IJABBR- 2014- eISSN: 2322-4827

International Journal of Advanced Biological and Biomedical Research

Journal homepage: www.ijabbr.com

Original Article

Anticonvulsant Activity of Aqueous Extract of Root and Stem Bark of *Newbouldia Laevis* Seem

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ARTICLE INFO

Article history:
Received: 05 June, 2014
Revised: 30 June, 2014
Accepted: 17 July, 2014
ePublished: 30 August, 2014

Key words:
Anticonvulsant
Metrazol
MES test
Newbouldia laevis,
Safety factor
Therapeutic index

ABSTRACT

Objective: *Newbouldia laevis* Seem, is an ubiquitous perennial plant belonging to the family Bignoniaceae with varying applicability in ethnomedicine. **Methods:** The anticonvulsant effect of the aqueous extracts of root and stem bark preparations was undertaken using standard techniques. **Results:** Results showed that the LD_{50} in mice was 489.8mg/kg body weight while 5.6mg/kg body weight protected mice against death due to minimum dose of leptazole which caused 100% mortality. The therapeutic index for *N. laevis* was 87.2 and its safety factor was 1.5. The extract of *N. laevis* appears to be more effective against drug-induced convulsion than electroshock seizures because the ED_{50} in metrazol test (5.6mg/kg body weight was significantly lower than the ED_{50} in MES test (63.1mg/kg body weight). We conclude that *N. laevis* is safe and will offer maximal benefit to patients suffering from seizures.

1.INTRODUCTION

Traditional African herbs contain many useful compounds which can be used for the treatment of chronic diseases. Numerous reports suggest that traditional herbs have potentials for preventing pathological outcome of some neurodegenerative diseases, cancers, metabolic disorders, among others and most of the active principles of some useful drugs have been initially isolated from plants. Furthermore, most of the herbal drugs are a mixture of a number of plants or parts of the same plant whose cumulative effect increases their efficacy in curing diseases as well as reducing toxicity (Krall et al, 1978; Manomani et al, 1995).

However, there are a number of plants well known for their medicinal values for centuries and are still being used by herbalists or ethnomedicine practitioners. *Newbouldia laevis* (P. Beaur) Seem, or boundary tree locally known as Ogirisi (Igbo), Aduruku (Hausa), Akoko (Yoruba) (Hutchinson and Daziel, 1963); is a medium sized angiosperm, it is an ubiquitous perennial plant belonging to the family Bignoniaceae, widely distributed in West Africa where it has a variety of medicinal uses which vary from country to country (Irvine, 1961). It grows to a height of about 7-8m, more usually as a shrub of 4m, many stemmed forming clumps of gnarled branches (Arbonier, 2004; Usman and Osuji, 2007). It is native to tropical Africa and grows on moist and well drained soil extending from Guinea Savannah to the

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dense forest zones (Tanko et al, 2008). It is being used in different ways by several groups of physicians. In Sierra Leone, the dried bark and young twigs are mashed with spices and dispensed as decoction or as infusion for the treatment of uterine colic, dysmenorrhoea and post partum haemorrhage (Dalziel, 1955). The plant has been claimed to be highly effective in the treatment of elephantiasis, dysentery, syphilis, piles and as a vermifuge for round worm infestation (Dalziel, 1955; Irvine, 1961)). The leaf, stem and fruits have also been used for febrifuge, wound dressing and stomach ache (Iwu, 2000) and recent studies have shown that ethanolic flower extract of the plant has antidiabetic properties (Tanko et al, 2008b) while the leaf extracts have been shown to be a good antibacterial source (Usman and Osuji, 2007). In Southeast Nigeria, *N. laevis* is given special attention because of its great ecological, environmental and ethnobotanical importance. The present study was designed to evaluate the anticonvulsant activity of the root and stem bark extract of *Newbouldia laevis* in different experimental models in mice.

2. MATERIALS AND METHODS

Plant materials: The roots and stem bark of *N. laevis* were obtained from Obukpa, near the University town of Nsukka, Enugu State, Nigeria; during the rainy season. The plant was identified by Ozioko A. of the International Centre for Ethnomedicine and Drug Development, Nsukka, Enugu State where a voucher specimen number: (BDCP/INTERCEDO/033) was deposited and authenticated by Prof. J. C. Okafor of Enugu Forestry Herbarium.

2.1. Extraction Method

Equal quantities (weight for weight) of the root and stem barks of *N. laevis* were cut into small pieces, after washing, air dried and pulverized. One kilogram (1000g) of the pulverized materials was extracted with water in a Soxhlet chamber. Concentration of the filtrate was accomplished by evaporating to dryness using the rotary evaporator at 40^{o}C under reduced pressure and the yield was 53.4g.

2.2. Animals

Adult albino mice of either sex weighing between 20 and 35g used for the study were obtained from the Departmental Animal House. The following experimental procedures were carried out.

(a) Phytochemical test: The aqueous extract of *N. laevis* was analyzed by thin-layer chromatography (TLC) using specific reagents according to the method described by (Marini-Bettolo et al, 1981) and (Farombi, 2003).

(b) Acute toxicity study in mice: Because of the potential value of *N. laevis* as an anticonvulsant agent, the acute toxicity study was carried out in line with relevant ethical guidelines in line with Principles of Laboratory Animal Care as contained in the Animal Welfare Act. They were fed on standard mice pellets and had free access to water *ad libitum*. Food was withdrawn 12h before the experiment. The mouse was used as the animal model. Albino mice of either sex weighing between 20 and 35g were divided into groups of ten and mice in each group received the same dose intraperitoneally (i.p.) of the extract of *N. laevis.* The number of deaths in each group 24h later was recorded and the percentage mortality calculated. The percentage mortality was plotted against log dose and the LD_{50} determined.

(c) Protective effect against leptazole-induced (Metrazol test) convulsion in mice: Preliminary studies were done to determine the minimal dose of leptazole that would cause 100% convulsion 18h after administering the drug, and also to determine if *N. laevis* would offer some protection against drug-induced convulsion in mice. When it was discovered from preliminary studies that *N. laevis* possessed some anticonvulsant activity against leptazole-induced toxicity in mice, it was decided to determine the safety factor (SF) and the therapeutic index (TI) of *N. laevis* against leptazole-induced toxicity. From the preliminary studies, it was found that the minimum dose of leptazole which caused 100% mortality in 18h was 70mg/kg body weight. The effects of various doses of *N. laevis* on mortality due to this dose of leptazole was determined as follows:

Mice weighing between 20 and 35g were divided into groups of 10. Each mice in any group received the same dose of *N. laevis* at 2.5-80mg/kg body weight, a few minutes before the dose of leptazole (70mg/kg body weight) was administered. The percentage mortality was recorded and the protection due to each dose of *N. laevis* was determined. The ED_{99} and the ED_{50} i.e the dose of *N. laevis* which respectively protects 99% and 50% mice from dying following treatment with leptazole (70mg/kg body weight) were determined. The LD_1 and LD_{50} i.e. the dose of the drug which would cause 1% and 50% deaths respectively were also determined. The safety factor (SF) i.e. LD_1/ED_{99} and the therapeutic index (TI) i.e. LD_{50}/ED_{50} were calculated.

(d) Electroshock-induced convulsion: The method of (Dikshit et al, 1972) was used with modifications. Another set of sixty mice of either sex weighing between 20 and 35g were randomly divided into 6 groups of 10 mice per group.

Mice in the first group (control) received 25ml/kg of normal saline intraperitoneally while mice in groups 2 to 6 received different doses of the extract (2.5-80mg/kg body weight) intraperitoneally. Thirty minutes after drug administration, each mouse was given an electroshock (Ugo Basil Electroconvulsive Treatment Unit ECT) using ear clip electrodes previously dipped in normal saline to make better conduction. The stimulation parameters used were 45mA, 0.2sec and 100Hz which produced maximum shock without being lethal. The animals in each experimental group was observed for 60minutes for seizures. An episode of clonic spasm that persisted for a minimum of 30 seconds was interpreted as threshold convulsion. Animal devoid of a threshold convulsion during the observatory period was considered protected and expressed as percentage (Brodie, 1990).

2.3. Statistical Analysis

Data are expressed as mean±SEM. Differences between groups were considered to be significant at $p<0.05$ using unpaired two tailed Student "*t*" test assuming equal variance at 95% confidence interval.

3. RESULTS AND DISCUSSION

Preliminary phytochemical analysis by thin layer chromatography using specific reagents showed that the extracts contained alkaloids, flavonoids glycosides, saponins and tannins. Acute toxicity in mice: The LD_{50} value following intraperitoneal administration of the extract of *N. laevis* in mice was 489.8 ± 1.9mg/kg body weight. It was also observed that the extract did not produce any external symptoms such as wet fur, oedema and the animals moved freely.

Figure 1 shows the log-dose response curve and gives the percentage protection by *N. laevis* (left panel) from death due to leptazole at 70mg/kg body weight. Leptazole at this dose would cause 100% mortality in the mice on its own. The graph also shows the percentage mortality due to *N. laevis* (right panel). The LD_1 and ED_{99} for *N. laevis* were 43.7mg/kg and 30.2mg/kg body weight respectively. The safety factor (SF) for *N. laevis* was therefore 1.45, similarly the LD_{50} and ED_{50} were 489.8mg/kg and 5.6mg/kg body weight respectively, and the therapeutic index (TI) was 87.2. The ED_{50} on electroshock (MES test) was 63.1mg/kg and it is about 11 times higher than the corresponding ED_{50} in the metrazol test (drug-induced). The difference was significant at $p<0.05$. The use of herbal medicines in the treatment of convulsion is a well known therapeutic approach in African folk medicine (Brodie, 1990; Porter, 1990). The present study has sought to provide evidence for the anticonvulsant activity of the water extract of the root and stem bark of *N. laevis.* However, in order to interpret the effectiveness of the herbal extract, an acute toxicity study was done to ensure its safety, the results obtained will serve as a guide in selection of doses for further studies. It was found that the LD_{50} in mice was 489.8mg/kg, the safety factor (SF) 1.5 and the therapeutic index (TI) 87.2. The therapeutic index is a measure of estimate of relative safety of drugs in animals which will offer idea of safety in man following extrapolation. This shows that (TI) provides a very crude measure of safety of a drug. Its main shortcomings being that it is based on animal toxicity data which may not reflect forms of toxicity that are important clinically in humans; and does not consider the idiosyncratic toxic reactions that accompany normal therapeutic doses. However, therapeutic window which is a more clinically relevant index of safety, describes the dosage range between the minimum effective therapeutic dose and minimum toxic dose (Porter, 1990). During the investigation, it was noted that *N. laevis* preparation was non-toxic and did not induce any toxic effect as animal behaviors, food and water intake were normal. The dose of the extract of *N. laevis* which achieved 50% reversal was about 1.1% of the LD_{50} in mice which shows that *N. laevis* has great potential value as an anticonvulsant agent. This is further strengthened by SF and TI values of 1.5 and 87.2 respectively. The SF of a drug depends on the degree of separation between the dose producing a desirable effect and the dose at which adverse events are elicited. However, an SF greater than 1.0 as in this study indicates that the dose effective in 99% of the population is less than that which would be lethal in 1% of the population while a value less than 1.0 is indicative of overlap between the maximally effective and minimally toxic doses. Furthermore, the extract with high LD_{50} (489.8mg/kg) was able to offer 100% protection against leptazole-induced (metrazol test) convulsion in mice with a subliminal dose of 80mg/kg or 16% of the LD_{50}. The TI and SF which are both indices for measuring and estimating the safety of a drug indicate favourable pharmacological outcome for the extract of *N. laevis*.

There is a significant difference ($p<0.05$) between the maximum protection in leptazole-induced (metrazol test) and that for electroshock-induced (MES test) convulsions in mice. The ED_{50} of the extract on metrazol test given as 5.6mg/kg body weight is significantly lower than its effective dose (ED_{50}) on MES test (63.1mg/kg body weight). This is an indication that the extract of *N. laevis* is more effective against leptazole-induced convulsions than electroshock convulsion. The low ED_{50} in metrazol test is indicative that *N. laevis* possesses efficacious controlling capacity on the biological events that occur during convulsion without producing any toxic effects.

CONCLUSION

Finally, as the clinical aspects of generalized seizures are highly correlated with experimental seizures induced by leptazole, it seems probable to infer that the extract of *N.*

laevis would offer better therapeutic efficacious benefits to patients with generalized than partial seizures.

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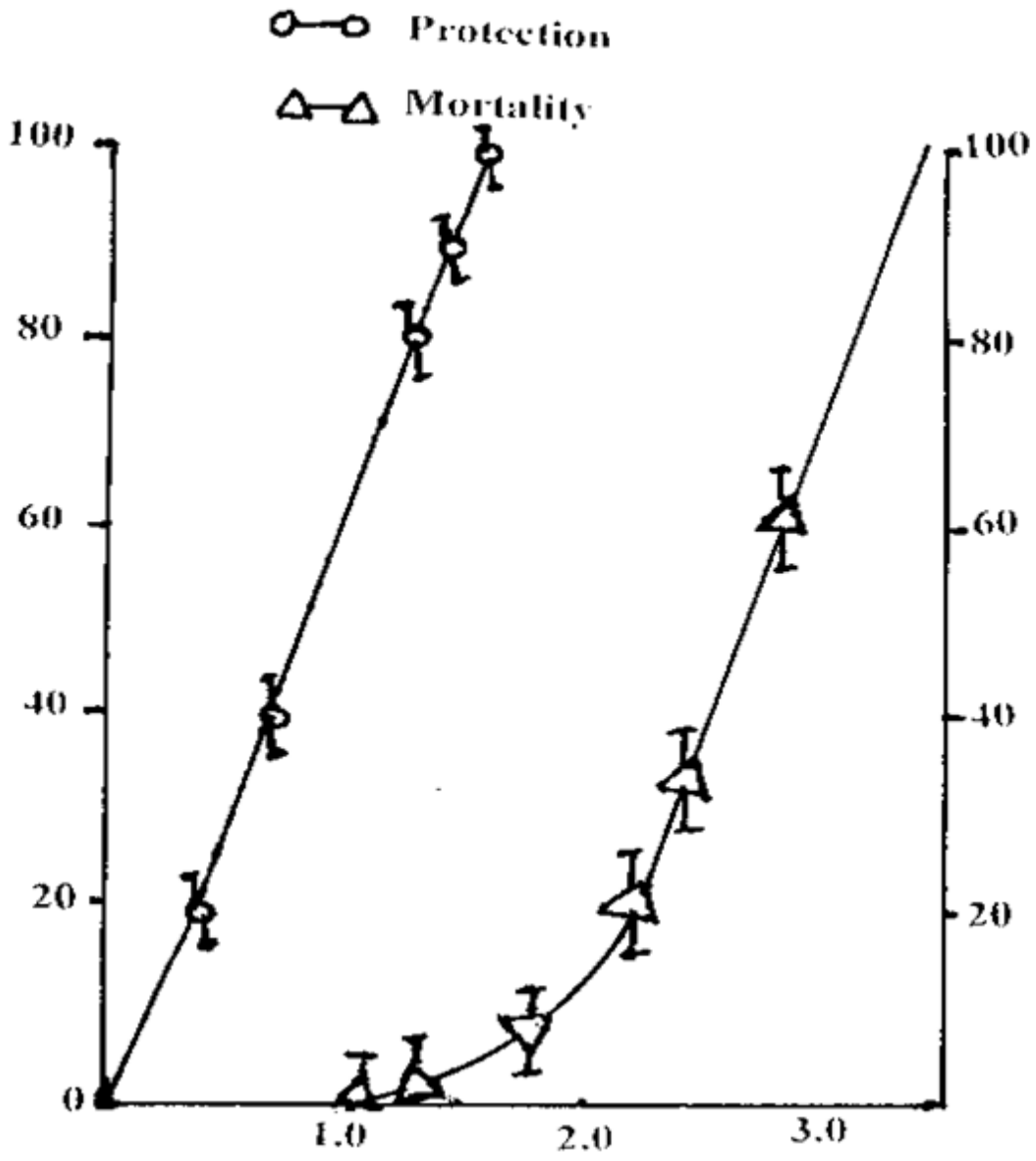

Figure 1: Log-dose response curve depicts percentage protection by *Newbouldia laevis* from death due to leptazole at 70mg/kg body-weight .