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Evaluation of the anticonvulsant and muscle relaxant effects of the methanol root bark extracts of *Annona senegalensis*

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ABSTRACT

Objective: To investigate the potentials of the root bark of Annona (*A.*) *senegalensis* in the control of seizure and related hypnotic and motor incoordination effects in mice using experimental models. **Methods**: The methanol extract (ME) of the root bark of *A. senegalensis* was studied in mice using pentylenetetrazole (PTZ) induced convulsions, phenobarbitone induced sleeping time and motor coordination test on rota-rod performance. Acute toxicity and lethality (LD_{50}) test as well as phytochemical analysis were also carried out. **Results**: The extract (200, 400, 800 mg/kg) exhibited a non- dose dependent significant ($P<0.05$) delay in the onset of both tonic and clonic phases of seizure induced by PTZ (60 mg/kg, s.c.) as well as offered a 100% protection (200 mg/kg) in mice from PTZ induced seizures. The extract significantly ($P<0.05$) decreased the latency and increased the duration of phenobarbitone induced sleeping time. At 200 mg/kg, the extract exhibited a significant ($P<0.05$) motor incoordination. The acute toxicity test revealed an oral LD_{50} of 1 296 mg/kg, while the phytochemical studies showed the presence of alkaloids, resins, glycosides, carbohydrate, reducing sugar, flavonoids, terpenoids, saponins and tannins. **Conclusion**: The extract of *A. senegalensis* possessed anticonvulsant activity with pronounced hypnotic and muscle relaxant effects.

1. Introduction

It has been estimated that more than 50 million people suffer from epilepsy worldwide and many of whom are refractory to treatment with standard antiepileptic drugs (AEDs) [1]. The use of standard antiepileptic drugs (AEDs) has been limited by side-effects as well as long-term toxicity [2]. Epilepsy is a life long disorder and therefore, there is the need for the development of better tolerated, potent AEDs with lesser toxicities. The use of herbal preparations is very common in many parts of the world in the management of various forms of epilepsies. In South-eastern Nigeria, herbal concoctions of the root bark of *Annona(A.) senegalensis* (Pers) (Annonaceae) is used by traditional medicine practitioners in the treatment of epilepsy and febrile convulsions. The objective of this study is to investigate the scientific basis for the folkloric use of *A. senegalensis* in the treatment of epilepsy. *A. senegalensis* has been reported to have antineoplastic and antiprotozoal activity in mice [3], antibacterial activity [4], antidiarrhoeal activity [5], anticonvulsant effects of the whole root extract[6], analgesic activity[7], trypanocidal activity[8] and antisnake venom activity [9]. Also essential oils from the leaves of *A. senegalensis* exhibited antibacterial activity [10]. *Annona senegalensis* has been shown to contain a lot of constituents which are responsible for its various pharmacological activities. Acetogenins have been isolated in the seeds, roots bark, stems and fruits of most annona species and appear to have great potential in anti-cancer treatments [11] and (-) Roemerine, an aporphine alkaloid [3].

2. Materials and methods

2.1. Plant material

Fresh roots of *A. senegalensis* were collected from Enugu-Ezike, Enugu State, Nigeria in the month of June 2007, and authenticated by Mr. A. O. Ozioko of Bioresources Development and Conservation Programme (BDCP) Center, Nsukka. The root-bark were peeled off, cut into pieces and allowed to dry. The dried root-bark was then pulverized into coarse powder. The powdered root-bark (600 g) was extracted with methanol by cold maceration for 48 hours [12], and filtered to obtain the methanol extract. This was evaporated using a rotary evaporator at reduced pressure to obtain a yield value of 64.11 g (10.67%w/w). The extract was subjected to phytochemical analysis using standard methods [12, 13].

2.2. Animals

Adult albino mice (18 - 30 g) bred in the Laboratory

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Animal Facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, were used in the studies. The animals were maintained under standard laboratory conditions and had free access to standard pellets (Guinea Feeds, Plc, Nigeria) and water. On transfer to the work area, animals were allowed two weeks of acclimatization before the commencement of the experiments.

2.3. Acute toxicity and lethality (LD_{50}) test

The acute toxicity and lethality tests (LD_{50}) of the methanol extract (ME) was determined through the oral route in mice using the method described by Lorke [14].

2.4. Pentylenetetrazole-induced convulsion test

Albino mice were randomly divided into five groups (n = 5/group). Group I (Control) received the vehicle (10 mL/kg, 10 % Tween 80 + olive oil, p.o). Groups II-IV received the extract (200, 400 and 800 mg/kg, p.o.) while group V received diazepam (Hoffman-la Roche, 3 mg/kg, i.p.). Thirty minutes later, pentylenetetrazole (PTZ) (Sigma, 60 mg/kg, s.c.) was administered to all the animals. The animals were observed for the time of onset of tonic and clonic seizures. Percentage protection of mice was also recorded in each group. Animals devoid of seizure/convulsion without subsequent death during the 60 minutes observation period were considered protected [15, 16].

2.5.Phenobarbitone induced sleeping time

Adult albino mice were randomly divided into 5 groups (n = 5/group). Control (group I) animals were treated with the vehicle (10 mL/kg, 10% Tween 80 + olive oil, p.o.). Mice in groups II-IV were treated with the methanol extract (200, 400, 800 mg/kg, p.o.), while group V received diazepam (Hoffman-la Roche, 3 mg/kg, i.p.). These treatments were carried out 20 minutes before the administration of phenobarbitone sodium (Renaudin, France, 35 mg/kg, i.p.) to all the groups. Each mouse was observed for the onset (latency) of sleep and the duration of sleep using the loss of righting reflexes as the criterion for onset of sleep and the duration of sleep or hypnosis as the time the animal presented a loss of postural reflexes [17, 18].

2.6. Motor coordination (Rota-Rod performance) test

This test was performed using Mouse Rota Rod (Ugo Basile, 47 600). Adult albino mice were randomly divided into four groups (n = 5/group). Group I (Control) received the vehicle (10 mL/kg, 10% Tween 80 + olive oil, p.o.). Groups II & III received the extracts (200 and 400 mg/kg, p.o.) while group IV received diazepam (Hoffman-la Roche, 3 mg/kg, i.p.). The animals had already been subjected to the revolution speed (9 RPM) for acclimatization before treatment with the extracts and drugs. Five mice were simultaneously placed on the (tread mill) rota rod 30 minutes post treatment and thereafter at interval of 30 minutes for the period of 90 minutes. The time lag before the animal fell off from the rotating rod during the 30 minutes run was recorded, which is the determinant of the animal endurance time [19, 20].

2.7. Statistical analysis

Data were analyzed using One Way Analysis of Variance (ANOVA, SPSS Version 13) and expressed as mean ± SEM. Differences between means were regarded significant at $P<0.05$ and post-hoc tests were then performed using the Dunnet test.

3. Results

3.1. Phytochemical constituents

The phytochemical studies revealed the abundance of carbohydrate, alkaloid, glycoside, reducing sugar and resins while, saponins, tannins, flavonoids, steroids and terpenoids occurred in trace amounts (Table 1).

Table 1
Phytochemical constituents of methanol extract.

Phytochemical Constituents	Extract (10.69% w/w)
Carbohydrate	+++
Alkaloid	++++
Reducing sugar	+++
Glycoside	+++
Saponins	+
Tannins	+
Flavonoids	++
Resins	++++
Steroids	+
Terpenoids	+

Value in parenthesis is the extractive yield, + = present, ++ = moderately present, +++ = highly present, ++++ = abundantly present.

3.2.Acute toxicity tests

The oral acute toxicity test (LD_{50}) of ME was calculated to be 1,296 mg/kg.

3.3. PTZ – induced Convulsion

The extract prolonged the onset of both tonic and clonic phases of seizures induced by PTZ. This effect though not those dependent was more at 200 mg/kg, at which 100% protection occurred (Table 2).

3.4. Phenobarbitone induced sleeping time

The extract significantly ($P<0.05$) reduced the latency for the onset of sleep and potentiated the duration of sleep at all the doses tested. The ME exhibited maximum effects of reduction of latency and potentiation of the duration of sleep at the 200 mg/kg dose. The effects were not dose dependent (Table 3).

3.5. Motor coordination test

The extract progressively decreased the motor coordination as evidenced by the poor performance of the mice on the horizontal rotating rod. The decrease in mean motor activity of the animals with the resultant muscle-relaxation was significant ($P<0.05$) up to the 90 minutes of treatment. The motor in-coordination or the decrease in the fatigue resistance exhibited by the ME was not dose dependent (Table 4).

Table 2
Pentylenetetrazole-induced convulsion.

Treatment	Dose (mg/kg)	Onset of seizure (min.)		% Protection
		Tonic phase	Clonic phase	
Control	–	3.20 ± 0.34	7.00 ±0.94	0
Diazepam	–	50.86 ±0.60*	60.00±0.00*	100
ME	200	5.76 ±0.45*	30.96 ±11.86*	100
ME	400	5.60±0.66*	11.10±1.95*	60
ME	800	4.46± 0.24	24.80±8.73*	80

All values are expressed as Mean ± SEM; *Significance (P<0.05, ANOVA post hoc Dunnet test) compared to control. ME = methanol extract, n = 5.

Table 3
Phenobarbitone induced sleeping time.

Treatment	Dose (mg/kg)	Sleep time (min.)	
		Onset	Duration
Control	–	24.40 ± 2.04	6.40 ± 0.87
Diazepam	–	9.40 ±0.75*	42.20 ±1.40*
ME	200	13.00 ± 0.95*	32.00 ± 0.71*
ME	400	22.76 ±0.36	16.16 ± 0.12*
ME	800	18.46 ± 0.40	20.40 ± 0.28*

All values are expressed as Mean± SEM, *Significance (P < 0.05, ANOVA post hoc Dunnet test), compared to control, ME = methanol extract, n = 5

Table 4
Motor coordination test.

Treatment	Time of fall		
	30 mins	60 mins	90 mins
Vehicle	60.00 ± 0.00	60.00 ± 0.00	60.00 ± 0.00
ME 200 mg/kg	41.00± 3.96*	16.00± 1.00*	16.20 ± 0.66*
ME 400 mg/kg	57.40± 2.60	30.20 ± 2.70	29.20± 2.03
Diazepam	32.80± 0.66	3.60± 2.49	2.80 ± 0.37

All values are expressed as Mean ± SEM; *Significance (P < 0.05, ANOVA post hoc Dunnet test) compared to control. ME=methanol extract, n = 5

4. Discussion

The results obtained in this study showed that the methanol extract of *A. senegalensis* possesses anticonvulsant, hypnotic and muscle relaxant effects. The extract significantly potentiated the onset of both tonic and clonic phases of seizure in mice. The effect on the PTZ-induced seizures is an indication of possible effectiveness of the extract against absence seizures as drugs that inhibit PTZ-induced convulsions are generally effective against absence seizures [1, 2]. The reduction in the latency time and prolongation of the duration of sleep is an indication of the central depressant effects of the extract. In a previous report, the anticonvulsant activity of the chloroform whole root extract of *A. senegalensis* has been attributed to its central depressant and neuro-pharmacological modulation [6]. Sedative together with the muscle relaxant effects of the ME tend to suggest central inhibition as its possible mechanism of action. Anticonvulsant drugs such as barbiturates and benzodiazepines exhibit their effects through enhancement of GABA receptor-mediated

inhibition path way in the central nervous system (CNS) [18, 21]. Benzodiazepines exhibit pharmacological actions like anticonvulsant, reduction of muscle tone, sedation and induction of sleep [22]. Since diazepam protects mice against PTZ-induced seizures, the extract might possibly be exhibiting its effects through similar specific mechanisms as diazepam. The active principle responsible for these effects is not yet identified and further work is going on in our laboratory to this effects. In conclusion, the root bark extract of A. senegalensis possesses anticonvulsant, sedative and muscle relaxant effects and these data collaborate with the folkloric and ethno-medicinal use of this plant. The active constituents might serve as a lead compound for agents that could be used in the management of generalized absence and myoclonic seizures [1].

Reference

[1]White HS. Clinical significance of Animal seizure models and Mechanism of Action: Studies of potential antiepileptic drugs. *Epilepsia* 1997; **38** (Suppl. 1): S9-S17.
[2]Rang HP, Dale MM, Ritter JM, Flower RJ. Antiepileptic drugs. In: *Rang and Dale's Pharmacology*. 6th ed. Elsevier: Churchill Livingstone; 2007, p. 575-86.
[3]You M, Wickramaratne DB, Silva GL, Chai H, Chagwedera TE, Farnsworth NR, et al. Roemerine, an aporphine alkaloid from *Annona senegalensis* that reverses the multidrug- resistance phenotype with cultured cells. *J Nat Prod* 1995; **58** (4): 598-604.
[4]Apak L, Olila D. The in vitro antibacterial activity of *Annona senegalensis*, *Securidacca longipendiculata and Steganotaenia araliacea*-Ugandan medicinal plants. *Afr Health Sci* 2006; **6** (1): 31-5.
[5]Suleiman MM, Dzenda T, Sani CA. Antidiarrhoel activity of the methanol stem-bark extract of *Annona senegalensis* Pers. (Annonaceae). *J Ethnopharmacol* 2008; **16** (1): 125-30.
[6]Ezugwu CO, Odoh UE. Anticonvulsant activity of the root extract of *Annona senegalensis*. *J Trop Med Plants* 2003; **4** (1): 51-5.
[7]Odoh UE, Ezugwu CO, Ajali U. Preliminary investigation of the analgesic activity of the chloroform root extract of *Annona senegalensis* L. *J Pharm And Allied Sciences* 2004; **2** (1): 169-72.
[8]Ogbadoyi EO, Abdulganiy AO, Adama TZ, Okogun JI. *In vivo* trypanocidal activity of *Annona senegalensis* Pers. Leaf extract against Trypanosoma brucei brucei. *J Ethnopharmacol* 2007; **112** (1): 85-9.
[9]Abzu B, Abubakar MS, Izebe KS, Akumka DD, Gamaniel KS. Effect of *Annona senegalensis* root bark extracts on Naja nigricotlis nigricotlis venom in rats. *J Ethnopharmacol* 2005; **96** (3): 507-13.
[10]Samie A, Obi CL, Bessong PO, Namrita L. Activity profiles of fourteen selected medicinal plants from Rural Venda communities in South Africa against fifteen clinical bacterial species. *Afr J Biotechnol* 2005; **4** (12): 1443-51.
[11]Pinto AC de Q, Cordeiro MCR, de Andrade SRM, Ferreira FR de C, Filgueiras HA, Alves RE, et al. *Annona species*. Southampton: International Centre for Underutilized Crops, University of Southampton,UK; 2005.
[12]Trease GE, Evans WC. *Text book of pharmacognosy*. 15th ed. London: Bailliere Tindall; 1989, p. 315-679.
[13]Harbone JB. Phytochemical Methods: a guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall; 1988, p. 55-6.
[14]Lorke D. A new approach to practical acute toxicity testing. *Arch Toxicol* 1983;**54**: 272-89.
[15]Akah PA, Nwaiwu JI. Anticonvulsant activity of roots and stem extracts of *Calliandra portoricensis*. *J Ethnopharmacol* 1988; **22**: 205-10.
[16]Okoye TC, Aguwa CN, Okoli CO, Akah PA, Nworu CS. Anticonvulsant and sedative effects of leaf extracts of *Stachytarpheta cayennensis*. *J Trop Med Plants* 2008; **9** (1): 17-22.
[17]Miya TS, Holok HGO, Yim GRW, Spratto GR. *Laboratory Guide in Pharmacology*. Minneapolis: Burgess Publishing Co.; 1973, p. 44-6.
[18]Nogueira E, Vassilieff VS. Hypnotic, anticonvulsant and muscle relaxant effects of *Rubus brasiliensis*. Involvement of GABA-system. *J Ethnopharmacol* 2000;**70**:275-80.
[19]Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rats and mice. *J Am Pharmaceut Assoc Scientific Edit* 1957; XLVI: No. 3.
[20]Mukherjee PK. *Quality control of herbal drugs*. New Delhi: Business Horizons Publishers; 2007, p. 573-74.
[21]McNamara JO. Drugs effective in the therapy of the epilepsics. In: Gilman AG, Limbird LE, Hardman JG, editors. *Goodman's and Gilman's the Pharmacological Basis of Therapeutics*. 10th ed. New York: McGraw-Hill; 2001, p. 521-47.
[22]Rang HP, Dale MM. Anxiolytic and hypnotic drugs. In: *Rang and Dale's Pharmacology*. 6th ed. Elsevier: Churchill, Livingstone; 2007, p. 538.

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