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RESEARCH ARTICLE OPEN ACCESS

Diospyros mespilifoemis hochst Modulates the Hippocampus and Prefrontal Cortex of Wistar Rat following Lithium chloride pilocarpine-induced Epilepsy

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Abstract
Objectives: Epilepsy is a disease with vast complexity and diverse clinical manifestations causing neuronal misfiring. Diospyros mespiliformis (DM) possesses anti-epileptic properties.
Methods: Fifty rats were used for this study. Group A received normal saline while groups B, C, D, and E received lithium chloride (127 mg/kg, i.p.) + pilocarpine (30 mg/kg, i.p.) to induce seizure. Following induction, C, D, and E received 50 mg/kg, 200 mg/kg DM and 10 mg/kg sodium valproate respectively (p.o) while B was left untreated. Rats were assessed for Open field and radial arm maze tests. Levels of SOD, MDA, catalase glutamate, GABA, IL-6 and TNF-alpha were assayed using appropriate protocols. Histological and immunohistochemistry tests were done.
Results: DM reduced glutamate levels in C, D and E when compared with A and B while there were no significant changes in the levels of GABA across groups. DM significantly reduced IL-6 in C, D and E but not in A and B while there were no significant changes in TNF alpha across groups. DM boosted catalase release than superoxide dismutase to the after-status epilepticus. In the open field test, DM reversed the altered activities in the epileptic rats. DM ameliorates neuronal vacuolation, disorientation and increased reactive gliosis. There was positive NeuN reactivity across groups except in D.
Conclusion: We concluded that DM (probably 1,2,3-Benzenetriol content) can be employed in managing epilepsy as evident in the mitigation of histoarchitecture and the maintenance of the levels of the neurotransmitters.

Keywords: Epilepsy, LiCl-pilocarpine, Animal Model, Diospyros mespiliformis, Immunohistochemistry

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Plain English Summary
Epilepsy is a health condition with high societal stigma. It is a result of an abnormal burst of the electrical components of the brain leading to seizures and consequent damage to the brain tissues if not well managed in good time. The use of medicinal plants in the management of this ailment has a long, yet unverified scientific claim. *Diospyros mespiliformis* (West African ebony) has some novel ingredients that possess anti-epileptic properties. The ability of these constituents to ameliorate the damage caused by epilepsy on the brain is what this study focuses on, to give hope to the affected subjects of this highly stigmatized ailment ravaging our world.

Introduction
Epilepsy is a complex disease with diverse clinical characteristics that affects the brain and causes frequent seizures. Seizures can be described as bursts of electrical activity in the brain that temporarily affect how the brain works. Around 50 million people worldwide have epilepsy, making it one of the most common neurological diseases globally (1). Nearly 80% of people with epilepsy live in low and middle-income countries (1). It is estimated that up to 70% of people living with epilepsy could live seizure-free if properly diagnosed and treated. The risk of premature death in people with epilepsy is up to three times higher than in the general population. Three-quarters of people with epilepsy living in low-income countries do not get the treatment they need. In many parts of the world, people with epilepsy and their families suffer from stigma and discrimination. Intractable form of epilepsy continues to be a major challenge worldwide, and despite an increasing number of medical therapies in the treatment of epilepsy, approximately 1 in 3 patients continue to have seizures (2).
Diospyros mespiliformis, also known as West African ebony is an evergreen tree with a rounded, dense crown; it varies considerably in height, sometimes flowering as a shrub when just 3 m tall, growing 12 - 15 m tall in drier areas of its range and 25 m or more (with reports of trees up to 50 m) in the wetter areas. The tree is an important source of food, medicine, and other commodities for the local people and is often kept intact when land is being cleared for farming (3, 4). The leaves are astringent, febrifuge, haemostatic, mildly laxative, stimulant and vermifuge. An infusion is used in the treatment of a range of conditions like infectious fevers, dysentery, pneumonia, syphilis, leprosy and yaws (4). The hippocampus situated in the temporal lobe of the brain is often the site of epileptic seizures and hippocampal sclerosis is one of the most commonly visible types of tissue damage in temporal lobe epilepsy (5). It is an important structure in the pathophysiology of convulsions and epilepsy. It is one of the most widely studied brain regions in both human and experimental epilepsy and hippocampal sclerosis is one of the most common pathologies (6).
The prefrontal cortex (PFC) on the other hand is associated with high-order cognitive and emotional functions including attention, decision-making, goal-directed behavior, and working memory (7, 8). Studies have shown that even when temporal lobe seizures do not propagate, they may cause remote dysfunction in other regions, leading to neuronal deficits (9, 10). Several neuroimaging studies using single photon emission computed tomography (SPECT) have revealed increased cerebral blood flow (CBF) associated with epileptic activity in the temporal lobe, but a simultaneous decrease in the frontoparietal neocortex (11, 12). Frontoparietal decrease in CBF during temporal lobe seizures in humans was correlated with deficits in consciousness (9). Neuro-inflammation has been reported to contribute primarily to epileptogenesis (13). Its involvement in epileptogenesis can be studied by measuring levels of pro-inflammatory cytokines. Reactive oxygen species (ROS) have been implicated in acting as mediators in the process of neuronal injury (14). The consequential structural alterations within the brain have been reported in epileptic individuals (15). Pro-inflammatory cytokines play a role in these processes not only because they are responsible for the aggravation of immune response, but also because they regulate pro and anticonvulsive neuronal excitability (16).
The present study hypothesized that DM will stop the progression of seizures in animal models of epilepsy and also improve the hippocampus and prefrontal cortex neurons and astrocytes in this model of epilepsy in Wistar rats.

Materials and Methods
Animal care and management
Fifty (50) male Wistar rats (100-120g) were used for this study. They were obtained from the animal house of Babcock University, Ilisan Remo Ogun State. Ethical approval was sought and obtained from the Health Research and Ethics Committee of Babcock University (BUHREC 969/21). The rats were randomly assigned into 5 groups (A, B, C, D, and E) n = 10 and were housed in clean plastic

cages in a clean environment of a natural day/night cycle. Rats in all groups were fed on standard laboratory rat chow and allowed access to water *ad libitum*.

Animal treatments
Group (group A) received 1 mg/ml of normal saline i.p. in the first 48 hours and then continued orally daily throughout the administration.
Rats in groups B, C, D and E were pretreated with lithium chloride (127 mg/kg, i.p.) before being given pilocarpine (30 mg/kg, i.p) 24 hours later to induce epilepsy (17). Seizures were allowed to last for 45 minutes (once the animal had displayed all the five features of Racine scales) and then were terminated by diazepam administration (10 mg/kg, i.m.) to reduce the mortality rate. Rats that displayed status epilepticus (stages 3 - 5) were only selected (18).
After the first seizure was achieved in groups B to E, rats in groups C and D were administered 50 mg/kg and 200 mg/kg leave extract of *Diospyros mespiliformis* (DM) orally daily, group E received sodium valproate (10mg/kg) while group B was left untreated. Treatment lasted for the chronic phase of epilepsy starting on the second day post-induction and ending on the 32nd day (30 days) post-induction.

Racine's scale
Classification of seizure severity was confirmed according to the modified Racine's scale (18).

Plant Identification, Authentication, and Extraction
Fresh leaves of DM were purchased from a local market in Oyo State and authenticated at the Forest Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria (FHL: 11331). The leaves were air-dried at room temperature and pulverized into powder using a mechanical blender with a known weight soaked with 1:1 Hyrdo-ethanol (v/v). The extract was concentrated using a rotary evaporator at 40 ^{O}C and constituted 10 mg/ml. Gas chromatography-mass spectrometry (GC-MS) analysis was carried out at the Shimadzu Training Centre for Analytical Instruments (STC) (GCMS/QP2010SD-SHIMADZU, JAPAN) following the standard methods (19).

Reagents
Lithium chloride pilocarpine was dissolved in normal saline and administered via intraperitoneal injections (i.p.) for all experimental groups. *Diospyros mespiliformis* and Sodium Valproate (an antiepileptic drug) were administered orally. Lithium chloride, pilocarpine, and Sodium Valproate were obtained from Sigma-Aldrich Chemicals, USA.

Neurobehavioral Tests
Rats were subjected to neurobehavioral tests on day 30 post-induction. Open field and Radial arm maze tests were done following epilepsy induction. All neurobehavioral tests were recorded using a webcam camera connected through a cable from a laptop to the behavioural apparatus and later scored manually by at least two independent trained observers.

Animal Sacrifice and Biochemical Studies
At the end of behavioural studies, rats were sacrificed by cervical dislocation and the whole brain was isolated. The brain was homogenized, and its supernatant was preserved at -20^{0}C for assays of brain glutamate, gamma-aminobutyric acid (GABA), interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α) using enzyme-linked immunosorbent assay (ELISA) technique. The absorbance of each sample was measured at 450 nm using a microplate reader. Superoxide dismutase (SOD), malondialdehyde (MDA) and catalase levels were also measured in the brain homogenates using the spectrophotometric method. The histological and immunohistochemical tissues were fixed in 10% neutral buffered formalin for paraffin wax embedding. H & E stain was used for the neuroarchitectural test while NeuN and Glia Fibrillary Acidic Proteins (GFAP) were used to determine neuronal degeneration and astrogliosis respectively. All stained sections were examined using a Leica DM750 microscope interfaced with a digital camera (Leica ICC 50).

Statistical Analysis
Data were expressed as Mean ± SEM and One-way analysis of variance (ANOVA) was used followed by the Student-Newman-Keus post hoc test. P value was set at $P<0.05$ while GraphPad Prism 6 software was the statistical package used.

Results
Table 1 and Figure 1 show the detected compounds and their bioactivity.

Table 1: GC-MS Chromatogram Shows compounds detected in DM

No	Retention time	Name of compound	Peak area %
1	10.601	1,2,3-Benzenetriol	46.70
2	13.107	n-Capric acid isopropyl ester	7.50
3	12.518	Benzoic acid, 4-hydroxy-	7.08
4	8.501	Benzofuran, 2,3-dihydro-	3.92
5	15.883	n-Hexadecanoic acid	3.57
6	17.384	9-Octadecenoic acid, (E)-	2.01

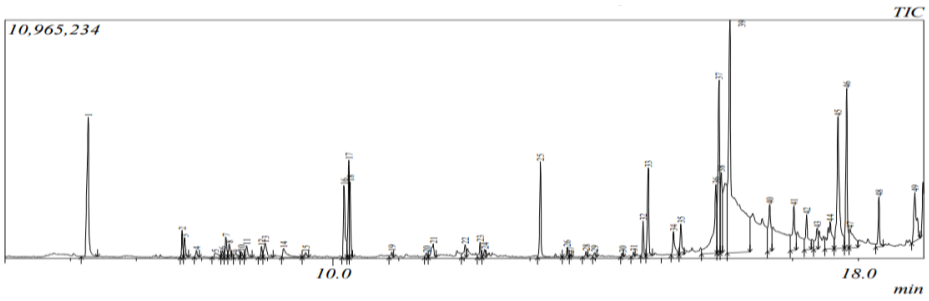

Figure 1: GC/MS Chromatogram shows Bioactivity of Compounds Detected Through Analysis of the Hydroethanolic Extract of DM Leaves

Neurotransmitters and Biochemical Results

Level of Glutamate

The result shows a significant decrease in the levels of glutamate in the epileptic-treated groups when compared with the untreated group (Figure 2).

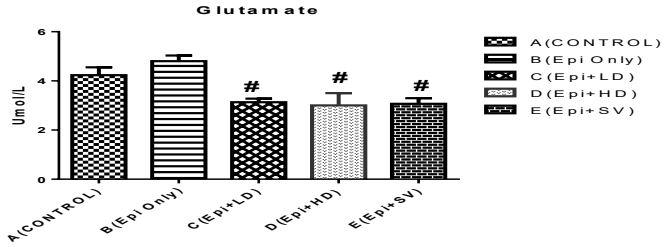

Figure 2: Bar Chart Showing the Effect of DM on the Level of Glutamate in Rats Subjected to Lithium Chloride -Pilocarpine-induced Epilepsy.
 # = significantly different from epilepsy only group. Values are expressed as Mean ± SEM. The figure shows that the epilepsy-only group was significantly higher than groups C, D, and E.

Level of GABA

There was no significant difference when the control was compared to group B and the rest of the treated groups (Figure 3).

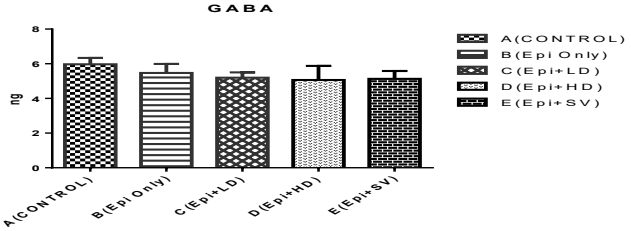

Figure 3: Bar Chart Showing the Effect of DM on the Level of GABA in Rats Subjected to Lithium Chloride -Pilocarpine-induced Epilepsy.

Level of interleukin 6

The figure shows that the epilepsy-only group was significantly higher than groups C, D, and E (Figure 4).

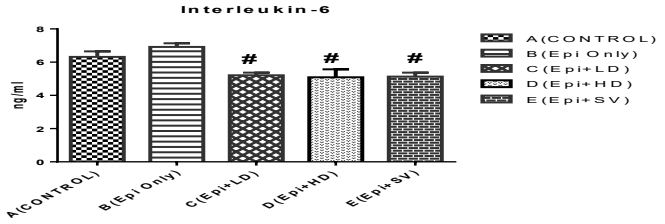

Figure 4: Bar Chart Showing the Effect of DM on the Level of Interleukin-6 in Rats Subjected to Lithium Chloride -Pilocarpine-induced Epilepsy.
 # = significantly different from epilepsy only group. Values are expressed as Mean ± SEM.

Level of Tumor Necrosis Factor-alpha

There was no significant difference when the control was compared to group B and the rest of the treated groups (Figure 5).

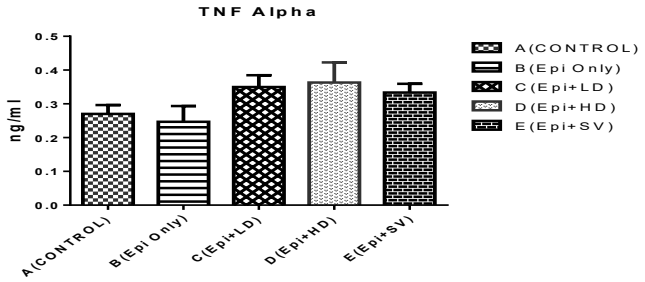

Figure 5: Bar Chart Showing the Effect of DM on the Level of Tumour Necrosis Factor Alpha in Rats Subjected to Lithium Chloride -Pilocarpine-induced Epilepsy.

Level of Superoxide dismutase

There was no significant difference when the control was compared to group B and the rest of the treated groups (Figure 6).

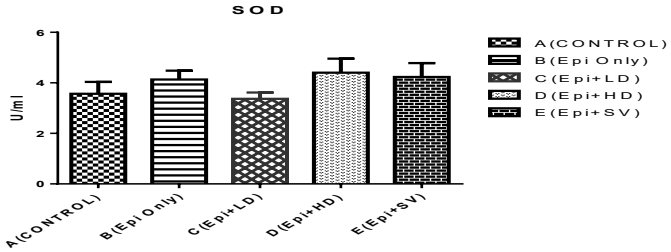

Figure 6: Bar Chart Showing the Effect of DM on the Level of Superoxide Dismutase in Rats Subjected to Lithium Chloride -Pilocarpine-induced Epilepsy.

Level of Catalase

The figure shows a significant increase in the epilepsy-only group when compared with the control group and groups C and D (Figure 7).

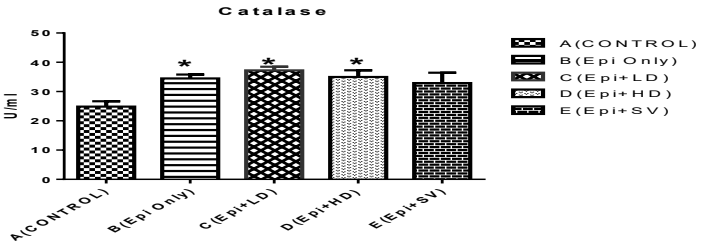

Figure 7: Bar Chart Showing the Effect of DM on the Level of Catalase in Rats Subjected to Lithium Chloride -Pilocarpine-induced Epilepsy.
*** = significantly different from the Control group. Values are expressed as Mean ± SEM.**

Level of Malondialdehyde

There was no significant difference when the control was compared to group B and the rest of the treated groups (Figure 8).

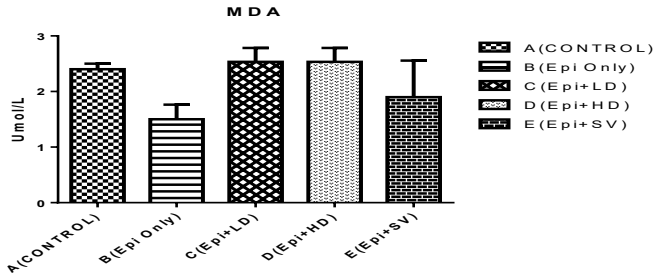

Figure 8: Bar Chart Showing the Effect of DM on the Level of MDA in Rats Subjected to Lithium Chloride -Pilocarpine-induced Epilepsy.

Neurobehavioral results
Radial arm maze: Rearing

There was no significant difference when the control was compared to group B and the rest of the treated groups (Figure 9).

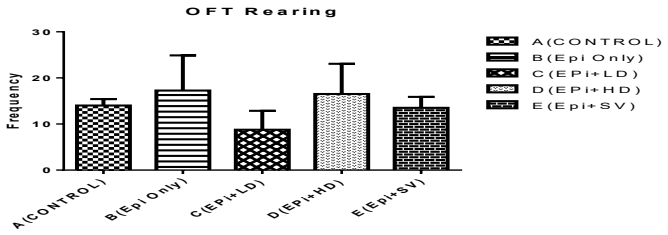

Figure 9: Bar chart showing the result of the Open field test for rearing behaviour of the Wistar rats Subjected to Lithium Chloride -Pilocarpine-induced Epilepsy and treated with DM

Radial arm maze: Grooming

There was no significant difference when the control was compared to group B and the rest of the treated groups (Figure 10).

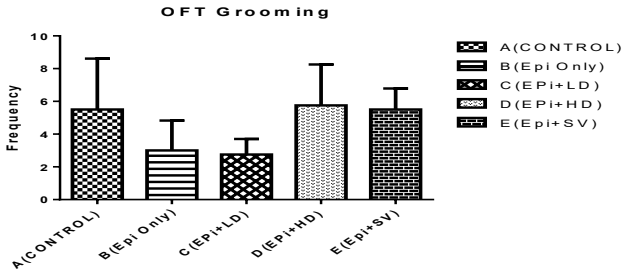

Figure 10: Bar chart showing the result of the Open field test for the Grooming behaviour of the Wistar rats Subjected to Lithium Chloride -Pilocarpine-induced Epilepsy and treated with DM

Open field: Number of Line Cross

The figure shows a significant increase in the control group compared to the epilepsy-only group and also a decrease in the epilepsy-only group when compared with group D (Figure 11).

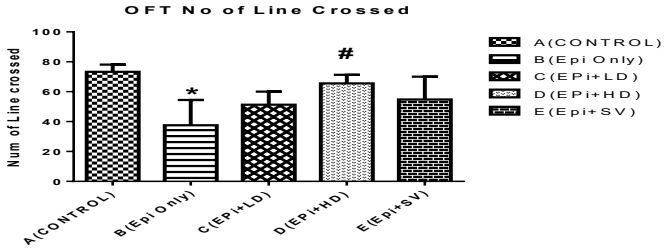

Figure 11: Bar chart showing the result of the Open field test for the number of line-crossed behaviours of the Wistar rats Subjected to Lithium Chloride -Pilocarpine-induced Epilepsy and treated with DM
***= significantly different from the Control group, # = significantly different from the epilepsy-only group, Values are expressed as Mean ± SEM.**

Open field: Freezing

The figure shows a significant decrease in the control group compared to the epilepsy-only group and also an increase in the epilepsy-only group when compared with groups C, D and E (Figure 12).

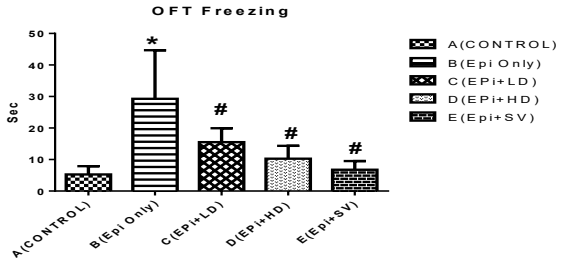

Figure 12. Bar chart showing the result of the Open field test for freezing time (s) behaviour of the Wistar rats Subjected to Lithium Chloride -Pilocarpine-induced Epilepsy and treated with DM
***= significantly different from the Control group, # = significantly different from the epilepsy-only group, Values are expressed as Mean ± SEM.**

Histological results

The Histological finds are presented in Plates 1 to 6.

Hematoxylin and Eosin Stain for the hippocampus

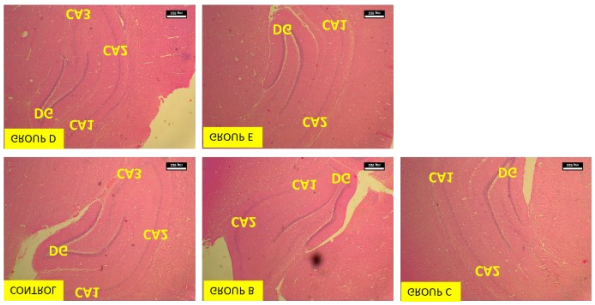

PLATE 1: Photomicrograph of the hippocampus of Wistar rat subjected to Lithium chloride-pilocarpine-induced epilepsy and treated with *Diospyros mespiliformis*. The plate shows the panoramic view of the hippocampus delineating the various subfields of the Cornus ammonis (CA1-CA3) and the dentate gyrus. H&E scale bar = 326µm

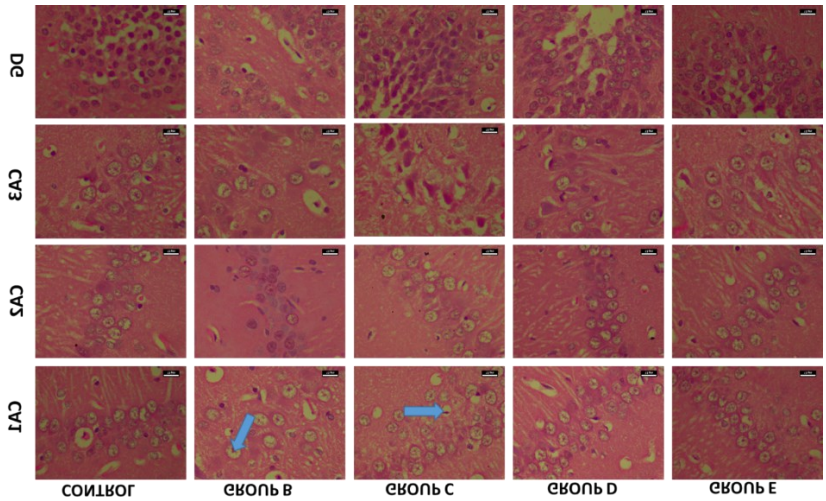

PLATE 2: Photomicrograph of the hippocampus of Wistar rat subjected to Lithium chloride-pilocarpine-induced epilepsy and treated with *Diospyros mespiliformis*. There is a sign of neuronal vacuolation (arrows) in the CA1 subfield of groups B and C when compared with the control and other treated groups. There is also the appearance of neuronal disorganization in the CA1 and CA3 of group B when compared with others. No observable neuronal aberrations were seen in the dentate gyri across the groups. H & E; scale bar = 13µm.

Hematoxylin and Eosin Stain for the Prefrontal cortex

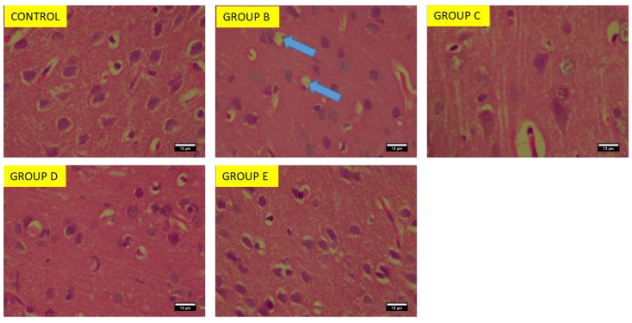

PLATE 3: Photomicrograph of the prefrontal cortex of Wistar rat subjected to Lithium chloride-pilocarpine-induced epilepsy and treated with *Diospyros mespiliformis.* The control shows normal histoarchitecture while there is a sign of vacuolation (arrows) in the neurons in group B when compared with the other groups. H & E; scale bar = 13µm

Immunohistochemistry results GFAP for the Hippocampus

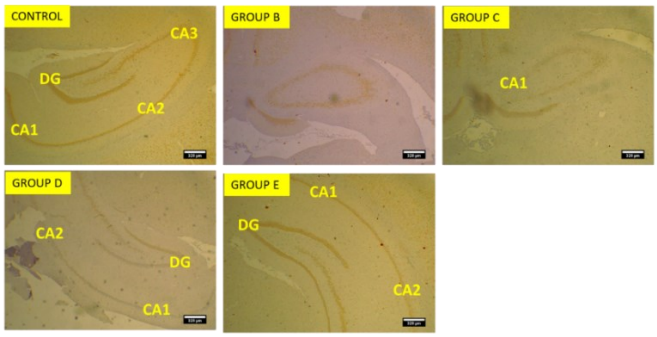

PLATE 4: Expression of GFAP in the hippocampus of rats subjected to Lithium chloride-pilocarpine-induced epilepsy and treated with *Diospyros mespiliformis*. The plate shows the panoramic view of the hippocampus delineating the various subfields of the Cornus amonis (CA1-CA3) and the dentate gyrus. Scale bar = 326µm

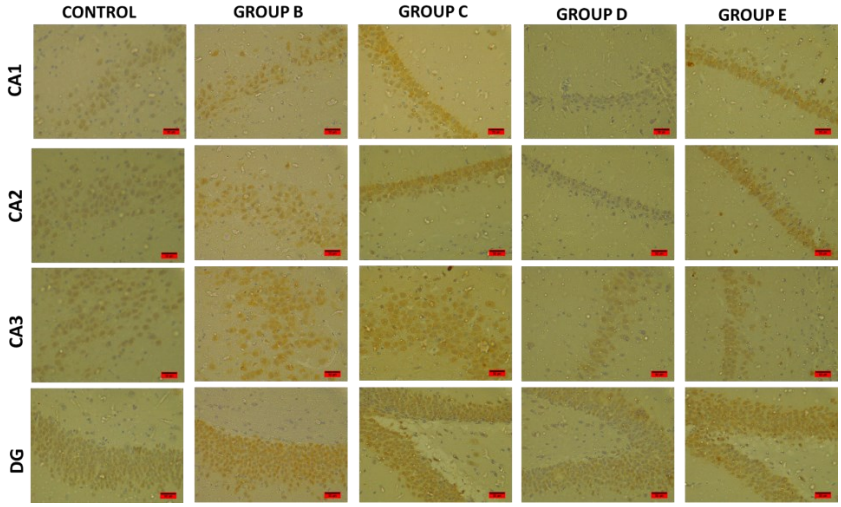

PLATE 5: Expression of GFAP in the hippocampus of rats subjected to Lithium chloride-pilocarpine-induced epilepsy and treated with *Diospyros mespiliformis*. The control shows negative reactivity as well as the CA1 and CA2 subfields of group D. However, groups B, C and E with the CA3 and DG subfields of group D show high levels of positive reactivity indicated by the brownish colouration. Scale bar = 13µm

NeuN for the Prefrontal Cortex

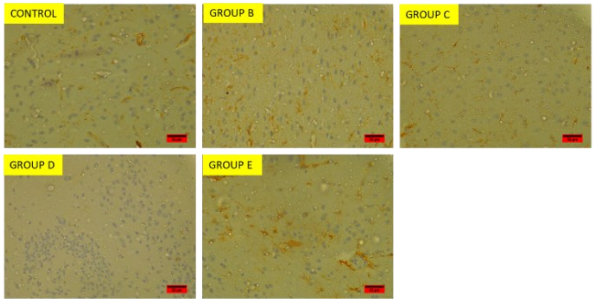

PLATE 6: Expression of NeuN in the prefrontal cortex of rats subjected to Lithium chloride-pilocarpine-induced epilepsy and treated with *Diospyros mespiliformis*. The control with groups B, C and E show signs of strong positive reactions while group D shows negative reactivity. Scale bar = 32µm

Discussion

The present study investigated if *Diospyros mespiliformis* (DM) will stop seizure progression in animal models of epilepsy and also improve the hippocampus and prefrontal cortex neurons and astrocytes in lithium chloride pilocarpine-induced epilepsy in rats.

Preliminary phytochemical screening of DM has been reported (20), and we investigated further the potential phytoconstituents using gas chromatography-mass spectrometry (GC-MS) (Figure 1). The potential phytocompounds present in the HE extract of DM identified through gas GC-MS includes 1, 2, 3-Benzenetriol (Peak area % (PA), 46.70), n-Capric acid isopropyl ester (PA, 7.5), Benzoic acid- 4-hydroxy (PA, 7.1), Benzofuran-2,3-dihydro (PA, 3.92), n-Hexadecanoic acid (3.5) and 9-Octadecenoic acid, (E) (2.0) respectively (Table 1). Interestingly, several of these phytocompounds have been shown to modulate in vivo cellular toxicity ranging from oxidants to induced DNA damage in animal models of seizures (21).

In the present study, glutamate level was increased in epileptic rats (Figure 2). Glutamate, a major excitatory neurotransmitter, is involved in initiating and spreading seizure activity, thereby playing a critical role in epileptogenesis (22). Previous studies have shown an increase in the extracellular concentration of glutamate levels before or during seizure onset, suggesting that it contributes to seizure initiation (23). Administration of DM significantly lowered glutamate levels in the epileptic rats as compared to the untreated group. The effects of low and high doses of DM administration on glutamate levels in the epileptic rats were comparable to that of sodium valproate, a standard antiepileptic drug, as glutamate levels in the epileptic rats administered with varied doses of DM had similar reductions in glutamate levels as with the sodium valproate group. This suggests that one or more constituents of DM possess potent anticonvulsant properties which lowered glutamate levels.

Interleukin-6 has been shown to play a role in epileptogenesis (24, 25). Activities of the immune system have been implicated in epilepsy as abnormalities in the expression of immune mediators and cells are documented in both human and animal models (26, 27). Acute seizures are associated with increased cytokine production which triggers inflammatory responses in the involved brain areas (28). In this present study, IL-6 levels were significantly higher in lithium-induced epileptic rats compared to normal rats (Figure 4). This is in line with the findings from previous studies in humans and animal models of epilepsy (28, 29). However, the administration of DM significantly reduced IL-6 levels in the treated when compared to the untreated. The effects of low and high doses of DM on IL- 6 levels in the epileptic rats are similar to that of sodium valproate, as IL- 6 levels in epileptic rats administered with 50mg/kg and 200mg/kg of DM respectively had similarly reduced IL- 6 levels as with the group administered 10mg/kg of sodium valproate. The findings show that DM possesses anti-inflammatory properties, due to a reduction in IL-6, known to be involved in the initiation of epilepsy.

Lowered catalase activity, glutathione level and total antioxidant capacity but higher malondialdehyde and nitric oxide levels have been observed in patients with status epilepticus (30). Other studies have reported increased levels of oxidative stress markers in pilocarpine-induced epilepsy in experimental animal models (31, 32). Increased production of superoxide anion, overloading of endogenous antioxidant system and oxidative damage of proteins, phospholipids and mitochondrial DNA have been reported (33). Most of these studies evaluated oxidative stress status within a short period from the time of epilepsy induction. In this study, the brain homogenate showed high levels of catalase activity thirty days after status epilepticus (Figure 7). The SOD and MDA levels were not different from the control at this time (Figures 6 and 8, respectively). The elevated catalase activity suggests persistent production of hydrogen peroxide in the rats. This finding is consistent with the observation by (34) of abnormal levels of hydrogen peroxide in the hippocampus of rats three months after status epilepticus. The animals treated with DM did not show a significant difference from the untreated animals, but treatment with sodium valproate reduced catalase activity.

The exploratory behavioural test has since been used to evaluate the energy balance, orientation as well as habituation in animal models of epilepsy (35). Results from this study showed persistent characteristics of epilepsy symptoms in the untreated rats. However, divided doses of DM effectively reversed the observed altered rearing (Figure 9), grooming (Figure 10), line crossing (Figure 11) and freezing time (Figure 12) when compared with control rats. Also, DM at a low dose was more effective in reversing epilepsy activity. Although, our study employed sodium valproate, the potential of DM as seen in this study supports its ethnobotanical application, however, the mechanistic role during the stages of epilepsy is not clear.

One of the prominent effects of epilepsy on hippocampal histoarchitecture is the loss of neurons, particularly in the subfields of the hippocampus such as the CA1, CA3, and dentate gyrus. This phenomenon, known as hippocampal sclerosis, involves the selective loss of pyramidal neurons and granule cells. Excitotoxicity (i.e. activation of glutamate receptors leading to neuronal damage) is a key factor in this process (36). In the same vein, research conducted on rat models of epilepsy has revealed that prolonged seizures can lead to alterations in dendritic complexity, such as reduced dendritic arborization and spine density in the prefrontal cortex (37). These changes affect the neural circuitry, impacting its information-processing capabilities. We observed neuronal vacuolation and disorientation in the CA1 and CA2 but not in the dentate gyri in groups B and C (Plates 1 and 2). The prefrontal cortex presents similar features in group B when compared with the others (Plate 3) which are in tandem with previous studies as quoted above (37). Epileptic activity can trigger neuroinflammatory responses in the hippocampus and the prefrontal cortex leading to the activation of microglia and astrocytes, and subsequent release of proinflammatory cytokines leading to altered histoarchitecture (29). The other treated groups remained histoarchitecturally stable possibly as a result of the anti-inflammatory and antioxidant properties of DM.

Glial fibrillary protein (GFAP) is a well-known marker for astrocytes, the predominant glial cell type in the brain. Astrocytes play multifaceted roles in maintaining brain homeostasis, including regulating neurotransmitter balance, providing metabolic support, and forming the blood-brain barrier (38). Astrocytes in the hippocampus of rats with epilepsy exhibit increased expression of GFAP, indicative of reactive gliosis—a process involving astrocytic hypertrophy (39). This aligns with the result from the present studies in which increased reactive gliosis was observed in the epileptic rats as indicated by the positive expression of GFAP (Plates 4 and 5). However, the negative reactivity in the CA1 and CA2 subfields of group D suggests a potential antioxidant and anti-inflammatory property of DM against lithium chloride-pilocarpine-induced epilepsy. In addition, it has been suggested that the upregulation of GFAP is often associated with structural and functional changes in astrocytes, potentially leading to alterations in synaptic transmission and neuronal excitability (40).

Epilepsy is often associated with neuronal damage and loss and expression of NeuN which has been employed to assess the extent of neuronal loss in the epileptic hippocampus, making it possible to quantify the severity of the disease (41). Results from our present study revealed negative reactivity of NeuN in the group D of the epileptic rat while the control and the remaining groups showed positive reactivity in the prefrontal cortex (Plate 6). This suggests that neurons in group D could not be protected by the extract. The loss of NeuN-positive neurons in the brain is indicative of neuronal death and dysfunction, which can contribute to hyperexcitability. This loss disrupts the intricate balance of excitatory and inhibitory circuits, potentially leading to increased susceptibility to seizures (42). On the other hand, while NeuN is a valuable tool for assessing neuronal loss, it's important to note that its expression might be influenced by factors beyond neuronal death (43).

Conclusion

It can be concluded that DM possesses vital compounds that can be employed in the management of epilepsy, which is evident in its ability to mitigate the histoarchitectural aberrations in the hippocampus and the prefrontal cortex and the maintenance to the bearable levels of the neurotransmitters and some other indicators and players in epileptic condition of the rats.

List of Abbreviations

GABA: Gamma-Aminobutyric Acid
SOD: Superoxide dismutase
GFAP: Glial fibrillary acid protein
TNF alpha: Tumor Necrotic Factor-alpha
NeuN: Neuronal nuclei

Declarations

Ethical approval and consent to participate

In this study, all animal procedures and experiments were performed by adopting international ethical guidelines of the National Institutes of Health on the care and use of laboratory animals, and the study protocol was approved by the Babcock University Ethical Research Committee (BUHREC 969/21).

Consent for publication

All authors gave consent for publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of data and materials

All essential data supporting the findings of this case are available within the article. Additional data are available upon request from the corresponding author.

Competing interests
The authors declared no conflicts of interest concerning the research.

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Authors' contributions
OSY: Conceptualization, coordination, arrangement and interpretation of immunohistochemistry; KOE: Carried out phytochemical screening, and manuscript editing; OOA: Did the biochemical assays, interpreted result and discussion; OJA: manuscript editing; EI: Manuscript editing and biochemical interpretation; AST: Photomicrography, slides interpretation and manuscript writing; OPO: General coordination and reviewed manuscript.

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