Preliminary Screening of Ethanolic Extracts of *Clausena anisata* for Anticonvulsant Activity

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Abstract

The ethanol extracts of leaves, root bark and stem bark of Clausena anisata were tested for their ability to control convulsion induced by pentylenetetrazole in mice. Test animals were given sublethal but convulsive doses of pentylenetetrazole subcutaneously. The extracts tested were administered intraperitoneally in varying doses 2 h before pentylenetetrazole was administered. Of the three extracts, two (leaves and stem bark) were found to be ineffective in protecting the animals, but showed some sedative properties. The third (root bark) exhibited partial protection at a dose level of 800 mg/kg of mice.

Keywords: *Clausena anisata*, Anticonvulsant activity, Ethanolic extracts of leaves, root bark, stem bark.

Introduction

Convulsion which represents the clinical symptoms of sudden periodic and excessive discharges of cerebral activity can manifest itself in many varied patterns and is of high incidence all over the world (Lewis, 1998). Despite the many significant advancements that have occurred in the development of synthetic anticonvulsant drugs, the use of medicinal plant products especially in the developing parts of the world in treating convulsion is still of importance. A number of medicinal plants have been used traditionally in the treatment of various ailments including bacterial infections, inflammatory disorders, convulsions and coronary heart diseases (Adedayo *et al*., 2001; Ekpendu *et al*., 1994; Olaniyi, 2000; Keli *et al*., 1996). A few of such plants used in Nigeria to treat convulsions include *Ehretia cymosa* leaves, *Senseviera senegambica* root, *Annona muricata* fruit, *Uvaris chamae* root, *Callindra porericansis* leaves and *Desmodium adcendes* root (Iwu, 2000). *Clausena anisata* has been used traditionally for different medicinal purposes including antidiabetic, antihypertensive, anti-inflammatory, gastrointestinal disorders, anticonvulsant and treatment of some mental disorders (Okunade and Olaife, 1987). Previous *in vivo* pharmacological study has demonstrated the hypoglycemic activity of the *Clausena anisata* (Ojewole, 2002). *Clausena anisata* however has not been evaluated for its potential to control convulsion. In the present work, we investigated the ability of the ethanolic extract of leaves, root bark and stem bark to control pentylenetetrazole-induced convulsion in mice.

Materials and Methods

Plant materials: The plant materials used in this study namely leaves, root bark and stem bark of *Clausena anisata* Hook (Rutaceae) were collected from Nsukka, Southeast of Nigeria in June 2007. The identification was carried out by A. J. Ezekwe, an ethnobotanist at the Botany Department of the University of Nigeria, Nsukka. The albino mice were obtained from the animal house of the Faculty of Veterinary Medicine of the University.

Preparation of crude ethanol extract of *C. anisata*: Each of the plant part was dried in a shade to a constant weight and pulverized. The powdered plant (10 g) was extracted with ethanol using Soxhlet apparatus. The resulting ethanol extract was evaporated to dryness on a water bath to give a dry extract.

Phytochemical screening: Phytochemical analysis was carried out on the dried crude material using standard reagents and procedures for the presence of alkaloids, flavonoids, saponins, tannins (Harbourne, 1973; Trease and Evans, 2003).

Acute toxicity tests: The oral acute toxicity test (LD_{50}) of the extracts in albino mice (15-25 g) was performed using a variation of the method of Lorke, 1983. The method involved an initial dose-range determination stage using nine animals (three animals per treatment group). The plant extract was suspended in 0.5 % w/v aqueous carboxmethylcellulose (CMC) and doses of 10, 100 and 1000 mg / kg calculated and administered to the appropriate groups of mice. The extracts were given orally and the animals observed for 24 h. On the basis of the first stage results, the second step was carried out in which doses of 900 and 1200 mg / kg (for leaves); 400, 900 and 1200 mg / kg (for root bark); 1500, 2900 and 5000 mg / kg (for stem bark) were administered to 8 groups of three animals per group. The LD_{50} was then calculated as the geometric mean of the dose killing none of the animals and that killing all. Neurological toxicity test was also performed for pentylenetetrazole using dosage range of 80, 85 and 95 mg / kg for 3 groups of three animals per group.

Anticonvulsant test: Anticonvulsant test was done using albino mice (20-30 g). The mice were housed in standard cages and had free access to food and water before and throughout the experiment. The anticonvulsant activity of each extract was evaluated by means of pentylenetetrazole seizure

threshold test (Glasser *et al*., 1971). The albino mice were divided into nine groups (n = 3). Each extract was suspended in 0.5 % w/v aqueous carboxymethylcellulose to give a concentration of 0.5 % w/v. The extracts were administered intraperitoneally to six groups of animals (n = 3) at a dose of 400 and 800 mg / kg for leaves and root bark respectively, 500 and 1000 mg / kg for stem bark. The seventh and eighth groups of animals received 1 mg / kg and 3 mg / kg respectively of a solution of diazepam (standard drug), while the ninth group received 1 ml / kg of 0.5 % w/v carboxymethylcellulose. Two hours after administration of the extract, standard drug and the vehicle, the mice were injected with pentylenetetrazole (85 mg / kg) subcutaneously. The mice were observed for seizures for 45 min. An episode of clonic spasm that persisted for a minimum of 5 sec was considered a threshold convulsion. Animals not exhibiting threshold convulsions during 45 min were considered protected.

Results and Discussion

The yields obtained from ethanol extracts of *Clausena anisata* are shown in Table 1. The high yield of the extracts shows that optimal extraction of the constituents requires use of polar solvent. The acute toxicity results are also presented in Table 1.

Table 1: Extraction yields and acute toxicity results (LD_{50}) of ethanol extracts of *Clausena anisata*

Plant extract	% yield	LD_{50} (mg/kg)
Leaves	15.4	950
Rook bark	17.1	950
Stem bark	21.8	1265

The high LD_{50} of the extracts indicates the nontoxic properties of the extracts. The results of the phytochemical tests (Table 2) revealed the presence of alkaloids, flavonoids, saponins and tannins in the dried crude extract.

Table 2: Phytochemical tests on the plant extracts

Test	Plant extracts		
	Leaves	Root bark	Stem bark
Alkaloids	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+

Legend: + = Present; - = Absent

The anticonvulsant activity of each extract evaluated by means of pentylenetetrazole seizure threshold test is given in Table 3. The results indicate that only the ethanol extract of the root bark of *Clausena anisata* exhibited some partial protection on the mice. The leaves and stem bark extracts had no protection against the induced convulsion, but showed some sedative effects on the animals. Diazepam, the control drug at a dosage level of 3 mg/kg, produced complete protection on the mice.

Table 3: Pentylenetetrazole seizure threshold tests in mice

Pentylenetetrazole Dose, mg/kg Subcutaneously (s.c)	Pretreatment intraperitonelly (i.p)	Number of animals protected 2h after administration
80	None	-
85	None	-
90	None	-
	Leaves extract, mg/kg	
85	400	0/3
	800	0/3
	Root bark extract, mg/kg	
85	400	0/3
	800	2/3
	Stem bark extract, mg/kg	
85	500	0/3
	1000	0/3
	Diazepam, mg/kg	
85	1	2/3
	3	3/3
	0.5 % w/v Carboxymethylcellulose	
85	1	0/3

Anticonvulsant activity of the extracts was assessed by including in the investigation diazepam known to provide significant protection against pentylenetetrazole induced seizures (Aguwa, 2004). The extracts were also found not to modify convulsive seizure threshold or duration caused by pentylenetetrazole stimulus. The neurologic status of each animal was evaluated prior to each test for anticonvulsant activity and toxicity was considered present if the animal failed to climb to the top of a1-liter beaker after being suspended from the rim by its forelimbs three times within one minute (Karnet *et al*., 1974). In conclusion, although the plant is traditionally used in the treatment of convulsion, the present study observed only a partial protection (66 %) against pentylenetetrazole-induced convulsion in mice by the ethanol root bark (800 mg / kg) and therefore failed to provide a scientific basis for its folkloric use as an anticonvulsant.

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