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Original article

Anticonvulsant studies on a traditional antiepileptic mixture used by the Hausa people of north-western Nigeria

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Abstract

Background and objectives: The use of herbal drugs in the treatment of many neurological disorders is gaining popularity in developing countries due to their fewer unwanted side effects, affordability and cultural acceptability. A mixture of three plants roots *Calotropis procera* (Asclepediaceae), *Combretum micranthum* (Combretaceae) and *Ficus abutilifolia* (Moraceae) has been reported in Hausa traditional treatment of epilepsy. We have reported the evaluation of the acute toxicity and anticonvulsant activity of the ethanol extract of this mixture. **Methods:** The intra-peritoneal medial lethal dose (LD_{50}) of the aqueous ethanol extract of the mixture as well as its anticonvulsive activity against pentylenetetrazole (PTZ), 4-amino pyridine (4-AMP) and maximum electric shock (MES) were evaluated. **Results:** The mixture at the doses of 25 to 100 mg/kg could not afford a significant protection to mice against PTZ and 4-AMP; however, it significantly delayed the mean onset and reduced the mean recovery time of the animals at the tested doses in 4-AMP test ($p<0.05$). Furthermore, it afforded a dose-depended protection to one-day-old cockerels against MES. The LD_{50} of the extract in albino mice was estimated to be greater than 1000 mg/kg. **Conclusion:** The present study suggest some level of protection by the extract of the traditional mixture against MES-induced seizure in chicks, thereby giving support to the traditional claim for its application in the treatment and/or management of convulsion and epilepsy.

Keywords: anticonvulsant, epilepsy, traditional medicine

Introduction

Epilepsy is a neurological disease arising from abnormal and uncontrollable electrical firings of a group of neurons appearing in the central nervous system. It contributes about 1% of the global burden of diseases and as high as 80% of the burden of developing countries [1]. The prevalence of epilepsy in Nigeria was estimated to be 8 to 13 per thousand people two decades ago [2]. As high as 30% of epilepsy may be refractory to existing anti-epileptic agents, which stimulate an intense research into newer molecules with novel mechanisms of action [3]. The inability of the existing anti-epileptic agents to affect epileptogenesis and their serious side effects has significantly compromised their usefulness [4,5]. There is therefore a clear

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medical need for new antiepileptic drugs with novel mechanisms of action to serve as alternate or adjunct therapy for the treatment of resistant or refractory epilepsy [6]. There is a reawakening interest in traditional medicine in the management of chronic conditions such as epilepsy [7]. Medicinal plants and their products have been used as sources of relief from diseases for more than five millennia, and to date, they still remain the almost exclusive sources of drugs for more than 60% of the world's population especially in developing countries (Nigeria inclusive), they also serve as important sources of new drugs, new drug leads, and new chemical entities [8,9]. African traditional medicine practitioners claim that their traditional remedies offer a huge potential to fight diseases, including epilepsy. The plants they use in their ethno-medicine contain mixtures of different chemical constituents [10] from one or more plants and sometimes including substances from mineral sources. Ethnobotanical information (Usman Mai-almajirai, personal communication, 2004), revealed the use of a herbal mixture (containing three plants roots namely: *Calotrpis procera* (Asclepediaceae), *Combretum micranthum* (Combretaceae) and *Ficus abutilifolia* (Moraceae) in the treatment of epilepsy.

Calotropis procera R. Br. (Asclepiadaceae) is a large, bushy shrub or small tree that may grow up to 4 m high. It exudes copious milk sap when cut or broken. It is common in the tropical and subtropical regions of Africa, Asia and Latin America. Traditionally, *C. procera* has been reported to be used as anti-diarrhoea, antidote for snake bites, stomatic and also in the treatment of elephantiasis, rheumatism, leprosy, sinus fistula, skin disease, and jaundice [11,12]. Extracts from the latex of *C. procera* have been reported to induce cytotoxicity and have shown anti-tumour, anti-ulcer and anti-inflammatory activities. A cardiotonic steroid coded UNBS1450 has also shown anti-cancer activity [13]. Further report also showed that the ethanol extract of the root-bark of *C. procera* has potential anticonvulsant activity [11].

Combretum micranthum G. Don (Combretaceae) is a bushy shrub when growing on rocky hills, but in woodlands it becomes a tree with stem up to 10 cm in diameter or a liana reaching 15-20 m long. Native to West African savannah forests, *C. micranthum* is distributed from Senegal to Nigeria and on into Gabon. It has been used in the traditional treatment of a number of illnesses including malaria, bronchitis, wounds, female sterility and indigestion [14]. Antibacterial, antifungal, antiviral, anti-malarial, anti-diabetic, anticonvulsant and immune-stimulating activities have been reported for different extracts of *C. micranthum* [15,16].

Ficus abutilifolia (Miq.) Miq. (Moraceae) is commonly called large-leaved rock fig or rock wild fig. It is a small to medium sized, deciduous to semi-deciduous tree that may grow up to 15 m high. Traditionally, the leaves have been used in promoting fertility and the latex to remove skin warts and the barks as a strengthening tonic. Anticonvulsant activity has also been reported against maximum electroshock for the ethanol extract of the root-bark of the plant [17].

The present study has investigated the acute toxicity as well as the anticonvulsant potential of the mixture of the three roots used traditionally by the Hausa Traditional healers of north-western Nigeria in the management of epilepsy.

Experimental

Collection, identification and preparation of the sample

Plant samples were collected in March-April, 2015 and identified at Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria (voucher specimen numbers 900219; 900257 and 900742 for *C. procera*, *C. micranthum* and *F. abutilifolia* respectively). The samples were cleaned, air dried and ground to powder. The mixture was prepared by mixing a known weight of the powdered plants material in a one to one ratio.

Experimental animals

Swiss albino mice of both sexes, weighing 18-25 g were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. One-day-old white Ranger cockerels were obtained

from National Animal Production Research Institute (NAPRI) Shika-Zaria, Nigeria. Animals were treated according to the standard guidelines to good practice in housing and handling laboratory animals [18]; kept under well-ventilated conditions, 12 h light/dark cycle, room temperature of 25 ± 2 ℃ and fed on standard feeds (Excel Feeds Plc) and had access to water *ad libitum.*

Extraction of plant material

Powdered mixture (500 g) was macerated with 2 L of 70% aqueous ethanol for four days. The extract was concentrated using rotary evaporator at 50 °C under reduced pressure and allowed to dry freely at room temperature. The extract was reconstituted in distilled water whenever the need for solution was arisen.

Acute toxicity studies (LD_{50})

LD_{50} determination was conducted using Lorke's method [19]. Nine mice were divided into 3 groups of 3 mice each. Mice in group 1 received the extract (*i.p.*) at a dose of 10 mg/kg body weight; group 2 received the extract at a dose of 100 mg/kg body weight whereas mice in group 3 received the extract at a dose of 1000 mg/kg body weight. Animals were observed for general signs and symptoms of toxicity including mortality over a period of 24 h.

In the second phase, four mice were divided into 4 groups of one mouse each. The extract was administered at the dose of 200, 400, 800, and 1600 mg/kg (*i.p.*) to groups 1, 2, 3 and 4 respectively. The final LD_{50} was calculated as the square root of the geometrical mean of highest non lethal dose and the lowest lethal dose [19].

Pentylenetetrazole–induced seizures in mice (Sc-PTZ)

Twenty-five albino mice were randomly divided into 5 groups of five mice per group. The first group served as negative control and was treated with normal saline (*i.p.*). Groups 2-4 received different doses of the extract (25, 50 and 100 mg/kg, *i.p*. respectively); while group 5 served as positive control and was treated with 20 mg/kg (*i.p.*) phenytoin (Sanofi synthelabo, France).

Thirty min later, 85 mg/kg of freshly prepared solution of pentylenetetrazole (Sigma, Germany) was administered subcutaneously to all mice. The animals were observed for the onset and incidence of seizures. An episode of tonic extension of the hind limbs or clonic spasm which persisted for a minimum of 30 s was taken as threshold of convulsion. Lack of threshold convulsion during 60 min of observation was regarded as protection. The number of mice protected was noted as the anticonvulsant properties of the extract [20].

4-Amino pyridine-induced seizure in mice (4-AMP)

Twenty-five albino mice were randomly divided into 5 groups of five mice per group. The first group which served as negative control was treated with normal saline. Groups 2-4 received different doses of the extract (25, 50 and 100 mg/kg, *i.p*, respectively). Group 5 which served as positive control was treated with 30 mg/Kg *i.p.* phenobarbitone (Pfizer, USA).

Thirty min later, 15 mg/kg of freshly prepared solution of 4-amino pyridine (BDH, UK) was administered subcutaneously to all mice. The mice were observed for 30 min for characteristic behavioral signs such as hyperactivity, trembling, intermittent forelimb extension, tonic seizure and death. Lack of threshold convulsion during 30 min of observation was regarded as protection. The number of mice protected was noted and the anticonvulsant properties of the extract expressed as percentage protection [21].

Maximal electroshock-induced seizures in chicks (MES)

Fifty one-day-old cockerels were randomly divided into 5 groups of 10 chicks per group. The first group served as negative control and was treated with normal saline (*i.p.*). Groups 2-4 received different doses of the extract (25, 50 and 100 mg/kg, *i.p*. respectively); while group 5 serves as positive control and was treated with phenobarbitone 20 mg/kg, (*i.p.*).

Thirty min later, maximal electroshock was delivered to all animals to induce seizures using the Ugo basile electroconvulsive machine (model 1801, Ugo Basile, Italy) with corneal electrodes place on the upper eyelid of the chick after dipping them in normal saline. The current, shock duration, frequency and pulse width were set and maintained at 80 mA, 0.6 s, 150 Hz and 0.8 m/s, respectively. An episode of tonic extension of the hind limbs of the chicks was considered as full convulsions. Lack of tonic extension of the hind limbs was regarded as protection [22,23].

Statistics analysis

Data was expressed as mean ± standard error of mean. Statistical analysis was carried out using one-way ANOVA, followed by Dunnetts test and Chi-square for percentage protection; $p<0.05$ was considered significant.

Results and Discussion

In the first phase of the acute toxicity determination, two of the three animals in group 3 (1000 mg/kg group) died while all other animals in the remaining two groups (1 and 2) survived; thus, in the second phase the selected doses were 200, 400, 800 and 1600 mg/kg [19]. From the data in table 1, the aqueous ethanol extract of the mixture gave an LD_{50} of approximately 1130 mg/kg body weight in mice when administered *i.p.*

With an LD_{50} of 1130 mg/kg in mice intraperitoneally, the ethanol extract of the mixture was regarded as relatively safe [19] also the doses used in the experiments were chosen to be less than one quarter which was considered to be pharmacologically safe.

The anticonvulsant studies results revealed that the extract could not afford a significant protection to the laboratory animals against the chemically induced convulsion of PTZ (tables 2) as well as 4-AMP (table 3). However, the extract of the mixture was noted to afford a dose-depended delay of the onset and also reduced the duration of convulsion to the laboratory animals in the 4-AMP test only. On the other hand, the mixture dose-dependently protected the animal from the electrically induced convulsion in MES test (table 4).

Table 1. Acute toxicity determination of aqueous ethanol extract of the mixture

	First Phase		Second Phase	
Group	**Dose (mg/kg)**	**No. died**	**Dose (mg/kg)**	**No. died**
1	10	0	200	0
2	100	0	400	0
3	1000	2	800	0
4	-	-	1600	1

No. died = number of animal that died; n=3 in the first phase and n=1 in the second phase

The extract did not offer outright protection against pentylenetetrazole-induced seizure in mice. The PTZ model detect agent that possess the capacity to raise the seizure threshold. Agents that act via the enhancement of the γ-amino butyric acid (GABA) system *e.g*. benzodiazepine, diminution of glutamatergic system *e.g*. felbamate and T-type calcium current have all been shown to be protective against PTZ induced seizure. The inability of the extract to offer any protection to the laboratory animals against the chemically-induced seizure of PTZ at the tested doses suggested its lack of ability to interact with any of the aforementioned mechanisms [24].

Similarly, the extract did not offer any protection to the animals against convulsive activity of 4-AMP; however, it delayed the onset of seizure induced by 4-aminopyridine, a potassium channel blocker. K^+ channels are involved in regulation of neuronal excitability, responsiveness to synaptic inputs, frequency adaptation and neurotransmitters release [6].

The 4-AMP model has the ability to detect agents that prevent seizure spread from an epileptic foci *e.g*. phenytoin [21]. Interestingly, most agents that offer protection in the MES model are active in the 4-AMP models due to the role of potassium channel in the regulation of neuronal activities. It is therefore, plausible to suggest that the anticonvulsant activity of the extract may involve the interaction with the potassium channels.

The extract also dose dependently protected the

Table 2. Effects of different doses of aqueous ethanol extract of the mixture on the convulsive activities of PTZ

Treatment	Mean onset of convulsion (min)	Mean duration of convulsion (min)	Quantal protection	Percentage protection	Percentage mortality
Normal saline	9.6 ± 6.35	12.67 ± 4.73	0	0	60
Mixture					
25 mg/kg	10.75 ± 1.50	11.50 ± 3.70	1	20	80
50 mg/kg	11.60 ± 4.40	8.25 ± 3.30	0	0	80
100 mg/kg	14.77 ± 3.10	5.25 ± 3.40	0	0	80
Phenobarbitone 20 mg/kg	-	-	5	100	0

Values are given as mean ± standard error of mean; No statistically significant different (p<0.05) from the control; n=5.

Table 3. Effects of different doses of aqueous ethanol extract of the mixture on the convulsive activities of 4-AMP

Treatment	Mean onset of convulsion (min)	Mean duration of convulsion (min)	Quantal protection	Percentage protection	Percentage mortality
Normal saline	9.4 ± 0.90	13.71 ± 2.37	0	0	100
Mixture					
25 mg/kg	12.20 ± 5.54	9.50 ± 1.79	0	0	100
50 mg/kg	13.40 ± 1.82*	7.25 ± 2.30*	0	0	100
100 mg/kg	15.40 ± 4.56*	5.33 ± 2.02*	0	0	100
Phenytoin 20 mg/kg	19.60 ± 7.44*	2.33 ± 0.22*	0	0	100

Values are given as mean ± standard error of mean; * significantly different (p<0.05) from the control; n=5.

Table 4. Effects of different doses of aqueous ethanol extract of the mixture on the convulsive activities of electroshock

Treatment	Mean Duration of convulsion (min.)	Quantal Protection	Percentage Protection	Percentage Mortality
Normal saline	8.25 ± 0.96	0	0	0
Mixture				
25 mg/kg	11.10 ± 0.79	5	50	0
50 mg/kg	9.67 ± 0.52	6	60	0
100 mg/kg	5.88 ± 0.74*	8	80	0
Phenytoin 20 mg/kg	9.00 ± 0.00	9	90	0

Values are given as mean ± standard error of mean; * significantly different (p<0.05) from the control; n=10

animals against maximal electroshock seizure with the highest protection of 80% produced at the dose of 100 mg/kg which was comparable to the standard drug phenytoin that gave 90% protection.MES test is a model of generalised tonic clonic seizure with no false negativity. Virtually, all currently available drugs in the management of generalised tonic clonic epilepsy suppressed tonic hind limb extension (THLE) in test animals. There is also an established consistency between behavioural and electrographic seizures generated in the MES test with that observed in human disorder [20]. It has the capacity to detect agents that prevent the spread of epileptic seizure from its foci [23].

Phytochemically, the presence of flavonoids, saponins, tannins, terpenoids and/or steroids has been reported in the ethanol extract of *F. abutilifolia* [17], *C. micranthum* [15] and *C. procera* [11]. The presence of the above chemical compounds in the ethanol extracts of the three plant roots may singly or in combination contribute to the observed anticonvulsant effect of the mixture of the roots.

Flavonoids are a group of phenolic compounds that are widely distributed in plants. They occur both in the free state and as glycosides. Chrysin, apigenin, wogonin, fisetin (free flavonoid aglycones), rutin and vitexin (glycosidic flavonoids) are some few examples of isolated flavonoids that have been reported to exhibit anticonvulsant activity [25]. The detection of

flavonoids in the root extracts of all the constituents of the mixture is therefore a good indicator to support the traditional use of the mixture in the treatment of epilepsy.

Saponins are another class of phytochemical components that were reported in the ethanol extracts of the mixture. Zhu and co-workers have reported anticonvulsant effect of a number of saponin containing plants including *Cynanchum otophyllum, Astragalus mongholicus* and *Ficus platyphylla* [25].

Terpenoids with their common five-carbon isoprene units are compounds that are widely distributed in the plant kingdom. These compounds with their diverse classes have exhibited antiepileptic activity. Citronellol and α-terpineol from the monoterpenoids class; bilobalide from the sequiterpenoids class; the cannabinols, abietic acid and phytol from the diterpenoids class as well as ursolic acid and baccoside A from the triterpenoids class , have demonstrated good anticonvulsant activitiy in different experimental laboratory models [25]. Thus, presence of the above secondary metabolites in the individual plant components of the mixture might singly or in combination be responsible for the reported anticonvulsant property of the mixture.

The present study showed that the extract from the mixture of the roots of *C. procera*, *C. micranthum* and *F. abutilifolia* used in Hausa traditional medicine for the treatment of epilepsy possessed anticonvulsant activity. The findings therefore provide some scientific rationale for the traditional use of the mixture of these roots in the management of epilepsy among the Hausa people of north western Nigeria.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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