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Antiepileptogenic and Anticonvulsant Actions of *Dalbergia saxatilis* (Hook, F.) in Sub-toxic Chemical Kindling and Toxic Convulsant Models

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Authors' contributions

This work was carried out in collaboration between all authors. Authors OKY and OOA designed the study, author OKY performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed the literature searches; authors OKY and OOA managed the analysis of the study. Both authors OKY and OOA read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: The aqueous root extract of *Dalbergia saxatilis* (DS) is used in traditional African medicine to manage convulsions and epilepsies. This study aimed at investigating DS action against models that mimic seizure development in the neurons of epileptics, the sub-toxic dose kindling models.
Study Design: Sub-toxic doses of strychnine and picrotoxin chemical kindling models; as well as single-dose toxic bicuculline convulsive models in mice.
Place and Duration of Study: Neuropharmacology Unit Laboratory, Department of Pharmacology, College of Medicine, University of Lagos, Lagos, Nigeria, between July 2006 and March 2008.
Methodology: Strychnine kindling was produced by a 48h interval, i.m administration of 1.5mg/kg strychnine for 9 trials. The mice were treated with 200mg/kg, p.o. DS, before strychnine thus: Group I: throughout the 1st - 9th kindling; group II: During the 1st - 5th

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kindling; and group III: during the 6th - 9th kindling trials. Control group received distilled water instead of DS throughout the 1st - 9th kindling trials. For picrotoxin study, a subconvulsant dose of 1.5mg/kg picrotoxin was injected i.p. 3 times a week for 10 weeks, 200mg/kg of DS was administered orally before picrotoxin thus: Group I: throughout the 1st - 30th kindling trials; group II: during the 1st - 12th kindling trials; group III: during the 13th - 30th kindling trials; control group received distilled water instead of DS throughout the 30 trials. Behavioural seizures were classified for seizure stages. In another study, DS (50-200 mg/kg, p.o.) was administered to mice, 30 min. before 10mg/kg, s.c. bicuculline and onset to seizures and time to death noted.

Results: DS significantly (*P*=.05) retarded the development and progression of strychnine kindling, but did not reverse already reached kindled state. Moreover, DS significantly (*P*=.05) retarded the development of picrotoxin kindling, decreased the scoring from kindling progression and prevented convulsion in fully picrotoxin-kindled mice. A significant delay of seizure onset, with complete protection at 200mg/kg DS was produced against bicuculline seizures in mice.

Conclusion: DS may attenuate development of seizures in both GABAergic and glycinergic mechanisms and be useful in the prevention of seizures as well as neuroprotection in epileptics, justifying its use in the folkloric management of epilepsies.

Keywords: Dalbergia saxatilis; kindling; strychnine; picrotoxin; bicuculline.

1. INTRODUCTION

Epilepsies are very common and present with neurological embarrassing problems with limited drugs to manage them; and where available, with serious side effects. Unfortunately, many so-called anticonvulsants may not manage the underlying neuronal problems in epilepsies. Therefore newer drugs or sources of drugs to either prevent or treat epilepsies are always sought and welcome. *Dalbergia saxatilis,* Hook, F. (Family: Leguminosae; sub-family: Papilionaceae) is a woody shrub widely distributed in the forest and savannah regions of West Africa, where the plant parts are employed for various medicinal uses [1]. Traditional medicine practitioners among the Yoruba people of Nigeria popularly employ the root decoction of the plant, among other various uses, in the management of epilepsies and convulsions. We have previously demonstrated the anticonvulsant effects of the aqueous root extract of *Dalbergia saxatilis* (DS) against picrotoxin- and strychnine- induced seizures [2]. To further ascertain the mechanism(s) of action of DS, its action against epileptogenesis, a chronic type of epilepsy is explorable. It is noteworthy that a considerable number of epileptic patients develop this condition as consequences of brain trauma and other neurologic disorders [3,4]. These cause progressive recruitment of cells with lowered seizure threshold, as well as propagation of excitable 'epileptic' neurons of seizure susceptibility resulting from repetitive electrical stimulation referred to as *kindling* effect, a phenomenon which affects specific parts of the brain like the hippocampus and amygdala, causing behavioural seizures [5]. The animal kindling model could satisfactorily mimic this clinical epileptogenicity that is similar to human epilepsies. Moreover, kindling method is used for the second screening of anticonvulsant drugs, and has been described as an extensively studied animal model of epilepsy, epileptogenesis and developed seizures [6,7]. It is also useful in determining anticonvulsant activity and has been reported to have many advantages in the screening of potential anticonvulsant/anti-epileptic drugs [8]. Kindled seizures can be produced by repeated administration of a subconvulsant dose of drugs like picrotoxin, pentylenetetrazole (PTZ) and cocaine [9-11] and strychnine [12]. Among the currently used antiepileptic drugs, benzodiazepines, phenobarbitone, valproic acid and

recently, levetiracetam have shown some antiepileptogenic effects [13-15]. In this study, we further examined the effect of DS against seizures in strychnine kindling and picrotoxin kindling models of epileptogenesis in mice. Moreover, bicuculline- induced convulsion, a model which has been established to be through $GABA_A$ receptor interaction was also studied.

2. EXPERIMENTAL DETAILS

2.1 Plant Material

Fresh root parts of *Dalbergia saxatilis* were collected from a secondary forest in Ibadan, Oyo State, Nigeria. It was authenticated by T.K. Odewo, an assistant chief superintendent of the Forestry Research Institute of Nigeria (FRIN) where a voucher specimen (FHI 106484) was deposited for reference and confirmed by Professor J.D. Olowokudejo of the Botany Department, University of Lagos, Nigeria.

2.2 Extract and Drug Preparation

Root, washed, ground dried and powdered was boiled in distilled water for 30min. It was left for 24h at room temperature for further extraction and filtered. The filtrate was evaporated to dryness in an oven at 40^{o}C (yield: 10.6 % was calculated as: $\frac{\text{weight of dried extract}}{\text{weight of powdered root.}} \times 100$)

The dried extract was stored in the refrigerator and dissolved in distilled water before use; the standard drug, phenobarbitone also was dissolved in distilled water, while the convulsant drugs- strychnine, picrotoxin and bicuculline were dissolved in normal saline immediately before each administration.

The doses selected for DS in this study were based on preliminary experiments. A dose of 200 mg/kg was the maximum effective anticonvulsant dose that did not cause any form of toxic manifestations or behavioural changes.

Phytochemical screening suggested the presence of saponins, tannins, oils, glycosides, sugars and phenols [2].

2.3 Animals

Adult albino mice of either sex, maintained on standard rodent feed and given water ad libitum, were obtained from the Laboratory Animal Centre of the College of Medicine of the University of Lagos, Nigeria.

2.4 Strychnine-Induced Kindling

The mice were divided into four groups (15 per group) to which 200mg/kg of the extract was administered p.o. before a subconvulsant dose of 1.5mg/kg, i.m. strychnine thus: Group I: throughout the 1st - 9th kindling trials; group II: during the 1st - 5th kindling trials; group III: during the 6th - 9th kindling trials, but group IV (control) mice were given distilled water orally, instead of DS, before strychnine [7] throughout the 1st - 9th kindling trials. Treatments, in each group, were done for nine times, at 48h-interval (18 days). The injected mice were placed in individual cages, and observed closely for 30 min. Behavioral seizures were rated for seizure

stages according to observations in our laboratory using a modified seizure progression of strychnine:

Stage 0 : normal, no seizure
Stage 1 : restlessness, grooming and biting
Stage 2 : intermittent forelimb myoclonus, without falling
Stage 3 : generalized tremors with jumping and falling
Stage 4 : tonic hind limb extension
Stage 5 : death following tonic seizure

All experiments were performed between 10a.m. and 6 p.m.

2.5 Picrotoxin-Induced Kindling

The mice were divided into three groups (20 per group) to which 200mg/kg of the extract was administered p.o. before a subconvulsant dose of 1.5mg/kg, i.p. picrotoxin thus: Group I: throughout the 1^{st} - 30^{th} kindling trials; group II: During the 1^{st} - 12^{th} kindling trials; and Group III: during the 13^{th} - 30^{th} kindling trials [7]. The control group mice were given distilled water orally, instead of DS, before picrotoxin, throughout the 30 trials. The injected mice were placed in individual cages, and observed closely for 30 min. Picrotoxin was injected three times a week (minimum 48-h intervals). Behavioral seizures were rated for seizure stages according to our previous observations [16]:

Stage 0 : No seizure.
Stage 1 : Initial reduced activity followed by hyperactivity and sniffing.
Stage 2 : Successive forelimb and head cloni with sharp noise.
Stage 3 : generalized body tremor without rearing and falling.
Stage 4 : Rearing, falling and periods of tonic seizures

All experiments were performed between 10a.m. and 6 p.m.

In another set of experiments, 200mg/kg, p.o. DS was administered to two different groups of fully picrotoxin-kindled mice: (i) for eight consecutive days, and (ii) once, with final administration at 30 min. before 1.5 mg/kg of picrotoxin, intraperitoneally.

2.6 Bicuculline–Induced Seizures

In another study, DS (50, 100 or 200 mg/kg) was administered p.o., to different groups of mice (n=8), 30 min. before 10mg/kg, i.p. bicuculline and onset to clonic and tonic seizures, as well as time to death was noted for those mice that convulsed. Mice that did not convulse within 30 min. were considered protected according to a modified method [17]. Control mice received distilled water instead of the extract, and phenobarbitone (40 mg/kg), being a standard drug previously found to be effective against bicuculline seizure [17], served as the reference drug.

2.7 Statistical Analysis

Results are expressed as mean $\pm$ S.E.M. Statistical analysis was performed by analysis of variance (ANOVA); when statistical significance was obtained with ANOVA, a post hoc

Fisher's PLSD test was performed for multiple comparisons. Values of *P* = .05 were considered significant.

3. RESULTS

3.1 DS on Strychnine-Induced Kindling

Repeated administration of the aqueous root extract of *Dalbergia saxatilis*, throughout the experiment from 1st to 9th trials in the treatment group I, did not prevent development, but significantly retarded strychnine-induced kindling progression to the end of the experiment (Fig. 1).

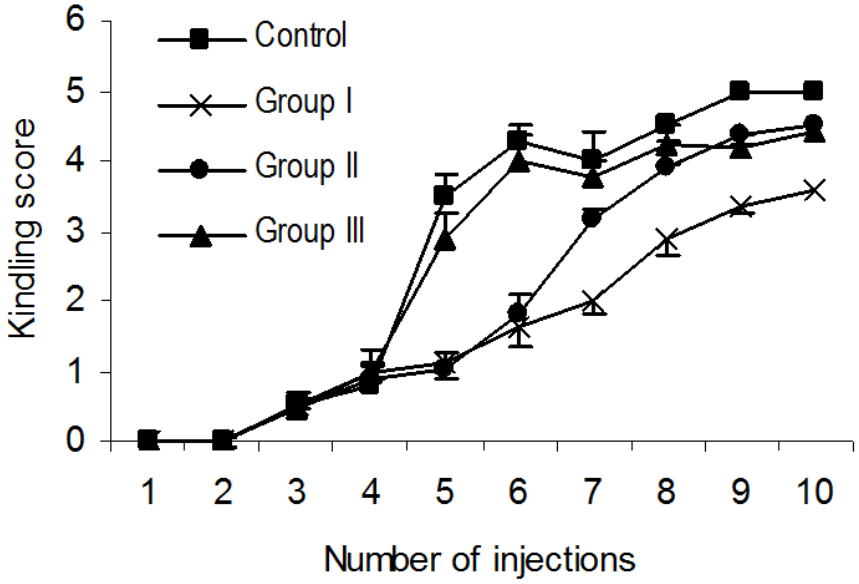

Fig. 1. Graph of kindling score versus number of injections after strychnine

Fig. 1. Represents the progression of kindling score with every 48h, intramuscular dosing of groups of mice. Group I: received DS (200mg/kg, p.o.) throughout, group II: received DS (200mg/kg, p.o.) during 1st – 5th trial; group III: received DS (200mg/kg, p.o.) during 6th – 9th trial; group IV (control) received distilled water (10ml/kg, p.o.). 30 min. before 1.5mg/kg strychnine (n =15). Score 0: normal, no seizure; score 1: restlessness, grooming and biting; score 2: intermittent forelimb myoclonus, without falling; score 3: generalized tremors with jumping and falling; score 4: tonic hind limb extension; score 5: death following tonic seizure.

The extract significantly (*P* = .05) retarded the development of strychnine kindling in mice, decreased the kindling progression and decreased the number of tonic-clonic convulsions. The administration of the extract from the 1st - 5th kindling trials in the treatment group II, retarded the progression of kindling, but there was a rebound progression of kindling from the 6th trial, where the extract was withdrawn and was almost reaching full kindling at the end of the trials (Fig. 1). The extract did not cause any reduction in score, but retarded the rate of progression of kindling till the end of the experiment, when the first administration of the extract was during the strychnine kindling trial, specifically on the 6th kindling trial in the treatment group III (Fig. 1).

3.2 DS on Picrotoxin-Induced Kindling

Repeated administration of the extract to treatment group I prevented kindling till the 16th injection of picrotoxin (1.5 mg/kg), after which the kindling score increased marginally until the end of chronic treatment with picrotoxin, but did not reach full kindling. The administration of extract from the 1st - 12th kindling trials (group II) prevented any development of kindling, but there was a progression of kindling from the 13th trial where the extract was withdrawn and almost reaching full kindling at the end of the trials. Where the first administration of the extract was during the picrotoxin kindling trial, specifically on the 13th kindling trial (group III), the extract caused a sharp decrease in the kindling score till the end of the experiment. The extract significantly (P = .05) retarded the development of picrotoxin kindling in mice, decreased the kindling progression and decreased the number of tonic-clonic convulsions (Fig. 2). The extract prevented the development of convulsion when administered daily for 8-consecutive days, but not when the same dose was administered once, in fully picrotoxin -kindled mice (Table 1).

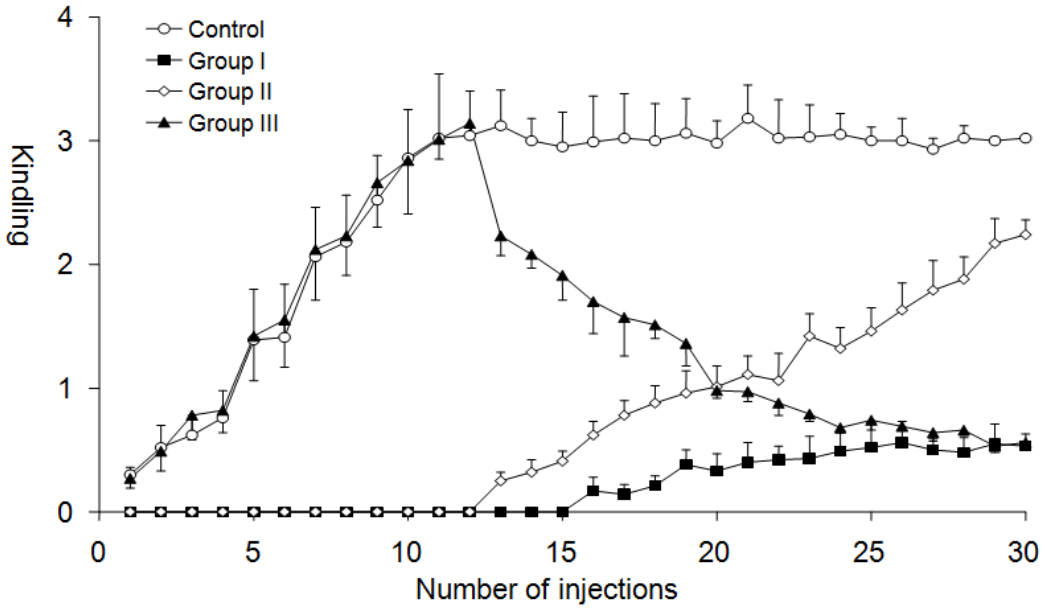

Fig. 2. Graph of kindling score versus number of injections after picrotoxin injections

Fig. 2 represents the progression of kindling score with three times a week (minimum 48-h intervals) 10 consecutive week (30 times) administration of 200mg/kg of the extract orally before picrotoxin thus: Group I: throughout the 1st - 30th kindling trials; group II: During the 1st - 12th kindling trials; group III: during the 13th - 30th kindling trials; group IV (control) received distilled water (10ml/kg, p.o.). 30 min. before 1.5mg/kg picrotoxin (n = 20). Score 0: Normal, no seizure; score 1: initial reduced activity followed by hyperactivity and sniffing; score 2: successive forelimb and head cloni with sharp noise; score 3: generalized body tremor without rearing and falling; score 4: rearing, falling and periods of tonic seizures.

Table 1. Effect of aqueous root extract of *D. saxatilis* on fully picrotoxin-kindled mice.

Substance administered	*Period of administration*	*Kindling score*
Control (distilled water)		*3.09 ±0.10*
200mg/kg Dalbergia saxatilis	*Once*	*2.87 ±0.27*
	8 days	*1.69 ±0.17**

*Dalbergia saxatilis (200mg/kg) and vehicle were singly administered 30 min before or administered for 8 consecutive days with final administration at 30 min before picrotoxin challenge in fully picrotoxin-kindled mice. Values are means ± S.E.M. (n=10). *Significant as compared with control at P =.05 (ANOVA).*

3.3 DS on Bicuculline–Induced Seizures

A significant (P = .05) dose-dependent delay of seizure onset up to 100mg/kg, and complete prevention at 200mg/kg of DS was produced against bicuculline seizures in mice (Table 2).

Table 2. Effect of *D. saxatilis* on bicuculline–induced seizures

Treatment & Dose (mg/kg)	**Onset to clonic seizure (min)**	**Onset to tonic seizure (min)**	**Time to death (min)**
Distilled water (10ml/kg)	12.55 ± 1.31	21.30 ± 2.71	23.13 ± 2.29
DS (50)	13.10 ± 1.64*	29.56 ± 2.09*	36.52 ± 3.12*
DS (100)	22.18 ± 2.63*	--	--
DS (200)	--	--	--
Phenobarbitone (40)	--	--	--

** Significant at P = .05, Student's t test.*

Table 2. showing latency to clonic and tonic seizures and time to death. A significant (P<0.05), dose related prolongation of onset and time to death were produced, but a complete prevention of seizure was produced by 200mg/kg of DS like phenobarbitone. DS = aqueous root extract of Dalbergia saxatilis; Control mice received 10ml/kg distilled water (n = 8).

4. DISCUSSION

The (anti-) kindling model is useful in determining anticonvulsant activity and has been reported to offer several advantages in the screening of potential anticonvulsant drugs [8]. The reliability of this model has been demonstrated and proven for epileptogenesis and developed seizures [6,7]. *Antiepileptogenesis* is a pharmacological phenomenon that describes the prevention of the biological processes that lead to chronic epilepsies [4]. Strychnine is an antagonist of the glycine inhibitory action in the central nervous system [18]. Therefore, the antiepileptogenic effect of strychnine induced kindling produced by DS, suggests that DS, can aid the retardation of the rate and/or extent of convulsions in susceptible patients. This is also the case for DS in the picrotoxin kindling, where the enhancement of GABA inhibition is suggested. In support of this, we have previously documented the anticonvulsant effects of DS in strychnine and picrotoxin [2] induced acute seizures. These effects, hereby support our previous report that the central inhibitory actions of the aqueous root extract of *Dalbergia saxatilis* might involve both GABAergic and glycinergic neurotransmissions [2]. It is however noteworthy that the concept of kindling is expected to alleviate neurodegeneration and enhance the protection of brain neurons, a term referred to as *neuroprotection* [19]. Based on this, it is thought that at least part of the mechanism of DS antiepileptogenic action could involve neuroprotection and're-raising' the seizure threshold in implicated brain disorders. The involvement of specific mechanism was

not ascertained in our previous studies, but bicuculline used as a convulsant in this study has been established to produce its convulsant action by antagonising the central inhibitory action of *gamma amino butyric acid* (GABA) at the $GABA_A$ receptor sites [18]; the GABAergic action of DS might involve this specific receptor site. In our laboratory, preliminary phytochemical screening on DS revealed the presence of saponins, tannins, soluble carbohydrates, glycosides and phenols [2]. Previous studies have identified saponins and glycosides as predominant components of plants in that family [20]. Specifically in a series of studies by one researcher, triterpenoid glycosides have been isolated from *Dalbergia saxatilis* root extract and found to be biologically active [21-23]. Furthermore, the influence of tannins and/or O-glycosides on the anticonvulsant effects of DS is presently being established in our laboratory.

5. CONCLUSION

In conclusion, these findings suggest that the aqueous root extract of *Dalbergia saxatilis* could protect against the development, progression and clinical manifestations of convulsions, particularly in epileptics, probably by promoting neuroprotection in susceptible patients which therefore, provide a justification for the use of the root extract to manage some forms of epilepsies in traditional medicine.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that “principles of laboratory animal care” (NIH publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the research and ethics committee of the college of medicine of the University of Lagos, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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