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ANTICONVULSANT STUDIES ON *COCHLOSPERMUM TINCTORIUM* AND *PAULLINIA PINNATA* EXTRACTS IN LABORATORY ANIMALS

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ABSTRACT

Cochlospermum tintorium (Cochlospermaceae) and *Paullinia pinnata* (Sapindaceae) are widely used medicinal plants in Northern Nigeria in the management of epilepsy. The hydroalcoholic root bark extract of *Cochlospermum tintorium* and methanolic stem bark extract of *Paullinia pinnata* were investigated for anticonvulsant activities using Maximal electroshock test in chicks, Pentylentetrazole and Strychnine-induced seizures, in mice. The *Paullinia pinnata* extract produced a dose-dependent protections (50%, 60% and 70%) against maximal electroshock at doses tested (125, 250 and 500 mg/kg) while the *C. tintorium* extract did not protect the animals at the doses tested (5, 10 and 20 mg/kg). The two extracts did not protect the animals against strychnine-induced seizure. Both extracts protected 20% of the animals against pentylentetrazole-induced seizure at the highest doses tested. This study suggests that the aqueous methanolic stem bark extract of *P. pinnata* contains bioactive constituents that may be beneficial in grand mal epilepsy and lend pharmacological credence to the ethnomedical claim for the use of the plant in the management of this epilepsy.

Key Words: *Paullinia pinnata*; *Cochlospermum tintorium*; Seizure; Maximal electroshock; Pentylentetrazole; Strychnine

INTRODUCTION

Epilepsy is a chronic brain disorder characterized by recurrent derangement of the nervous system due to sudden excessive disorderly discharge from the cerebral neurons (Kabir *et al.*, 2005). It is the second most common neurological disorder attended to by neurologists (Sridharan, 2002). The estimated

prevalence of epilepsy in Nigeria is 8 to 13 per thousand people (Azubuike and Nkanginieme, 1996). About 30% of epilepsy is refractory to treatment (WHO, 2001). Many people in developing countries may not receive basic treatment due to high cost, unavailability and untoward effects associated with the available

Antiepileptic drugs. Therefore, many people in the developing countries still rely on traditional healing practices and medicinal plants for their basic health care needs. WHO encourages the inclusion of herbal medicines of proven safety and efficacy in the healthcare programs of developing countries (Amos *et al.*, 2001). Scientific research is needed to provide evidences of the safety and efficacy of beneficial medicinal plants. *Cochlospermum tinctorium* and *Paullinia pinnata* are commonly used medicinal plants in traditional medicine practice in Nigeria.

Cochlospermum tinctorium (Cochlospermaceae) is a bushy plant attaining about 50 cm in height. It has widespread occurrence in the savannah and shrub land throughout the drier areas of West Africa region. The common vernacular names in Nigeria include *rawaya*, *kyamba* (Hausa), *obazi*, *abanzi* (Igbo) and *sewutu* (Yoruba). The root decoction is used for management of epilepsy (Burkill, 2000).

Paullinia pinnata (Sapindaceae) is a woody or sub-woody climber at damp site and stream banks of the forest and jungle regrowth in the savannah zone. The common vernacular names in Nigeria include *goron dorina*; *yatsa biyar* (Hausa) *edefina*; *aliligo* (Igbo) and *ogbe-okiye* (Yoruba). Decoction of the leafy twigs is used in West Africa for jaundice, yellow fever and heart irregularities while the leaves decoction is used for diarrhoea, dysentery and colic (Burkill, 2000). The root is reported to be used in northern part of Nigeria in the management of convulsion (Personal Communication: Ado Ibrahim of Science Secondary School, Dawakin Tofa, Kano State).

To our knowledge, there is no report in the literature on the anticonvulsant

activities of these medicinal plants. This study, therefore, aims at evaluating the anticonvulsant properties of the aqueous methanolic root bark extract of *C. tinctorium* and stem bark extract of *P. pinnata* in laboratory animals.

MATERIALS AND METHODS

Preparation of plant material and extracts

Fresh stem bark of *Paullinia pinnata* were collected from Sakani village along New Jos Road, Kaduna state, Nigeria while fresh root part of *Cochlospermum tinctorium* was collected from a secondary forest in New Bussa, Niger state, Nigeria in January 2008. The collections were authenticated by Malam Musa Mohammed and Umar Galla of the Herbarium Section in the Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria-Nigeria. Voucher specimens (number 2314 for *C. tinctorium* and 427 for *P. pinnata*) were deposited at the herbarium for future reference. The root bark of *C. tinctorium* and the stem bark of *P. pinnata* were air dried separately under shade until constant weights were obtained. They were then size-reduced into coarse powder with a pestle and mortar. Extraction was carried out using cold maceration with occasional shaking for 72 hr using 500 mL of 70% aqueous methanol for 100 mg of each powdered materials. The extracts were concentrated *in vacuo* and subsequently referred to as *Paullinia pinnata* extract (PPE) and *Cochlospermum tinctorium* extract (CTE). Fresh aqueous solutions of the extracts were prepared for each study.

Experimental animals

Swiss albino mice (18-30 g) of either sex were obtained from the animal house facility of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria. Day old Rangers cockerels were obtained from the National Animal Production Research Institute (NAPRI), Shika, Kaduna 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 approved by the University animal ethics committee. The experiments were conducted in a quiet laboratory between the hours of 900 h to 1600 h.

Acute Toxicity studies: LD₅₀

Intraperitoneal median lethal doses of CTE and PPE were determined using the method of Lorke (1983). Briefly; the method was divided into two phases. In the initial phase, 3 groups of three mice each were treated with the methanol root bark extract of the plant at doses of 10,100 and 1000mg/kg body weight *i.p.* and were observed for signs of toxicity and death for 24 hours. In the second phase, 4 groups each containing one mouse was injected with four more specific doses of the extract. The LD₅₀ value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1).

Maximum electroshock-induced convulsion in chick

The method described by Swinyard and Kufferberg (1985) as modified by Sayyah *et al.* (2002) was employed in this study. Eighty (80) one day old white cockerels were randomly divided into eight groups each containing ten chicks.

The first group received normal saline (10 ml/kg) *i.p.*; second, third and fourth groups were treated with PPE (125 mg/kg, 250 mg/kg and 500 mg/kg), *i.p.* The fifth, sixth and seventh groups received CTE (5 mg/kg, 10 mg/kg and 20 mg/kg). The last group was administered with phenytoin (20 mg/kg), *i.p.* Thirty minutes after pre-treatment, maximal electroshock was administered to induce seizure in the chicks using Ugo basile electroconvulsive machine (Model 7801) connected to Claude Lyons stabilizer with corneal electrodes placed on the upper eyelids of the chicks. The shock duration, frequency and pulse width were set and maintained at 0.80s, 200 pulse per second and 0.8ms respectively. A current of about 90mA, which produced tonic seizures in 90% of the control chicks, was used throughout the study. Seizures were manifested as tonic hind-limb extension (THLE). The ability to prevent this feature or prolong the latency and or onset of the THLE was considered as an indication of anticonvulsant activity (Sayyah *et al.*, 2002).

Pentylentetrazole-induced Seizure in mice

The method of Swinyard *et al.*, (1989) was employed. Forty mice were divided into eight groups each containing five mice. The first group received normal saline (10 ml/kg) *i.p.*; second, third and fourth groups were treated with PPE (125 mg/kg, 250 mg/kg and 500 mg/kg), *i.p.* The fifth, sixth and seventh groups received CTE (5 mg/kg, 10 mg/kg and 20 mg/kg). The last group was treated with 20mg phenobarbitone per kg, *i.p.* (positive control). Thirty minutes post treatment, mice in all the groups received 85mg pentylentetrazole per kg *s.c.* and were observed over a period of

30 minutes. Absence of an episode of clonic spasm of at least 5 seconds duration indicated a compound's ability to abolish the effect of pentylenetetrazole on seizure threshold.

Subcutaneous Strychnine-induced Seizure in mice

The first group received normal saline (10 ml/kg) *i.p.*; second, third and fourth groups were treated with PPE (125 mg/kg, 250 mg/kg and 500 mg/kg), *i.p.* The fifth, sixth and seventh groups received CTE (5 mg/kg, 10 mg/kg and 20 mg/kg). The last group was treated with 20mg phenobarbitone per kg, *i.p.* (positive control). Thirty minutes post - treatment, mice in all the groups received 1.2mg strychnine per kg *s.c.* The proportion of mice presenting convulsions as well as the onset of tonic convulsions was recorded. Abolition of tonic extensor jerks of the hind limbs within 30 minutes after strychnine administration was considered an indicator that the testing material could prevent strychnine-induced convulsion.

Statistical analysis

The results were analyzed for statistical significance using one-way ANOVA

followed by Dunnet's test (SPSS-15). A P value <0.05 was considered significant.

RESULTS

The median lethal dose values for PPE and CTE were found to be 2346.42 mg/kg and 118.32 mg/kg, respectively. PPE produced a dose-dependent protections (50%, 60% and 70%) against maximal electroshock test at doses tested (125, 250 and 500 mg/kg) while CTE extract did not protect the animals at the doses tested (5, 10 and 20 mg/kg) (Figure 1). There was no significant decrease in the mean recovery time in the unprotected chicks in both PPE and CTE groups (Table 1). The two extracts did not protect the mice against strychnine-induced seizure (Table 2). Both extracts protected 20% of the mice against pentylenetetrazole-induced seizure at their highest doses. While the standard drug used, phenobarbitone (20 mg/kg) afforded 100% protection (Figure 2). There was no significant difference in the mean onset of seizure in mice treated with the extracts when compared with the control (Table 3).

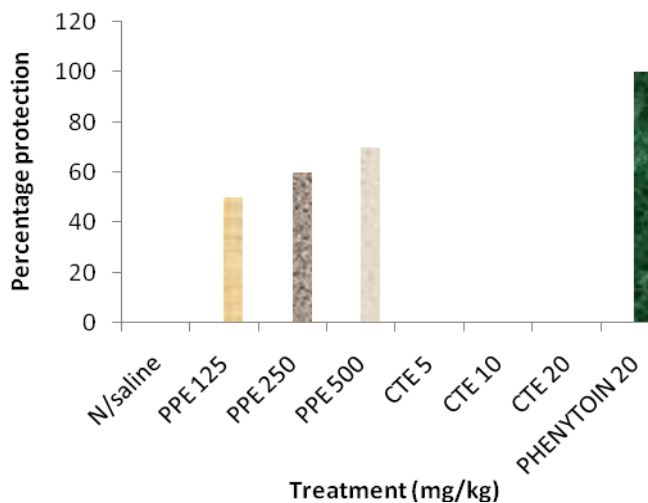


Fig 1: Effect of methanolic extracts of *P. pinnata* and *C. tinctorium* on MEST in chick

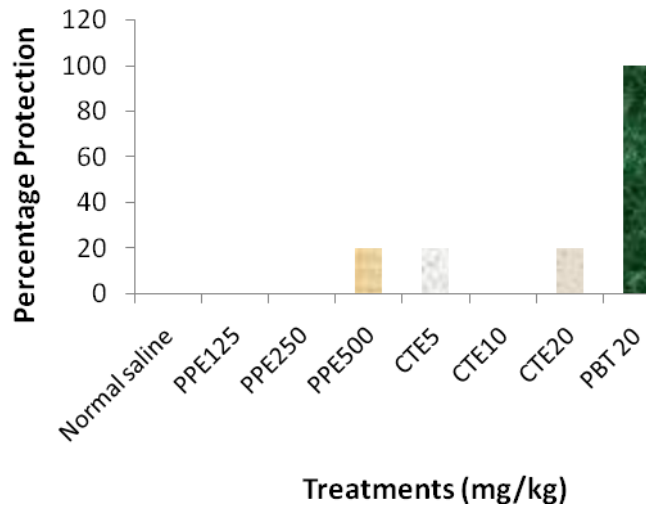


Fig 2: Effect of methanolic extracts of *P. pinnata* and *C. tinctorium* on PTZ induced seizure in mice (n=5) CTE: *Cochlospermum tinctorium* extract; PPE: *Paullinia pinnata* extract; PBT: Phentobarbitone

Table 1: Effect of methanolic extracts of *C. tinctorium* and *P. pinnata* on MEST in chicks

Groups	Doses	Mean Recovery time (mins)
N/Saline	10ml/kg	5.5 ± 0.34
CTE	5	6 ± 0.93
CTE	10	5.2 ± 1.2
CTE	20	4.5 ± 1.27
PPE	125	5.2 ± 0.37
PPE	250	4.5 ± 0.65
PPE	500	11 ± 5.03

CTE: *Cochlospermum tinctorium* extract; PPE: *Paullinia pinnata* extract

Table 2: Effect of methanolic extracts of *C. tinctorium* and *P. pinnata* on Strychnine-induced Seizure in mice

Groups	Doses	Quantal protection	Mean onset time (mins)
N/Saline	10ml/kg	0/6	3.0 ± 0.77
CTE	5	0/6	2.8 ± 0.2
CTE	10	0/6	2.0 ± 0.2
CTE	20	0/6	2.0 ± 0.00
PPE	125	0/6	2.2 ± 0.24
PPE	250	0/6	3.0 ± 0.31
PPE	500	0/6	4.0 ± 0.55
PBT	20	6/6	-

CTE: *Cochlospermum tinctorium* extract; PPE: *Paullinia pinnata* extract; PBT: Phenobarbitone

Table 3: Effect of methanolic extracts of *C. tinctorium* and *P. pinnata* on Pentylentetrazole-induced seizure in mice

Groups	Doses	Mean onset time (mins)
N/Saline	10ml/kg	3.0 ± 0.77
CTE	5	6.0 ± 0.00
CTE	10	1.2 ± 0.2
CTE	20	2.0 ± 0.00
PPE	125	1.6 ± 0.2
PPE	250	1.75 ± 0.25
PPE	500	2.25 ± 1.25

CTE: *Cochlospermum tinctorium* extract; PPE: *Paullinia pinnata* extract

DISCUSSION

The MES and PTZ models are widely believed to be predictive of activity in common form of human epilepsy (Wickenden, 2002). There is no false negative in MES test and all currently available AEDs that are clinically effective in the management of generalized tonic-clonic and partial seizures such as phenytoin, carbamazepine and lamotrigine all suppresses the hind limb tonic extension (HLTE) in MES test (Browning, 1992; Rho and Sankar, 1999). They act by modulating neuronal voltage gated sodium channels (Wickenden, 2002). Thus, the ability of a compound to inhibit HLTE suggests anticonvulsant activity for the treatment of generalized tonic clonic and partial seizures. Compound that protect against MES are known to prevent the spread of seizure. Therefore, the ability of the PPE to inhibit the HLTE suggests anticonvulsant activity which may be beneficial in generalized tonic-clonic and partial seizures.

Anticonvulsant activity in PTZ test identifies compounds that can raise seizure threshold in the brain (White *et al.*, 1998). The inability of PPE and CTE to protect the mice against PTZ-induced seizure suggests they may not be

effective in the therapy of absence or myoclonic seizures.

Strychnine is a competitive antagonist of the inhibitory amino acid glycine (Larson, 1969). The inability of the extracts to protect against strychnine-induced seizure suggests non-involvement of glycine receptors in their activity.

The results of this study suggest that the aqueous methanolic stem bark extract of *Paullinia pinnata* (PPE) contains bioactive constituents which possess anti-convulsant activity that may be beneficial in the management of grand mal epilepsy and lend credence to the use of the plant in the management of epilepsy in traditional medicine. Further work is going on in our laboratory to determine the constituent(s) responsible for these observed effects.

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