



Anticonvulsant effect of the aqueous extract of *Xeromphis nilotica* in mice

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ABSTRACT

Xeromphis nilotica is used traditionally to treat epilepsy. The freeze dried aqueous extract of the plants' rootbark was tested for anticonvulsant activity against pentylenetetrazol (PTZ) induced seizures and pentobarbital induced sleep in mice. Results showed that the extract significantly ($p < 0.05$) reduced the onset and severity of the PTZ-induced seizure and prolonged the duration of sleep induced by pentobarbital dose dependently. The results show that the extract has depressant effect, supporting the claimed ethnomedical usage in controlling seizure.

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INTRODUCTION

Epilepsy is a central nervous system (CNS) disorder characterized by recurrent seizures (Sander and Shorvon, 1996). The available synthetic anti-epileptic drugs are not within reach in rural Africa. Medicinal plant agents have been used traditionally to treat the disease (Raza et al., 1999), with some of the plant materials having anticonvulsant effect (Achliya et al., 2004; Nguenefack et al., 2006). *Xeromphis nilotica* is used in ethnomedical practice as an anti-epileptic agent (Hedberg et al., 1983). The plant is also reputed to be useful in the treatment of stomach pain, dropsy, fever, abdominal pain, asthma, and to induce labour (Hedberg et al., 1983; Chhabra et al., 1991; Bashir, 1996). In this study, we investigated the ethnomedical use of *Xeromphis nilotica* as an epileptic agent, by

testing the anticonvulsant effect of the aqueous extract of the plant's rootbark using two standard experimental models: pentylenetetrazol (PTZ) induced seizure and pentobarbital induced sleep. The acute toxicity potential (LD₅₀) and phytochemical constituents of the extract were also tested.

MATERIALS AND METHODS

Plant extract

The plant material (*Xeromphis nilotica* (Stapf) (Rubiaceae) was collected in May 2005 from Funtua, Katsina State, northwestern Nigeria. The plant was authenticated at the Department of Biological Sciences, Ahmadu Bello University, Zaria, Kaduna State, Nigeria where a voucher specimen (no. 2867) was also deposited for reference. The roots were dried under shade,

powdered and 200g of the powdered material was macerated in 1L of distilled water, for 24h with occasional stirring. It was then filtered over a filter paper using a vacuum pump (ABM, Germany) and the filtrate freeze-dried using Lyovac, GT2 (Germany). The phytochemical constituents of the extract were tested using standard procedures (Harborne, 1998).

Animals

Swiss albino mice obtained from the Animal Facility Centre (AFC), National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria and Animal House, Department of Pharmacology and Therapeutics, Mbarara University of Science and Technology, Mbarara, Uganda were used. The mice were housed in standard polypropylene cages with saw dust as beddings, and have access to food and water *ad libitum*. The animals were used in accordance with the ethical norms of 'Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85 – 23, The National Academy Press, Washington, DC (1985)'.

Drugs

Sodium pentobarbital, pentylenetetrazol, diazepam (Sigma Chemical Co., USA) and sodium chloride (BDH, England) were used.

Acute toxicity test

The acute toxicity potential of the extract was evaluated by determining the LD₅₀ using Lorke's (1983) method. The extracts were administered at the doses of 10, 100, 1000 and 2000mg/kg i.p. to four groups of mice. Another group was given normal saline to serve as the control. They were all kept under same conditions, and mortality was recorded in each group within 24 h. Symptoms and signs of toxicity were noted and the LD₅₀ estimated from as the square of the lowest lethal dose and the highest non-lethal dose from the second stage of dosing (Vongtau et al., 2004).

Pentylenetetrazol (PTZ) induced seizure

This test was carried out using the Parmar et al. (1974) technique as modified for our local laboratory setting (Adzu et al., 2002). Mice were grouped into four (n = 5). Groups 1 – 3 were treated with the extract (50,

100 and 200mg/kg, i.p.); Group 4 received normal saline (10ml/kg, i.p.) and served as the control. They were all injected with PTZ (70 mg/kg, i.p.) 30 min later and were observed for occurrence of seizures. Two parameters were evaluated that include: onset of tonic seizure which was the latency between PTZ injection and first convulsion characterized by falling and jerking; and tonic clonic with generalized seizure and hind-limb extension.

Pentobarbital induced sleep

The test was to assess the ability of the extract to prolong pentobarbital-induced sleep (Fujimori, 1965). Twenty five mice were used. They were grouped into five (n = 5) and treated with extract (50, 100 and 200mg/kg, i.p.), saline (10ml/kg, i.p.) and diazepam (1mg/kg, i.p.). The mice received sodium pentobarbital (25 mg/kg, i.p.) 30 min after the pentobarbital injection. The index of hypnotic effect was recorded as follows: time between the injections of pentobarbital until loss of righting reflex was taken as the onset of sleep, while the time from this loss of reflex and its recovery was taken as the duration of sleep (Adzu and Gamaniel, 2003).

Data analysis

Results were expressed as mean ± SEM. Analysis was performed using ANOVA followed by Dunnett's test using GraphPad Prism Version 4.00 for Windows, GraphPad software, San Diego California USA, (www.graphpad.com).

RESULTS AND DISCUSSION

The aqueous extract of *Xeromphis nilotica* gave positive tests for saponins, tannins, glycosides, alkaloids and flavonoids, which are medicinally useful phytochemicals. The LD₅₀ of the extract was established to be 1549 mg/kg, i.p. in mice, indicating that the experimental doses used (50, 100 and 200mg/kg, p.o.) were within safe margin. The extract prolonged the onset and reduced the severity of seizures induced by PTZ (Table 1). Systemic administration of PTZ in rodents is used as an acute experimental model of seizure to screen anticonvulsant drugs (Parmar et al., 1974). The drug produces convulsion through antagonizing the γ -aminobutyric acid (GABA)-benzodiazepines receptor complex (Corda et al., 1990), by diminishing the

Table 1: Effect of *X. nilotica* aqueous extracts on pentylenetetrazol (70 mg/kg) induced seizures.

Treatment	Dose (mg/kg, i.p.)	Onset of seizures (s)	
		Tonic seizures	Tonic clonic seizures with hind-limb extension
Saline	10 ml/kg	14.2 ± 2	76 ± 3
	50	31.2 ± 3	135 ± 5*
	100	54.4 ± 6*	191 ± 6*
Extract	200	125.4 ± 2*	259.2 ± 1*

Tabulated values are mean ± SEM (n = 5); * Significant difference; F [(3, 19 = 3.27; p < 0.05].

Table 2: Effect of *X. nilotica* aqueous extract on pentobarbital induced sleep.

Treatment	Dose (mg/kg, i.p.)	Onset of sleep (min)	Duration of sleep (min)
Saline	10 ml/kg	5.4 ± 1	42.2 ± 2
	50	4.4 ± 1	54.4 ± 3*
	100	4.3 ± 1	59.6 ± 2*
Extract	200	4 ± 1	66 ± 2*
	Diazepam	1	3.2 ± 1

Tabulated values are mean ± SEM (n = 5); * Significant difference; F [(4, 24 = 3.02; p < 0.05].

benzodiazepine site (Rehavi et al., 1982), or increasing the central noradrenergic activity (De Porter et al., 1980). Agents positive on the PTZ test is considered useful in humans (Goodman et al., 1953). The model however cannot clearly separate sedative from anticonvulsant properties of a substance (Andrews et al., 1989) both activity being useful in seizures. The extract also showed potency on the pentobarbital induced sleep, by prolonging the duration of sleep (Table 2). This effect was shown to be due to sedative and/or hypnotic property often attributed to inhibition of pentobarbital metabolism or central mechanism involved in regulation of sleep (Fujimori, 1965; N'Gouemo et al., 1994), suggesting central depressant effect, which is a known mechanism of anticonvulsant action (MacDonald and Kelly, 1994). These results show that the aqueous extract has potent agents, useful against seizure. The plausible mechanism of action is not clear at this stage, but the extract tend to act similar to the standard control (diazepam) used in the experiment. Diazepam antagonizes PTZ competitively (Coleman et al., 1985). In conclusion, the aqueous extract of *Xeromphis nilotica* contains useful phytochemicals, relatively safe and has anticonvulsant effect

which is likely due to the extracts' depressant effect on the CNS.

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