Original Research Article

PHYTOCHEMICAL AND ANTI-EPILEPTIC STUDIES OF *BOSWELLIA DALZIELII* (FRANKINCENSE TREE) ETHANOL STEM BARK EXTRACT

Abstract

Aim: This research work aimed to establish scientific basis for the use of *Boswellia dalzielli* stem bark, in traditional medicine as anti-epileptic medication.
Study design: The study is of two (2) phases. Phase I (Chemical Analysis): The stem bark of *Boswellia dalzielii* is to be extracted, screened for phytochemicals, Phase II (Pharmacological studies): The extract is to be subjected to toxicity study using Lorke's method and Pentylenetetazole-induced convulsion in rats and maximal electroshock induced seizure (MEST) in Chick.
Place and duration of Study: The study was carried in the Department of Pharmacology laboratory, Faculty of Pharmacy, University of Maiduguri, Borno State between January, 2018 to June, 2018.
Methodology: The ethanol stem bark of the plant was extracted and screened for phytochemicals. Acute toxicity study was carried out using Lorke's method and the antiepileptic activity was evaluated using maximal electroshock induced seizure test in day-old chicks and pentylenetetrazole (PTZ) using mice.
Result: Phytochemical screening of ethanol stem bark extract of *B. dalzielii* revealed the presence of saponins, tannins, flavonoids and steroids/terpenoides. The intrapertoneal median lethal dose value (LD50) of BDE in mice was 2592.3 mg/kg, indicating the stem bark extract is relatively safe. The extract at the dose of 500 mg/kg body weight protected 40% of animals against PTZ-induced convulsion and also protected 20% of chicks against Tonic Hindlimb Extension (THLE) phase of the Maximal Electroshock Test (MEST) significantly ($p<0.05$).
Conclusion: The antiepileptic investigation suggests that ethanol stem bark extract of *B. dalzielii* has antiepileptic activity.

Keywords: *B. dalzielii, Phytochemical antiepileptic, pentylenetetrazole (PTZ), maximal electroshock induced seizure (MEST)*

INTRODUCTION

Epilepsy is a common chronic neurological disorder. Around 50 million people in the world have epilepsy and approximately 5% of the general population experience at least one seizure (excluding febrile seizure) during their lifespan [1]. Currently available antiepileptic drugs (AED) are synthetic molecules that have serious adverse effects such as weight gain, hepatotoxicity, teratogenicity and withdrawal symptoms [2]. Pharmacotherapy of epilepsy with available AED is symptomatic as these drugs inhibit seizure and do not cure the underlying disease process in the brain [3]. Globally, about 2.4 million people are diagnosed with epileptic condition each year [4]. In spite of the introduction of valuable antiepileptic drugs, there is no known cure for epilepsy and relapse is still extremely high [5]. This has necessitated the search within the plant kingdom for a new drugs and lead compounds in the treatment of many neurological disorders including epilepsy [6].

Boswellia dalzielii is popular plant in the Northern part of Nigeria due to its ethno medicinal importance. The decocted root bark is used traditionally by the Hausa-Fulanis in Sokoto, Nigeria to treat diabetes, the bark is boiled up in large quantity to make a wash for fever, rheumatism etc., the fluid is taken internally for gastrointestinal troubles, the Fulanis use a cold infusion for snake bite, the fresh bark of the root is eaten in Adamawa State, Nigeria, to cause vomiting after a few hours and thus relieves symptoms of giddiness and palpitations as well as antidotes to arrow-poison [7,8,9]. This research aimed at establishing a scientific fact regarding the use of *B. dalzielii* for the treatment and management of epilepsy by the local people in Northern Nigeria with the view of identifying an anti-epileptic lead plant.

MATERIALS AND METHODS

Plant collection

The fresh stem bark of *Boswellia dalzielii*, was collected from Lassa, Askira-Uba Local Government Area, Borno State. The plants were identified and authenticated by Professor S.S. Sanusi; a Taxonomist with the Department of Biological Sciences, University of Maiduguri, Borno State Nigeria. A voucher specimen number of 012A was assigned to the plant and was deposited for future reference.

Preparation of the Plant Extract

The stem bark of *B. dalzielii* was air dried at room temperature for 2 weeks and was size-reduced into coarse powder using pestle and mortar the powdered plant material (200 g) each) was defatted with petroleum ether (400ml) for 24 hours using soxhlet extractor. The marc was air dried and macerated with (400ml) of ethanol (99% $^{v}/_{v}$) for 4 days with occasional shaking. The filtrate was evaporated to dryness *in vacuo* at 40^{o}C and stored in a desiccator. The extract was subsequently referred to as *Boswellia dalzielii* extract (BDE*).* A fresh aqueous suspension of the extract in 2% tween-80 was prepared for each study.

Phytochemical screening

The screening was done in accordance with the standard protocol describe by Evans [10]. The extract was screened for the presence of alkaloids, tannins, flavonoids, saponins, anthraquinones, terpenoids, cardiac glycosides, and carbohydrate.

Animals

Male and female Swiss albino mice (19-21 g) were maintained at the Animal House of the Department of Pharmacology and Toxicology, University of Maiduguri and used for the study. They were housed in a well-ventilated cage, fed with standard laboratory feed (wheat offal). All experiments were conducted in accordance with the National Institute of Health Guidelines for the Care and use of Laboratory Animals (NIH Publications No.80-23) as revised in 1996.

Drugs and Drug Solutions

Pentylenetetrazole was purchased from Sigma Chemical Co. (St. Louis. USA). Sodium valproate (Fawdon Manufacturing Centre, Newcastle-upon-Tyne, UK) and Phenytoin (Manfes Pharmaceutical Limited, Nigeria). The drug solutions were prepared fresh for each day's experiment to maintain stability of the drugs used. The solutions were kept in air-tight, amber coloured containers and stored in the refrigerator ready for use.

Routes of Drug Administration

The extract, phenytoin and sodium valproate were administered intraperitonealy while pentylenetetrazole was administered subcutaneously

Pharmacological Studies

Acute Toxicity Study

The acute toxicity of the BDE crude extract was investigated in mice using intra-peritoneal route. The method used was as described by Lorke [11]. The study was carried out in two phases, in the initial phase 3 groups of three mice each were treated with the extract of the plant at doses of 10, 100 and 1000 mg/kg body weight *i.p* and observed for signs of toxicity and death for 24 hours. In the second phase, 3 group each containing one mouse was injected with four more specific doses of the extract based on result obtained in phase I. The LD_{50} value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived ($^0/_1$ and $^1/_1$).

Pentylenetetrazole-Induce Seizure in Mice

Twenty-five mice (18-21g) were divided into five groups each containing five mice. The first three groups received BDE (100, 250 and 500 mg/kg) the fourth group (valproic acid 200mg/kg) and the fifth group received 10 mL normal saline per kg body weight intraperitoneally respectively. Thirty minutes later, mice in all the groups received 60 mg/ kg of pentylenetetrazole subcutaneously and were observed over a period of 30 minutes. Absence of a clonic spasm of at least 5 seconds duration indicated a compounds ability to abolish the effect of pentylenetetrazole on seizure threshold [12].

Maximum Electroshock Induced Seizure in Chick

The BDE was administered to groups of chicks (23-32g) in doses ranging from 100-500 mg/kg *i.p*. Group one received vehicle while the second group received phenytoin (20 mg/kg *i.p*) as a reference standard. Thirty minutes after pretreatment, maximal electroshock was administered to induce seizure in the chicks using Ugobasile Electroconvulsive machine (model 7801) connected to Claude Lyons stabilizer with corneal electrodes placed on the upper eyelids of the chicks. The current, shock duration, frequency and

pulse width were set and maintained at 90 mA, 0.80 second, 200 pulse/second and 0.8 m/seconds respectively. The ability to prevent this feature or prolong the latency and/or onset of the tonic hindlimb extension was considered as an indication of an anticonvulsant activity [13].

Statistical Analysis

The results pharmacological investigations were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. A $p<0.05$ was considered significant.

Results

Extraction Profile

The extractive values of *B. dalzielii* stem bark was found to be 12.16 g with yield of 6.08%, (Table 1).

Table 1: Extraction profile of *Boswellia dalzielii* ethanol stem bark extract

Plant	Yield (g)	Percent (%) yield
B. dalzielii	**12.16**	**6.08**

Phytochemical screening

Phytochemical screening of ethanol stem bark extract of *B. dalzielii* revealed the presence of saponins, tannins, flavonoids and steroids/ terpenoides among other phytochemical constituents (Table 2).

Table 2: Phytochemical Screening of *B. dalzielii*

S/No.	Test	Inference
1.	Test for carbohydrate	
	Test for free reducing sugar-Fehling's test	+++
2.	Test for soluble starch	-
3.	Test for tannins	
	Ferric chloride test	++
	Lead acetate test	++
4.	Test for Anthraquinones	-
	Test for combined anthraquinone	-
5.	Test for cardiac glycosides	
	Salkowski test	++
	Liebermann-Burchard test	+++
6.	Test for terpenoids	+++
	Frothing test	++
7.	Test for saponins	
	Frothy test	+++
8.	Test for flavonoid	
	Lead acetate test	++
	Sodium hydroxide test	-
9.	Test for alkaloid	-
	Dragendorff's reagent	-

Key: (+) present in low concentration, (++) present in moderate concentration, (+++) present in higher concentration, (-) absent.

Acute Toxicity

The signs and symptoms observed in the test animals injected intraperitoneally with crude extract of *B. dalzielii* were decreased mobility, decreased activity and death. The intraperitoneal median lethal dose value (LD_{50}) of BDE in mice was 2592.3 mg/kg bogy weight (Table 3).

Table 3: LD_{50} value of ethanol stem bark extract *B. dalzielii* in mice

Group	Mice	Weight (g)	Dose mg/kg	Death
PHASE I				
1	M1	19	1000	0/3
	M2	21		
	M3	19		
2	M1	20	2000	0/3
	M2	19		
	M3	21		
3	M1	18	3000	3/3
	M2	21		
	M3	19		
PHASE II				
1	M1	19	2200	0/1
2	M2	20	2400	0/1
3	M3	19	2800	1/1

Key: 0/1 & 0/3 (no death), 1/1 & 3/3 (death)

$LD_{50} = \sqrt{2400 \times 2800} = 2592.3$ mg/kg *i.p*

Effect of Ethanol Stem Bark Extract of *B. Dalzielii* on Pentylenetetrazole-Induced Seizure in Mice

To determine the dose dependent effect of ethanol stem bark extract of *B. dalzielii* on pentylenetetrazole-induced convulsion, mice were injected (*i.p*) with 100, 250 and 500 mg/kg of the extract for 30 min before pentylenetetrazole administration.

The crude ethanol stem bark extract of *B. dalzielii* at 100 mg/kg b.d wt. protected 20 % of mice against clonic spasm induced by pentylentetrazole. It also decreased onset of seizure of convulsed mice from 4.40±0.24 min in normal saline treated group to 3.75±0.14 min. Similarly, the extract at the dose of 250 mg/kg body weight protected 40% of the mice were protected. It also significantly ($p<0.05$) increased

onset of seizure of convulsed mice from 4.40±0.24 min in normal saline treated group to 5.33±0.25 min. In addition, the extract at the dose of 500 mg/kg body weight protected 40% of the mice. It also significantly ($p<0.05$) increased onset of seizure of convulsed mice from 4.40±0.24 min in normal saline treated group to 6.00±0.13 min. Valproic acid (200 mg/kg) protected all the mice (100%) against clonic spasm induced by pentylenetetrazole. (Table 4)

Table 4: Effect of ethanol extract of *B. dalzielii* on pentylenetetrazole induced seizure in mice

Treatment (mg/kg)	Onset of convulsion Mean time ±SEM	Quantal Protection	Protection (%)
Control (Vehicle)	4.40±0.24	0/5	0
BDE 100 mg/kg	3.75±0.14	1/5	20
BDE 250 mg/kg	5.33±0.25*	2/5	40
BDE 500 mg/kg	6.00±0.13*	2/5	40
Sodium valproate	0	5/5	100

Values are expressed as Mean±S.E.M., *$p<0.05$

Effect of Ethanol Extract of *B. Dalzielii* on Maximal Electroshock Test (MEST) in Chicks

The ethanol extract of *B. dalzielii* at the dose of 100 mg/kg body weight did not protect chicks against tonic hindlimb extension (THLE) in maximal electroshock test. It however increased the mean recovery time from 7.60±0.70 min (normal saline group) to 9.70±0.73 minutes. Similarly, the extract at the dose 250 mg/kg body weight did not protect the chicks against tonic hindlimb extension (THLE) in maximal electroshock test. It decreased the mean recovery time 7.60±0.70 min (normal saline group) to 6.10±0.35 min. The extract at the dose of 500 mg/kg body weight protected 20% of the chicks against tonic hindlimb extension (THLE) in maximal electroshock test and significantly ($p<0.01$) decreased the mean recovery time from 7.60±0.70 min to 4.75±0.33 min. Phenytoin (20 mg/kg body weight) used as positive control produced 100% protection of the chicks against THLE in MEST (Table 5).

Table 5: Effect of ethanol extract of *B.dalzielii* on Maximal Electroshock Test (MEST) in chicks

Treatment (mg/kg)	Mean recovery time (min)	Quantal Protection	Protection (%)
Control (Vehicle)	7.60±0.70	0/5	0
BDE 100 mg/kg	9.70±0.73	0/10	0
BDE 250 mg/kg	6.10±0.35	0/10	0
BDE 500 mg/kg	4.75±0.33*	2/10	20
Sodium valproate	-	10/10	100

Values are expressed as Mean±S.E.M., *p<0.05, n=10

Discussion

The preliminary phytochemical screening of the ethanol stem bark extract of *B. dalzielii* revealed the presence of saponins, tannins, flavonoids, terpenoids. these results were in accordance with that obtained by Nwinyi et al. [14] and Mamza et al. [15] that reported the presence of tannins and the absence of alkaloids and anthraquinones in the same plant.

Several medicinal plants with reported antiepileptic effect such as *Ficus platyphylla* [6], *Carissa edulis* [16] and *Peristrophe bicalyculata* [17] amongst several others, are largely attributed to the presence of phytochemicals.

Saponins have been shown to produce anticonvulsant activity in animal models [18]. The flavonoid, hispidulin has been reported to act as a positive allosteric modulator across a range of gamma-aminobutyric acid receptor sub-type A ($GABA_A$) [19]. Woo *et al.* [20] also reported the sedative effects of saponins and flavonoids obtained from *Ziziphus spina-christi* seeds. It is therefore, possible that the anticonvulsant activity of BDE may be due to the presence of saponins and flavonoids among others which have been shown to be present in the extract.

The acute toxicity study revealed a relatively high median lethal dose (LD_{50}) of 2592.3 mg/kg of BDE, suggesting that *B. dalzielii* is relatively safe.

The antiepileptic investigation suggests that ethanol stem bark extract of *B. dalzielii* has antiepileptic activity. The extract at the dose of 500 mg/kg body weight protected 40% of animals against PTZ-induced convulsion. The result demonstrates that *B. dalzielii* stem bark may have the potential of raising seizure threshold. It may, therefore be beneficial in the treatment of myoclonic and absence seizures [21]. Pentylentetrazole is a known convulsant and anticonvulsant activity in subcutaneous PTZ test identifies compounds that can raise the seizure threshold in the brain [22]. It may be exerting its convulsant effect by inhibiting the activity of GABA at $GABA_A$ receptors [23]. GABA is the major inhibiting transmitter which is implicated in epilepsy. The enhancement and inhibition of transmission of GABA will attenuate and enhance convulsion, respectively [24]. Antiepileptic drugs (AEDs) are effective in the therapy of generalized seizures of (absence or myoclonic) petitmal type such as ethosuximide (ETX), valproic acid (VPA), phenobarbitone (PHB), and benzodiazepine (BDZ) exhibit dose-dependent suppression of various seizure patterns induced by PTZ [21]. At the cellular level, one of the basic mechanisms of actions of AEDs such as ETX and VPA is the suppression of T-type calcium current in thalamic neurons [25,26].

The ethanol stem bark extract of *B. dalzielii* at the dose of 500 mg/kg body weight protected 20% of animals against THLE phase of the MEST and significantly ($p<0.05$) decreased the mean recovery time of THLE phase of the MEST. The moderate activity of BDE against MEST suggests that it possesses the ability to abolish MEST seizure spread. MEST is a standard AED test that evaluates the testing material's ability to protect against hindlimb tonic extension phase of the MEST [27].

The MEST is a model for generalized tonic clonic seizure which is highly reproducible with a consistent endpoint [28]. AEDs that suppresses the THLE in MEST are effective in the therapy of generalizes tonic seizures and partial seizures.

Protection against tonic hindlimb extension (THLE) in the maximal electroshock test (MEST) predicts anticonvulsant activity of antiepileptic drugs that prevent the spread of the epileptic seizure discharges

from an epileptic focus during seizures. Compounds such as phenytoin, cabamazapine, oxcarbamazapine and lamutrigine suppresse THLE in MEST [29].

Conclusion

This study suggests that *Boswellia dalzielii* ethanol stem bark extract contains phytochemicals which might be responsible for the anticonvulsant properties and leads credence to the traditional use of the plant in the management of epilepsy.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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