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Full Length Research Paper

Neuropharmacological evaluation of *Annona senegalensis* leaves

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The neuropharmacological activities of methanol leaf extract (ME) of *Annona senegalensis* Pers (Annonaceae) and its bioactive fractions (MF and F_7) were studied in rodents using pentylenetetrazol (PTZ)-induced seizures, pentobarbitone-induced sleep, apomorphine-induced stereotypy, open field, elevated plus maze (EPM) and rotarod performance tests. The extract and fractions inhibited PTZ-induced seizures, prolonged pentobarbitone-induced sleep, reduced stereotypic behaviour induced by apomorphine, decreased the frequency of line crossing and centre square entries and increased rearing in the air in the open field. The frequency of grooming and rearing against the wall were decreased, whereas the duration of grooming increased. Also, the extract and fractions increased the duration of stay in the open arm when compared to the closed arm of the EPM, and reduced the average time spent on the rotarod. Acute toxicity test showed an oral LD_{50} of ME greater than 5 g/kg in mice. Phytochemical analysis showed that ME tested positive for carbohydrates, reducing sugar, resins, saponins, tannins, steroids, terpenoids, alkaloids, flavonoids and glycosides; MF tested positive for saponins, steroids, terpenoids, alkaloids, carbohydrates, reducing sugar, flavonoids and glycosides; while F_7 tested positive for flavonoids. These findings suggest that leaves of *A. senegalensis* possess anticonvulsant, central depressant and anxiolytic-like properties attributable to flavonoids.

Keywords: *Annona senegalensis,* anticonvulsant, anxiolytic, sedative, stereotypy.

INTRODUCTION

Annona senegalensis Pers (Annonaceae) commonly known as “Wild Custard Apple” is a shrub or small tree widely distributed in Africa (Adzu et al., 2005; Ogbadoyi et al., 2007). It has aromatic flowers which are used to flavour food. The ripe fruit is yellow in colour and has a sweet edible jelly with pleasant odour.

In Nigeria, *A. senegalensis* is variously known as “Gwandar daji” in Hausa, “Abo” in Yoruba, “Uburu ocha” in Ibo, and “Ikpokpo” among the Idoma speaking people in the Middle Belt region of Nigeria. It is widespread in the Savannah area and near streams and enjoys great reputation for its immense medicinal value and hence, ethnomedicinal uses. The plant decoction is used in the treatment of sleeping sickness in Northern Nigeria (Igwe and Onabanjo, 1989) and in folkloric treatment of cancer (Durodola, 1975; Gbile and Adesina, 1985; Graham et al., 2000; Abubakar et al., 2007), chest pain, coughs, anaemia, urinary tract infections (Burkill, 1985; Muanza et al., 1994), intestinal troubles, stomach ache (Dalziel, 1955), diarrhoea, bloody stool, dysentery (Muanza et al., 1994; Ekpendu et al., 1998; Kudi and Myint, 1999), arthritis, rheumatism (Audu, 1989), intestinal and guinea-worms (Watt and Breyer-Brandwick, 1962; Alawa et al., 2003), venereal diseases (Durodola, 1975; Bhat et al., 1990; Tabuti et al., 2003), head and body ache (Arnold and Gulumian, 1984; Chhabra et al., 1987), leishmaniasis (Akendengue et al., 1999), trypanasomiasis (Atawodi et

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Abbreviations: ME, Methanol leaf extract; **PTZ,** pentylenetetrazol; **EPM,** elevated plus maze; **CNS,** central nervous system; **TLC,** thin layer chromatography; **MF,** methanol fraction; **HF,** hexane fraction; **EF,** ethyl acetate fraction; **F_7,** fraction 7.

al., 2003), lice infestation (Hirschmann and Rojas De Arias, 1990), eyelid swelling (Klaus and Adala, 1994) and snakebites (Durodola, 1975; Kela, 1990; Selvanayahgam et al., 1994). It is also used as an anthelmintic by local livestock farmers in Nigeria (Nwude and Ibrahim, 1980; Ibrahim et al., 1983). In southeastern Nigeria, the leaves are used for the treatment of convulsion.

Review of documented literature showed that the antidiarrheal (Suleiman et al., 2008), antimicrobial (More et al., 2008), anticancer (Sowemimo et al., 2007), trypanocidal (Ogbadoyi et al., 2007), antimalarial and cytotoxic (Ajaiyeoba et al., 2006), anticonvulsant (Ezugwu and Odoh, 2003), analgesic, anti-inflammatory (Adzu et al., 2003), antiulcer/antacid, smooth muscle relaxant (Langason et al., 1994), antibacterial (Muanza et al., 1994; Magassouba et al., 2007), antitumor (Fatope et al., 1993; Sahpaz et al., 1994), antiprotozoal (Igwe and Onabanjo, 1989), molluscicidal (Sofowora and Adewunmi, 1980) and hormone-mimetic (Jacobson et al., 1975) activities have been reported. The plant has also been shown to be beneficial in the treatment of snake bite (Adzu et al., 2005). The isolation of monotetrahydrofuran and bis-tetrahydrofuran acetogenins (Sahpaz et al., 1994) and two cytotoxic monotetrahydrofuran acetogenins (Sahpaz et al., 1996) from this plant are also documented.

Anticonvulsant agents affect diverse centrally-mediated functions due to their interference with a variety of mechanisms and structures in different regions of the central nervous system (CNS). In line with the use of leaves of this plant in the treatment of convulsion and the documented anticonvulsant activity of its roots (Ezugwu and Odoh, 2003), we studied the neuropharmacological activities of the leaves and identified the centrally-active constituents using bioactivity-guided technique employing pentylenetetrazol (PTZ)-induced seizures as activity-guide.

MATERIALS AND METHODS

Animals

Adult Swiss albino rats (150 - 200 g) and mice (19 - 22 g) bred in the laboratory animal facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka were used for the study. The animals were maintained freely on standard pellets and water. All animal experiments were in compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85 – 23, revised 1985).

Equipment

Equipment
The open field apparatus consisted of a plexiglass box measuring 72 × 72 cm with 36 cm high walls, the walls and the floor were painted white. Blue lines, drawn under the clear plexiglass floor with a marker, divided the floor into 16 squares (18 × 18 cm). A central square of equal size was drawn in the middle of the maze; elevated plus maze (EPM) consisted of two open arms (40 × 10 cm each) and two closed arms (40 x 10 x 10 cm each) radiating from a central platform (10 x 10 cm) arranged in such a way that the two arms of each type were opposite to each other. The maze was elevated 100 cm above the floor. The maze floor and walls were constructed with wood; a rotarod apparatus (Ugo Basile, 01778; Comerio-Va-Italy) consisting of a motor-driven aluminum rod (6 - 8 cm diameter) divided into five segments by circular aluminum plates which served to limit lateral movements of the animals on the rod; rotary evaporator (Staffordshire, ST 150BG; England) and video camera.

Plant material and preparation of extract

Fresh leaves of *A. senegalensis* were collected in March 2006 from Nsukka, Enugu State, Nigeria. The plant material was identified and authenticated by Mr. A. Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State, where a voucher specimen is deposited (specimen number: BDCP/INTERCEDD 64). The leaves were dried under the sun for 5 days and pulverized to coarse powder using an electric blender. The powdered leaf (2.5 kg) was extracted with methanol by cold maceration for 48 h. Concentration of the filtrate in a rotary evaporator at 40 - 50°C under reduced pressure afforded 131.4 g of the methanol extract (ME; 5.26% w/w).

Acute toxicity tests

The acute toxicity and lethality of ME was studied in mice using the method described by Lorke (1983). Briefly, nine mice of both sexes randomly divided into three groups (n = 3) received oral administration of one of 10, 100, and 1000 mg/kg of ME and were observed for 24 h for death. Since no death was recorded, further doses of 1,600, 2,900 and 5000 mg/kg of ME were administered to a fresh batch of animals (n = 1) and the number of deaths in 24 h was recorded. The LD_{50} was calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose (Lorke, 1983).

Solvent-guided fractionation of ME and bioactivity-guided studies

The methanol extract (100 g) was subjected to solvent-guided fractionation in a silica gel (60 - 120 mesh size) column (60 cm in length and 7.5 cm in diameter), successively eluted with n-hexane, ethyl acetate and methanol. The fractions were concentrated under reduced pressure in a rotary evaporator (40 - 50°C) to obtain the hexane fraction (HF; 0.2 g; 0.2% w/w), ethyl acetate fraction (EF; 2.5 g; 2.5% w/w) and methanol fraction (MF; 70.26 g; 70.3% w/w). Bioactivity-guided studies on the extract and fractions using the PTZ-induced seizure model as activity-guide showed that MF caused the highest delay in the onset of tonic-clonic seizures and also afforded 100% protection against seizure-induced deaths. Subsequently, MF (27.6 g) was separated in a silica gel column (60 cm in length and 7.5 cm in diameter) eluted with gradient mixtures of dichloromethane and methanol and the fractions were collected in aliquots of 10 ml in test tubes. The collected fractions were subsequently pooled into eight broad fractions, $F_1 – F_8$, based on the similarity of constituents visualized on silica gel pre-coated thin layer chromatography (TLC) plates developed with mixtures of methanol and dichloromethane. Further activity-guided studies on the fractions showed that F_7 (0.56 g; 2.03% w/w) caused the

greatest delay in onset of tonic-clonic seizures with 60% protection.

Consequently, ME, MF and F_7 were screened for effects on sedation, paradigms of anxiety and depression, stereotype behavior and motor coordination. Phytochemical tests on the extract and fractions for constituents identification was performed using standard procedures (Harborne, 1973; Trease and Evans, 1983).

Pentylenetetrazole-induced seizures in mice

Male mice (20 - 39 g) were randomly divided into groups (n = 5) to receive oral administration of one of ME, MF (100, 200 and 400 mg/kg) or F_7 (400 mg/kg) suspended in Tween 80 (3% v/v). Control animals received either the vehicle (10 ml/kg p.o) or phenobarbitone (35 mg/kg i.p). Thirty minutes later, seizure was induced by intraperitoneal administration of pentylenetetrazole (70 mg/kg). The animals were observed for seizures. The onset and duration of seizures as well as quantal protections were recorded for each group. An episode of clonic spasm that persisted for a minimum of 30 s was taken as threshold convulsion. Animals devoid of threshold convulsion and without subsequent death during 60 min of observation were considered protected (Akah et al., 1998).

Pentobarbitone-induced sleeping time test in rats

Male rats were randomly divided into five groups (n = 5) to receive intraperitoneal administration of one of ME, MF or F_7 (50, 100, or 200 mg/kg) suspended in Tween 80 (3% v/v). Control groups received either the vehicle (2 ml/kg p.o) or diazepam (1 mg/kg i.p). Thirty minutes later, sleep was induced by intraperitoneal injection of pentobarbitone sodium (35 mg/kg). Each animal was observed for onset and duration of sleep. The time from induction of sleep to loss of righting reflex was considered as onset of sleep, while that between loss and recovery of righting reflex was recorded as the duration of sleep (Wambebe, 1985).

Apomorphine-induced stereotypic behavior test

The effect of the extract and fractions on apomorphine-induced stereotypic behavior was evaluated as earlier described (Kenneth and Kenneth, 1984). Male mice were divided into groups (n = 5) to receive intraperitoneal administration of one of ME, MF or F_7 (50, 100, or 200 mg/kg) suspended in Tween 80 (3% v/v). The control groups received either chlorpromazine (2 mg/kg i.p) or the vehicle (10 ml/kg p.o). Thirty minutes later, stereotype behavior was induced by subcutaneous injection of apomorphine (1 mg/kg). Signs of stereotypic behavior, which include mainly sniffing and gnawing, were observed and scored as follows: Absence of stereotypy = 0; occasional sniffing = 1; occasional gnawing = 2; frequent gnawing = 3; continuous gnawing = 4; gnawing intensively and staying at the same spot = 5. Stereotypic behavior was measured and scored for 5 min, immediately after induction (0 min) and at 30 min intervals for up to 120 min.

Open field test

The effect of the extract and fractions on locomotor activity, exploration and grooming was studied in the open field (Archer, 1973). Briefly, male mice (19 - 30 g) selected at random were divided into groups (n = 5) to receive oral administration of one of ME, MF or F_7 (200 or 400 mg/kg) suspended in Tween 80 (3% v/v). The control groups received either diazepam (1 mg/kg i.p) or the vehicle (10 ml/kg p.o). Thirty minutes after treatment, each mouse was placed in the centre square of the open field and observed for 5 min with the aid of video camera. Behavioral parameters recorded include line crossing, centre square entries, rearing (in the air and against the wall) and stereotypy as shown by frequency and duration of grooming. The floor of the open field was cleaned with 70% ethanol and allowed to dry between tests.

EPM

Male mice were divided into groups (n = 5) to receive one of ME, MF or F_7 (50, 100 or 200 mg/kg) suspended in Tween 80 (3% v/v) and administered intraperitoneally. The control groups received either diazepam (1 mg/kg i.p) or the vehicle (10 ml/kg p.o). The mice were placed at the junction of the open and closed arms, facing the open arm opposite to where the experimenter was. A video camera set at 45° between one open and one closed arm was then started to track and record the activity of the rodent on the maze for 5 min (Pellow et al., 1985; Lister, 1987) and was scored on video playback. An observer sat on an elevated platform to observe the behavior. Parameters observed included duration of stay and number of fecal boli in the open, closed and mid arms of the EPM.

Motor coordination test

The effect of the extract and fractions on motor coordination in mice was studied using the rotarod test. Thirty-five male mice (20 - 35 g) were selected at random and divided into 7 groups (n = 5) to receive one of ME, MF or F_7 (200 or 400 mg/kg) suspended in Tween 80 (3% v/v) and administered orally. The control group received either diazepam (1 mg/kg i.p) or the vehicle (10 ml/kg p.o). The mice were allowed to acclimatize for 3 min on the rotarod beam before administration of the extract and fractions. Thirty minutes after treatment, each mouse was placed on one of the five rotarod beams facing the opposite direction of the beam's motion. All mice started from a non-motion beam and then the rotarod was turned on. When a mouse fell from the beam into the chamber, it remained there until all other mice had either completed 3 min on the beam or fell off. After one-minute break, the mice were lifted and placed on the beam once more. Each mouse went through 10 trials and was tested for time spent on the rod during each trial. The average total time each animal stayed on the rotating rod was recorded. The increase in time the animal remained on the rod was taken as an index of motor coordination/learning.

Statistical analysis

Data obtained was analyzed using one way analysis of variance (ANOVA) and subjected to least significant difference (LSD) post hoc test for multiple comparisons. Differences between means were accepted to be significant at $P < 0.05$ and the results expressed as mean ± SEM.

RESULTS

Phytochemical constituents of extract and fractions

Phytochemical analysis showed that ME tested positive for carbohydrates, reducing sugar, resins, saponins, tannins, steroids, terpenoids, alkaloids, flavonoids and

Table 1. Phytochemical constituents of the extract and fractions.

Phytochemical constituents	Extract and fraction		
	ME (5.26% w/w)	MF (70.3%w/w)	F_7 (2.03% w/w)
Alkaloids	+++	+	-
Carbohydrates	+++	+++	-
Flavonoids	+++	+++	+++
Glycosides	+++	+++	-
Reducing sugar	+++	+++	-
Resins	++	-	-
Saponins	+++	+++	-
Steroids	+++	+++	-
Tannins	+	-	-
Terpenoids	+++	+++	-

Values in parenthesis are extractive yields. ME, methanol extract; MF, methanol fraction; F_7, fraction 7; +++, conspicuously present: ++, moderately present; +, present; -, absent.

Table 2. Effect of extract and fractions on PTZ-induced seizures.

Treatment	Dose (mg/kg)	Onset of seizure (min)	Duration of seizure (min)	Quantal protection	Protection (%)
Control	-	1.2 ± 0.1	1.6 ± 0.7	0/5	0
ME	100	2.9 ± 1.0	11.7 ± 4.5	2/5	40
	200	2.8 ± 0.1	19.6 ± 4.1*	2/5	40
	400	2.6 ± 0.6	19.4 ± 4.6*	2/5	40
MF	100	2.3 ± 0.5	13.5 ± 4.4*	3/5	60
	200	4.0 ± 1.1	11.5 ± 1.6	3/5	60
	400	3.1 ± 0.2	18.4 ± 2.4*	5/5	100
F_7	400	2.6 ± 0.8	5.2 ± 1.9	3/5	60
Phenobarbitone	35	13.33 ± 1.45*	0.1 ± 0.4	5/5	100

n, 5; *$P < 0.05$ compared to control (ANOVA; LSD post hoc); ME, methanol extract; MF, methanol fraction; F_7, fraction 7.

glycosides; MF tested positive for saponins, steroids, terpenoids, alkaloids, carbohydrates, reducing sugar, flavonoids and glycosides, while F_7 tested positive to flavonoids (Table 1).

Acute toxicity and lethality (LD_{50}) of ME

Oral administration of ME of up to 5 g/kg caused no death in mice. Therefore, the oral LD_{50} of ME in mice was >5 g/kg.

Effect of extract and fractions on PTZ-induced seizure

The ME, MF and F_7 caused significant ($P < 0.05$) and non-dose-related inhibition of PTZ-induced seizures characterized by increased seizure latency, prolonged duration of seizure and increased survival time or protection against seizure-induced deaths (Table 2).

Effect of extract and fractions on pentobarbitone-induced sleeping time

The ME and MF significantly ($P < 0.05$) shortened the sleep onset time (sleep latency) and prolonged sleeping time in a dose-related manner. F_7 significantly ($P < 0.05$) prolonged sleep time but increased sleep latency in a dose-related manner. The magnitude of potency of effect was of the order F_7 > MF > ME (Table 3).

Effect of extract and fractions on stereotypy induced by apomorphine in mice

Treatment with the extract and fractions significantly ($P <$

Table 3. Effect of extract and fractions on pentobarbitone induced sleeping time.

Treatment	Dose (mg/kg)	Sleep time (min)		
		Onset	Duration	Prolongation (%)
Control	-	24.1 ± 0.8	94.9 ± 7.0	-
ME	50	24.0 ± 13.6*	56.8 ± 8.9*	NP
	100	22.0 ± 10.3	109.2 ± 17.4	15.07
	200	15.2 ± 13.4*	201.2 ± 3.3	112.01
MF	50	11.8 ± 1.1*	94.0 ± 6.5*	NP
	100	5.4 ± 0.6*	128.2 ± 3.0*	35.09
	200	3.2 ± 0.5*	178.3 ± 2.4*	87.88
F_7	50	34.8 ± 0.5	102.0 ± 2.7*	7.48
	100	40.0 ± 7.1*	198.2 ± 4.1	108.85
	200	34.2 ± 0.5	252.5 ± 5.1*	166.07
Diazepam	1	12.9 ± 0.8	192.9 ± 31.5	103.27

n = 5; *$P<0.05$ compared to control (ANOVA; LSD post hoc); ME = methanol extract; MF = methanol fraction, F_7 = fraction 7; NP = no prolongation; prolongation (%) was calculated relative to the control.

0.05) reduced stereotypic behaviour in a dose-related manner (Table 4).

Effect of extract and fractions on mice in the open field

The extract and fractions decreased the frequency of line crossing in a dose-related manner. However, F_7 produced a higher frequency of line crossing with increased dose, which was still lower than that of control animals. Although F_7 (400 mg/kg) slightly increased centre square entries, ME produced a decrease, while MF caused no change. On rearing, ME and the fractions increased the frequency of rearing in the air with MF and F_7 showing greater effect. However, with the exception of MF (200 mg/kg), extract and fractions decreased the number of rearing against the wall. The extract and fractions treated groups had reduced frequency and increased duration of grooming (Table 5).

Effect of extract and fractions on the behavior of mice on the EPM

The ME and fractions significantly ($P < 0.05$) increased the duration of stay in the open arm and decreased the duration of stay in the closed arm. There was little or no change in fecal boli produced by treated and control groups in the mid, closed and open arms. The magnitude of increase in duration of stay in the open arm was of the order of potency F_7 > MF > ME (Table 6).

Effect of extract and fractions on rotarod performance

Treatment with the extract and fractions significantly ($P < 0.05$) reduced the average total time spent by rats on the rotarod. The magnitude of reduction in permanence on the rod was of the order ME > MF > F_7 (Table 7).

DISCUSSION

In this study, evaluation of the neuropharmacological activities of *A. senegalensis* showed that the leaf extract and bioactive fractions possess anticonvulsant, sedative/anxiolytic and central depressant properties. Assessment of the anticonvulsant activity revealed increased seizure latency, prolonged duration of seizure and protection of treated mice from seizure-induced deaths. PTZ induces convulsions by antagonizing the γ-aminobutyric acid ($GABA_A$) receptor Cl^- channel complex (Corda et al., 1990) which attenuates GABA-dependent inhibition. Thus, the anticonvulsant activity of leaves of this plant and its usefulness in traditional treatment of convulsion may be derived in part from enhancement of GABAergic mechanisms. Agents that protect against tonic–clonic seizures induced by PTZ are considered useful in controlling myoclonic and absence seizures in humans (Nisar et al., 2008).

Studies on the sedative activity revealed reduced latency of induction and increased duration of pentobarbitone-induced sleep. Although the pentobarbitone-sleeping time test is not clearly specific because

Table 4. Effect of extracts and fractions on stereotypy induced by apomorphine in mice.

Treatment	Dose (mg/kg)	Stereotypic behaviour score (min)				
		0	30	60	90	120
Control	-	1.19 ± 0.29	1.07 ± 0.47	0.97 ± 0.15	1.03 ± 0.08	0.96 ± 0.09
ME	50	0.44 ± 0.41* (63.03)	0.72 ± 0.30 (32.71)	0.60 ± 0.45 (38.14)	0.32 ± 0.27* (68.93)	0.04 ± 0.84* (95.83)
	100	0.32 ± 0.41 (73.11)	0.88 ± 0.18 (17.76)	0.36 ± 0.36 (62.89)	0.12 ± 0.27* (88.35)	0.04 ± 0.84* (95.83)
	200	0.36 ± 0.49 (69.75)	0.64 ± 0.29 (40.19)	0.32 ± 0.18 (67.01)	0.00 ± 0.0 (100)	0.00 ± 0.00 (100)
MF	50	0.40 ± 0.40* (66.39)	1.00 ± 0.00 (6.54)	0.61 ± 0.55 (37.11)	0.80 ± 0.36 (22.33)	0.60 ± 0.55 (37.50)
	100	0.32 ± 0.22* (73.11)	0.80 ± 0.45 (25.23)	0.44 ± 0.26 (54.64)	0.44 ± 0.38* (57.28)	0.12 ± 0.27* (87.50)
	200	0.00 ± 0.00 (100)	0.36 ± 0.26* (66.36)	0.68 ± 0.28 (29.90)	0.72 ± 0.23 (30.10)	0.04 ± 0.09* (95.83)
F_7	50	0.80 ± 0.00 (32.77)	1.00 ± 0.00 (6.54)	0.88 ± 0.18 (9.28)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)
	100	0.40 ± 0.28* (66.39)	0.92 ± 0.18 (14.02)	0.52 ± 0.44 (46.39)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)
	200	0.16 ± 0.26* (86.55)	0.60 ± 0.28 (43.93)	0.56 ± 0.36 (42.27)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)
Chlorpromazine	2	0.20±0.41 (83.19)	0.10±0.30 (90.65)	0.00±0.00 (100)	0.00±0.00 (100)	0.00±0.00 (100)

n = 5; *$P < 0.05$ Compared to control (ANOVA; LSD post hoc); ME = methanol extract; MF = methanol fraction; F_7 = fraction 7. Values in parenthesis represent inhibition of stereotypic behaviour calculated relative to control.

Table 5. Effect of extract and fractions on mice in the open field.

Treatment	Dose (mg/kg)	Locomotor activity		Exploratory activity		Grooming	
		Line crossing	Centre square entries	Rearing in the air	Rearing against a wall	Frequency	Duration (s)
ME	200	32.6 ± 18.4	1.2 ± 0.6	0.8 ± 0.8	2.2 ± 1.4*	1.8 ± 0.7	6.4 ± 1.8*
	400	3.8 ± 2.8*	0.0 ± 0.0	0.2 ± 0.2	0.8 ± 0.8*	0.8 ± 0.2*	4.6 ± 1.9
MF	200	46.6 ± 26.7	1.4 ± 0.9	1.0 ± 1.0	16.4 ± 8.4	1.6 ± 0.8	8.0 ± 3.7
	400	7.6 ± 5.6*	0.0 ± 0.0	0.4 ± 0.4	1.6 ± 1.6*	1.0 ± 0.4*	5.0 ± 3.9
F_7	200	32.8 ± 15.0	0.6 ± 0.6	1.0 ± 1.0	9.4 ± 4.6	1.8 ± 0.9	5.4 ± 3.0
	400	41.8 ± 19.9	1.6 ± 1.2	0.0 ± 0.0	4.8 ± 2.3	2.0 ± 1.4	8.0 ± 5.8
Diazepam	1	7.3 ± 5.0	0.0 ± 0.0	0.0 ± 0.0	3.7 ± 2.03	2.0 ± 0.58	0.6 ± 0.44
Control	-	60.4 ± 12.4	1.4 ± 0.9	0.0 ± 0.0	15.4 ± 3.1	2.8 ± 0.9	5.0 ± 1.3

n = 5; *$P < 0.05$ Compared to control (ANOVA; LSD post hoc); ME = methanol extract; MF = methanol fraction; F_7 = fraction 7.

compounds that interfere with biotransformation of pentobarbital by cytochrome P-450 complex can exhibit the same effects (Goloubkova et al., 1998), the result possibly indicates sedative/anxiolytic or central depressant activities.

Evaluation of the effect of the extract and fractions on paradigms of anxiety and depression showed that treated mice exhibited decreased locomotor and exploratory activities likely due to

Table 6. Effect of extract and fractions on mice on the elevated plus maze.

Extract/ fraction	Dose (mg/kg)	Number of fecal boli			Duration of stay (s)		
		Mid arm	Closed arm	Open arm	Mid arm	Closed arm	Open arm
Control	-	0.5 ± 0.6	0.2 ± 0.3	0.2 ± 0.3	8.3 ± 3.4	251.6 ± 16.1	88.9 ± 41.9
ME	50	0.2 ± 0.5	0.4 ± 0.6	0.2 ± 0.5	5.4 ± 3.2	180.4 ± 73.1	114.2 ± 74.3
	100	0.0 ± 0.0	0.2 ± 0.5	0.0 ± 0.0	10.2 ± 6.2	191.0 ± 89.5	98.6 ± 88.2
	200	0.2 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	4.2 ± 3.9	157.4 ± 91.9	138.4 ± 89.9
MF	50	0.2 ± 0.5	0.2 ± 0.5	0.0 ± 0.0	6.6 ± 8.3	130.8 ± 31.5*	162.6 ± 30.6*
	100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	16.2 ± 3.0*	106.0 ± 18.5*	177.8 ± 19.6*
	200	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	9.6 ± 5.6	107.0 ± 43.6*	163.4 ± 51.0*
F_7	50	0.2 ± 0.5	0.2 ± 0.5	0.2 ± 0.5	20.8 ± 9.5	135.6 ± 44.4*	143.2 ± 48.9*
	100	0.2 ± 0.5	0.2 ± 0.5	0.2 ± 0.5	24.6 ± 15.8	76.2 ± 64.3*	199.2 ± 73.9*
	200	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	13.0 ± 8.8	79.0 ± 99.7*	206.2 ± 91.3*
Diazepam	1	0.0 ± 0.0	0.2 ± 0.5	0.0 ± 0.0	8.9 ± 4.2	128.4 ± 35.2	173.0 ± 18.4

n = 5; *$P < 0.05$ compared to control (one way ANOVA; LSD pos hoc); ME = methanol extract; MF = methanol fraction; F_7 = fraction 7.

Table 7. Effects of the extract and fractions on motor co-ordination on rotarod.

Treatment	Dose (mg/kg)	Duration of stay on the rotarod (s)
Control	-	98.1 ± 15.4
ME	200	14.6 ± 4.5*
	400	22.0 ± 5.2*
MF	200	26.9 ± 19.0*
	400	36.4 ± 24.3*
F_7	200	66.1 ± 25.5
	400	96.7 ± 36.7
Diazepam	1	32.6 ± 0.66*

n = 5; *$P < 0.05$ compared to control (ANOVA; LSD post hoc); ME = methanol extract, MF = methanol fraction, F_7 = Fraction 7.

central depression. The open field test is used to measure not only anxiety-like behaviors, but also sedative (Prut and Belzung, 2003) as well as non-specific effects of drugs on locomotor activity (Choleris et al., 2001). Treatment of mice with the extract and fractions reduced the frequency of grooming but caused little or no change in the duration which suggests reduced stress and anxiolytic-like effect. The mice also exhibited reduced frequency of rearing against the wall, whereas rearing in the air was increased. In a novel environment as in the open field, anxious rodents exhibited thigmotaxic behavior, which is a spontaneous preference for periphery/walls of the open field to the central parts. Thus, the animals exhibited reduced thigmotaxic behaviour as shown by reduced preference for walls of the open field which is consistent with central depressant activity. Decrease in spontaneous motor activity such as locomotor activity (horizontal activity) and rearing (vertical activity) results from reduced excitability of the central nervous system and sedation (Ozturk et al., 1996; Perez et al. 1998; Prut and Belzung, 2003). Rearing is a function of the excitability levels of the CNS (Cunha and Masur, 1978), while grooming is modulated by various neurotransmitters (Traber et al., 1988) particularly, dopamine (Drago et al., 1999; Serafim and Felício, 2001; Gomes et al., 2008).

Consistent with the anxiolytic-like activity is the effect on the EPM. In the EPM, treatment with the extract and fractions caused anxiolytic-like effect by increasing exploration and time spent in the open arms and reduces the number of entries and time spent in the enclosed arms. The EPM is widely used to measure anxiety in rodents (Pellow et al., 1985; Lister, 1987). Naive mice spend more time in the enclosed arm which may reflect an aversion towards the open arms caused by fear of open spaces (Rodgers and Dalvi, 1997). Drugs that increase the open arms exploration are considered anxiolytics and the reverse is also true for anxiogenic compounds (Handley and McBlane, 1993). Anxiolytic compounds promote exploration and reduce the animal's natural aversion for

the open arms. Several plants used to reduce anxiety in folk medicine have been shown to increase exploration in the open arms of the EPM (Helli´on-Ibarrola et al., 2006).

The extract and fractions also reduced stereotypic behavior induced by apomorphine in mice. This effect is an indication of neuroleptic potentials and antidopaminergic properties. Agents which inhibit apomorphine-induced stereotypy are known to antagonise dopamine receptors in the nigrostriatal system (Chindo et al., 2003; Tarsy and Baldessarini, 1986). In mice, apomorphine increases the intensity and duration of stereotypic behavior by acting directly on the post-synaptic dopamine D_2 receptors (Stolk and Rech, 1970). Thus, inhibition of the effects of apomorphine reverses the hyperactivity and stereotypic behavior suggesting interference with central dopaminergic neurotransmission by the extract and fractions. This action is consistent with the effect of the major tranquillizers which cause profound central depression by interfering with central dopaminergic neurotransmission as a result of blockade of D_2 receptors (Baldessarini and Tarazi, 2001).

In addition, interference with dopaminergic neurotransmission may partly account for the decrease in permanence of treated mice on the rotarod as is also seen with major tranquillizers which cause motor incoordination (Baldessarini and Tarazi, 2001).The rotarod test designed to assess motor coordination, balance and equilibrium is used to evaluate the pharmacological actions of psychotropic agents on the central or peripheral nervous system (Dunhan and Miya, 1957). Impairment of the rotarod performance has been thought to reflect, at least in part, a behaviorally depressive state. However, it is well known that the riding time on the rotarod is also decreased by a relaxation or weakness of the muscles or motor dysfunction. Thus, in addition to central inhibition, the ability of the extract and fractions to affect motor coordination may also indicate peripheral blockade of the neuromuscular system (Perez et al., 1998; Amos et al., 2001). Several studies have shown similar results with diazepam, a known skeletal muscle relaxant. It is well known that benzodiazepines act as anxiolytics (at low doses), anticonvulsants and also produce sedation and myorelaxant effect at higher doses (Melo et al., 2006). It is likely that the activities of the extract and fractions are mediated by central and peripheral mechanisms.

Biological activity-guided technique was employed to relate activity to constituents and revealed F_7 as the most active anticonvulsant fraction. Phytochemical tests for constituent identification showed that F_7 may be a flavonoid compound. Further neuropharmacological studies showed that F_7 possesses varying levels of activity in the different tests and interestingly, had the least effect on motor-coordination. Flavonoids with anxiolytic activity attributed to their affinity for the central benzodiazepine receptor have also been described in many plant species used in folk medicine to depress the central nervous system (Medina et al., 1993; Medina et al., 1997; Griebel et al., 1999; Paladini et al., 1999; Rocha et al., 2002). It is likely that further separation of constituents of F_7 would yield a neuropharmacologically-active flavonoid compound.

In conclusion, findings from this study suggest that constituents of leaves of *A. senegalensis* possess anticonvulsant, central depressant and anxiolytic-like properties and justify the folkloric use of the leaves for the treatment of convulsions. These neuropharmacological activities may be attributed to flavonoids in the leaves. Further activity-guided studies are ongoing to isolate the flavonoid(s) responsible for these activities.

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