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Methanol Leaf Extract of *Diospyros mespiliformis* Hochst. offers Protection against Some Chemoconvulsants

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ABSTRACT

Diospyros mespiliformis Hochst (Ebenaceae) is reported to have wide ethnomedical application notably in the management of fever, whooping cough, wounds, pneumonia, syphilis, leprosy and epilepsy among others. This study examined the anticonvulsant activity of its methanol leaf extract at the doses of 50, 100 and 200 mg/kg in pentylenetetrazole, maximal electroshock, strychnine, picrotoxin and 4-aminopyridine induced seizure tests. The intraperitoneal and oral median lethal doses of the extract were estimated in mice, rats and chicks. The extract at doses of 50, 100 and 200 mg/kg protected the mice (50%, 66.67% and 66.67%, respectively) against pentylenetetrazole induced seizures. The extract at all doses tested did not offer protection against maximal electroshock and 4-amino pyridine induced seizures. The extract significantly prolonged the onset of seizure induced by strychnine. The extract protected 83.3% of the mice against picrotoxin-induced seizure at the highest dose tested (200 mg/kg) and significantly ($P < 0.05$) increased the onset of seizure in the unprotected animals at the lower doses (50 and 100 mg/kg). The median lethal dose (LD_{50}) values of *D. mespiliformis* methanol leaf extract was found to be 774.6 mg/kg *i.p.* and greater than 5000 mg/kg *p.o.* in both rats and mice. In chick, the LD_{50} was found to be >5000 mg/kg, intraperitoneally. These results suggest that *D. mespiliformis* leaf extract possesses anticonvulsant activity and provide some scientific justification for the ethnomedicinal use of the leaves of the plant in the management of epilepsy.

Introduction

Epilepsy is a chronic neurological disorder which affect about fifty million people worldwide and forty million of those affected are in developing countries including Nigeria. [1] Major causes of epilepsy include meningitis, tumours and trauma especially road traffic accidents where Nigeria and East African countries present the highest rate of automobile accidents in the world with attendant increases in post-traumatic epilepsy. [2] About 30% of all seizures are said to be provoked by central nervous system (CNS) disorders or insults (e.g. meningitis, trauma, tumours and exposure to toxins. [3] Studies have shown increased prevalence of epilepsy as people age [4, 5] and higher frequency of occurrence in infants and the elderly - the incidence rates are highest in childhood, plateau from the age of 15 to 65 years and rises again among the elderly. [6, 7]
Epilepsy as with other neurological disorders is usually managed but not cured with medications (anti-epileptic drugs) and over 30% of people with epilepsy do not have seizure control even with the best available medication hence surgery may be considered in difficult and refractory cases. [8] It is also worth noting that currently available antiepileptic drugs (AEDs) are usually associated with serious adverse effects on cognition and behavior [9] and this could be problematic since the management of epilepsy requires chronic pharmacotherapy. Though alot of people in developing countries where epilepsy is more prevalent still depend on traditional healing practices and medicinal plants for their daily healthcare needs, [10] a number of these plants with ant-epileptic activity abound that are yet to be scientifically evaluated.

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Diospyros mespiliformis is a tall upright tree that can reach a height of 30 m with over 2 m in girth found in moist places of the Guinean and Sudanian woodlands, throughout the West African region and generally widespread in such localities across Africa except in the Congo Basin. The plant has been reported to have wide applications in traditional medicine which include the use of leaf decoction as a remedy for fever, whooping cough and for wound healings. [11, 12] Stem barks and roots of *Diospyros mespiliformis* are used for microbial infections such as malaria, pneumonia, syphilis, leprosy and dermatomycoses, as an antihelmintic and to facilitate parturition. [13] In Nigeria, a leaf infusion is taken as a mild laxative and as a vermifuge, for fever, dysentery and is applied to wounds as a haemostatic agent. The Hausas chew the leaf and fruit or apply an infusion for gingivitis and toothache [14] and locally in Dembo village, Zaria Kaduna State northern Nigeria, the plant is claimed to be useful in combination with the leaves of *Annona senegalensis* in the management of convulsive disorders (Personal Communication,17th January 2011). The present study investigated the anticonvulsant potential of the methanol leaf extract of *Diospyros mespiliformis* in animals.

Materials and Methods

Collection and identification of plant materials
The leaves of *Diospyros mespiliformis* were collected from Dembo village in Zaria Local Government area of Kaduna State, Northern Nigeria. The plant was identified and authenticated by Mallam Umar Gallah a taxonomist in the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria by comparison with already deposited specimen with voucher specimen number 1611.

Preparation of plant extract
The leaves of *Diospyros mespiliformis* were cleaned, air dried in a shade for 7 days and then crushed into fine powder with a pestle and mortar. The powdered sample (1326 g) was macerated with 7 L of methanol (98%) for 72 hours with occasional mixing. The mixture was filtered using a filter

paper and the filterate was evaporated to constant weight using a water bath set at 50ºC.

Phytochemical screening
The methanol leaf extract of *Diospyros mespiliformis* was subjected to preliminary phytochemical screening to test for the presence of alkaloids, saponins, flavonoids, tannins, anthraquinones cardiac glycosides and carbohydrates according to standard procedures. [15, 16]

Study Animals
Three species of animals-rats of both sexes (weighing 156-247 g), mice (25-34 g) and day old chicks (24-33 g) were employed in this study. The rodents used were obtained from the Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, while the day-old chicks were obtained from the National Animal Production Research Institute (NAPRI) hatchery, Ahmadu Bello University, Shika Zaria. All experimental protocols were in accordance with the Ahmadu Bello University Research policy, and ethic and regulations governing the care and use of experimental animals as contained in "Principles of laboratory animal care" (NIH Publication no. 85-23, revised 1985). Ethical Approval (DAC/IW-OT/009-15) was obtained from Institutional Animal Use and Ethics Committee.

Acute toxicity study
Estimation of the oral and intraperitoneal median lethal doses of extract were carried out in mice, rats and chicks. Briefly, the method was divided into phase I and II. In phase I three groups of three animals each were administered 10, 100 and 1000 mg/kg of the extract respectively to ascertain the extent of toxicity of the extract. The animals were observed for any sign of toxicity or death within 24-hour period. In phase II which depended on the outcome of the first phase, other specifically graded doses were administered to four different sets of rats, mice and chicks using both oral and *i.p* routes as the case apply and observed for sign of toxicity or death within another 24-hour period. From the outcome of phase II, LD_{50} value was determined by calculating the geometric mean of lowest dose that causes death and the highest dose for which the animals survived. [17]

Anticonvulsant Study
General study design
The study was carried out using five groups each containing ten or six animals as the case may be. Groups 1 and 5 served as negative and positive controls using normal saline and a standard drug respectively while groups 2, 3 and 4 received graded doses of the extract. Results obtained for each experiment were recorded accordingly.
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Pentylenetetrazole induced seizure test
Thirty mice of either sex were divided into five groups of six mice each. Mice in group 1 were treated with equivolume of distilled water per kg body weight intraperitoneally, the second, third and fourth group were treated with 50, 100 and 200 mg of the extract per kg body weight *i.p*., the fifth group were treated with 200 mg Sodium valproate per kg body weight *i.p*. Thirty minutes later, mice in all the groups were treated with 90 mg/kg of freshly prepared pentylenetratrazole subcutaneously. The mice were observed for presence or absence of clonic spasm of at least 5 seconds duration, hind limb extension or death. [18]

Maximal electroshock test
The method of Toman *et al.* [19] as modified by Swinyard and Kupferberg [20] was employed. Fifty day old chicks were divided randomly into five groups of 10 chicks per group. The first group was treated with distilled water (10 mL/kg) *i.p*., second, third and fourth groups were treated with 250, 500 and 1000 mg of the extract per kg *i.p*. respectively and the fifth group was treated with 20 mg phenytoin per kg *i.p*. as positive control. Thirty minutes later, maximal electroshock was administered to induce seizure in the chicks using Ugo Basile electroconvulsive machine (Model 7801) with corneal electrodes placed on the upper eyelids of the chicks. The current, shock duration, frequency and pulse width used were maintained at 90 mA, 0.8 s, 100 pulse per second and 0.8 ms respectively. The chicks were observed for hind limb tonic extension which was considered as convulsion while abolition of hind limb tonic extension was considered as protection against electrically induced convulsion.

Strychnine induced seizure test
The method of Porter *et al.* [21] was employed. Thirty mice of either sex were divided into five groups of six mice each. Mice in group 1 were treated with 10 mL/kg of distilled water per kg body weight *i.p*., the second, third, and fourth group were treated with 50, 100 and 200 mg of the extract per kg body weight *i.p*., and the fifth group was treated with 30 mg /kg phenobarbitone *i.p*. as positive control. Thirty minutes later, mice in all the groups were treated with 2.5 mg of freshly prepared strychnine per kg *s.c*. The mice were observed for tonic extensor jerks of the hind limb which was considered as convulsion and abolition of such was considered as protection.

Picrotoxin-induced seizure in mice
Five groups of mice (n=6) were treated with normal saline (10 ml/kg), extract (50, 100 and 200 mg/kg) or phenorbarbitone (30 mg/kg), intraperitoneally. 30 minutes post-treatment, seizure was induced in each mouse by administration of picrotoxin (10 mg/kg), subcutaneously. Absence of tonic hind limb extension or prolongation of the latency of the hind limb tonic extension was considered as an indication of anticonvulsant activity. [22]

4-Aminopyridine induced seizure test
The method of Yamaguchi and Rogawski [23] was employed. Thirty mice of either sex were divided into five groups of six mice each. Mice in the first group were treated with 10 mL normal saline per kg body weight *i.p*. The second, third and fourth groups were treated with 50, 100 and 200 mg of the extract per kg body weight *i.p*. respectively. The fifth group was treated with 30 mg phenobarbitone per kg body weight *i.p*. Thirty minutes later, mice in all the groups were treated with 14 mg of freshly prepared 4-aminopyridine per kg *s.c*.. The mice were observed for presence or absence of hind limb tonic extension, onset of episodes of convulsion and possibly death, abolition of any of these was considered as protection.

Statistical Analysis
Results were expressed as Mean ± Standard Error of Mean. Statistical analysis was performed by analysis of variance (ANOVA); when a statistically significant result was obtained with ANOVA, a post hoc Dunnets t-test was performed for multiple comparisons. Values of $p < 0.05$ were considered significant.

Results and Discussion

Diospyros mespiliformis leaves enjoys wide patronage among traditional practitioners in Northern Nigeria. The present study attempted to provide pharmacological rationale for the ethnomedicinal use of the leaves of *Diospyros mespiliformis* in the management of epilepsy. The preliminary phytochemical studies on the methanol leaf extract of *Diospyros mespiliformis* revealed the presence of alkaloids, saponins, flavonoids, tannins, carbohydrates, cardiac glycosides and the combined anthracene type of anthraquinones (Table 1). Flavonoids, tannins and saponins are phytoconstituents that have been reported to modulate central nervous system activities. [24, 25] Similarly, some alkaloids (nantenine derivatives) have been found to be effective in inhibiting pentylenetetrazole induced seizure and maximal electroshock-induced seizures [26] hence, it can be inferred that the observed pharmacological activity shown by the extract may be due to the presence of the afore mentioned phytoconstituents. The LD_{50} values of the extract in mice and rats when administered via oral route were above 5000 mg/kg each while for the intraperitoneal route of administration, the LD_{50} was found to be 774.6 mg/kg for both mice and rats. In chicks, the LD_{50} value was found to be above 5000 mg/kg body weight via the intraperitoneal route of administration (Table 2).
The extract protected the animals against PTZ-induced seizure. At 50 mg/kg of the extract, the percentage protection was found to be 50% which then increased to 66.7% protection at a dose of 100 mg/kg, additional protection was not observed at higher doses. Sodium valproate (200 mg/kg) produced 100% protection against seizure and mortality as well. There was no statistically significant difference in the mean onset of seizure between the control group and extract-treated groups (Table 3). Studies have shown that pentylenetetrazole is a chemoconvulsant agent that induces seizure by the non-competitive blockage of the major inhibitory pathways mediated by the predominant inhibitory-neurotransmitter GABA, at all levels of the central nervous system [27] and is widely accepted experimental model for absence seizure. [28] The GABAergic system represents the most successful target for the rational design of novel antiepileptic compounds. [29] It has been shown that seizures induced by pentylenetetrazole, can be blocked by drugs such as ethosuximide that reduces T-type Ca^{2+} currents [30] while drugs such as phenytoin and carbamazepine that were found to be effective against maximal electroshock seizure are ineffective against pentylenetetrazole induce seizure. [31] Activation of NMDA receptor system appears to be

Table 1: Phytochemical constituents of Methanol Leaf Extract of *Diospyros mespiliformis*

Chemical Constituents	Inference
Alkaloids	Present
Saponins	Present
Flavonoids	Present
Tannins	Present
Carbohydrates	Present
Cardiac Glycosides	Present
Anthraquinones (combined anthracene)	Present
Anthraquinones (free)	Absent

Table 2: Median Lethal Dose Values of Methanol Leaf Extract of *Diospyros mespiliformis* via Intraperitoneal and Oral routes of administration

Species	Route of Administration	LD_{50} Values (mg/kg)
Mice	Intraperitoneal	774.6
	Oral	>5000
Rats	Intraperitoneal	774.6
	Oral	>5000
Chicks	Intraperitoneal	>5000

Table 3: Effect of Methanol Leaf Extract of *Diospyros mespiliformis (DM)* and Sodium Valproate on Pentylenetetrazole (PTZ) induced seizure in mice

Treatment (mg/kg)	Mean onset of seizures (min)	Quantal Protection	Protection against seizure (%)	Mortality rate (%)
N/saline (10 ml/kg)	9.00±1.00	1/6	16.67	83.33
DM 50	11.00± 3.46	3/6	50.00	50.00
DM 100	6.50 ±1.50	4/6	66.67	33.33
DM 200	10.00 ±4.00	4/6	66.67	33.33
VA 200	0.0	6/6	100.0	0.00

Values are presented as mean ± SEM, n=6 per group, DM=*Diospyros mespiliformis* Sodium valproate.

Table 4: Effect of Methanol Leaf Extract of *Diospyros Mespiliformis* (DM) and Phenytoin (PHT) on maximal electroshock (MES) – induced seizure in chicks

Treatment (mg/kg)	Mean Time of Recoveryfrom seizures (min)	Quantal Protection	Protection against seizure (%)	Mortality rate (%)
N/saline (10 mL/kg)	14.8 ±5.09	0/10	0.00	0.00
DM 250	11.90± 4.88	0/10	0.00	0.00
DM 500	9.10 ±1.54	0/10	0.00	0.00
DM 1000	11.90 ± 3.30	0/10	0.00	0.00
PHT 20	17.0	9/10	90.00	0.00

Values are presented as mean ± SEM, n=10 per group DM= *Diospyros Mespiliformis* PHT=Phenytoin.

Table 5: Effect of methanol leaf extract of *Diospyros mespiliformis* (DM) and phenobarbitone (PHB) on strychnine induced seizure in mice

Treatment (mg/kg)	Mean onset of seizures (min)	Quantal Protection	Protection against seizure (%)	Mortality rate (%)
N/saline (10 mL/kg)	2.67±0.33	0/6	0.00	100.00
DM 50	5.33±0.71	0/6	0.00	100.00
DM 100	6.00±0.37	0/6	0.00	100.00
DM 200	5.50±0.50	0/6	0.00	100.00
PHB 20	0.0	5/6	83.33	16.67

Values are presented as mean ± SEM, n=6 per group, DM = *Diospyros mespiliformis*, PHB = phenobarbitone. There is no statistically significant difference in the mean onset of seizure between the control (Normal saline) group and the treated groups given 50, 100 and 200 mg/kg of the extracts at **p*<0.05 (Dunnet's post hoc test for multiple comparison).

Table 6: Effect of methanol leaf extract of *Diospyros mespiliformis* (DM) and phenobarbitone (PHB) on Picrotoxin-induced seizure in mice

Treatment (mg/kg)	Quantal protection	Mean onset of Seizure in Unprotected Animals
N/saline (10 ml/kg)	0/6	9.91 ± 0.52
DM 50	2/6	13.3 ± 0.62*
DM 100	3/6	12.9 ± 1.2*
DM 200	5/6	10.0
PHB 30	6/6	-

Values are presented as mean ± SEM, n=6 per group, DM= *Diospyros Mespiliformis*, PHB =phenobarbitone. **p*<0.05 (Dunnet's post hoc test for multiple comparison).

Table 7: Effect of methanol leaf extract of *Diospyros mespiliformis* (DM) and phenobarbitone (PHB) on 4-amino pyridine induced seizure in mice

Treatment (mg/kg)	Mean onset of seizures (min)	Quantal Protection	Protection against seizure (%)
N/saline (10 mL/kg)	13.67±1.69	0/6	0.00
DM 50	20.67± 3.21	0/6	0.00
DM 100	15.33 ± 2.96	0/6	0.00
DM 200	16.00 ±2.49	1/6	16.67
PHB 30	0.0	6/6	100.00

Values are presented as mean ± SEM, n=6 per group, DM = *Diospyros mespiliformis*, PHB = Phenobarbitone. There is no statistically significant difference in the mean onset of seizure between the control (normal saline) group and the groups given 50, 100 and 200 mg/kg of the extract respectively at *p*<0.05 (Dunnet's post hoc test for multiple comparison).

involved in the initiation and propagation of pentylenetetrazole-induced seizures. [32] It has also been reported that agents that block glutamatergic excitation mediated by NMDA receptors such as felbamate also showed anticonvulsant activity against pentylenetetrazole-induced seizure. [33] It is therefore plausible to suggest that the observed anticonvulsant activity shown by the extract against pentylenetetrazole induced seizure might be due to either activation of GABA neurotransmission or blockade of NMDA mediated glutamatergic neurotransmission in the central nervous system. Anticonvulsant activity in the subcutaneous PTZ test identifies compounds that can raise seizure threshold in the brain [33] and that agents such as phenorbabitone and benzodiazepines that were found to be effective in the management of generalized seizures of (absence or myoclonic) petil mal type are capable of raising seizure threshold induced

by PTZ. [28] The extract therefore can be said to possess some bioactive phytoconstituents that may be effective in the management of absence or myoclonic seizures.

The extract did not confer protection against MES-induced seizure in chicks at all the doses of the extract tested, while phenytoin (20 mg/kg) demonstrated 90% protection against seizure. There was no statistically significant difference observed in the mean recovery time from seizure between the control (normal saline) group and treated groups (Table 4). The maximal electroshock test is probably the best validated preclinical test that predicts drugs effective against generalized seizure of the tonic clonic (grand mal) type. [34-36] The model permits evaluation of the ability of a substance to prevent seizure spread through neural tissue of the central nervous system. [20, 28, 36] Antiepileptic agents that act via this pathway are able to limit the repetitive firing of action potentials by slowing the rate of recovery of voltage-activated sodium channels from inactivation and suppress hind limb tonic extension in maximal electroshock seizures. [30] In other words, the maximal electroshock tests predict drugs acting on sodium channels e.g. carbamazepine and phenytoin [37, 38] and these agents are ineffective against PTZ induced seizure. [31] The findings that the extract showed activity against pentylenetetrazole induced seizure and did not show any activity against maximal electroshock-induced seizures agrees with previous works which showed that agents that are effective against pentylenetetrazole induced seizure do not alter maximal electroshock (MES) thresholds. [31] The inability of the extract to protect the chicks against maximal electroshock seizure possibly signifies that the extract does not act on sodium channels and hence may not be effective in generalized tonic clonic and partial seizures.

Against strychnine, the extract demonstrated statistically significant ($P < 0.05$) difference in the latency of death by prolonging the onset of seizure at the tested doses. However, the extract did not protect the animals against seizure and lethality at all the tested doses (Table 5). The ability of the extract to delay onset of seizure in the strychnine test showed that the extract has the ability to raise strychnine-induced seizure threshold. Strychnine and related alkaloids such as brucine and thebain induce generalized convulsion by selectively and competitively blocking postsynaptic neurotransmission mediated by glycine, an important inhibitory neurotransmitter to motor neurones and interneurones in the spinal cord. [39] Agents that reverse the action of strychnine (an antagonist of glycine) have been shown to have antiepileptic effects. [40] The ability of the extract to exert activity by delaying the onset of strychnine induced convulsion and death may be a strong indication of glycine activation in the central nervous system.

The extract protected 83.3% of the mice against picrotoxin-induced seizure at the highest dose tested (200 mg/kg). There was also a significant ($P < 0.05$) increase in the onset of seizure in the unprotected animals at the lower doses (Table 6). Picrotoxin, a non-competitive $GABA_A$ receptor antagonist selectively blocks chloride channels. The ability of the extract to protect against picrotoxin-induced seizure suggests that its anti-convulsant action may involve interaction with picrotoxin sites on $GABA_A$-chloride ion channel complex. [41]

The extract did not offer significant protection (16.67%) against 4-aminopyridine (4-AP) (Table 7). 4-AP induces tonic-clonic convulsions by blocking potassium channels, [23] conversely agents that activate potassium channels have anticonvulsant effects in some experimental seizure models [42] especially MES. It is unexpected therefore that the extract did not protect against both MES and 4-AP seizures. Potassium channels play a vital role in the control of neuronal excitability and seizure susceptibility, and would be of importance for the suppression of seizure initiation and spread. [43] Failure of the extract to protect against 4-AP suggests that it may not be acting via the potassium channels.

Conclusion

Based on the findings of the present study, it could be said that the use of the leaves of *Diospyros mespiliformis* as a traditional remedy for convulsive disorders have some scientific justification. Further study will involve isolating the bioactive components responsible for the observed activities.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

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

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