Pharmacological Evidence for the Anticonvulsant Properties of the Leaf of *Newbouldia laevis* Seem (Bignoniaceae)

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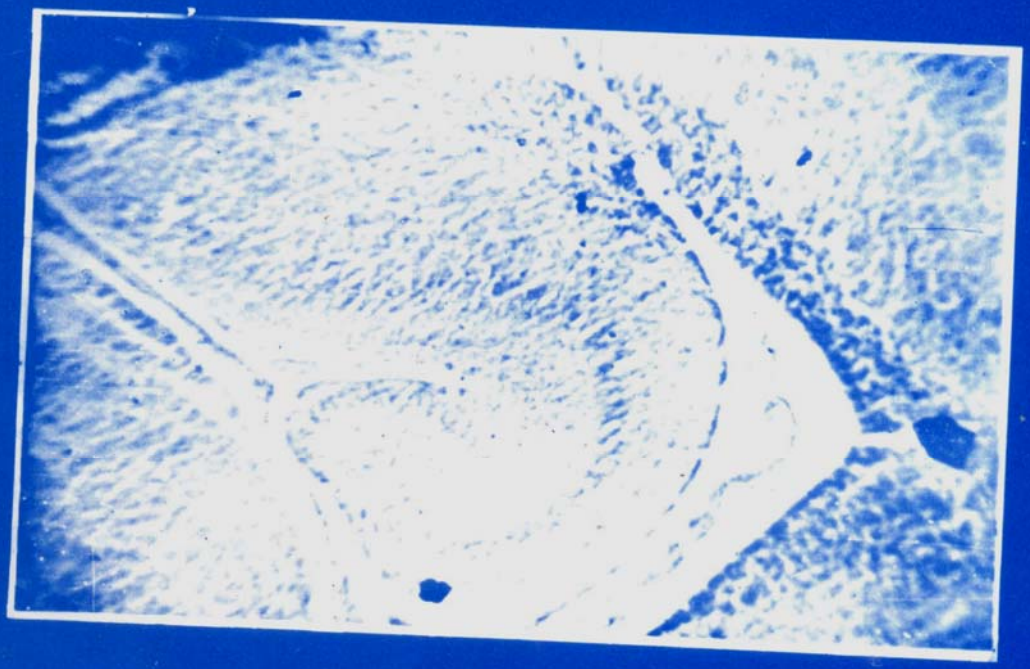

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Pharmacological Evidence for the Anticonvulsant Properties of the Leaf of *Newbouldia laevis* Seem (Bignoniaceae)

P. C. Ihekwereme*[1], P. A. Akah[1], D. N. Akunyili[2], C. O. Okoli[1] and S. V. Nwafor[1]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka, Nigeria.

[2]Department of Pharmacology and Therapeutics, College of Medicine, University of Nigeria, Nsukka, Nigeria.

Abstract

Methanol extract, n-hexane and chloroform fractions of the leaf of *Newbouldia laevis* were investigated for anticonvulsant activity. The extract/fractions were tested for effect on pentobarbitone-induced sleeping time, pentylenetrazole (PTZ)-induced convulsion, maximal electroshock stimulation, behavioural profile, and isolated tissue preparations. Acute toxicity test and phytochemical screening were performed on the methanol extract. The methanol extract exhibited an i.p LD_{50} of 3.8 mg/kg. Both fractions and the methanol extract displayed significant potentiation of pentobarbitone-induced sleeping time ($P < 0.05$); with the methanol extract being most potent. They also delayed the onset of PTZ and maximal electroshock induced-convulsion. Their effect on behavioural profile indicated CNS depressant activity. The contractions induced by different agonists (acetylcholine, serotonin and histamine) were inhibited to varying degrees by the extract/fractions. The results indicated that the anticonvulsant effect of *N. laevis* may be ascribed to enhancement of the central inhibitory activity of GABA and/or depression of voltage-activated calcium currents.

Keywords: *Newbouldia laevis,* anticonvulsant activity, central nervous system depression.

Introduction

Newbouldia laevis (Biognoniaceae) is a glabrous erect shrub known in major Nigerian languages as 'ogirisi' (Igbo), 'ako'ko' (Yoruba) and 'aduruku' (Hausa). It grows as high as 15 meters high and 5 - 7 cm in girth. The morphological description has been documented (Oliver, 1960, Gledhiu, 1972). It is widely distributed in West Africa and in South Eastern Nigeria, it is found in many residential compounds where it occurs as a domestic string of trees constituting a fence (Nielsen, 1965), or boundary demarcation.

N. laevis is highly valued by herbalists because of its multiple medicinal uses. In Guinea, a decoration of the root is used as a vermifuge for roundworms and also for syphilis. The bark is used as a stomachic and a remedy for colic. In Cote d'Ivoire, it is administered to pregnant women as leaves in palm oil soup to promote an easy delivery as well as enhance

*Corresponding author

the flow of breast milk; the chewed leaves are applied to snakebite and the wound sucked to extract the poison (Dalziel, 1937). The decoction or infusion of dried bark and young twigs is also used for uterine colic and dysmenorrhoea (Iwu, 1993) while that of the roots is claimed to posses aphrodisiac properties (Iwu, 1993; Burkill, 1985).

A decoction of the leaves is commonly used as treatment for convulsive seizures in South-Eastern Nigeria and this claim has been pharmacologically validated using the aqueous leaf extracts (Akah et al., 1997). The n-butanol fraction has been reported to produce significant inhibition of intestinal movement while the methanol extract exhibited anti-inflammatory, antipyretic, analgesic and anticonvulsant activities and potentiated pentobarbital-induced sleeping time (Olajide et al., 1997).

The root of the plant has been demonstrated to contain compounds characterized as withdomine, newbouldine, lapachol and their respective derivatives (Adesanya et al., 1994; Houghton, et al., 1994).

This work is aimed at further investigating the possible mechanism(s) involved in the anticonvulsant property of the leaf extract.

Materials and Methods

Collection

Fresh leaves of *N. laevis* were collected in September, 2000 within the University of Nigeria, Nsukka (UNN) campus. The leaves were identified by Mr. A. Ozioko of the Department of Botany, UNN. A specimen of the plant has been deposited in the University's Herbarium.

Extraction/Fractionation

Fresh leaves of the plant were air-dried for five days and milled to a coarse powder. The powder (400 g) was extracted for 48h with methanol using cold maceration. The percentage yield was then determined. A given weight (20 g) of the crude methanol extract (ME) was successively fractionated using n-hexane and chloroform to get the corresponding n-hexane (HF) and chloroform (CF) fractions.

Phytochemical

The crude methanol extract was chemically tested for the presence of chemical constituents using standard methods (Trease and Evans, 1983).

Animals

White albino mice (15 – 30 g) and guinea pigs (250 – 500 g) bred in the Department of Pharmacology and Toxicology, UNN, were used in the studies. The animals had free access to food and water before the commencement of the experiment.

In Vitro Pharmacological Studies

Segments of the guinea pig ileum of about 2 cm long were suspended in an aerated 30 ml organ bath filled with Tyrode solution, maintained at 37±1 ^{0}C. The experiment was set up as described by the Staff of the Department of Pharmacology, University of Edinburgh (1974). The composition of the Tyrode solution was

(g/L): NaCl 8.00, KCl 0.20, $CaCl_2$ 0.20, $NaHCO_3$ 1.00, $NaHPO_4$ 0.05, $MgCl_2$ 0.10 and glucose 1.00. The preparation was set up under a resting tension of 0.5 g and allowed to equilibrate for 30 min., during which the bathing fluid was changed every 10 minutes to prevent the accumulation of toxic metabolites.

Non-cumulative dose-response relationship to acetylcholine, histamine and serotonin were established; after which, the effects of the extract and fractions on the responses elicited by these agonists were investigated. The responses were recorded using isotonic transducer, 7006 (Ugo Basile, Italy) connected to a 2-channel recorder "Germini" 7070. Each investigation was done in triplicate.

In vivo Pharmacological screenings

Acute toxicity test

The intraperitonal median lethal dose (LD_{50}) of the crude methanol extract was determined in mice using the method described by Lorke (1983).

Pentobarbitone-induced sleeping time

Fifteen albino mice were randomly divided into three groups of 3 animals per group. Pentobarbitone sodium (20 mg/kg i.p) was administered to the first group while the second and last groups received 200 and 400 mg/kg of the extract i.p respectively 30 min before the administration of pentobarbitone sodium (Akah and Odita, 2001). The same procedure was repeated for each of the fractions. Each animal was observed for the onset and duration of sleep using loss of righting reflex as the criterion for sleep (Miya et al., 1973, Olajide et al., 1997).

Maximal electroshock seizure test

Twelve albino mice of either sex were randomly divided into three groups of four animals per group. The first group was administered 3% Tween 85 (20 ml/kg i.p) while the second and third groups received the extracts 200 and 400 mg/kg i.p respectively. Thirty minutes later each animal was subjected to the stimulation parameters (45 mA, 0.25 ms, 108 Hz) which were found to produce maximal shock without being lethal (Akah and Nwaiwu, 1988; Nwaiwu and Akah, 1986). The duration and onset of convulsion were recorded for each group. Animals that did not have seizures during the 30 min observation period were considered protected (Akah et al., 1998).

Pentylenetetrazole (PTZ)-induced seizure test

The procedure and method are same as that employed in maximal electroshock-induced seizure test, the difference being that in this case, the animals were subjected to chemoconvulsion using pentylenetetrazole at a dose of 70 mg/kg i.p (Akah et al., 1998 , Amos et al., 2003)

Neuropharmacological profile

The method described by Turner (1965) and Sofowora (1982) were used. For each of the behavioural patterns investigated, 12 mice divided into four groups of three animals per group were used. Each animal in the first group received the

vehicle (20 ml/kg of 3% Tween 85). The second, third and forth groups of animals received the methanol extract, n-hexane and chloroform fractions at the dose of 400 mg/kg i.p respectively. The behavioural patterns of the animals were observed. The scores were on the scale of 0 to 8 with a base of a normal response as 4. Abnormal responses like convulsion were scored 0 to 4 (Akah et al., 2002; Sandberg, 1967).

Statistical analysis

Significance between pairs of mean values was determined by Student's t-test and $P < 0.05$ was considered significant for all the analysis.

Results

The percentage yield (w/w) of the crude methanol extract was 7.18% while those of n-hexane, and chloroform fractions were 2.81 and 0.45% respectively. The result of the phytochemical analysis is shown in Table 1. Acute toxicity test indicated an i.p LD_{50} of 3.8 g/kg for the methanol extract. The methanol extract and all the fractions significantly potentiated pentobarbitone-induced sleeping time ($P < 0.05$). The methanol extract exhibited the longest duration both at 200 and 400 mg/kg doses while the shortest duration was noted for n-hexane fraction at 200 mg/kg (Fig. 1).

The extract/fractions delayed the onset of convulsion by maximal electroshock stimulation. Chloroform fraction at doses of 200 mg/kg and 400 mg/kg, produced the longest onset and shortest duration of convulsion respectively (Figs 2 and 3). The methanol extract, n-hexane and chloroform fractions prolonged the onset of PTZ-induced convulsion but the effect of chloroform fraction was most pronounced at the two dose levels (Fig. 4). The fractions and extract did not alter if the duration of PTZ-induced convulsion. The result of the neuropharmacological profile is shown in Table 2. The result indicated that methanol extract and n-hexane fraction demonstrated signs of CNS depression whereas no remarkable change was noted for the chloroform fraction.

The extract/fractions did not evoke contractions in the guinea pig ileum at concentrations tested (25 – 800 µg/ml). The agents inhibited to varying degrees, the maximal contractions induced by acetylcholine, serotonin and histamine in guinea pig ileum; however, the n-hexane fraction potentiated the maximal contraction induced by histamine (Table 3).

Tables 1: Phytochemical constituents of the methanol extract/fractions

Constituents	Presence/absence
Glycosides	++
Cardiac glycosides	++
Anthracene glycosides	-
O-and C-glycosides	-
Alkaloids	++
Flavonoids	+
Proteins	++
Carbohydrates	++
Amino acid	+
Reducing sugars	+
Saponins	+
Steroidal aglycones	++
Tannins	++

- = absent; + = moderately present; ++ = abundantly present

Table 2: Neuropharmacological profile of the extract and fractions at 400 mg/kg

Behavioural activity	Control (20 mg/kg Tween 85)	ME	HF	CF
Mood				
Vocalization	0	1	0	0
Restlessness	4	2	2	3
Grooming	4	3	2	6
Awareness				
Alertness	4	3	2	6
Passivity	0	2	3	0
Motor activity				
Inquisitiveness in unfamiliar environment	4	3	2	3
Touch response	4	2	2	5
Motor incordination	0	-1	2	0
Central excitation				
Response to loud noise	4	3	3	5
Degree of Straub response	4	4	2	5
Convulsion	0	0	0	0
Tremour	0	0	0	0
Others				
Grip strength	4	3	3	5
Writhing	0	0	0	0

ME = methanol extract, HF = n-hexane fraction, CF = chloroform fraction. All the test agents were administered at 400 mg/kg dose level by i.p route.

Table 3: Effect of 50µg/ml of extract/fractions on maximal contractions induced by different agonists in guinea pig ileum.

Agonists	Extract/Fractions	Percentage maximal response
Acetylcholine	Methanol extract	30.00 ± 2.16
Acetylcholine	n-Hexane fraction	75.76 ± 5.32
Acetylcholine	Chloroform fraction	34.52 ± 3.54
Histamine	Methanol fraction	34.59 ± 4.01
Histamine	n-Hexane fraction	166.67 ± 7.54
Histamine	Chloroform fraction	35.56 ± 5.41
Serotonin	Methanol extract	27.00 ± 1.05
Serotonin	n-Hexane extract	78.34 ± 2.89
Serotonin	Chloroform extract	42.15 ± 3.74

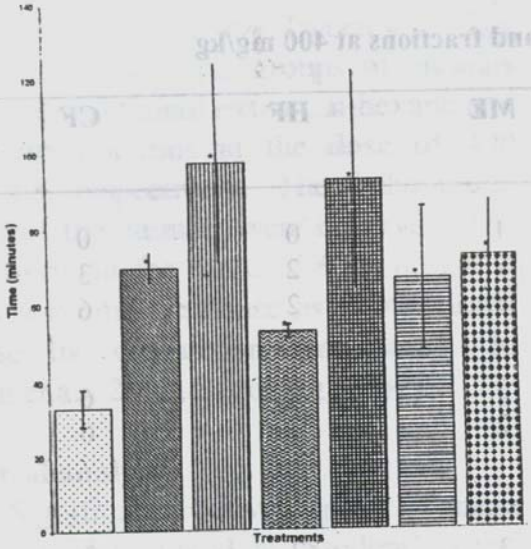

Fig. 1: Effect of extract/fractions on pentobarbitone-induced sleeping time. $P < 0.05$

control (20 ml/kg Tween 85)
methanol extract (400 mg)
n-hexane fraction (400 mg)
chloroform fraction (400 mg)
methanol extract (200 mg)
n-hexane fraction (200 mg)
chloroform fraction (200 mg)

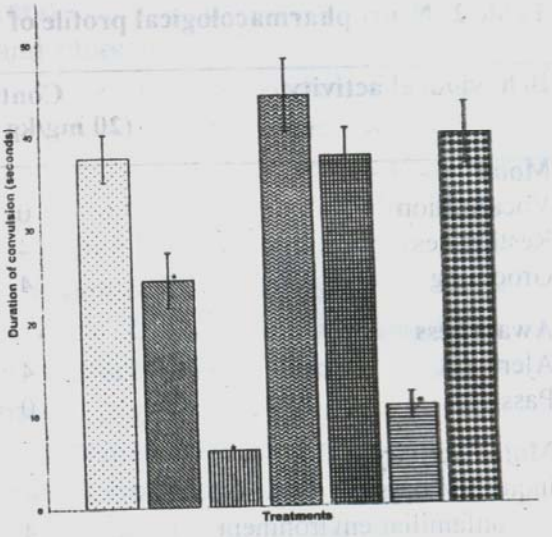

Fig. 3: Duration of convulsion induced by maximal electroshock stimulation. $P < 0.05$

control (20 ml/kg Tween 85)
methanol extract (400 mg)
n-hexane fraction (400 mg)
chloroform fraction (400 mg)
methanol extract (200 mg)
n-hexane fraction (200 mg)
chloroform fraction (200 mg)

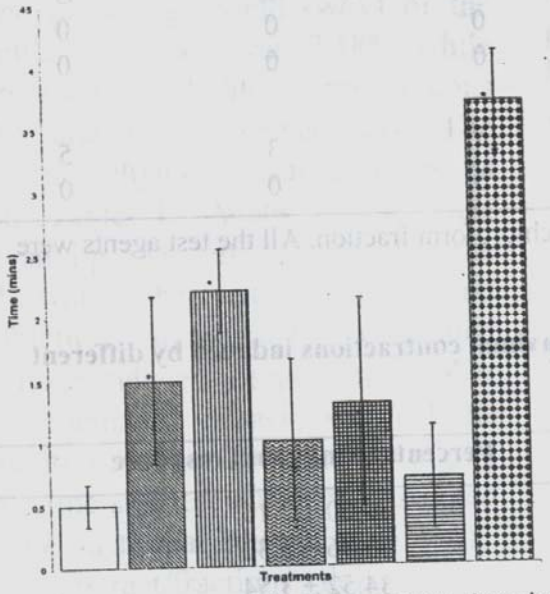

Fig. 2: Onset of convulsion induced by maximal electroshock stimulation. $P < 0.05$

control (20 ml/kg Tween 85)
methanol extract (400 mg)
n-hexane fraction (400 mg)
chloroform fraction (400 mg/kg)
methanol extract (200 mg)
n-hexane fraction (200 mg)
Chloroform fraction (200 mg/kg)

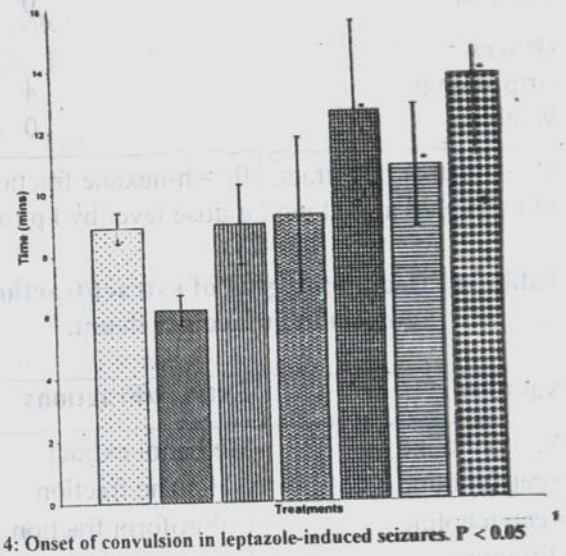

Fig. 4: Onset of convulsion in leptazole-induced seizures. $P < 0.05$

control (20 ml/kg Tween 85)
methanol extract (400 mg)
n-hexane fraction (400 mg)
chloroform fraction (400 mg)
methanol extract (200 mg)
n-hexane fraction (200 mg)
chloroform fraction (200 mg)

Discussion

The results indicated that the actions of agents that stimulate the central nervous system (leptazole and maximal electroshock stimulation) were antagonized whereas the action of CNS depressants (pentobarbitone sodium) was evidently potentiated. These results, and the effect on behavioural profile, tend to suggest that the extract/fractions possessed CNS depressant action. The result agrees with the sedative property of the methanol extract earlier reported by Amos et al (2002). Classical anticonvulsant agents such as barbiturates and benzodiazepines are CNS depressants (Charney et al., 2001). Barbiturates cause CNS depressant effects by activating the inhibitory gamma aminobutyric acid A ($GABA_A$) receptors and inhibiting excitatory α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtype of glutamate receptors (Charney et al., 2001; Saunders and Ho, 1990). Pentobarbital, a barbiturate, potentiates GABA-induced increases in chloride conductances and depresses voltage-activated Ca^{2+} currents (Ffrech-Mullen et al., 1993). Since the extract/fractions potentiated pentobarbitone-induced effects, it is likely that they exhibit anticonvulsant activity by potentiating GABA-induced inhibitory events in the CNS.

Methanol extract and chloroform fraction inhibited the contractions induced by acetylcholine, histamine and serotonin. Such non-specific antagonism of agonist-induced contractions is an indication of agents that act non-specifically by inhibiting the mobilization of Ca^{2+} into the intracellular compartments through the voltage-activated Ca^{2+}-channels (Godfrainds and Kaba, 1969; Quitana, 1978). Thus even though the above three endogenous neurotransmitters have little or no role to play in the etiology and treatment of epilepsy (McNamara, 1996; Craig, 1995, Porter and Meldrum, 2001), the ability of the methanol extract and chloroform fraction to antagonize in a non-specific manner, the contractions induced by these agonists may indicate depression of the voltage-activated calcium currents, an important mechanism through which barbiturates cause their anticonvulsant effect (Ffrench-Mullen et al, 1993).

Since only the ME and CF but not HF were potent at inhibiting contractions induced by histamine, acetylcholine and serotonin, the bioactive component of *N. laevis* inhibiting the induced contractions is likely polar in nature.

In the *in vivo* experiment, the ME displayed consistent, predictable and dose-dependent activity at increasing the time of onset and decreasing the duration of induced seizures more than other fractions, a further indication that the bioactive anticonvulsant principles may be polar in nature. However, the bioactive constituent responsible for the observed activities is not precisely known.

In conclusion, the extract and fractions of *N. laevis* exhibited activities against induced-convulsions. The anticonvulsant activities may be attributed

to potentiation of GABA-induced inhibitory effect at the central axis and/or depression of the voltage-activated calcium currents.

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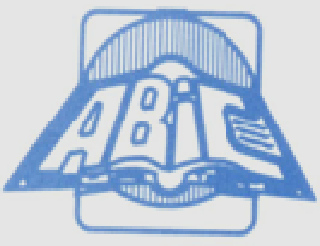

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