and filtrate was concentrated to dryness in vacuo at 40^0c using rotary evaporator.

Animals

Swiss albino mice (17-30g) of either sex were obtained from the Animal Facility Centre (AFC) Department of Pharmacology, Bayero University, Kano, Nigeria, while day old cockerels were obtained from National Animal Production Research Institute of Nigeria (NAPRI), Zaria, Nigeria. Animals were maintained in a well ventilated room and fed with standard feeds and water provided *ad libitum*. We certify that all experiments were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of Laboratory Animals {NIH Publications No.80-23} revised in 1996. The study was approved by Departmental Animal Ethics committee.

Phytochemical screening

The phytochemical screening was performed on the dried stem bark extract of *Trichilia roka* using standard procedure [11].

Acute toxicity testing

The intraperitoneal LD_{50} was determined in mice. The study was carried out in two phases. In the first phase, nine mice of either sex were randomly divided into three groups of three mice each and were administered 10, 100 and 1000 mg/kg of the extract intraperitoneally. Mice were observed for signs and symptoms of toxicity including death over a period of 24 hours. In the second phase of the study, 600, 1000, 1600 and 2900 mg/kg body weight of the extracts were given to four different groups of one mouse each intraperitoneally (*i.p.*) based on the result of the first phase. The LD_{50} was estimated by calculating the geometric mean of the lowest dose that caused death (1/1) and the highest dose that animal survived (0/1) [12].

Pentylenetetrazole induced seizures in mice

Mice were divided into five groups of six mice each. Group 1 received normal saline (10 ml/kg), group 2, 3 and 4 received the extract at the doses of 7.5, 15 and 30 mg/kg of body weight respectively, while group 5 received standard drug sodium valproate at a dose of 200 mg/kg intraperitoneally. Thirty minutes later, mice in the groups received 100 mg/kg PTZ subcutaneously (CD_{100}). Animals were observed for the presence or absence of threshold seizures (an episode of clonic spasm of at least 5 second duration), mean onset of convulsion, quantal protection and percent protection, number of convulsions and time to death according to the method of Swinyard (1989) [13].

Maximal electro shock test (MEST) in chicks

The apparatus used was Ugo Basile Electroconvulsive Machine (Model 7801) with corneal electrodes placed on the upper eyelid of the chicks after dipping them in normal saline. The current, shock duration, pulse width and frequency were set and maintained at 80 mA, 0.6 sec, 0.6 ms and 100 pulses per second respectively. Fifty day old cockerels were grouped into five groups of ten chicks each. Group 1 was pretreated with normal saline (10 ml/kg *i.p.*), group 2, 3 and 4 were administered with extract at doses of 7.5, 15, 30 mg/kg body weight intraperitoneally, while group 5 was treated with phenytoin sodium 20 mg/kg body weight intraperitoneally. Thirty minutes post treatment, electroshock was administered to each animal to induce convulsion. Results were recorded as either positive or negative depending on whether tonic hind limb extension (THLE) was produced. The time of recovery of convulsed chicks were recorded and the percentage of convulsed animals calculated [14].

Strychnine induced seizures in mice

Mice were grouped into 5 groups of 6 mice each. Group 1 received 10 ml/kg of normal saline, group 2, 3 and 4 were administered with the extract at doses of 7.5, 15 and 30 mg/kg body weight respectively while group 5 received phenobarbitone at a dose of 30 mg/kg body weight intraperitoneally. Thirty minutes later, mice were administered 1.2 mg/kg body weight of strychnine subcutaneously and observed for incidence of convulsions. Prevention of tonic hind limb extensor jerk was considered as protection against seizures induced by strychnine [15].

Picrotoxin induced seizures in mice

Thirty mice were grouped into five each consisting of six mice. The mice in first group received normal saline (10 ml/kg), the second, third and fourth groups were injected with the extract at doses of 7.5, 15 and 30 mg/kg body weight respectively. The fifth group received phenobarbitone 30 mg/kg body weight, all via intraperitoneal route. Thirty minutes later, mice in all the groups were given picrotoxin subcutaneously 5 mg/kg. They were then observed for hind limb tonic extension over 30 minutes period. Absence of tonic hind limb extension or prolongation of the latency of the hind limb tonic extension was considered as an indication for anticonvulsant activity [16].

Statistical analysis

Results were expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis was done by analysis of variance (ANOVA), a Dunnet's post hoc test was done, when statistically significant result was obtained with ANOVA. Values of $p \leq 0.05$ were considered significant.

RESULTS

Acute toxicity testing

The intraperitoneal median lethal dose LD_{50} was calculated to be 118.32 mg/kg body weight in mice. Respiratory depression, decreased locomotor activities were observed in the animals prior to death.

Phytochemical constituents

Preliminary phytochemical screening had shown the presence of flavonoids, alkaloids, saponins, steroids, anthraquinones, tannins and glycosides (Table, 1).

Anticonvulsant studies

The ethanol stem bark extract of *Trichilia roka* at doses of 15 and 30 mg/kg significantly ($p \leq 0.05$) delayed the latency of seizures induced by PTZ when compared with normal saline treated groups. Similarly, sodium valproate a standard drug significantly ($p \leq 0.01$) prolonged the mean onset of seizures when compared with normal saline treated groups (Table, 2).

The ethanol stem bark extract of *Trichilia roka* had no effect on the mean recovery time of convulsed animals after maximal electroshock at all the tested doses (7.5, 15 and 30 mg/kg) when compared with normal saline treated group. Conversely, phenytoin a standard drug at a dose of 40 mg/kg significantly ($p \leq 0.05$) protected all the animals from tonic hind limb extension induced by MEST throughout the 30 minutes observation period (Table 3).

The ethanol stem bark extract of *Trichilia roka* significantly (P≤ 0.05) delayed the mean onset of seizures at doses of 7.5 and 15 mg/kg when compared with normal saline, in strychnine induced seizures. Phenobarbitone a standard drug produced significant (P≤ 0.001) delay at a dose of 30 mg/kg body weight (Table 4).

In the picrotoxin induce seizure model, the ethanol stem bark extract of *Trichilia roka* at all the doses tested (7.5, 15 and 30 mg/kg) did not have any effects on the mean onset of convulsions in mice. However, phenobarbitone (30 mg/kg) significantly (P≤ 0.001) delayed the mean onset of convulsions in the animals (Table 5)

Table 1: Phytochemical constituents of ethanol stem bark extract of *Trichilia roka*

CONSTITUENTS	INFERENCE
FLAVONOIDS	
a. Sulphuric acid test	-
b. Lead acetate test	+
c. Shinoda test	+
TANNINS	
a. General test	-
b. Ferric chloride test	+
c. Phlobatannins	-
SAPONINS	
a. Frothing test	+
ALKALOIDS	
a. Dragendorff's test	+
b. Mayer's test	+
c. Wagner's test	+
GLYCOSIDES	
a. Salkowski's test	+
b. Keller-Kelliani's test	-
STEROIDS/TERPENOIDS	
a. Lieberman Burchard test	+
ANTHRAQUINONES	+

KEY: + PRESENT, - ABSENT

Table 2: Effect of Ethanol Stem Bark Extract of *Trichilia roka* on PTZ induced Seizures in Mice

TREATMENT	DOSE (MG/KG)	ONSET OF SEIZURES (MIN)	MEAN NO. OF CLONIC SPASM	QUANTAL PROTECTION	TIME TO DEATH (MIN)
NORMAL SALINE	10	2.83±0.30	2.60±0.40	0/6	9.75±2.95
TR 7.5MG/KG	7.5	5.40±1.12	1.80±0.37	1/6	10.60±1.43
TR 15MG/KG	15	5.83±0.70*	1.50±0.22	0/6	7.50±0.28
TR 30MG/KG	30	6.00±0.68*	3.75±2.75	0/6	8.16±2.16

Data presented as Mean ±SEM, quantal protection, n=6, TR=*Trichilia roka* *P≤0.05, **P≤0.001 one way ANOVA followed by Dunnette post hoc test.

Table 3: Effect of ethanol stem bark extract of *Trichilia roka* on MEST induced seizures in chicks

TREATMENT	DOSE (MG/KG)	MEAN RECOVERY TIME (MIN)	QUANTAL PROTECTION
NORMAL SALINE	10ML/KG	5.44 ± 0.89	1/10
PHENYTOIN	40	-	10/10
TR 7.5	7.5	8.60 ± 1.62	0/10
TR 15	15	14.50 ± 8.17	0/10
TR 30	30	6.30 ± 0.59	0/10

Data presented as Mean ± SEM, % protection, n=10, TR= *Trichilia roka*, one way ANOVA

Table 4: Effect of Ethanol Stem Bark Extract of *Trichilia roka* on Strychnine Induced Seizures in Mice

Treatment	Dose (mg/kg)	Mean onset of seizures (Min)	Quantal protection
N/saline	10 ml/kg	4.00±0.51	0/6
Phenobarbitone	30	21.00±2.00**	4/6
TR 7.5	7.5	7.50±0.56*	0/6
TR 15	15	7.33±1.17*	0/6
TR 30	30	6.33±0.76	0/6

Data expressed as Mean ± SEM, quantal protection, n=6, TR= *Trichilia roka*,*P≤ 0.05, ** P≤0.001, one way ANOVA followed by Dunnette post hoc test.

Table 4: Effect of ethanol stem bark extract of *Trichilia roka* on MEST induced seizures in chicks

Treatment	Dose (mg/kg)	Onset of seizures (Mean ± SEM)	Quantal protection
N/saline	10 ml/kg	10.33 ± 1.23	0/6
Phenobarbitone	30 mg/kg	29.00 ± 0.00*	4/6
TR 7.5	7.5 mg/kg	13.44 ± 0.96	0/6
TR 15	15 mg/kg	13.67 ± 0.99	0/6
TR 30	30 mg/kg	12.83 ± 1.05	0/6

Data expressed as Mean ± SEM, one way ANOVA, n=6, TR = *Trichilia roka* followed by Dunnette post hoc test *p≤0.001

DISCUSSION

The LD_{50} value obtained suggested that the ethanol stem bark extract is slightly toxic [12]. The two preliminary screening methods for screening antiseizure drugs are MEST and PTZ models. Drugs such as phenytoin, lamotrigine and carbamazepine have been shown to abolish tonic hind limb extension in MEST test. Primarily, they act by prolonging the inactive state of Na^+, consequently, preventing the repetitive firing of the neurons. In addition, felbamate also block aspartate receptors. Antiseizure drugs that abolish or depress MEST seizure act by preventing the spread of seizures in the brain and the spinal cord [2, 17]. Consequently, the absence of activity in the extract of *Trichilia roka* against MEST seizures clearly demonstrated that it is ineffective in generalized tonic clonic seizures.

On the other hand, the PTZ seizure model screens agents with activity against petit mal epilepsy. Antiseizure drugs such as phenobarbitone, benzodiazepines ethosuximide, sodium valproate are active against seizures induced by PTZ. Drugs that abolish petit mal epilepsy act by enhancing $GABA_A$ inhibitory action and block T-type Ca^{2+} current [17, 18]. The ethanol stem bark extract of *Trichilia roka* showed a dose dependent prolongation in the mean onset of seizures in the PTZ model and therefore, suggested that the extract is effective in absence seizures.

Strychnine is a competitive antagonist of glycine in the central nervous system [19]. Compounds that are effective in seizures induced by strychnine, act by enhancing the inhibitory action of glycine. *Trichilia roka* demonstrated a significant activity against this seizure model, and suggested that the extract mediates its anticonvulsant effect via enhancing the action of glycine.

Picrotoxin is a non competitive antagonist of GABA. Antiseizure drugs such as benzodiazepines, barbiturates, tiagabine, valproate, gabapentin modulates their effect by enhancing $GABA_A$ interaction with picrotoxin sites in $GABA_A$-Chloride channel complex [20]. The lack of effect against picrotoxin induced seizure demonstrated that the extract is not interacting with picrotoxin sensitive site on $GABA_A$ receptor in mediating its anticonvulsant action.

CONCLUSION

The result obtained above clearly showed that the ethanol stem bark extract of *Trichilia roka* contained bioactive substances that are useful in the treatment of absence seizures and further justifies the use of this plant part in the management of epilepsy in northern Nigeria.

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