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Research Article

Anticonvulsant activity of methanol stem bark extract of *Boswellia dalzielii* Hutch. (Burseraceae) in mice and chicks

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Background: *Boswellia dalzielii* is a widely used medicinal plant in African traditional medicine. The efficacy of its stem bark extract in management of convulsions is well acclaimed among communities of Northern Nigeria.

Objective: To evaluate the anticonvulsant potentials of methanol stem bark extract of *Boswellia dalzielii* in mice and chicks.

Methodology: Phytochemical screening, elemental analysis and acute toxicity studies was carried out. The extract was evaluated for anticonvulsant activity against electrically-induced seizures in chicks and against pentylenetetrazole, strychnine, picrotoxin and 4-aminopyridine-induced seizures in mice at doses of 20, 40 and 80 mg/kg.

Results: The intraperitoneal LD₅₀ was estimated to be 280 and 570 mg/kg in mice and chicks respectively. The extract at 20 mg/kg provided 40% protection and significantly ($p < 0.05$) increased the mean onset of seizure in MEST. A dose-dependent and significant ($p < 0.05$) increase in the mean onsets of pentylenetetrazole and strychnine-induced seizures were produced by the extract at 80 mg/kg. Similarly, a dose-dependent and significant increase ($p < 0.05$ and $p < 0.01$) in latency to picrotoxin-induced convulsions was observed at 40 and 80 mg/kg respectively.

Conclusion: These findings suggests the methanol stem bark extract of *Boswellia dalzielii* possesses anticonvulsant activities and thus supports the ethnomedical rationale for its use against convulsions.

Keywords: Anticonvulsant, *Boswellia dalzielii*, Epilepsy, Pentylenetetrazole, Picrotoxin

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1. Introduction

Epilepsy is one of the most common neurological disorders affecting people of all ages, race and social class; and imposing significant economic implications in terms of health care needs, premature death and loss of work productivity (WHO 2017). There are an estimated 50 million people with epilepsy globally, of which 75-80% of such people live in low and middle-income countries with little or no access to medical services or

treatment (Ngugi et al, 2010; WHO 2017). The prevalence of epilepsy in Nigeria is about 6.2 per 1000 (Banerjee et al, 2009). Although several anticonvulsants are used in management of epilepsy, 30% of epileptic patients do not have seizure control even with the best anticonvulsants (Yemitan and Adeyemi, 2013). The use of such drugs are associated with not only drug interactions but also debilitating adverse reactions including allergies, sedation, blood dyscrasias, teratogenesis, changes in mood and memory problems

(Loscher, 2002; Johannessen Landmark and Patsalos, 2010). Therefore, development of new, affordable and accessible pharmacological agents with better safety and efficacy profiles has become paramount in epilepsy research (Yaro et al, 2015).

Natural products have remained the keystone in drug discovery and will still maintain an important role in this area in the future (Newman and Cragg, 2012, Malami et al, 2016). Many medicinal plants are known for their anticonvulsant activity and their extracts can be important source of chemicals for the development of better and safer drugs for the treatment of epilepsy (Quintans Junior et al, 2008; Kumar et al, 2012). Similarly, the use of herbal medicine in the management of epilepsy is widely accepted in most communities in Nigeria and their efficacies are well acclaimed. Some of these plants remain to be investigated for their value as sources of antiepileptic drugs and therefore, research is needed to validate the folkloric claims of these medicinal plants so as to provide scientific evidence of their safety and efficacy.

Boswellia dalzielii Hutch. is a member of Burseraceae family, a tree that grows up to 13 meters high with a characteristic ragged pale papery bark. It is locally abundant from Northern Ivory Coast to Northern Nigeria and into Cameroun and Central African Republic. It is commonly known as frankincense tree and vernacular names include "Hano" (Hausa) and "Andakehi" (Fulfulde) (Burkill, 1985). In Northern Nigeria, the bark has common applications in treating fever, arthritis, rheumatism and gastro-intestinal problems. The fresh bark is used as an emetic and to relieve symptoms of giddiness and palpitations (Burkill, 1985). Decoction of the stem bark is also used as tranquilizer, in the treatment of convulsions and mental derangement (Ibrahim et al, 2007). To the best of our search, there is no report on the anticonvulsant activity of *Boswellia dalzielii* stem bark in literature and this study therefore was aimed at evaluating its anticonvulsant activity using animal models of epilepsy.

2. Materials and Methods

2.1 Test drugs and chemicals

Pentylentetrazole, Strychnine, Picrotoxin (Sigma Chemical Co., USA) and 4-aminopyridine (Merck-Schuchardt, Germany) were used for the induction of seizure in the experimental animals. Standard drugs used were Phenytoin sodium (Parker-Davis and Co Ltd. Detroit), Phenobarbitone (Lab Renaudin, France) and Sodium Valproate (Sanofi-aventis, UK). Solvents used include Methanol, Petroleum ether, Chloroform and Ethyl acetate (Sigma Chemical Co., USA).

2.2 Animals

Albino mice (18-24 g) of either sex obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria were used for the study. One-day old ranger cockerels (30-40 g) were obtained from the National Animal Production and Research Institute, Shika, Zaria. The animals were maintained in a well-ventilated room under ambient temperature and fed on animal feeds (Feeds Masters, Ilorin) and water *ad libitum*. The experiments

performed in this study followed the principles of laboratory animal use and care policy outlined by the ethical committee of Ahmadu Bello University. The experimental protocols were approved by the University Animal Ethics Committee with the approval number: **DAC/IW-OT/013-13**.

2.3 Plant material

The stem bark of *Boswellia dalzielii* was collected in March, 2013, from Galadimawa, Kaduna State, Nigeria. The plant was identified and authenticated by a taxonomist in the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria, and a voucher specimen (Number **2448**) was deposited for future reference.

2.4 Preparation of the plant extract

The stem bark was air dried under shade and then pulverized to coarse powder using pestle and mortar, of which 400 g was extracted with petroleum ether in a soxhlet apparatus. The marc was air dried and then re-extracted to exhaustion with 800 ml of absolute methanol. The extract was then concentrated under reduced pressure to give solid residue and later stored in a dessicator until required in the main study.

2.5 Phytochemical screening

Thin layer chromatographic analysis of the extract was carried out using standard methods (Wagner and Bladt, 1996). The solution of the extract was spotted on silica gel-coated thin layer chromatographic (TLC) plates (4 cm×8 cm) and the plates were developed in a solvent system (Chloroform: Ethylacetate, 5:3). Each plate was sprayed with a specific visualizing reagent to screen for the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins and tannins.

2.6 Elemental analysis

The dried stem bark and methanol extract of *Boswellia dalzielii* were digested by wet digestion method. Briefly, 0.5 g of each of the sample was weighed into a beaker and digested with 10 ml of concentrated nitric acid and concentrated hydrochloric acid in the ratio 3:1 at 200°C. The solution was then filtered into a 50 ml volumetric flask and made up to the mark with distilled water. The digest was transferred to plastic bottles and later analyzed for the presence and levels of Magnesium, Copper, Calcium, Zinc, Nickel, Iron, Cadmium and Manganese using Varian AA240FS spectrophotometer following standard method (AOAC, 1995).

2.7 Acute toxicity testing

The intraperitoneal median lethal dose (LD₅₀) was determined in mice and chicks (Lorke, 1983). The study was carried out in two phases. In the initial phase, three groups each containing three animals (mice or chicks) received the methanol stem bark extract at doses of 10, 100 and 1000 mg/kg body weight and then observed for signs of toxicity and death within 24 hrs. In the second phase, four animals (mice or chicks) were treated with more specific doses (200, 400, 800 and 1600 mg/kg) of the extract respectively and observed for signs of toxicity and death within 24 hours. The LD₅₀

was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose.

2.8 Anticonvulsant studies

Maximal electroshock induced convulsion test in chicks

The methods of Swinyard and Kupferberg (1985) and of Browning (1992) were employed using fifty day-old cockerels which were divided into five groups of 10 chicks each. The apparatus used was Ugo Basile Electroconvulsive Machine (Model 7801, Italy) connected to a stabilizer with corneal electrodes placed on the upper eyelids of the chicks. A day-old chick has an underdeveloped blood brain barrier, thereby facilitating easy passage of drugs and current into the brain. A current (80 mA) which induced tonic seizures in 90% of the control group of chicks was selected. The shock duration, frequency and pulse width was set at 0.8 sec, 100 pulse/sec and 0.6 ms respectively, and was used throughout the study.

The second group of chicks were pretreated with 20 mg/kg of phenytoin, while the third, fourth and fifth groups were pretreated with 20, 40 and 80 mg/kg of the extract respectively all through the intraperitoneal route. Thirty minutes later, these chicks were subjected to maximum electroshock as in the control group. Seizures were manifested as hind limb tonic extension (HLTE). The ability to prevent this feature or prolong the latency and or onset of the HLTE was considered an indication of anticonvulsant activity (Swinyard, 1969).

Pentylenetetrazole (PTZ)-induced convulsion test in mice

The method of Swinyard et al, (1989) was employed. Thirty mice were divided into five groups of six mice each. The first group was pretreated with normal saline (10 ml/kg *i.p.*). The second, third and fourth groups were pretreated with 20, 40 and 80 mg/kg of the extract respectively, while the fifth group was pretreated with 200 mg/kg body weight of sodium valproate *i.p.* Thirty minutes later, mice in all the groups were injected with a convulsive dose of PTZ (90 mg/kg) subcutaneously and were observed for a period of thirty minutes. The absence of a clonic spasm of at least five seconds duration indicates the extract's ability to abolish the effect of PTZ on seizure threshold.

Strychnine-induced convulsion test in mice

The method described by Porter et al, (1984) was employed. Thirty mice were divided into five groups of six mice each, with the first group was pretreated with normal saline (10 ml/kg *i.p.*). The second, third and fourth groups were pretreated with 20, 40 and 80 mg/kg of the extract respectively while the fifth group received 20 mg/kg of phenobarbitone all through the *i.p.* route. Thirty minutes later, mice in all the groups were injected with a convulsive dose of strychnine (1.0 mg/kg) subcutaneously. Abolition of tonic extension jerks of the hind limbs within 30 min after strychnine administration was considered as an indication of anticonvulsant activity.

Picrotoxin-induced convulsion test in mice

In this test, thirty mice were randomly divided into five groups containing six mice each. The first group served as negative control and was pretreated with normal saline 10 ml/kg. The second, third and fourth groups were pretreated with 20, 40 and 80 mg/kg of the extract respectively, while the fifth group was pretreated with 20 mg/kg phenobarbitone all through the intraperitoneal route. Thirty minutes post treatment all mice were treated with 4 mg/kg picrotoxin by subcutaneous route. Immediately after picrotoxin injection mice were observed for the following symptoms during the next 30 min: clonic seizures, tonic seizures and death (Vogel, 2008).

4-aminopyridine (4-AP)-induced convulsion test in mice

Thirty mice were randomly divided into 5 groups each containing six mice. The first group served as control and was pretreated with normal saline 10 ml/kg *i.p.* The second, third and fourth groups were pretreated with 20, 40 and 80 mg/kg of the extract respectively, while the fifth group was pretreated with 20 mg/kg of phenobarbitone all through the intraperitoneal route. Thirty minutes post treatment 4-AP was administered at a dose of 14 mg/kg subcutaneously to each mouse. The mice were observed for 30 min for characteristic behavioural signs, such as hyperactivity, trembling, intermittent forelimb extension, tonic seizures and death. Ability of the extract to protect the mice from lethality within the 30 min observation period was considered as an indication of anticonvulsant activity (Yamaguchi and Rogawski, 1992).

2.9 Statistical analysis

Data were expressed as percentages and as mean \pm standard error of mean (S.E.M.). Difference between means was analyzed by one way analysis of variance (ANOVA); when statistical significance was obtained with ANOVA, Dunnett's post hoc test was performed. Values of $p < 0.05$ were considered significant.

3. Results

Percentage yield of methanol stem bark extract of *Boswellia dalzielii* was 8.66% w/w. Thin layer chromatographic analysis revealed the presence of cardiac glycosides, flavonoids, saponins and tannins.

The elements investigated were found in stem bark of *Boswellia dalzielii* with calcium occurring in the largest proportion (**Table 1**).

Acute toxicity studies

In the first phase of the study, two mice and two chicks died at 1000 mg/kg of the extract following respiratory depression. In the second phase of the study, the highest lethal dose was 400 mg/kg in mice; however, no death of chicks was recorded. The intraperitoneal LD₅₀ of the crude stem bark extract of *Boswellia dalzielii* in mice and chicks was thus estimated to be 280 and 570 mg/kg respectively.

Table 1: Mineral content of the powdered stem bark and methanol stem bark extract of *Boswellia dalzielii*

Elements	Stem bark (ppm)	Methanol extract (ppm)
Magnesium	3.475	5.761
Copper	0.020	0.102
Calcium	56.294	32.505
Zinc	0.360	0.382
Nickel	0.026	0.158
Iron	0.991	0.591
Manganese	0.108	0.051

ppm = parts per million

Effect of methanol stem bark extract of *Boswellia dalzielii* on electroshock-induced convulsions in chicks

The extract at 20, 40 and 80 mg/kg protected 40%, 10%, and 30% of chicks respectively against HLTE induced by the electroshock. This effect was not dose dependent. The extract at 20 mg/kg significantly ($p < 0.05$) increased the mean onset of seizure compared to normal saline group.

However, there was no significant difference in the mean recovery periods. The standard drug phenytoin provided 90% protection against HLTE (Table 2).

Effect of methanol stem bark extract of *Boswellia dalzielii* on PTZ-induced convulsion in mice

Boswellia dalzielii extract produced a dose dependent increase in the mean onset of seizures with significant difference ($p < 0.05$) at 80 mg/kg compared to the saline control group. The 80 mg/kg also afforded 33.33% protection against seizures.

The standard drug, sodium valproate provided 100% protection against PTZ-induced convulsion in mice (Table 3).

Effect of methanol stem bark extract of *Boswellia dalzielii* on strychnine-induced seizures in mice

Boswellia dalzielii extract provided 33.33 % protection against strychnine-induced seizure at 40 mg/kg while at 20 and 80 mg/kg, 16.67 % protection was provided. The extract also significantly ($p < 0.05$) increased the latency of convulsion at 20 and 80 mg/kg compared to control group. The standard drug, phenobarbitone produced 83.33% protection against seizures (Table 4).

Effect of methanol stem bark extract of *Boswellia dalzielii* on picrotoxin-induced seizures in mice

The extract at 40 mg/kg protected 50% of the mice against picrotoxin induced convulsion. It also produced a dose dependent increase in latency to convulsions with significant difference ($p < 0.05$) at 40 mg/kg and $p < 0.01$ at 80 mg/kg compared to the saline control group. The standard drug, phenobarbitone produced 100% protection (Table 5).

Effect of methanol stem bark extract of *Boswellia dalzielii* on 4-AP-induced convulsion in mice

The extract had no effect against 4-AP-induced seizures. However, the standard drug phenobarbitone provided 100% protection against seizure (Table 6).

Table 2: Effect of methanol stem bark extract of *Boswellia dalzielii* on electroshock-induced convulsion in chicks

Treatment (mg/kg)	Mean onset of seizures (sec)	Mean duration of seizure (min)	Quantal protection	% Protection against seizure
NS 10 ml/kg	4.63 ± 0.28	6.11 ± 0.70	1/10	10.00
MEBD 20	7.03 ± 1.04*	5.00 ± 0.37	4/10	40.00
MEBD 40	4.32 ± 0.18	5.56 ± 0.58	1/10	10.00
MEBD 80	4.17 ± 0.42	5.57 ± 0.65	3/10	30.00
PH 20	5.75 ± 0.00	4.00 ± 0.00	9/10	90.00

Values are Mean ± S.E.M., n=10, * denotes significant difference from normal saline group $F \{(3, 27) = 6.15; p < 0.05\}$ - One way ANOVA followed by Dunnett's test, NS = Normal saline, MEBD = Methanol stem bark extract of *Boswellia dalzielii*, PH- Phenytoin

Table 3: Effect of methanol stem bark extract of *Boswellia dalzielii* on PTZ-induced convulsions in mice

Treatment (mg/kg)		Mean onset of seizures (min)	Quantal protection	% Protection against seizure
NS	10 ml/kg	05.00 ± 0.73	0/6	0.00
MEBD	20	06.00 ± 1.79	1/6	16.67
MEBD	40	09.50 ± 2.01	0/6	0.00
MEBD	80	10.75 ± 2.01*	2/6	33.33
SV	200	-	6/6	100.00

Values are Mean ± S.E.M., n=6, * denotes significant difference from normal saline group F {(3, 17) = 2.59; p< 0.05} - One way ANOVA followed by Dunnett's test, NS= Normal Saline, MEBD=Methanol stem bark extract of *Boswellia dalzielii*, SV=Sodium valproate

Table 4: Effect of methanol stem bark extract of *Boswellia dalzielii* on strychnine-induced convulsions in mice

Treatment (mg/kg)		Mean onset of seizures (min)	Quantal protection	% Protection against seizure
NS	10 ml/kg	06.33 ± 0.49	0/6	0.00
MEBD	20	13.00 ± 2.02*	1/6	16.67
MEBD	40	10.00 ± 1.35	2/6	33.33
MEBD	80	10.60 ± 0.75*	1/6	16.67
PBT	20	21.00 ± 0.00*	5/6	83.33

Values are Mean ± S.E.M., n=6, * denotes significant difference from normal saline group F {(3, 16) = 5.51; p< 0.05} - One way ANOVA followed by Dunnett's test, n=6, NS=Normal Saline, MEBD- Methanol stem bark extract of *Boswellia dalzielii*, PBT- Phenobarbitone

Table 5: Effect of methanol stem bark extract of *Boswellia dalzielii* on picrotoxin-induced convulsions in mice

Treatment (mg/kg)		Mean onset of seizures (min)	Quantal protection	% Protection against seizure
NS	10 ml/kg	11.50 ± 0.56	0/6	0.00
MEBD	20	15.67 ± 1.02	0/6	0.00
MEBD	40	19.00 ± 4.00*	3/6	50.00
MEBD	80	20.50 ± 1.59**	0/6	0.00
PBT	20	-	6/6	100.00

Values are Mean ± S.E.M., n=6, significant difference from normal saline group F {(3, 17) = 7.16, MEBD 40 mg/kg *p< 0.05, MEBD 80 mg/kg **p< 0.01} - One way ANOVA followed by Dunnett's test, NS=Normal Saline, MEBD=Methanol stem bark extract of *Boswellia dalzielii*, PBT= Phenobarbitone

Table 6: Effect of methanol stem bark extract of *Boswellia dalzielii* on 4-aminopyridine-induced convulsions in mice

Treatment (mg/kg)		Mean onset of seizures (min)	Quantal protection	% Protection against seizure
NS	10 ml/kg	12.17 ± 1.01	0/6	0.00
MEBD	20	16.60 ± 1.81	1/6	16.67
MEBD	40	12.67 ± 2.51	0/6	0.00
MEBD	80	16.83 ± 2.02	0/6	0.00
PBT	20	-	6/6	100.00

Values are mean ± S.E.M., n=6, no significant difference F {(3, 19) = 1.67} - One way ANOVA, NS = normal saline, MEBD = methanol stem bark extract of *Boswellia dalzielii*, PBT = Phenobarbitone

4.0 Discussion

Phytochemical analysis of the stem bark extract of *Boswellia dalzielii* revealed the presence of phytochemical constituents known to be responsible for

different pharmacologic activities. Flavonoids and saponins for example have been reported to possess anticonvulsant activity (Kavvadias et al, 2004). Thus, the anticonvulsant activities of the stem bark extract of *Boswellia dalzielii* may be due to the presence of

flavonoids and saponins among other phytoconstituents.

Trace elements like manganese, copper and zinc are essential micronutrients and have a variety of biochemical functions. Calcium for example, is an essential macro element required for critical biological functions such as nerve conduction, muscle contraction, cell adhesiveness, mitosis, blood coagulation and structural support of the skeleton (Miller et al, 2001). Hypocalcaemia has been reported to be responsible for 20 to 34% of convulsions especially in children (Cockburn et al, 1973). Studies by Robertson and Smith, (1975) on early neonatal hypocalcaemia had shown that infants who developed clonic convulsions simultaneously with the hypocalcaemia did not develop further convulsions after oral calcium supplements had raised the serum calcium to normal. It is plausible the calcium content of *Boswellia dalzielii* had played a role in the anticonvulsant activities observed.

The acute toxicity studies suggest that the methanol stem bark extract of *Boswellia dalzielii* is moderately toxic in mice following intraperitoneal administration according to classification by Corbett et al, (1984). The doses of the extract used in this study were lower than 30% of the LD₅₀. These doses are relatively safe for ethnopharmacological research (Vongtau et al, 2004).

Boswellia dalzielii extract showed weak protection against maximal electroshock test (MEST). MEST is a standard anti-epileptic drug (AED) test that evaluates the testing material's ability to protect against HLTE phase of the MEST (DeLorenzo et al, 2001; Magaji et al, 2013; Nazifi et al, 2015). Such protection projects anticonvulsant activity of AEDs that prevent the spread of the epileptic seizure discharge from an epileptic focus during seizure activity (Raza et al, 2001). Ability of the extract to significantly prolong the mean onset of seizure and protect the chicks against MEST suggests that it might possess compounds with ability to abolish seizure spread. This further suggests that it may be of value in the treatment of generalized tonic clonic and partial seizures.

PTZ is a known convulsant and anticonvulsant activity in PTZ test identifies compounds that can raise the seizure threshold in the brain (White et al, 1998). PTZ has been shown to interfere with GABA neurotransmitter and the GABA receptor complex (Bum et al, 2001). Antagonism of PTZ induced seizure suggests potentiating effect on GABAergic neurotransmission. Standard antiepileptic drugs like phenobarbitone, sodium valproate and benzodiazepines produce a dose dependent suppression of PTZ-induced seizure (McNamara, 2006). The dose dependent increase in the latency of seizures by the extract against threshold seizure induced by PTZ suggests the presence of bioactive compounds that could be effective in the therapy of absence or myoclonic seizures.

The convulsive action of strychnine is due to its ability to inhibit spinal reflexes of glycine (Sayin et al, 1993) which is an important inhibitory transmitter to motor neurons and interneurons in the spinal cord. Strychnine sensitive postsynaptic inhibition in higher centers of the central nervous system is also mediated by glycine. The significant increase in the latency of strychnine-induced

seizure by the extract may therefore be due to glycine inhibitory potentiation mechanisms. This suggests that the extract may contain compounds that interact with the glycine receptors probably as agonists or enhancing the binding of glycine to its receptors.

Picrotoxin has been reported to cause convulsion by blocking GABA_A receptor-linked chloride ion conductance into the brain cells (Leonard, 2000; Nicoll, 2001). The fact that the extract of *Boswellia dalzielii* protected animals against picrotoxin-induced convulsion further suggests that the plant extract contains compound(s) that facilitate GABA transmission.

4-aminopyridine is a known potassium channel blocker that interferes with all aspect of neuronal excitability, including resting membrane potential, responsiveness to synaptic inputs, frequency adaptation and neurotransmitters release (Wickenden, 2002). Drugs like phenytoin, which block seizure spread are effective antagonists of seizures induced by K⁺ channel blockade, while those with specific actions on other cellular targets may be weak or inactive, presumably because they are unable to attenuate the spread of intense (non-NMDA receptor mediated) excitation evoked by 4-aminopyridine (Yamaguchi and Rogawski, 1992). The absence of anticonvulsant activity against 4-aminopyridine-induced seizures suggests *Boswellia dalzielii* extract may not be interacting with potassium channels in producing its anticonvulsant activity.

5.0 Conclusion

The results obtained suggests the methanol stem bark extract of *Boswellia dalzielii* contains bioactive components with potential anticonvulsant properties, thus supporting its ethnomedicinal use in the treatment of convulsions.

Conflict of Interest declaration

The authors declare no conflict of interest.

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