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Phytochemical and Anticonvulsant Screening of the Ethanolic Flower Extracts of *Newbouldia laevis* (Bignoniaceae) in Mice

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Abstract: The anticonvulsant effects of the ethanolic flower extract of *Newbouldia leavis* (Bignoniaceae) were studied in mice. Preliminary phytochemical analysis of ethanolic flower extract revealed the presence of cardiac and saponins glycosides, flavonoids, steroids and tannins. The ethanolic flower extract had an intraperitoneal (i.p.) LD_{50} of 1264.9 mg kg^{-1} body weight in mice. Anticonvulsant studies were carried out on pentylenetetrazole (PTZ)-induced and 4-amino pyridine (4-AP)-induced seizures in mice. The results showed that the extract under study possesses slight dose-dependent anticonvulsant activities between 40-60% (50-200 mg kg^{-1} body weight) protection against PTZ-induced convulsion; and also a dose-dependent delay on the onset of convulsion was observed in 4-AP-induced convulsion in mice ranging from 8.0±0.45 to 11.2±1.31 min (50-200 mg kg^{-1} body weight). The data obtained correlate to the traditional claim of this plant in the treatment of convulsion due to petit mal seizure.

Key words: 4-amino pyridine, anticonvulsant, flower, *Newbouldia leavis*, pentylenetetrazole, phenobarbitone, phytochemistry

INTRODUCTION

Epilepsy is a major neurological disorder characterized by recurrent seizures with a lifetime prevalence of 5% (Sander and Shorvon, 1996; Raza *et al*., 2001). Even though, the modern conventional antiepileptic drugs (AEDs) are effective in approximately 50% of patients, many cases still remain resistant to AED treatment (Heinemann *et al*., 1994; Shorvon, 1996; Raza *et al*., 2001). These drugs are associated with vast array of side effects including chronic toxicity, teratogenecity, adverse effects on cognition and behaviour among others (Raza *et al*., 2001). Thus, due to aforementioned reasons and others; it is pertinent to look for affordable and convenient alternative medicine with view to providing a better protection and activities-particularly medicinal plants. Moreover, medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects and have been used in the discovery and development of new drugs (Farnsworth, 1994; Cragg *et al*., 1997).

The plant *Newbouldia laevis* (P. Beauv) Seem or boundary tree is locally called as: *Aduruku* in Hausa, *Ogirisi* in Igbo and *Akoko* in Yoruba languages (Hutchinson and Dalziel, 1963) is a medium sized angiosperm which belongs to the Bignoniaceae family. It grows to a height of about 7-8 (up to 15) m, more usually as shrub of 2-3 m, many-stemmed forming clumps of gnarled branches (Arbonnier, 2004; Usman and Osuji, 2007). It is native to tropical Africa and grows on moist and well-drained soils; extends from Guinea Savannahs to the dense Forests zones.

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In Nigeria, it has been found useful as a remedy for earache, sore feet, chest pain, epilepsy and children's convulsion (Akunyili, 2000). The leaf, stem and fruits have also been used for febrifuge; wound dressing and stomach ache (Iwu, 2000). Recent study has shown the leaf to be a good antibacterial source (Usman and Osuji, 2007).

Earlier studies on the leaves and bark of Congolese *Newbouldia leavis* revealed the absence of flavonoids; saponins, quinones, terpenes or steroids (Oliver-Bever, 1986). Recent phytochemical studies on the root, root bark and stem of this plant revealed the presence of alkaloids, quinoid and phenylpropanoid amongst others (Gafner *et al*., 1997; Aladesanmi *et al*., 1998; Germann *et al*., 2006). There was no extensive report on the isolation of compounds from the leaves (Usman and Osuji, 2007) and flowers of this species.

In this investigation, the ethanolic flower extract of this plant was subjected for the first time to phytochemical and anticonvulsant study, with the view to evaluating its efficacy as a source of potential antiepileptic agents.

MATERIALS AND METHODS

Plant Material

The flowers of *Newbouldia laevis* for this work were collected from Kudingi Village-Samaru Zaria, in the month of August 2005. The studies were carried out in May 2007 at the Research laboratory, Department of Chemistry, University of Maiduguri, Maiduguri and Department of Pharmacology and Clinical Pharmacy, ABU Zaria-Nigeria. The plant specimen was identified by Mr. U.A. Gallah of the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria - Nigeria, where a voucher specimen was deposited.

Extraction of Plant Material

The flowers of *Newbouldia laevis* was dried under shade for several days and then pulverized into fine powder. About 200 g of the powdered form was extracted exhaustively with 95% (v/v) ethanol in water using continuous soxhlet apparatus. The extract was concentrated under reduced pressure to yield a dark green mass which weighed 38.51 g (19.26% w/w). The crude extract was then coded NFE-Newbouldia leavis flower extract.

Animals

A total of 63 adult male Swiss albino mice weighing between 18-28 g were obtained from Animal house, Department of Pharmacology and Clinical Pharmacy, ABU Zaria-Nigeria. The animals were kept under well-ventilated conditions with 12 h light/dark cycle (6 am-6 pm) at room temperature, fed on Standard feeds (Excel feeds Plc. Kaduna, Nigeria) and allowed water *ad libitum*.

Drugs

Pentylenetetrazole (PTZ), Phenobarbitone (PHB) (Pfizer, USA) and 4-amino pyridine (4-AP) BDH reagent (Poole, UK). All drugs/chemicals were freshly prepared to the desired concentration with distilled water just before use.

Phytochemical Screening

The phytochemical analyses of the crude ethanolic extract of *Newbouldia leavis* flower was carried out in order to ascertain the presence of its constituents utilizing standard conventional protocols (Markham, 1982; Harborne, 1993; Sofowora, 1993; Trease and Evans, 2002).

Acute Toxicity Studies (LD_{50})

LD_{50} determination was conducted using the method of Lorke (1983). In the initial phase mice were divided in to 3 groups of three mice each and treated with ethanol extract at doses of 10, 100 and 1000 mg kg^{-1} i.p and observed for 24 h. In the final phase, 3 mice were divided in to 3 groups of one mouse each and ethanol extract administered at doses of 1600, 2900 and 5000 mg kg^{-1} i.p and the final LD_{50} value calculated.

Anticonvulsant Activity

Pentylenetetrazole-Induced Seizure in Mice

The method of Swinyard *et al.* (1989) was employed. Twenty five mice were divided into five groups of five mice each. The first, second and third groups received 50, 100 and 200 mg extract kg^{-1} body weight. The fourth group served as control and was given normal saline equivalent to vehicle given with the extract; while the fifth group was given 20 mg phenobarbitone (PHB) kg^{-1} body weight. The drug and extract were administered by intraperitoneal (i.p.) route. Thirty minutes post treatment, mice in all the groups received 85 mg pentylenetetrazole kg^{-1} subcutaneously (s.c.). Mice were then observed over a period of 30 min. Absence of an episode of clonic spasm of at least 5 sec duration indicated a compound's ability to abolish the effect of pentylenetetrazole on seizure threshold.

4-Amino Pyridine-Induced Seizure in Mice

The method adopted for this study was as described by Rogawski and Porter (1990) and Yagamuchi and Rogawski (1992). Male Swiss albino mice were randomly divided in to 5 groups each containing five mice. They were allowed to acclimatize with free access to food and water for a 24 h period before testing. The first, second and third groups received 50, 100 and 200 mg extract kg^{-1} body weight. The fourth group was given normal saline equivalent to the vehicle given the extract while the fifth group received 30 mg PHB kg^{-1} body weight. The drug and extract were administered by intraperitoneal route. 15 min post treatment; 4-AP was administered at a dose of 15 mg kg^{-1} body weight to each mouse s.c. The mice were observed for 30 min for characteristic behavioural signs, such as hyperactivity, trembling, intermittent forelimb extension, tonic seizures and death. The ability of the extract to protect the mice from lethality within 30 min observation period was considered as an indication for anticonvulsant activity (Yagamuchi and Rogawski, 1992).

Statistical Analysis

All values were expressed as mean±SEM. The data of PTZ and 4-AP tests were analysed statistically by one way ANOVA. The differences between means were considered significant.

RESULTS

Phytochemical Screening

The preliminary phytochemical analysis of the flower extract of *Newbouldia leavis* revealed the presence of cardiac and steroidal glycosides, flavonoids, tannins while other phytochemicals such as alkaloids, saponins was not detected as presented in Table 1.

Acute Toxicity Studies

The LD_{50} in mice was found to be 1264.9 mg kg^{-1} i.p.

Anticonvulsant Activity

Effects of Ethanolic Flower Extract on PTZ-Induced Seizures

The NFE exhibited slight dose-dependent protection on animals against seizures induced by PTZ (Table 2) but did not delay the onset of seizures in non-protected animals (Normal saline group). At

Table 1: Phytochemical constituents of the crude ethanolic flower extract of *Newbouldia laevis*

Constituents/test	Inference
Alkaloids	
Dragendorff's test	-
Mayer's test	-
Wagner's test	-
Carbohydrates	
Molisch's test	+
Barfoed's test	+
Fehling (reducing sugar) test	+
Fehling (combine reducing sugar) test	+
Glycosides	
Legal's test	+
Keller-Killiani's test	+
Flavonoids	
Shinoda's test	+
Lead acetate test	+
NaOH test	+
$FeCl_3$ test	+
Saponins	
Frothing test	-
Steroidal nucleus	
Salkowski test	+
Liebermann-Burchard's test	+
Keller-Killiani's test	+
Tannins	
$FeCl_3$ test	+
Lead acetate test	+

+ = Present, - = Absent

Table 2: Effect of ethanolic flower extract of *Newbouldia laevis* on PTZ-induced seizure mice

Treatments	Dose (mg kg^{-1})	Mean onset of convulsion (min)	Quantal protection	Protection (%)	Mortality (%)
Normal saline	-	6.80±0.37	0/5	0.0	10.00
Extract	50	10.33±2.33*	2/5	40.0	40.00
Extract	100	10.33±2.70*	2/5	40.0	10.00
Extract	200	13.00±3.00*	3/5	60.0	10.00
Phenobarbitone	20	0.00±0.00	5/5	100.0	0.00

One way ANOVA df = 3,12; F = 1.985; P = 0.187; n = 5. * No significant effect on mean onset of convulsion ($p<0.05$), since the number of convulsed animals are few

Table 3: Effect of ethanolic flower extract of *Newbouldia laevis* on 4-AP-induced seizure in mice

Treatments	Dose (mg kg^{-1})	Mean onset of convulsion (min)	Quantal protection	Protection (%)	Mortality (%)
Normal saline	-	5.40±2.44	0/5	0.00	100.00
Extract	50	8.00±0.45*	0/5	0.00	100.00
Extract	100	9.80±0.66**	0/5	0.00	100.00
Extract	200	11.20±1.31***	0/5	0.00	100.00
Phenobarbitone	30	16.00±0.00	4/5	80.00	10.00

One way ANOVA df = 3, 19; F = 10.273, n = 5. *, **, *** as $p<0.05$, $p<0.01$, $p<0.001$, respectively

the dose of 100 mg kg^{-1} body weight i.p., the NFE protected 40% of mice against seizures; while 200 mg kg^{-1} body weight exhibited 60% protection in mice.

Effects of Ethanolic Flower Extract on 4-Ap-induced Seizures

The NFE exhibited significant dose-dependent delay on the onset of seizures against 4-AP-induced convulsion seizures. The dosages 50 and 200 mg kg^{-1} body weight significantly delayed the onset of 8.0±0.45 and 11.2±1.31, respectively. The extract possesses weak anticonvulsant activity against 4-AP, since it can only delay the onset significantly (Table 3).

DISCUSSIONS

The present study shows that the ethanolic flowers extract of *Newbouldia laevis* have anticonvulsant activity in PTZ and 4-AP-induced seizures in mice. The toxicity index of the extract in mice indicated that it was moderately toxic.

The PTZ is a known convulsant and anticonvulsant activity in PTZ (s.c) test identifies compounds that can raise the seizure threshold in the brain (White *et al*., 1998; Raza *et al*., 2001). Agents (such as phenobarbitone, valproic acid, benzodiazepines) affecting the PTZ test can inhibit absence or myoclonic seizures (Vida, 1995; Hosseinzadeh and Khosravan, 2002). Earlier study has shown that the leaf ethanolic extract exhibit protection against PTZ-induced convulsion in mice (Akunyili, 2000). In line with these findings, this study showed that the extract possesses strong anticonvulsant activity against PTZ-induced seizure in mice.

The K^+ channel antagonist-4-amino pyridine is a powerful convulsant in animals and man. The drug readily penetrates the blood-brain barrier and it is believed to induce seizure activity by enhancing spontaneous and evoked neurotransmitter. The profile of drugs effective in this seizure model is distinct from other chemoconvulsant models and more similar to those that prevent tonic hind limb extension in maximal electroshock seizure test. The test is useful to differentiate the mode of action of anticonvulsant drugs (Vogel, 1997).

One potential anti-epileptic mechanism that has been exploited is K^+ channels, which play a major role in the control of all aspect of neuronal excitability, including resting membrane potential, responsiveness to synaptic inputs, frequency adaptation and neurotransmitter release (Wickenden, 2002). Drugs like phenytoin which block seizure spread are effective antagonist of seizures induced by K^+ channel blockade while those with specific actions on other cellular targets may be weak or inactive, presumably because they are unable to attenuate the spread of intense (non-N-methyl-D-aspartate (NMDA) receptor mediated) excitation evoked by 4-amino pyridine (Yagamuchi and Rogawski, 1992). The results of this research indicated that this extract possesses weak anticonvulsant activity against 4-amino pyridine-induced seizure.

CONCLUSIONS

This study therefore, suggests that since earlier studies had shown that the leaf and roots extracts contained potential anticonvulsants, it is hence, imperative to say that the extract of the flower of the same plant could be yet another source.

We therefore, conclude that the anticonvulsant potency of ethanolic flower extract from this plant could favourably suggest the presence of bioactive phytochemicals effective in the therapy of absence seizures and thus help in the control of petit mal; more so, the extract could not be useful in the management of grand mal seizures.

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REFERENCES

Akunyili, D.N., 2000. Anticonvulsant activity of the ethanolic extract of *Newbouldia leavis*. 2nd NAAP Scientific Conference, pp: 155-158.

Aladesanmi, A.J., R. Nia and A. Nahrstedt, 1998. New pyrazole alkaloids from the root bark of *Newbouldia leavis*. Planta Medica, 64: 90-91.

Arbonnier, M., 2004. Trees, Shrubs and Lianas of West African Dry Zones. CIRAD, Margraf Publishers GMBH MNHN, Cote D'Ivorie, pp: 194.

Cragg, G.E., D.J. Newman and K.M. Snader, 1997. Natural products in drug discovery and development. J. Nat. Prod., 60: 52-60.

Farnsworth, N.R., 1994. Ethnopharmacology and Drug Development. In: Ethnobotany and The Search for New Drugs. Ciba Foundation Symposium, Prance, G.T. and J. Marsh (Eds.). John Wiley and Sons: Chichester, 185 pp: 42-59.

Gafner, S., J.L. Wolfender, M. Nianga and K. Hostettmann, 1997. Phenylpropanoid glycosides from *Newbouldia laevis* roots. Phytochemistry, 44: 687-690.

Germann, K., M. Kaloga, D. Ferreira, J.P. Marais and H. Kolodziej, 2006. Newbouldioside A-C phenylethananoid glycosides from the stembark of *Newbouldia leavis*. Phytochem, 67: 805-811.

Harborne, J.B., 1993. Phytochemistry. Academic Press, London, pp: 89-131.

Heinemann, U.A., E. Draguhn, J. Ficker, Stabel and C.L. Zhang, 1994. Strategies for the development of drugs for pharmacological resistant epilepsies. Epilepsia, 35: S10-S21.

Hosseinzadeh, H. and V. Khosravan, 2002. Anticonvulsant effects of aqueous and ethanolic extracts of *Crocus sativus* L. stigmas in mice. Arch. Irn. Med., 5: 44-47.

Hutchinson, J. and J.M. Dalziel, 1963. Flora of West Tropical Africa Vol. II. Crown agents for oversea government and administration 4, Millbank, London, S.W. 1, pp: 435-436.

Iwu, M.M., 2000. Handbook of African Medicinal Plants. CRC Press, Inc., London, pp: 19.

Lorke, D., 1983. Approach to acute toxicity testing. Arch. Toxicol., 54: 275-87.

Markham, K.R., 1982. Techniques of Flavonoids Identification. Academic Press. New York, USA., pp: 1-113.

Oliver-Bever, B., 1986. Medicinal Plants in Tropical West Africa. Cambridge University Press, London, pp: 117-168.

Raza, M.F., M.I. Shaheen, A. Choudhary, A.U. Suria, S. Rahman, Sombati and R.J. Delorenzo, 2001. Anticonvulsant activities of the FS-1 sub-fraction isolated from roots of *Delphinium denudatum*. Phytother. Res., 15: 426-430.

Rogawski, M.A. and R.J. Porter, 1990. Antiepileptic drugs pharmacological mechanisms and clinical efficacy with consideration of promising developmental stage compounds. Pharmacol. Rev., 42: 223-286.

Sander, J.W.A.S. and S.D. Shorvon, 1996. Epidemiology of epilepsies. J. Neuro Surg. Psychiat, 61: 433-443.

Shorvon, S.D., 1996. The epidemiology and treatment of chronic and refractory epilepsy. Epilepsia, 37: S1-S3.

Sofowora, A., 1993. Medicinal Plants and Traditional Medicines in Africa. Spectrum Books limited Ibadan, Nigeria, pp: 150-153.

Swinyard, E.A., J.H. Woodhead, H.S. White and M.R. Franklin, 1989. General Principles: Experimental Selection, Quantification and Evaluation of Anticonvulsants. In: Antiepileptic Drugs, Levy, R.H., B. Mattson, J.K. Melrum and F.E. Dreifuss (Eds.). 3rd Edn., Raven Press, New York, pp: 85-103.

Trease, G.E. and M.C. Evans, 2002. Textbook of Pharmacognosy. 14th Edn., Balliere Tindall, London, pp: 81-90, 269-275, 300.

Usman, H. and J.C. Osuji, 2007. Phytochemical and *in vitro* antibacterial assay of the leaf extract of *Newbouldia leavis*. Afr. J. Trad. CAM., 4: 476-480.

Vida, J.A., 1995. Anticonvulsants. In: Principles of Medicinal Chemistry, Foye, W.O., T.L. Lemke and D.A. Williams (Eds.). London, Williams and Wilkins, pp: 184-198.

Vogel, H.G., 1997. Antiepileptic Activity. In: Drug Discovery and Evaluation: Pharmacological Assays, Vogel, H.G. and H.W. Vogel (Eds.). Springer-Verlag, Berlin Heidelberg, Germany, pp: 262.

White, H.S., H.H. Wolf, J.H. Woodhead and H.J. Kupferberg, 1998. The Nutritional Institute of Health Anticonvulsant Drug Development Program: Screening for Efficacy. In: Antiepileptic Drug Development: Advances in Neurology, French, J., I.E. Leppik and M.A. Dichter (Eds.). Lippincott-Raven Publishers; Philadelphia, 76: 29-39.

Wickenden, A.D., 2002. Potassium channels as antiepileptic drug targets. Neuropharmacology, 43: 1055-1060.

Yagamuchi, S. and M.A. Rogawski, 1992. Effects of anticonvulsant drugs on 4-amino pyridine-induced seizure in mice. Epilepsy Res., 11: 9-16.