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Article *in* Bollettino chimico farmaceutico · November 2002

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Sedative effects of the methanolic leaf extract of *Newbouldia laevis* in mice and rats

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Pervenuto in Redazione il 25 marzo 2002
Accettato il 25 luglio 2002

ABSTRACT

The effect of the methanolic extract of *Newbouldia laevis* seem on the central nervous system of rats and mice was investigated. The extract was tested on spontaneous motor activity, exploratory behaviour, apomorphine induced climbing behaviour in mice and pentobarbital induced hypnosis in rats. The extract caused considerable reductions of exploratory activity, spontaneous motor activity and prolonged pentobarbitone-induced hypnosis in rats. It was also found to attenuate apomorphine climbing in mice. The results suggest that the methanolic extract of *Newbouldia laevis* may contain principles that have sedative properties.

KEY WORDS: *Newbouldia laevis*, spontaneous motor activity, climbing behaviour, pentobarbital sleep, sedation

INTRODUCTION

Newbouldia laevis Seem. (Bignoniaceae) is a plant widely used especially in West Africa for a wide range of conditions and ailments. The plant part have been used in the treatment of scrotal elephantiasis, orchitis, dysentery, rheumatic swellings and aching limbs, persistent headaches, uterine colic and dysmenorrhoea. Available records also show that it has been used for syphilis, sinusitis and coryza, constipation, piles breast tumours and control of post partum bleeding (1, 2). More recently, Azuine et al (3) reported the anti-inflammatory and anticancer activity. Akunyili (4) recorded the anticonvulsant activity of *Newbouldia laevis*. To our knowledge there are no record of the pharmacological evaluation on the central nervous system. This study was designed to evaluate the sedative properties of the methanolic extract of the leaves of *Newbouldia laevis*.

EXPERIMENTALS

Animals

All experiments performed on laboratory animals in this study followed the "Principles of laboratory animal care" (NIH publication No. 85–23, revised (1985). Swiss albino mice (20–30g) and Wistar rats (180–200g) of either sex were used. All animals were maintained at the Animal Facility Center of NIPRD at standard conditions of temperature (25+2°C) and light (12 light/dark) and feed with standard diet (Ladokun feeds, Ibadan) and water *ad libitum*.

Drugs

Diazepam and Nitrazepam were obtained from Roche Nigeria Ltd, while Pentobarbital, Apomorphine were obtained from Sigma Chemical company, USA. All drugs were freshly prepared. Parallel control experiments were done in each case to correct possible effects caused by the vehicle alone.

Plant material

The stem bark of *Newbouldia laevis* growing in its natural habitat were collected from Suleja, Niger state, Nigeria between the months of October and December 2000. The plant was identified and authenticated by the late A. Oheari and I. Muazzam of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, where a voucher specimen has been deposited in the herbarium for future reference. After the plant material was washed and air-dried, they were pulverized using a motar and pestle into a coarse powder. 200 g of the powder was then cold macerated with 2 litres of methanol for a period of 36 h with continuous shaking using the GMB shaker. The filtrate was concentrate *in vacuo* using the rotary evaporatory, and dried completely over water bath to give a solid residue (12.5% w/w).

Preliminary phytochemical test

Phytochemical screening for the presence of saponins, alkaloids, flavonoids, antracenes, tannins, glycosides etc, were carried out following standard procedures (5)

Acute toxicity studies

The Acute toxicity (LD_{50}) was determined following the method described by Lorke, (6). Animals were divided randomly into six groups of six mice each. The extract was administered intraperitoneally in the range of doses 1, 10, 100, 1000, 2000 and 4000 mg/kg. The animals were observed for signs and symptoms of toxicity and the number of deaths in each group within 24 h recorded.

Studies on exploratory activity in mice

In this study, mice were divided into five groups of six mice each. Groups 1,2 and 3 were treated with the extract at doses of 25, 50 and 100 mg/kg i.p, respectively, while group 4 received normal saline (10ml/kg) which served as control. Animals in group 5 were treated with nitrazepam (2 mg/kg i.p.) After 30 min animals were placed individually on an automatic Letica board with 16 evenly spaced holes with a counter (Letica LE 3333). The number of head dips by the mice into the holes over a period of 5 min was automatically counted (7, 8).

Studies on spontaneous motor activity

Adult mice were randomly divided into four groups of 6 mice each. Groups 1, 2 and 3 received the extract at doses of 25, 50 and 100 mg/kg i.p, while group 4 received normal saline (10 ml/kg p.o). Motor activity of the mice was recorded using a Letica activity cage (LE 886) connected to a multicount (LE 3806), which automatically counts the animals' movements across the bar on the cage floor. The animals were singly placed in the cage and their activity was recorded for 6 min at 30 min intervals for a period of 120 min (9). In another experiment, the effect of amphetamine 2 mg/kg i.p was recorded. The effects of the extract on amphetamine-induced hyperactivity were compared to that of chlorpromazine 2 mg/kg i.p.

Studies on pentobarbital-induced sleep

The method followed that described by Amos et al, (10). Adult rats were divided into five groups of 6 rats each. Groups 1, 2 and 3 received 25, 50 and 100 mg/kg i.p of the extract and group 3 received normal saline (10 ml/kg, p.o) received Diazepam 1 mg/kg i.p was administered to animals in group 4. The rats were injected with pentobarbital sodium (35 mg/kg i.p), 30 min after drug treatment. Then, the onset and the duration of the loss of righting reflex (sleeping time) were recorded (11).

Studies on apomorphine-induced climbing in mice

Adult mice were randomly divided into four groups of 10 mice each. The first group received normal saline (10 ml/kg, orally) and served as control. Groups 2, 3 and 4 received the extract at doses of 25, 50 and 100 mg/kg p.o. Thirty minutes after treatment, all mice were treated with apomorphine (3 mg/kg s.c). Readings were taken at 10 20 and 30 min after apomorphine administration. The mice were observed for climbing and scored as follows: 0 = four paws on the floor, 1 = fore feet holding the vertical bars, 2 = four feet holding the bars (12).

Statistical analysis

Results are represented as mean ± SEM. Statistical significance of difference between groups was evaluated by ANOVA followed by Dunnet's post hoc test.

RESULTS

The phytochemical screening test revealed the presence of alkaloids, flavonoids, glycosides, saponins and tannins. The methanolic extract of *N. laevis* was found to be relatively safe as no lethality was observed at even 1000 mg/kg i.p in mice. The LD_{50} was calculated to be 3800 mg per kg i.p. (3600-4200 mg/kg within 95 % confidence limits)

Effect on exploratory activity in mice

N. laevis at doses of 25, 50 and 100 mg/kg were found to dose-dependently inhibit exploratory activity in mice. The effect was similar to that of Nitrazepam (2 mg/kg). The observed effect were statistically significant ($P<0.05$) from normal saline that served as control (Table I).

Effect on spontaneous motor activity

N laevis (25, 50 and 100 mg/kg) caused a significant ($P<0.05$) dose-dependent decrease in spontaneous motor activity in mice. The decrease in activity was also time dependent (Table II). Similarly, amphetamine induced hyper motility was reduced dose dependently an action similar to the effects of chlorpromazine (Table III).

Effect on pentobarbital sleeping time

N. laevis (25, 50 and 100 mg/kg) did not affect the onset of sleep, but significantly ($P<0.05$) prolonged the duration of pentobarbital sleep at the doses tested. The increase in duration of pentobarbital sleep was found to be dose-dependent and comparable to diazepam (Table IV).

Table I. Effect of the methanolic extract of *Newbouldia laevis* on exploratory activity in mice

Treatment	Dose (mg/kg)	Mean Score ± SEM
Normal Saline	10 ml/kg	45.0±2.6
Newbouldia laevis	25	34.2±2.8*
	50	20.7±2.4*
	100	12.0±2.1*
Nitrazepam	2	13.4±2.5*

Values are expressed as mean ± SEM. Scores of head dips were recorded 30 min after drug administration. *Significant difference between control and treated groups; $p<0.05$. N=6 in each group.

Table II. Effect of methanolic extract of *Newbouldia laevis* on spontaneous motor activity in mice

Treatment	Time (min)				
	0	30	60	90	120
N. Saline 10 ml/kg	136.8±5	130.1±4.2	128±4.9	124.1±4.3	118±4.4
N. laevis 25 mg/kg	135±4.2	97±2.6*	78±3.2*	70.2±3.6*	65±2.1*
N. laevis 50 mg/kg	136±4.8	65.7±4.1*	52.4±3.4*	42.3±4.6*	25.0±2.0*
N. laevis 100 mg/kg	137±5.2	40.0±3.4*	28.0±2.8*	18.0±2.0*	10.2±1.8*

Values are expressed as mean counts ± SEM. *Significant difference between control and treated groups ; p<0.05. (ANOVA followed by Dunnet's post hoc test). N=6 in each group.

Table III. Effect of methanolic extract of Newbouldia laevis on amphetamine (Amp) induced hyperactivity in mice

Treatment	Time (min)				
	0	30	60	90	120
N. Saline 10 ml/kg	137.6±5	134.1±4.6	128±4.9	125.1±4.3	115±4.4
Amp. 2 mg/kg	135±4.2	158.7±5.6	178±5.3	168.4±3.6	165±2.1
N. laevis 50 mg/kg	136±4.8	84.7±4.1*	56.4±4.0*	43.3±4.6*	28.0±2.0*
N. laevis 100 mg/kg	137±5.2	65.4±3.4*	31.5±2.8*	19.5±2.0*	15.4±1.8*

Values are expressed as mean counts ± SEM. *Significant difference between control and treated groups; p<0.05. (ANOVA followed by Dunnet's post hoc test). N=6 in each group.

Table IV. Effect of the methanolic extract of Newbouldia laevis on pentobarbital induced hypnosis in rats

Treatment	Dose (mg/kg)	Mean Duration of sleep ± SEM
Normal Saline	10 ml/kg	64.5±2.6
Newbouldia laevis	25	120±2.8*
	50	147.0±3.6*
	100	172.2±4.6*
Diazepam	1	87.4±1.6*

Values are expressed as mean duration ± SEM. *Significant difference between control and treated groups ; p<0.05. (ANOVA followed by Dunnet's post hoc test). N=6 in each group.

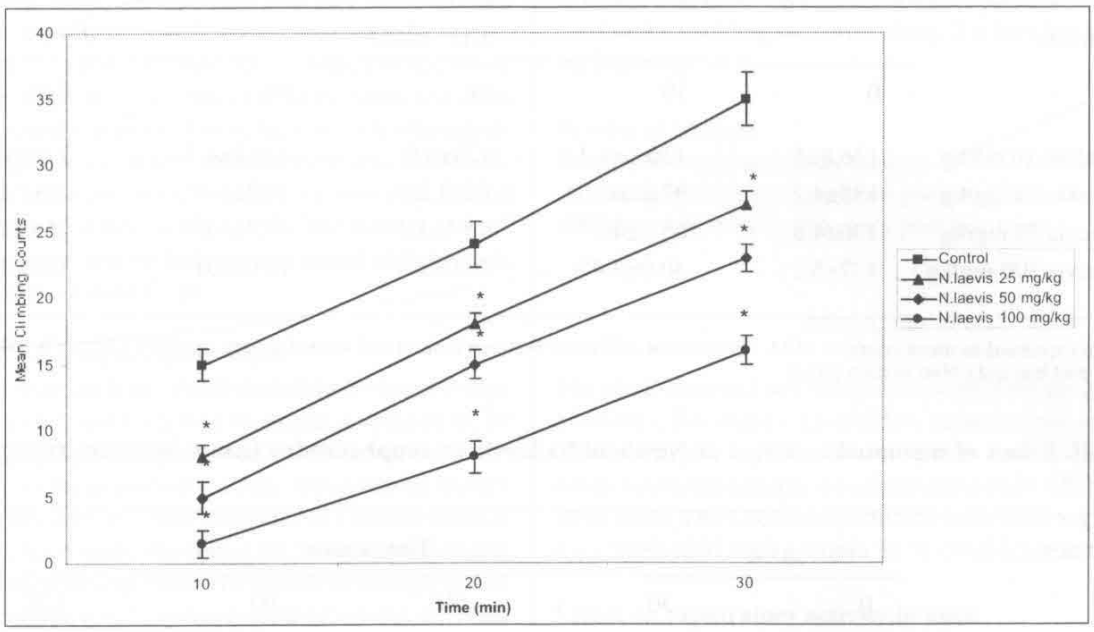
Effect on apomorphine-induced climbing in mice

N. laevis (25, 50 and 100 mg/kg) dose-dependently inhibited apomorphine-induced climbing. This was found to be time dependent (Figure 1)

DISCUSSION

The results of the study revealed that the methanolic extract of the leaves of *Newbouldia laevis* has sedative activities on the central nervous system of rodents. The extract was found to decrease exploratory activity. File and Wardill (13), have shown that the hole-board experiment is a measure of exploratory behavior in animals. A decrease in this parameter reveals sedative (14), which have also been accepted as a parameter for evaluating anxiety conditions in animals (15). The decrease in exploratory activity by the extract gives an indication of sedative activity. The extract was found to significantly reduce the spontaneous motor activity in mice. Spontaneous motor activity gives an indication of the level of excitability of the central nervous system and this decrease in activity exhibited by the extract may be closely related to sedation resulting from depression of the central nervous system (16). The extract prolonged the duration of pentobarbital sleeping time in rats. The decrease in spontaneous motor activity and potentiation of pentobarbital induced sleeping strongly suggest central depressant activity

Fig. 1. Effect of Newbouldia laevis on apomorphine - induced climbing in rats



of the extract (7), thus suggesting that the extract might be acting as a mild neurosedative drug (17) or might be acting on the central mechanisms involved in the regulation of sleep (18). The ability of a drug to antagonize apomorphine induced climbing behavior in mouse has been correlated with neuroleptic potential (12, 19) and such inhibitions could be mediated via D_1 and D_2 receptor (20). The potential benefit of traditional remedies might depend upon a combination of constituents. For instance, alkaloids and saponins have been reported to show potent sedative activities. They have also been shown to have antagonistic activity on amphetamine and inhibit motor activity in mice (9, 21, 22). It is therefore, likely that the alkaloid and saponin components of this plant might be contributing in part to the observed pharmacological effects. The overall data presented revealed that the methanolic extract of *Newbouldia leavis* posses sedative activity centrally. Further studies are in progress in our laboratory to isolate the useful active components.

Acknowledgement

The authors are grateful to Hauwa Abdullahi and Sunday Dzarma for their technical assistance and to Charles Balogun for secretarial assistance. The work is supported by a grant from NIPRD to S. Amos.

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NEWS OF THE WORLD

28th Meeting of the Committee for Orphan Medicinal Products

Five positive opinions on the designation of orphan medicinal products, were adopted by the Committee during this meeting, for the following conditions:

- Glioma (2 *x opinions)*
- Graft versus host disease
- Low flow priapism
- Cystic fibrosis

One oral explanation took place during the meeting. The COMP noted that two applications for orphan medicinal product designation were withdrawn by sponsors.

The European Commission granted one decision on orphan designation since the last COMP meeting on 11-12 September 2002, see Annex I. The status of orphan designation procedures, as of 9 October 2002, is summarised in the table below:

Intent to file notified	*Applications submitted*	*Application withdrawn*	*Positive COMP Opinions*	*Negative COMP Opinions*	*Designations granted by Commission*
52	212	60	123	3	115

Further information on designated orphan medicinal products is publicly available in the form of summarised COMP Opinions, which the Agency routinely publishes following adoption of the respective decisions on orphan designation by the European Commission. The Committee discussed and endorsed a revised version of the Commission guideline (ENTR/6283/00) on the format and content of applications for designation as orphan medicinal products. Section D.3 of the guideline titled "Justification of significant benefit", has been updated and now notes that the COMP may take into account the potential availability of a medicinal product to patients when assessing whether it will be of 'significant benefit'. In preparing an application for orphan medicinal product designation, sponsors are requested to follow this guideline.

Medicinal products Designated as Orphan Medicinal Products since the September 2002 COMP Meeting

Active substance	Doxorubicin carbon/iron magnetically targeted microparticles
Sponsor	Interface International Consultancy Limited
Orphan Indication	Treatment of hepatocellular carcinoma.
Opinion receipt date	24/07/2002
Date of Commission Decision	11/9/2002