EVALUATION OF ANTICONVULSANT ACTIVITY OF THE HYDROALCOHOLIC STEM BARK EXTRACT OF RANDIA NILOTICA STAPF. IN MICE AND CHICKS


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ABSTRACT

Medicinal plants have been used in the development of new drugs and continue to play an invaluable role in the drug discovery process. Randia nilotica Stapf. is a lowland shrub used in ethnomedicine for treatment of convulsion and other mental disorders. The aim of this work therefore was to screen for the anticonvulsant effect of Randia nilotica Stapf. The test systems selected were maximal electroshock test (MEST) in chicks, pentylenetetrazole (PTZ) induced seizure and strychnine (STN) induced seizure tests in mice. In MEST, the stem bark extract of Randia nilotica protected chicks against hind limb tonic extension by 90 % at 20 mg/kg body weight, which is similar to the effect obtained when phenobarbitone (20 mg/kg) was used as standard reference. At 5 mg/kg and 10 mg/kg body weights, there was a 50 % protection. On the other hand, the stem bark extract of Randia nilotica did not protect mice against PTZ and STN induced seizures. The mean number of myoclonic body twitches was significantly (p<0.05) reduced by the extract in the PTZ test as 5 mg/kg body weight showed a 27 % decrease while 10 and 20 mg/kg showed 59 % and 61 % reduction respectively. Phenobarbitone (30 mg/kg) used as control showed a 95 % decrease. The results obtained indicated potential anticonvulsant activity of the stem bark extract of Randia nilotica Stapf.

Keywords: Randia nilotica, MEST, pentylenetetrazole, strychnine, myoclonic body twitches

INTRODUCTION

Epilepsy is a major neurological disorder characterized by recurrent seizures and has a lifetime prevalence of 5% (Sander and Shorvon, 1996; Raza et al., 2001). Common causes include infectious, traumatic, metabolic or tumoral conditions or it may be idiopathic, that is unrelated to any underlying cause other than a possible hereditary predisposition (Engel, 2001). Falciparum malaria however is a common cause of seizures in children living in malaria endemic areas (Ogutu and Newton, 2004), while in cerebral malaria over 80% of children are admitted with a history of convulsions (Molyneux et al., 1989). Furthermore, seizures in malaria are
associated with a poor outcome. Prolonged seizures in children with malaria are associated with a neurological, cognitive and language deficits and the development of epilepsy (Holding et al., 1999; Carter et al., 2003). It can be postulated therefore, that sub-Saharan Africa that is malaria endemic may have a higher prevalence of epilepsy. Patients with epilepsy fail to experience adequate control of their seizures despite optimal use of available antiepileptic drugs- AEDs (Stables and Kupferberg, 1997). Synthetic AEDs are effective only in approximately 50% of patients and many refractory cases of epilepsy still remain highly resistant to their treatment (Heinemann et al., 1994; Shorvon, 1996). Furthermore, AEDs are associated with side effects, including teratogenicity and adverse effects on cognition and behaviour (Samren et al., 1997; Raza et al., 2001). According to Meldrum (1997), plant extracts can be an important source of natural and safer drugs for the treatment of epilepsy. *Randia nilotica* is a lowland shrub or tree widespread in Northern Nigeria and reported from lowland habitats in Central and East Africa as well as Cameroun and the Sudan (Lemmich et al., 1995). The plant is commonly known as ‘Barbaji’, ‘Tsibra’ or ‘Kwanarya’ by the Hausas in Northern Nigeria and the Fulanis call it ‘Gial goti’ (Dalziel, 1937). A decoction of *Randia nilotica* is used orally for treatment of mental breakdown and convulsions (Chhabra et al., 1991). In Tanzania dried root is used against epilepsy and for treatment of madness (Hedberg, 1983). Scientific evidence for the use of *Randia nilotica* in treatment of epilepsy is lacking and therefore this study was conducted to evaluate these claims. Three models of epilepsy were used including maximal electroshock test (MEST), pentylenetetrazole (PTZ) induced seizure and strychnine (STN) induced seizure tests. The electroshock assay is used primarily as an indication for compounds which are effective in grand mal epilepsy while PTZ induced seizure test identifies primarily compounds that raise seizure threshold and is a fairly good index of effectiveness against absence seizures – petit mal (Rang and Dale, 1995). Compounds which reverse the action of strychnine (an antagonist to glycine) have been shown to have antiepileptic effects (Raza et al., 2001). The effect of *Randia nilotica* in affecting behavior and consequently central nervous system was investigated in mice and reported (Danjuma et al., 2008).

**MATERIALS AND METHODS**

**Animals**

Swiss albino mice (18-25 g) of either sex maintained at the animal house of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University Zaria were used. In addition day old Black Ranger cockerels obtained from the National Animal Production and Research Institute Shika, Zaria were used. These were housed under standard conditions of temperature (25±2°C), 12/12 hour light/dark cycle and fed on standard diet (Feed Masters Plc. Ilorin, Nigeria) and water ad libitum. All experiments performed in this study followed the principles of laboratory animal care outlined by the ethical committee of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria. The study was conducted in the first quarter of 2007.

**Plant material**

*Randia nilotica* Stapf. stem bark was collected in Saye village in the outskirts of Zaria, Kaduna State, Nigeria. The plant was
identified and authenticated by a taxonomist with the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria by comparing with a voucher specimen (No. 2867) deposited for reference at the herbarium section of the Department.

**Preparation of the extract**

The stem bark was carefully removed washed and cut into pieces. The bark was air dried and ground into powder form using mortar and pestle and then sieved. The powdered material was macerated in hydro alcoholic solution (ethanol 70% - water 30%) with occasional shaking for 24 h and then filtered. The filtrate was then evaporated to dryness in vacuo at 60°C.

**Test drugs and chemicals**

Hydroalcoholic stem bark extract, pentylenetetrazole (Sigma Chem. Comp., USA), strychnine (Sigma Chem. Comp., USA) were prepared by dissolving the powder in deionised water prior to administration. Phenobarbitone was supplied in ampoules and appropriate dilutions were made with deionised water prior to use.

**Electroshock - induced convulsion test in chicks**

The methods of Swinyard and Kupferberg (1985) and of Browning (1992) were employed. The apparatus used was the Ugo Basile electroconvulsive test machine (Model 1801) Milano, Italy with corneal electrodes placed on the upper eyelid of the chicks after dipping them in normal saline. A current (95 mA) which induced tonic seizures in 80-90 % of a control group of chicks was selected. The shock duration (0.8 sec), pulse width (0.8 mm) and frequency (150 Hz) determined, were maintained for all other groups. A second group of 10 chicks was pretreated with phenobarbitone (20 mg/kg) intraperitoneally (i.p.). Thirty minutes later these chicks were subjected to electrical stimulation as in untreated controls. Test chicks were then pretreated in groups of 10 with 5, 10 and 20 mg/kg of *Randia nilotica* extract i.p. before being electrically shocked 30 min later. Results were recorded as either positive or negative depending on whether tonic hind limb extension was produced or not.

**Pentylenetetrazole - induced convulsion test in mice**

Mice were injected with a convulsive dose (CD$_{90}$), that is the dose that produced convulsion in about 90 % of the animals (90 mg/kg subcutaneous pentylenetetrazole) – Raza *et al.*, 2001. They were then observed for the presence or absence of threshold seizures (an episode of clonic seizure of at least 5 sec duration). PTZ seizures were further characterized into different patterns that are one or more generalized myoclonic body twitches (MBT), generalized body seizure with loss of righting reflex, loss of righting reflex with tonic forelimb extension and loss of the righting reflex with tonic forelimb and hind limb extensions (Loscher *et al.*, 1991). Three groups of six mice each were pretreated with the extract at 5, 10 and 20 mg/kg body weight i.p. Two other groups were given normal saline (1 ml/kg) and phenobarbitone (20 mg/kg) respectively and served as controls. Thirty minutes later, all the mice in the groups were injected with a convulsive dose of PTZ subcutaneously. They were then observed as described earlier.

**Strychnine - induced convulsion test in mice**

Mice were injected with a convulsive dose of STN (Raza *et al.*, 2001) – 1.2 mg/kg body
weight. They were then observed for the presence or absence of convulsion. Abolition of tonic extensor jerks of the hind limbs was considered an indication that the extract prevented STN-induced seizures (Porter et al., 1984). Five groups of six mice each were used for the study. The first group received normal saline while three other groups received Randia nilotica extract at 5, 10 and 20 mg/kg body weight. The fifth group was injected i.p. with phenobarbitone at 30 mg/kg. Thirty minutes later, all mice in the groups were injected with convulsive dose of STN and observed within thirty minutes for signs of convulsion.

**Statistical analysis**

Data were presented as percentages and as mean ± S.E.M. Results were analyzed using student’s t-test and difference between means by one way analysis of variance (ANOVA). Values of \( p \leq 0.05 \) were considered significant.

**RESULTS**

**Effect of *R. nilotica* on maximal electroshock test in chicks**

The crude hydroalcoholic stem bark extract (5, 10, 20 mg/kg) protected the chicks (50 %, 50 %, 90 % respectively) against hind limb tonic extension (HLTE) in this test (Fig. 1). Phenobarbitone (20 mg/kg) used as positive control had the same effect (90 % protection) with *R. nilotica* at 20 mg/kg.

**Effect of *R. nilotica* on pentylenetetrazole induced seizure in mice**

The hydroalcoholic stem bark extract of *R. nilotica* did not protect mice against PTZ induced seizure (Table 1). The number of myoclonic body twitches was significantly \((p \leq 0.05)\) reduced by the extract in this test (Fig. 2). The extract at 5 mg/kg showed a 27 % decrease while 10 and 20 mg/kg showed 59 % and 61 % reduction respectively. Phenobarbitone (control) exhibited a 95 % decrease.

**Effect of *R. nilotica* extract on strychnine induced seizure in mice**

The hydroalcoholic stem bark extract of *R. nilotica* did not protect mice against strychnine induced seizures in mice (Table 2). Phenobarbitone used as control showed 100 % protection.
Figure 1: Effect of hydroalcoholic stem bark extract of *Randia nilotica* (RNBE) and phenobarbitone (PBT) on hind limb tonic extension phase in chicks using maximum electroshock test. N/Saline = Normal Saline
Figure 2: Anticonvulsant effect (myoclonic body twitches) of the stem bark extract of *Randia nilotica* (RNBE) on subcutaneous pentylenetetrazole-induced seizures in mice

Significant difference between control and treated groups: \( p \leq 0.05(a) \) and \( p \leq 0.001(b) \) - one factor analysis of variance followed by Dunnet’s and Scheffe’s post hoc tests

\( N=6 \) in each group
Table 1: Anticonvulsant Effect of Stem Bark Extract of *Randia nilotica* (RNBE) and Phenobarbitone (PBT) on Subcutaneous Pentylentetrazole-induced Seizures in Mice

<table>
<thead>
<tr>
<th>TREATMENT (mg/kg)</th>
<th>PROTECTION</th>
<th>% PROTECTION</th>
</tr>
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<tbody>
<tr>
<td>Normal Saline</td>
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<tr>
<td>RNBE 20</td>
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<tr>
<td>PBT 20</td>
<td>6/6</td>
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Results are presented as % protection; Phenobarbitone (PBT) showed 100% protection

Table 2: Anticonvulsant Effects of Stem Bark Extract of *Randia nilotica* (RNBE) and Phenobarbitone (PBT) on Strychnine-induced Seizures in Mice

<table>
<thead>
<tr>
<th>TREATMENT (mg/kg)</th>
<th>PROTECTION</th>
<th>% PROTECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Saline</td>
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<td>PBT 20</td>
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Results are presented as % protection. Phenobarbitone showed 100% protection
DISCUSSIONS

Several medicinal plants have shown potential for use as anticonvulsants. For example Musa et al., (2006) reported strong anticonvulsant effect of Ficus thoningii Blums. The results obtained in this study had also demonstrated potential anticonvulsant property of R. nilotica extract. The extract at 20 mg/kg was able to inhibit mice from HLTE by 90% which compare favorably with that of PBT. Inhibition of HLTE is the common feature of maximal electroshock in rodents and the response of the rodents to the anticonvulsants is similar to that of humans (Raza et al., 2001). There are no false negatives in the MEST and all the currently available AEDs that are clinically effective in the treatment of generalized tonic and partial seizures such as phenobarbitone e.t.c. also suppress HLTE in MEST (Kupferberg and Schmutz, 1998). Protection against HLTE also indicates the ability of the extract of Randia nilotica to inhibit or prevent seizure discharge and spread within the brainstem seizure substrate (Browning, 1992). The strong inhibitory effects of the hydroalcoholic stem bark extract of Randia nilotica suggested that it possesses potent anticonvulsant activity that may be of value in the treatment of generalized tonic clonic and partial seizures. Pentylenetetrazole is a known convulsant and therefore anticonvulsant activity in the sc-PTZ test has been used to identify compounds that can raise the seizure threshold in the brain (White et al., 1998). PTZ has been shown to interact with the neurotransmitter, gamma amino butyric acid (GABA) and the GABA receptor complex (Bum et al., 2001). Standard AEDs such as diazepam and phenobarbitone produce their effects by enhancing GABA mediated inhibition in the brain. The absence of anticonvulsant activity in the sc-PTZ induced seizure test suggested that, the compounds present in the stem bark of Randia nilotica may not interact with the GABA receptor complex or with GABA. The significant reduction in total number of myoclonic jerks however, showed some anticonvulsant activity of R. nilotica probably through dopaminergic mechanism or suppression of t-type calcium currents (Rho and Sankar, 1999). Strychnine is a competitive antagonist of the inhibitory amino acid glycine. Absence of anticonvulsant activity in the sc-STN test suggested that the compounds present in hydroalcoholic stem bark extract of Randia nilotica may not interact with the glycine receptors. The overall result of this study provided some scientific basis for the use of this plant in traditional medicine in the treatment of all types of epilepsy.

REFERENCES


