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Protective Effects of Crude Extract and Fractions of *Newbouldia laevis* Leaves in Chemoconvulsant-Induced Seizures

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ABSTRACT

Millions of people around the world suffer from the burden of neurologic and neuropsychiatric disorders, including epilepsy. *Newbouldia laevis* is a medicinal plant used for the management of epilepsy in some developing nations. However, studies directed at validating its efficacy are few. In this study, the efficacy of crude extract (NLE), n-butanol fraction (BPE), n-hexane fraction (HPE) and ethylacetate fraction (EAPE) of *N. laevis* to modulate chemoconvulsant-induced seizures was investigated in mice. Picrotoxin, pentylenetetrazole, and strychnine models of convulsion were used for the assessment of the anticonvulsant property of the plant. Following oral pretreatment of mice with graded doses (150 – 600 mg/kg b.w) of crude extract and fractions of *N. laevis*, seizure was induced by intraperitoneal administration of the picrotoxin, pentylenetetrazole, or strychnine. Seizure latency, duration of seizure, and mortality were recorded thereafter. Possible receptor targets for *N. laevis* were evaluated using flumazenil and naloxone. In the picrotoxin and pentylenetetrazole models, the crude extract and fractions caused a significant increase ($p<0.05$) in seizure latency, and a decrease in duration of seizure and mortality compared to the control, but no significant changes were observed in these parameters in the strychnine-induced convulsion. Flumazenil and naloxone antagonized the protective effects of NLE against chemoconvulsant-induced seizures, but NLE was not effective against seizures induced by strychnine.
Findings from this study indicate that *N. laevis* leaves possess anticonvulsant effects, and these effects are likely mediated through GABAergic and opioidergic transmission systems.

Keywords: Seizures, Chemoconvulsants, *Newbouldia laevis*, Fractions, Mice.

Introduction

Neurologic and neuropsychiatric disorders are among the leading causes of disability and low quality of life in many places around the world.[1] One of the most prevalent of these disorders is epilepsy. Globally, epilepsy is a disorder that affects 1% of all people by age 20, and 3% by age 75. It is not limited to specific geographical area, social status, race or gender.[2] It is estimated that 50 – 60 million people around the world have the disorder, and majority of these people are in developing countries. It is also reported that 60 – 70% of those who are epileptic in the developing nations have no access to the required medical care.[3] Many epileptic patients are gainfully employed, while others require prolonged hospitalization. Epilepsy does not generally shorten life, but neurological, psychiatric or cognitive impairments can reduce the quality of life. The term epilepsy is used interchangeably with seizure and is derived from a Greek word 'epilambanein' (επιλαμβάνειν) which means 'to seize'. It is a group of chronic disorders characterized by the occurrence of seizures. A seizure is an episode or attack in which there is a loss or disturbance of consciousness, sometimes accompanied by convulsions.

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It is not all seizures that fall in the category of epilepsy, and not everyone who has a seizure could be regarded as being epileptic.[4] Furthermore, a single seizure does not diagnose epilepsy. Seizures are often preceded by an 'aura' which is a sensory experience that correlates to the epileptic event, and is usually unique to a particular patient. Such patients experience the same sensory manifestations every time before the onset of seizures. There are different kinds of auras that may precede seizures and these include somatic sensations like rising epigastric sensations, various hallucinations such as visual, gustatory, and olfactory hallucinations. Other patients experience headache, paresthesias and psychiatric phenomena such as déjà vu or jamais vu, etc. However, not all patients experience an aura prior to an epileptic event.[5]

Epilepsy is a disease with several distinctive forms in which repeated self-limiting episodes of paroxysmal alterations of brain function occur.[6] Many neoplastic, infectious, traumatic, ischemic, toxic, metabolic, developmental and degenerative factors can lead to epilepsy, but in many patients no antecedent conditions can be identified.[7] In young children, pyrexia from any cause can trigger isolated convulsive seizures. In focal epilepsy, attacks are usually preceded by 'auras' with alterations of mental, motor, and sensory functions which vary in complexity, pattern, and duration from patient to patient. Even without an aura, the patient may develop a generalized grand mal seizure, lose consciousness, and collapse, usually in a tonic spasm which includes respiratory muscles.[5]

In all forms of epilepsy, repeated firing occurs in localized groups of primary driver neurons with subsequent spreading, widespread recruitment of follower neurons, and derangement of normal brain function. Repeated firing of large groups of neurons may result from a variety of events which interfere with the normal resting polarization of their membranes. These events may occur at synapses or may

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involve other regions of dendritic and axonal membranes. Decreased inhibitory control or increase in excitatory neurotransmission at synapses leads to excessive neuronal firing. Changes in ionic flux such as increased entry of calcium or decreased exit of potassium through the neuronal membranes also lead to abnormal neuronal firing.[8] Altered extracellular ionic environments secondary to abnormal astrocytic function may increase the likelihood of neuronal firing. Neurons which fire repeatedly are subjected to increased metabolic stress while they produce energy to drive membrane ionic pumps essential for the maintenance of their internal milieu and electrical integrity. It is likely that such stressed neurons are at risk of metabolic exhaustion with subsequent loss of differential between their internal ionic constitution and that of the exterior, a situation that may result in their death.[9]

Since many of the antiepileptic drugs available in the market have side effects and are contraindicated in certain situations, there are a need for new drugs to be discovered and made available to epileptic patients.[10] In addition to their side effects, some of these drugs are not accessible and affordable to many patients in rural areas. As a result, herbal preparations are common alternative therapy for the management of epilepsy.[11]

Newbouldia laevis (P. Beauv) Seemann ex. Bureau is one of the medicinal plants employed for the treatment of epilepsy in many West African countries, including Nigeria, Republic of Benin, and Ghana[12]. *Newbouldia laevis* is an angiosperm of medium size and belongs to the family *Bignoniaceae*. Its common names are 'African Border Tree' and 'Fertility Tree'. In Nigeria, it is known locally among the Yoruba tribe as 'Akoko'. Despite the widespread use of the leaves of *Newbouldia laevis* as antiepileptic remedy, its efficacy as anticonvulsant agent has not been validated. In this study, the effects of crude extract and fractions of *Newbouldia laevis* leaves on picrotoxin-, pentylenetetrazole-, and strychnine-induced seizures were investigated.

Materials and Methods

Preparation of crude extract and fractions

Leaves of *Newbouldia laevis* were collected from Oroki Estate area of Osogbo in February, 2020. The plant was authenticated by a taxonomist in Forestry Research Institute of Nigeria (FRIN), Ibadan (Voucher number: FHI 208426). Pulverized leaves (800 g) were extracted in 2 L of 80% ethanol using a Soxhlet apparatus and concentrated by a rotary evaporator (Heidolph-Rotacool, Germany) to give the crude extract (NLE). Then, solvent-solvent partition was carried out using *n*-hexane, *n*-butanol, and ethyl acetate to give *n*-hexane (HPE), *n*-butanol (BPE) and ethyl acetate (EAPE) fractions.

Experimental animals

Male Swiss mice (20-25 g) were obtained from the Animal Holding Unit of the Department of Pharmacology and Therapeutics, Ladoke Akintola University of Technology (LAUTECH). The mice were kept in polypropylene cages in a well-ventilated section of the laboratory. They were maintained under standard laboratory conditions of temperature (22 ± 2°C), relative humidity (55-65%) and 12 h light/dark cycle. The animals were fed with standard animal diet and clean water *ad libitum*.

Ethical Consideration

All experimental procedures and protocols were as outlined in the "Guide for the Care and Use of Laboratory Animals" published by the National Research Council.[13] All procedures were carried out as approved by the laboratory animal use committee of Department of Pharmacology and Therapeutics, Ladoke Akintola University of Technology, Ogbomoso (Ethical Approval Number: PT20/007).

Novelty-induced rearing and grooming

The effect of NLE on novelty-induced rearing and grooming behavior was evaluated in mice between 10:00 hr and 16:00 hr under similar environmental conditions by the open field method previously described.[14] The open field is a rectangular wooden box (36 x 36 x30) placed in a quiet room. Graded doses of NLE (150, 300 and 600 mg/kg b.w), and 0.2 mL distilled water were orally administered to four groups of mice. One hour later, each mouse was placed in the box for observation of behavior. Frequency of grooming (face washing, body scratching, and licking of limb, tail and genital area) and rearing (standing with hind limbs, with the fore limb against the wall of the observation box or free in the air) was counted every 5 min in a 30 min session. The observation box was cleaned with 70% alcohol before another mouse is placed in it to eliminate any olfactory bias from the previous mouse.

Phenobarbital-induced hypnosis

Twenty-four mice were made to fast for 12 hours and assigned to 4 groups of six mice each. Groups 1 – 3 were pretreated with NLE (150, 300, and 600 mg/kg b.w), and group 4 with distilled water (10 mL/kg b.w) 30 minutes before intraperitoneal administration of sodium phenobarbital (45 mg/kg b.w). The onset and duration of sleep (time interval between loss and gain of righting reflex) were noted.[15,16] In separate studies, effects of the partition extracts (*n*-hexane, *n*-butanol and ethyl acetate extracts) on phenobarbital-induced hypnosis were also investigated by the same method.

Chemoconvulsant-induced seizures

Three experimental models were used. They are picrotoxin, pentylenetetrazole (PTZ), and strychnine models.[17-19] Mice were assigned into 5 groups of six mice each and treated per oral as follows: Group I received distilled water (10 mL/kg b.w), group II was treated with diazepam (2 mg/kg b.w), and group III, IV, and V were given 150, 300, and 600 mg/kg b.w of *N. laevis* (crude extract or fraction) respectively. This pretreatment was done 30 min before intraperitoneal administration of picrotoxin (10 mg/kg b.w), PTZ (85 mg/kg b.w), or strychnine (2 mg/kg b.w). Then, each mouse was placed in a separate transparent observation chamber. The animal was observed for 30 minutes after the administration of the convulsant to determine seizure latency and seizure duration. The number of animals that died within 24 hr was also recorded.

Assessing the effect of flumazenil on the anticonvulsant activity of N. laevis

Mice were assigned to six groups of 6 animals each. They were treated as follows:

Group I: Distilled water {10 mL/kg b.w (p.o)}, Group II: Flumazenil {2 mg/kg b.w (i.p)},

Group III: Diazepam {2 mg/kg b.w (i.p)}, Group IV: Diazepam {2 mg/kg b.w (i.p) + flumazenil (2 mg/kg b.w (i.p)}, Group V: Flumazenil {2 mg/kg b.w (i.p) + NLE (600 mg/kg b.w (p.o)}, Group VI: NLE {600 mg/kg b.w (p.o)}. All drugs were administered 30 minutes before the administration of pentylenetetrazole {85 mg/kg b.w (i.p)}. Seizure latency, seizure duration, and the number of death within 24 hr were recorded.[20, 21]

Assessing the effect of naloxone on the anticonvulsant activity of N. laevis

Mice were randomly divided into four groups of 6 animals per group. Group I, II, and III received distilled water {10 mL/kg b.w (p.o)}, naloxone {5 mg/kg b.w (i.p)}, and NLE {600 mg/kg b.w (p.o)} respectively. Group IV was treated with Naloxone {5 mg/kg b.w (i.p) + NLE (600 mg/kg b.w (p.o)}. Thirty minutes later, pentylenetetrazole {85 mg/kg b.w (i.p)} was administered. Seizure latency, seizure duration, and the number of mice that died within 24 hr were recorded.[22]

Statistical analysis

Data obtained from the experiments are expressed as mean ± standard error of mean (SEM). The data were subjected to one-way analysis of variance (ANOVA) and Student's - Newman-Keul test to determine the statistical significance of differences between groups. Differences were considered to be significant when $p < 0.05$.

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Results and Discussion

The results of novelty-induced rearing and grooming experiments showed that the frequency of rearing and grooming behaviors in mice was significantly reduced ($p < 0\ 05$) in the animals treated with 300 and 600 mg/kg b.w of crude ethanol extract of *N. laevis* compared with the control. The results are presented in Figures 1 and 2. In rodents, rearing is considered to be a manifestation of excitatory activity in the central nervous system, while grooming is generally seen as an index of behavioral adaptation to stress since this behavior increases with anxiety and fear.[23,24] Exposure to unfamiliar environment imposes a measure of stress on rodents and this induces grooming behavior. This behavior is thought to be linked to the activation of dopaminergic pathway through D_1 receptors.[25] The results showed that *N. laevis* ethanol extract (NLE) has inhibitory effect on the central nervous system. Such inhibition is characteristic of anticonvulsant drugs. The results of the effects of the crude extract and fractions of *N. laevis* on phenobarbital-induced hypnosis are presented in Figures 3 and 4. The results also showed that the extracts of *N. laevis* facilitated the inhibitory system of the central nervous system in mice. Phenobarbital is a barbiturate which has GABA-mimetic and GABA-facilitatory properties. It increases the duration of opening of chloride channels, allowing more and more chloride ion to move into the cells. This results in reduction of neural activity and inhibition of the central nervous system.[26,27] The significant decrease in sleep latency and the prolongation of sleep duration observed following pretreatment of mice with *N. laevis* extracts indicate that the inhibitory effect of phenobarbital was potentiated by the extracts, indicating that there is possibility of *N. laevis* possessing anticonvulsant property.

In picrotoxin-induced convulsion, the seizure latency was significantly increased in mice treated with the extracts of *N. laevis* compared with mice treated with distilled water. The extracts increased the seizure latency in a dose-dependent manner. With the administration of distilled water (control), the seizure latency was 1.18 ± 0.06 min. Administration of NLE, HPE, BPE, and EAPE at 600 mg/kg b.w significantly increased seizure latency ($p < 0.01$) to 11.90 ± 94, 9.88 ± 0.44, 12.50 ± 1.25, and 8.45± 0.35 min respectively. The effects of NLE and BPE are comparable to that of diazepam (standard drug) which produced seizure latency of 12.83 ± 1.02 min. Seizure duration in distilled water-treated mice was 26.32 ± 3.16 sec. This was significantly reduced ($p < 0.05$) in the groups of extract-treated and diazepam-treated mice. Mortality within 24 hr after the experiment was 100% in the distilled water-treated group and 50% in the group treated with diazepam. With administration of 600 mg/kg b.w of NLE and BPE, mortality was also reduced by 50%. These results are presented in Tables 1-3. Picrotoxin is a potent convulsant drug which prevents the action of gamma amino butyric acid (GABA) in opening a chloride ionophore. It is a compound of picrotoxinin and picrotin. Picrotin is known to be inert, and as such, the convulsant activity of picrotoxin is attributable to its picrotoxinin moiety.[28] Some compounds that antagonize picrotoxin effect are known to be effective anticonvulsant agents. For example, benzodiazepines are effective antagonists of convulsions produced by picrotoxin, but they are not effective against convulsions induced by strychnine and other agents that operate independently of GABA system. Substances which increase conductance of chloride or potassium ions through membranes tend to increase their polarization, thus stabilizing them and inhibiting discharge and synaptic excitatory transmission.[29] Gamma aminobutyric acid is one of the monocarboxylic amino acids which increase chloride ion conductance.[30] GABA is present in small interneurons in many parts of the brain and its release from these cells is important for inhibition of neurotransmission in short range negative feedback circuits. Reduction in the number of symmetrical inhibitory synapses and the level of GABA-synthesizing enzyme, glutamic acid decarboxylate (GAD) are found in experimental epileptogenic foci. GAD has also been found to be diminished in brain biopsies from humans with epilepsy.[31, 32] These findings support the notion that activation of GABA receptors inhibits neuronal firing activity, and inhibition of these receptors typically enhances excitatory activity.

In the pentylenetetrazole convulsion model, administration of NLE (600 mg/kg b.w), BPE (300 and 600 mg/kg b.w), and diazepam (2 mg/kg b.w) protected mice against seizure in 100 % of the animals. HPE and EAPE at 150, 300, and 600 mg/kg b.w increased seizure latency significantly ($p < 0.05$, $p < 0.05$, and $p < 0.01$ respectively) compared to the control. The extracts also reduced seizure duration significantly ($p < 0.01$) compared with the control. Mortality was 100 % in the control group and 0 % in the groups treated with diazepam (2 mg/kg b.w), NLE (600 mg/kg b.w), EAPE (600 mg/kg b.w), and BPE (300 and 600 mg/kg b.w). Mortality also decreased by 50 % following treatment with NLE (300 mg/kg b.w) and BPE (150 mg/kg b.w). Tables 1-3 give a summary of these results.

Pentylenetetrazole also inhibits chloride conductance by binding to picrotoxin sites on GABA receptor complex. It also acts as benzodiazepine receptor antagonist.[33]

Pretreatment of mice with crude and fractions of *Newbouldia laevis* leaves significantly increased seizure latency in convulsions induced by picrotoxin and pentylenetetrazole. This effect, as well as the significant decrease in seizure duration or protection against seizure observed in these experiments indicates that *N. laevis* extracts possess anticonvulsant activity. The anticonvulsant effect was more pronounced with the ethanol crude extract and *n*-butanol fraction. This suggests that the secondary metabolites responsible for the antiseizure activity are in higher concentrations in these extracts. It could also mean that the secondary metabolites are more synergistic in NLE and BPE.[34]

The results from strychnine-induced convulsion showed that there were no significant differences between the groups of rats treated with the extracts or diazepam and the control ($P > 0.05$) as presented in Tables 1-3. NLE and the fractions (150 – 600 mg/kg b.w) did not significantly prolong seizure latency and duration of seizure was not significantly reduced. The extracts did not protect mice against strychnine-induced convulsion as all the animals convulsed, and mortality was 100% in all the groups including the diazepam-treated group. This suggests that *N. laevis* does not interact with glycine receptors which is the binding site for strychnine.[35]

Pretreatment with flumazenil (2 mg/kg b.w) before NLE administration caused an inhibition of seizure latency prolongation observed when the animals were treated with only NLE. There was a significant difference ($p < 0.001$) between seizure latency in the group of mice treated with NLE (600 mg/kg b.w) and the group of mice pretreated with flumazenil. The increase in seizure latency observed with diazepam was also significantly inhibited by flumazenil pretreatment (Table 4). Both NLE (600 mg/kg b.w) and diazepam (2 mg/kg b.w) offered 100% protection against PTZ-induced convulsion and mortality in the absence of flumazenil. Inhibition of the anticonvulsant effect of *N. laevis* by flumazenil, a benzodiazepine antagonist, in PTZ-induced convulsion suggests that its modulatory effect is likely mediated through GABA receptor complex since there are benzodiazepine receptors in this complex.[36]

Administration of NLE (600 mg/kg b.w) protected mice from seizure induced with PTZ but this was inhibited in the presence of naloxone. In the group of mice pretreated with naloxone before administration of NLE, seizure latency was significantly reduced ($p < 0.001$) compared to the group treated with NLE alone. NLE offered 100% protection against mortality in PTZ-treated mice but this was reduced to 33.33% with naloxone pretreatment (Table 5).

Since naloxone is an opioid antagonist, these results suggest that opioid receptors could also be involved in the anticonvulsant effect of the plant. It has been reported that central opioidergic system exhibits convulsion-modulating properties.[37] Morphine, which is an opioid receptor agonist, has been reported to have anticonvulsant effect in low doses, but in high doses, it increases the susceptibility of rodents to seizures induced by convulsant drugs.[38] Additionally, the link between GABAergic system and the central opioidergic system in seizure modulation has been established.[39]

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Table 1: Effect of *N. laevis* on seizure latency (min) in chemoconvulsant-induced seizures

	Dose	Picrotoxin	Pentylenetetrazole	Strychnine
DW	[10]	1.18 ± 0.06	1.84 ± 0.24	1.87 ± 0.04
DZP	[2]	12.83 ± 1.02[b]	∞[c]	2.24 ± 0.32
NLE	[150]	8.95 ± 0.31[a]	8.51 ± 1.21[a]	1.92 ± 0.08
	[300]	10.24 ± 0.72[b]	10.20 ± 1.93[b]	1.80 ± 0.11
	[600]	11.90 ± 0.94[b]	∞[c]	2.13 ± 0.21
HPE	[150]	6.75 ± 0.60[a]	6.25 ± 1.88[a]	2.16 ± 0.06
	[300]	7.63 ± 0.58[b]	8.16 ± 1.40[a]	2.20 ± 0.04
	[600]	9.88 ± 0.44[b]	10.28 ± 1.92[b]	2.28 ± 0.03
BPE	[150]	9.76 ± 0.83[b]	10.84 ± 1.62[b]	2.62 ± 0.22
	[300]	10.65 ± 1.03[b]	∞[c]	2.53 ± 0.35
	[600]	12.50 ± 1.25	∞[c]	3.30 ± 0.23
EAPE	[150]	6.81 ± 0.66[a]	7.41 ± 1.04[a]	2.33 ± 0.16
	[300]	7.06 ± 0.70[b]	8.22 ± 1.33[a]	2.39 ± 0.30
	[600]	8.45 ± 0.35[b]	9.77 ± 1.70[b]	3.06 ± 0.42

Values represent mean ± SEM (n = 6); [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ compared with distilled water-treated mice. DW = distilled water (10 mL/kg b.w), DZP = diazepam (2 mg/kg b.w), NLE = crude ethanol extract of *N. laevis*, HPE = *n*-hexane partition extract of *N. laevis*, BPE = *n*-butanol partition extract of *N. laevis*, EAPE = ethyl acetate partition extract of *N. laevis*. Doses of extracts are in mg/kg b.w; ∞ indicates there were no seizures.

Table 2: Effect of *N. laevis* on duration of seizure (s) in chemoconvulsant-induced seizures

	Dose	Picrotoxin	Pentylenetetrazole	Strychnine
DW	[10]	26.32 ± 3.16	22.66 ± 2.53	2.64 ± 0.52
DZP	[2]	11.54 ± 1.42[b]	0.00 ± 0.00[c]	3.53 ± 0.65
NLE	[150]	24.25 ± 2.64	14.03 ± 1.80[a]	2.81 ± 0.24
	[300]	19.69 ± 3.23[a]	8.55 ± 0.76[b]	2.92 ± 0.60
	[600]	14.78 ± 2.61[a]	0.00 ± 0.00[c]	3.31 ± 0.43
HPE	[150]	19.63 ± 2.06[a]	17.91 ± 1.65[a]	2.68 ± 0.40
	[300]	17.50 ± 2.73[a]	11.46 ± 1.32[a]	2.62 ± 0.55
	[600]	16.13 ± 2.11[a]	9.63 ± 0.86[b]	2.98 ± 0.42
BPE	[150]	16.42 ± 2.31[a]	8.84 ± 0.41[b]	2.87 ± 0.71
	[300]	13.81 ± 1.84[a]	0.00 ± 0.00[c]	3.20 ± 0.33
	[600]	12.92 ± 2.22[b]	0.00 ± 0.00[c]	3.80 ± 0.25
EAPE	[150]	17.46 ± 2.86[a]	18.92 ± 1.61[a]	2.68 ± 0.30
	[300]	15.79 ± 2.08[a]	15.16 ± 1.20[a]	2.75 ± 0.67
	[600]	16.36 ± 2.60[a]	11.70 ± 1.44[b]	2.78 ± 0.53

Values represent mean ± SEM (n = 6); [a]$p < 0.05$ compared with distilled water-treated mice, [b]$p < 0.01$ compared with distilled water-treated mice. [c]$p < 0.001$ compared with distilled water-treated mice. DW = distilled water (10 ml/kg b.w), DZP = diazepam (2 mg/kg b.w), NLE = crude ethanol extract of *N. laevis*, HPE = *n*-hexane partition extract of *N. laevis*, BPE = *n*-butanol partition extract of *N. laevis*, EAPE = ethyl acetate partition extract of *N. laevis*. Doses of extracts are in mg/kg b.w.

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Table 3: Effect of *N. laevis* on mortality (24 hr) in chemoconvulsant-induced seizures

	Dose	Picrotoxin	Pentylenetetrazole	Strychnine
DW	[10]	6/6	6/6	6/6
DZP	[2]	3/6	0/6	6/6
NLE	[150]	6/6	6/6	6/6
	[300]	5/6	3/6	6/6
	[600]	3/6	0/6	6/6
HPE	[150]	6/6	6/6	6/6
	[300]	5/6	4/6	6/6
	[600]	4/6	2/6	6/6
BPE	[150]	4/6	3/6	6/6
	[300]	4/6	0/6	6/6
	[600]	2/6	0/6	6/6
EAPE	[150]	5/6	4/6	6/6
	[300]	5/6	2/6	6/6
	[600]	4/6	1/6	6/6

Values represent mean ± SEM (n = 6); $^{a}p < 0.05$ compared with distilled water-treated mice, $^{b}p < 0.01$ compared with distilled water-treated mice. DW = distilled water (10 ml/kg b.w), DZP = diazepam (2 mg/kg b.w), NLE = crude ethanol extract of *N. laevis*, HPE = *n*-hexane partition extract of *N. laevis*, BPE = *n*-butanol partition extract of *N. laevis*, EAPE = ethyl acetate partition extract of *N. laevis*. Doses of extracts are in mg/kg b.w; ∞ means there were no seizures.

Table 4: Effect of flumazenil on the antiseizure activity of NLE in PTZ-induced convulsion

Treatment	Seizure latency (min)	Duration of seizure (s)	% Protection in 30 min	Mortality in 24 hr
DW (10 ml/kg)	1.52 ± 0.08	28.83 ± 2.64	0	6/6
FLZ (2 mg/kg)	1.39 ± 0.09	23.71 ± 2.67	0	6/6
Diazepam (2 mg/kg)	∞**	$0.00 \pm 0.00^{*}$	100	0/6
Diazepam (2 mg/kg) + FLZ (2 mg/kg)	1.96 ± 0.13	34.21 ± 2.53	0	5/6
NLE (600 mg/kg)	∞**	$0.00 \pm 0.00^{*}$	100	0/6
NLE (600 mg/kg) + FLZ (2 mg/kg)	$3.61 \pm 0.42^{*\neq}$	23.92 ± 2.68	0	4/6

Values represent mean ± SEM (n = 6). $^{*}p < 0.01$ compared with control, $^{**}p < 0.001$ compared control. $^{\neq}p < 0.001$ compared with NLE (600). DW = distilled water (control), NLE = crude ethanol extract of *N. laevis*, FLZ = flumazenil, PTZ = pentylenetetrazole. ∞ indicates there were no seizures.

Table 5: Effect of naloxone on the antiseizure activity of NLE in PTZ-induced convulsion

Treatment	Seizure latency (min)	Duration of seizure (s)	% Protection in 30 min	Mortality in 24 hr
DW (10 ml/kg)	2.20 ± 0.32	25.26 ± 2.74	0	6/6
Naloxone (5 mg/kg)	2.80 ± 0.21	31.45 ± 2.82	0	6/6
NLE (600 mg/kg)	∞**	$0.00 \pm 0.00^{*}$	100	0/6
NLE (600 mg/kg) + Naloxone (5mg/kg)	$3.16 \pm 0.55^{\neq}$	28.94 ± 2.61	16.66	5/6

Values represent mean ± SEM (n = 6). $^{*}p < 0.01$, $^{**}p < 0.001$ compared with control, $^{\neq}p < 0.001$ compared with NLE (600). DW = distilled water (control), NLE = ethanol extract of *N. laevis*, PTZ = pentylenetetrazole. ∞ indicates there were no seizures

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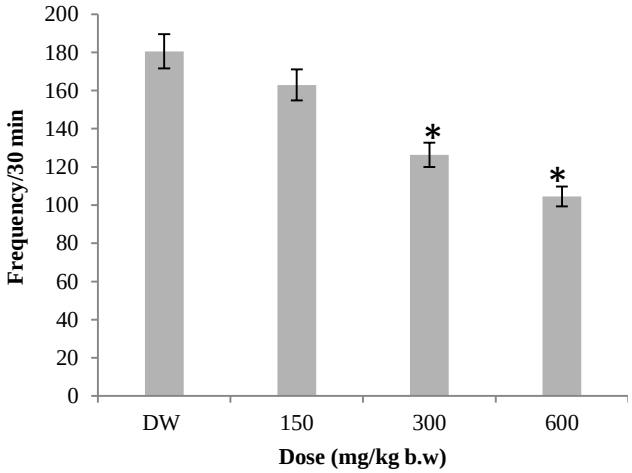

Figure 1: Effect of crude ethanol extract of *N. laevis* leaves on rearing behavior in mice.
Values represent mean ± SEM (n = 6), *p < 0.05 compared with the control. DW = distilled water (10 mL/kg b.w).

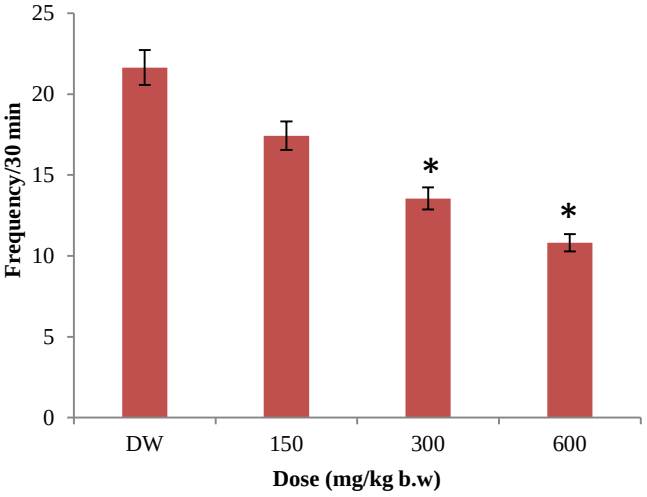

Figure 2: Effect of crude extract of N. laevis leaves on grooming behavior in mice.
Values represent mean ± SEM (n = 6), *p < 0.05 compared with the control. DW = distilled water (10 mL/kg b.w).

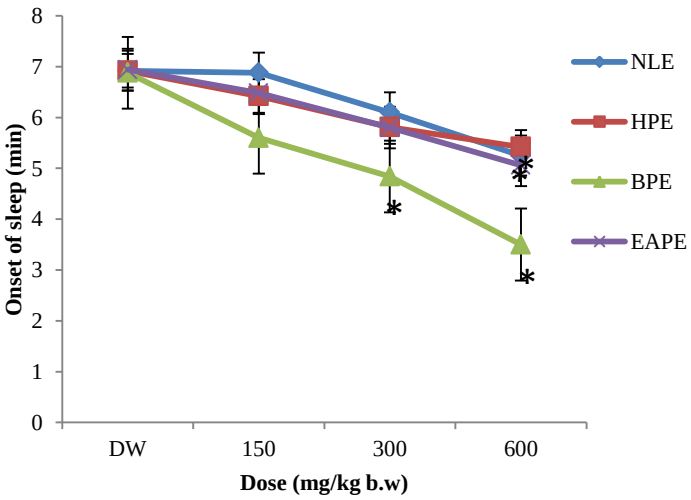

Figure 3: Effects of *N. laevis* extracts on onset of sleep in phenobarbital-induced hypnosis.
Values represent mean ± SEM (n = 6); *p < 0.05 compared with the control. DW = distilled water, NLE = crude ethanol extract, HPE = *n*-hexane partition extract, BPE = *n*-butanol partition extract, EAPE = ethyl acetate partition extract.

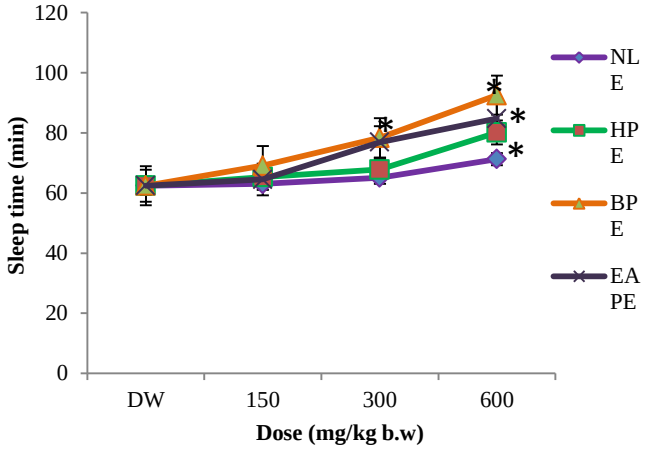

Figure 4: Effects of *N. laevis* extracts on sleep time in phenobarbital-induced hypnosis.
Values represent mean ± SEM (n = 6); *p < 0.05 compared with the control. DW = distilled water, NLE = crude ethanol extract, HPE = *n*-hexane partition extract, BPE = *n*-butanol partition extract, EAPE = ethyl acetate partition extract.

Conclusion

From the results obtained, it is concluded that crude and partition extracts of *N. laevis* modulate neuronal activity involved in seizures through interactions with GABA and opioid receptors. It is a promising medicinal plant from which a novel antiepileptic drug could be developed.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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