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Antioxidant and Anticonvulsant Effect of Dennettia Tripetala on Rat Model of Isoniazid-induced Seizure

Elizabeth Finbarrs-Bello[1], Ignatius Ikemefuna Ozor[1], Samsudeen G. Ojuolape[1,2*] and Abonyi Ernest Obinna[1]

[1]Department of Anatomy, Faculty of Basic Medical Sciences, Enugu State University of Science and Technology, Parklane, Enugu State. Nigeria.
[2]Department of Anatomy, St. Francis University College of Health and Allied Sciences, Tanzania.

Authors' contributions

This work was carried out in collaboration among all authors. Authors EFB and AEO designed the study, performed the statistical analysis, wrote the protocol. Author SGO wrote the first draft of the manuscript. Authors IIO and EFB managed the analyses of the study. Author SGO managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: This study aimed at investigating the antioxidative, anticonvulsive and histological effects of ethanolic fruit extract of *Dennettia tripetala* on the pre-frontal lobe of the brain in isoniazid-induced (300 mg/kg, i.p) seizure in adult wistar rat.
Introduction: Neuronal hyper-excitability and excessive production of free radicals have been implicated in the pathogenesis of a considerable range of neurological disorders, including epilepsy. The high rate of oxidative metabolism, coupled with the low antioxidant defenses and the richness in polyunsaturated fatty acids, makes the brain highly vulnerable to free radical damage.
Study Design: This is an original research conducted in Department of Anatomy, Faculty of Basic Medical Sciences, Enugu State University of Science and Technology (ESUT), Parklane, Enugu State, Nigeria, between June and August, 2019.

**Corresponding author: E-mail: samsudeeng84@gmail.com;*

Methodology: A total number of twenty four wistar rats were used for this experiment, the animals were grouped into six groups with four animals per group, Group I served as the negative control, Group II served as the positive control, Group III received the standard drug as well as the Isoniazid, while group IV, V and VI were treated with ethanolic extract of *Dennettia tripetala* at different dosages; 250 mg/kg, 500 mg/kg and 750 mg/kg, intraperitoneally (i.p) respectively and its effects compared with a standard drug (Pyridoxine) treated group.
Results: The extract significantly prolonged the onset of seizure at high dose administration (750 mg/kg) but completely prevented seizure occurrence at low and medium dose administration (250 mg/kg and 500 mg/kg, i.p) when induced with isoniazid (300 mg/kg, i.p.).
Conclusion: The results obtained from this work suggest that ethanolic extract of *Dennettia tripetala* has anticonvulsant activity, and this supports the use of the formulation traditionally in the treatment of convulsions, thus should be considered for clinical trials.

Keywords: Dennettia tripetala; antioxidant; anticonvulsant; seizure; ethanolic extract.

1. INTRODUCTION

Neuronal hyper-excitability and excessive production of free radicals have been implicated in the pathogenesis of a considerable range of neurological disorders, including epilepsy. The high rate of oxidative metabolism, coupled with the low antioxidant defenses and the richness in polyunsaturated fatty acids, makes the brain highly vulnerable to free radical damage [1]. The increased susceptibility of the brain to oxidative damage highlights the importance of understanding the role of oxidative stress in the pathophysiology of seizures [1]. The role of oxidative stress in seizure induction and propagation has provided and established the link between oxidative stress and seizures. The ability of antioxidants for reducing the seizure manifestations and the accompanying biochemical changes (i.e., markers of oxidative stress) further supports a role of free radicals in seizures and highlights a possible role of antioxidants as adjuncts to antiepileptic drugs for better seizure control (The experimental and clinical data suggest a putative role of oxidative stress in the pathophysiology of certain seizure types).

The use of animal models has made important contributions to our understanding of seizures [2]. There is evidence that suggests that antioxidant therapy may reduce lesions induced by oxidative free radicals in some animal seizure models. Animal studies about the effect of phenytoin on brain lipid peroxidation initiated by a free radical generating mechanism have shown that phenytoin treatment prevents the occurrence of convulsive. Isoniazid-induced seizure, often described as Status Epilepticus (SE), is an emergency condition characterized by repeated convulsive episodes that responds poorly to the currently available anticonvulsant drugs [3]. Status Epilepticus (SE) is one of the major side effects of isoniazid, a first line drug used for the treatment of tuberculosis. The sustained seizure produced by isoniazid is due to the inhibition of glutamate decarboxylase, the enzyme that control the formation of GABA, a chemical that reduce the firing rate of nerve cells in the brain. The major sign of SE in patients with isoniazid poisoning is repeated convulsions, which often leads to the formation of toxic substances that damage the brain cells. Although isoniazid-induced seizure is known to respond poorly to currently available anticonvulsant drugs, intravenous diazepam is still used to control the seizure episodes in the absence of pyridoxine [4,5,6,7]. On this basis, diazepam and pyridoxine, serving as reference drugs, were compared with the current study test substance. However, pyridoxine is reported as the only effective antidote for isoniazid toxicity and should be given in doses equivalent to the amounts of the ingested isoniazid in order to be effective [5,6,7].

Dennettia tripetala a tradition herbal remedy is acclaimed to possess antioxidant and anticonvulsant properties. The preliminary phytochemical analysis and vitamin profile revealed potent active agent and high profile of antioxidant vitamin like vitamins C and E in addition to vitamins B6 and B12. This is likely to has better prognosis in the treatment of seizure in alternative /complementary medicine and provide raw source of new effective pharmacotherapy for convulsion. But, the herb required further scientific evaluation to validate the claim.

1.1 Aims of the Study

This is aimed at, firstly, to phytochemically evaluate its antioxidant property Secondly, to

evaluate its anticonvulsant effect by determining the level of its prophylasis against the seizure. Thirdly, to compare anticonvulsant effect using pyridoxine and sodium valproate as standard treatment for comparison with the extract. Fourth, is to investigate histological changes in the frontal and temporal lobes using routine Haematoxylin and Eosin. It is expected that the extract should give protection against seizure and curb the generated oxidant species better than the standard. Experimental curiosity generated from this study will be suggested to further research evaluation.

2. MATERIALS AND METHODS

2.1 Collection and identification of Plant Materials

The fruits of *Dennettia tripetala* were obtained from a farm land within Nsukka, Enugu State Nigeria in the month of April, 2018. The fruits were taken to a plant curator in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. The name was confirmed on the world Botanical plant list. a voucher specimen was deposited at the herbarium with reference number UNH 8^{C} for reference.

2.2 Extraction of Plant Material

Dennttia tripetala fruits were examined for signs of pathology or pesticide damages hence only freshly haruart fruit which showed no sign of insect bites and mature or ripe deep colored fruits were selected for use and were washed, air-dried for 7 days. The fruits were hence pulverized into fine powder using a milling machine, the powder sample were weighed and 800g of the powder were macerated in 2.2 liters of 90% ethanol and allowed to stand for 48 hours with continous starring. Thereafter the sample were then be filtered through a mesh work and whatman paper No.1, The filtrate were left in the open space for the ethanol to evaporate leaving residue whose percentage yield is 4.25515% which were stored in the refrigrator at 4ºC.

2.3 Phytochemical Analysis of *Dennettia tripetala*

400 g of the dry pulverized fruits of *Dennettia tripetala* were collected and sent to Department of Pharmaceutical Chemistry Ahmedu Bello University (ABU), Zaria, Kaduna State, Nigeria for Phytochemical analysis. Dry pulverized fruits of *Dennettia tripetala* were collected and sent to NARICT UV-Visible analysis centre Zaria Kaduna State Nigeria for vitamin analysis.

2.4 Drugs and Chemicals

Isonaizid (Isonamede, India) and pyridoxine (Pauco Vitamin B6, Nigeria) were procured from a registered pharmacoly store. The stock solution of INH was prepared by dissolving 300 mg of the tablets in 10 ml of distilled water at room temperature. The stock solutions were prepared freshly prior to administration adopted from [8].

2.5 Animal Procurement and Husbandry

A total of 24 rats were used for this study, and each rat were weighed accordingly before use. The animals were obtained from the animal house in the department of Animal Scince in the University of Nigeria, Nsukka, and they were kept under constant environmental and nutritional conditions. The rats were also kept in their respective cages where food and water were allowed *ad libitum*; and left to acclamatize for 2 weeks before the commencement of the experiment.

2.6 *In vitro* Study Design and Drug Treatment Schedule

2.6.1 Experimental design and animal grouping

The experimental animal were grouped into six groups composing of 4 animals in each as vix: Group I as the negative control, Group II as the positive control received normal saline, Group III for the experimental animal were treated with vitmain B6 (pyridoxin). Group IV to VI were composed of experimental animal treated with different dosing of ethanolic fuits extract of *Dennettia tripetala* as adopted from Akpakpan et al.[9] and modified. Thirty minutes after administration of water to group II, test drug (extract) to groups IV to VI and 30 minutes after pyridoxine administration to group III; Group II to VI were treated with 300 mg/kg-bw INH by intraperitonial route. Immediately after INH injection, each rat was kept in separate cages and observed for the next 2 hrs to record latency to clonic, duration of seizure and mortality [10], if in an animal; convulsion not occur within 30 min, it was considered as full protected [11].

Animal sacrifice, tissue collection and processing: All the Wistar rats were sacrificed

after 2hours of observation under chloroform anesthesia, the cranium of each animal were opened using brain forceps. The tissues were then fixed in 10% neutral formal saline and processed for histological observation using routine Haematoxylin and Eosin staining techniques according to the methods of Bancroft and Gamble [12].

2.7 Statistical Analysis

Statistical analysis was done using the Statistical Package for Social Sciences (SPSS version 21) for Windows (SPSS Inc., Chicago, USA). Results were presented as mean ± standard deviation (SD). Data were compared by one way analysis of variance (ANOVA). Statistical significance will be taken at $p<0.05$.

3. RESULTS

3.1 Phytochemical Analysis

Qualitative analysis: The qualitative phytochemical analysis of the fruit of *Dennettia tripetala* showed the presence of the following phytochemical compounds.

Vitamins analysis: The vitamin analysis of the dry fruits of *Dennetia tripetala* showed the presence of the following vitamins.

Table 1. Qualitative and quantitative phytochemical analysis of the Fruits of *Dennettia tripetala*

Metabolites	Inferences	Average % of metabolite
Carbohydrate	+	
Tannins	+	6.00
Saponins	+	4.43
Alkaloids	+	6.13
Steroids and triterpenes	+	
Cardiac glycosides	+	
Flavonoids	+	7.99
Glycosides	+	
Phenol	+	8.00
Anthraquinones	_	

Key: + = detected; - = not detected

Anticonvulsant activity: In Table 3, in Group VI; a statistical significant increase ($P<0.05$) was observed when compared with Group II. Group I, III, IV and V could not be statistically compared due to the absence of seizure.

Table 2. Results of vitamin screening of the fruits of *Dennettia tripetala*

S/n	Sample ID	Type	Conc	Wavelength name	Wavelength (nm)
1	Vitamin C (Ascorbic acid)	unknown	0.340	WL478.5	0.082
2	Vitamin E (Tocopherols, Tovotrienol)	unknown	14.035	WL295.0	0.458
3	Vitamin B1 (Thiamin)	unknown	0.280	WL478.5	0.068
4	Vitamin B9 (Folic acid)	unknown	0.297	WL0.282	4.897
5	Vitamin B 6 (Pyridoxine)	unknown	2.129	WL530.0	0.112
6	Vitamin B12 (Cobalamin)	unknown	2.129	WL530.0	0.113

Table 3. Shows effect of *Dennettia tripetala*, pyridoxine and isoniazid on latency to seizure on induced seizure model

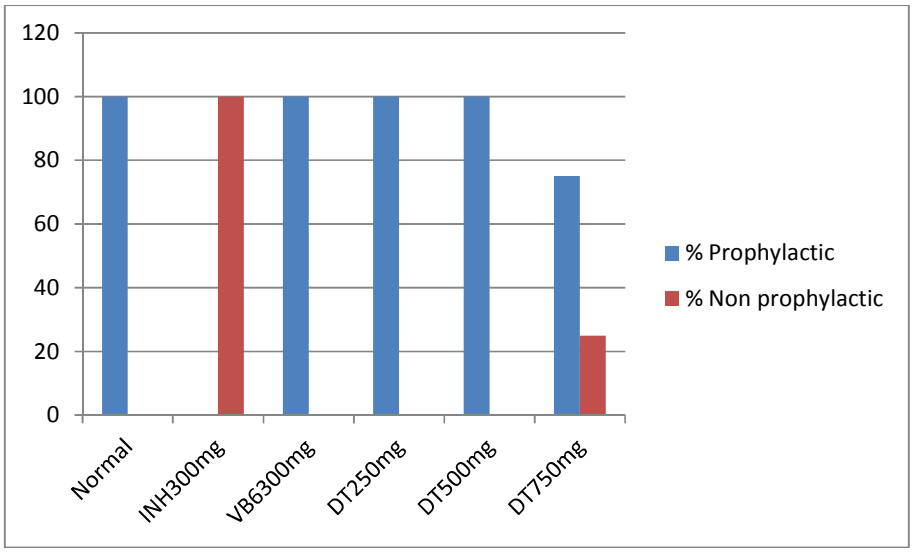
Group	Prophylatic	Non-prophylactic	Mean±SD	P-value
I	4/4 (100%)	0/4	Absence of seizure	
II	0/4	4/4 (100%)	16.165±7.700	0.013
III	4/4 (100%)	0/4	Absence of seizure	
IV	4/4 (100%)	0/4	Absence of seizure	
V	4/4 (100%)	0/4	Absence of seizure	
VI	3/4 (75%)	1/4 (25%)	59.615±23.904	0.013

Mean ± Std. Dev. Values for Latency Period

In the Table 4, the mean standard deviation value for the duration of seizure could not be compared due to absence of seizure in Groups I, III, IV, V and also due to mortality in Group VI.

Table 4. Shows effect of *Dennettia tripetala*, pyridoxine and isoniazid on the duration of seizure on induced seizure model

Group	Number of convulsed animal	Mean ±standard deviation
I	0/4	Absence of seizure
II	4/4	47.448±3.528
III	0/4	Absence of seizure
IV	0/4	Absence of seizure
V	0/4	Absence of seizure
VI	4/4	49.280 (3 convulsed till death)



Graph 1. Measurement of entries of prophylactic and non prophylactic from Seizure

Histological Analysis

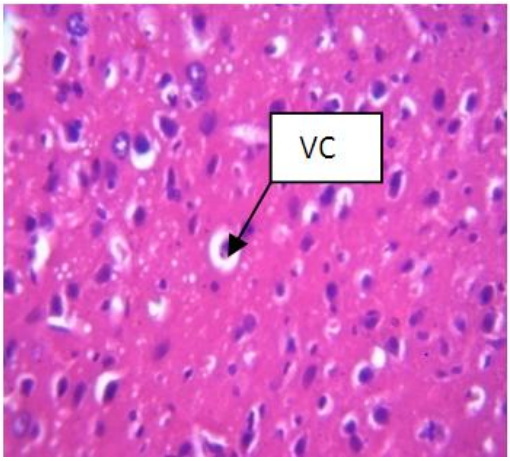


Plate 1. Photomicrograph of negative control section brain administered with normal saline shows frontal lobe with normal neuronal cell (NNC) X400 (H&E)

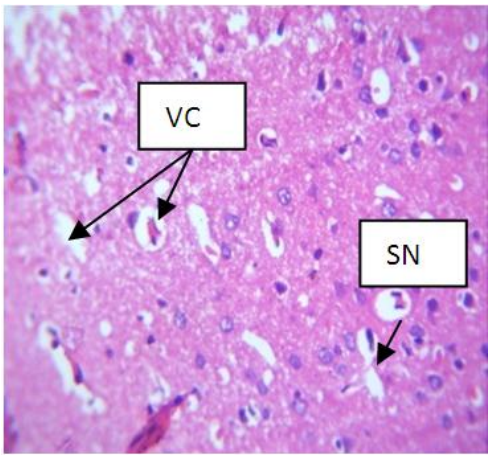


Plate 2. Photomicrograph of positive control section brain administered with isoniazid shows frontal lobe with severe degeneration with well developed vacuolated cytoplasm (VC),shrinking of the neurons (SN) and dilated perivascular spaces (DPS) X400 (H&E)

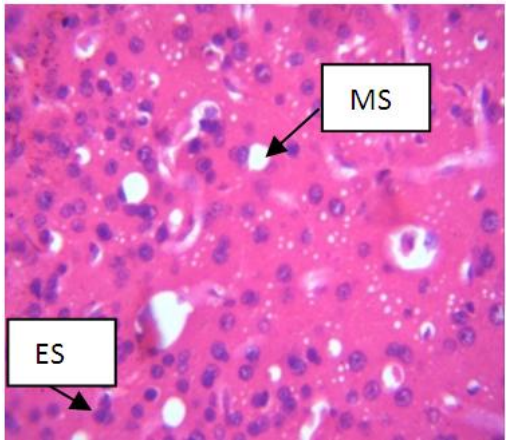


Plate 3. Photomicrograph of standard control section brain administered Isoniazid and pyridoxine shows frontal lobe with mild microcytic spaces (MS) and eosinophilic substance (ES) X400 (H&E)

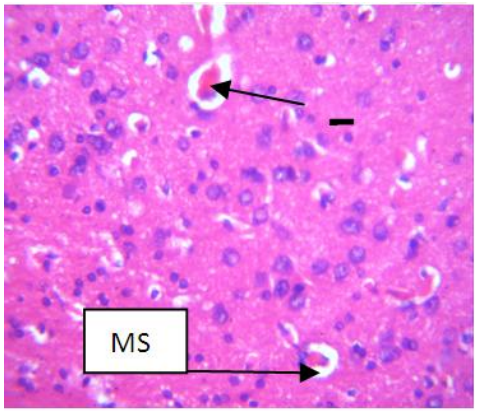


Plate 4. Photomicrograph of standard control section brain administered with Isoniazid and pyridoxine shows pre-frontal lobe with mild microcytic spaces (MS) X400 (H&E)

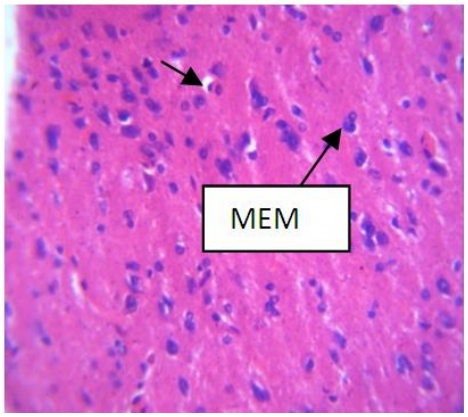


Plate 5: Photomicrograph of treatment control section brain administered 250 mg/kg-bwt *of Dennettia tripetala* and Isomazid only presents with frontal lobe with no vacuolated cytoplasm (NVC) with mild eosinophilic material (MEM) X400 (H&E)

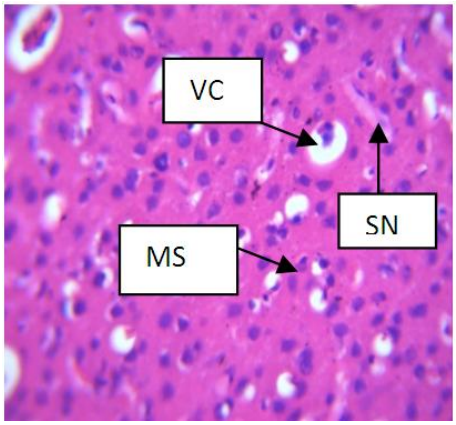


Plate 6: Photomicrograph of treatment control section brain administered with 500 mg/kg-bwt of *Dennettia tripetala* and 300 mg/kg-bwt of Isoniazid only shows frontal lobe with presence of the vacuolated cytoplasm (VC) shrinking of neurons (SN) microcytic space (MS) X400 (H&E)

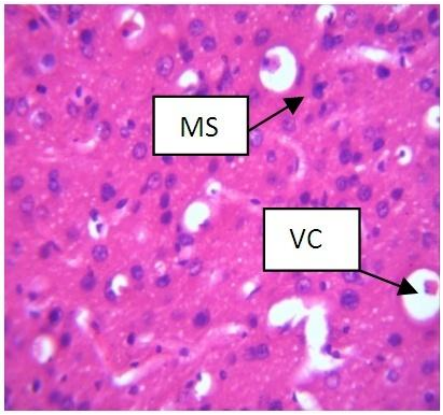


Plate 7: Photomicrograph of treatment control section brain administered with 750 mg/kg-bwt of *Dennettia tripetala* and 300 mg/kg-bwt of Isoniazid only shows frontal lobe with presence of the vacuolated cytoplasm (MVC) with mild shrinking of the neurons (SN) and microcystic spaces(MS) X400. (H&E)

4. DISCUSSION

The phytochemical analysis of the *Dennettia tripetala* fruits was carried out in accordance with the methods of Anwar et al. [13]. The result showed the presence of Saponins, Alkaloids, Tannins, flavanoid, cardiac glycosided, carbohydrate, steroids and triferpenes and glycosides; which is in contrary to the results of the qualitative phytochemical analysis reported by Egharevba and Idah [14] which showed the absence of saponin in the fruits of *Dennettia tripetala*, this diferences may be due to different geographical variations from where the fruits were collected, physiological, environmental, genetical and evolutionary factors [15]. These constituents provide a scientific bases for the use of *Dennettia tripetala* in traditional medicine, tannins, and flavonoids, for instance, are effective against diabetes, they also possess antimicrobial, anti-inflammatory and antioxidant properties, Cardiac glycosides on the other hand have been used in the treatment of asthma (Sylvia., 2015). Alkaloids (6.13%), flavonoids (7.99%), and saponins (4.43%) which are significantly present on the dry fruits of *Dennettia tripetala* on analysis have been reported to have anticonvulsant activitie [16]. The presence of these metabolites could impact anticonvulsant effect on its therapeutic use.

The result of the vitamin analysis of the dry pulverized fruits of *Dennettia tripetala* indicated the presence of Vitamin C (ascorbic acid), Vitamin E (Tocopherols, Tocotrienols) Vitamin B1 (Thiamin) Vitamin B9 (Folic Acid), vitamin B12 and Vitamin B6 (Pyridoxine). The extract contained higher concentration of Vitamin E (14.035) (tocopherols, tocotrienols) followed by Pyridoxine (2.129). According toLheureux *et al.* [17] pyridoxine has been severally justified by clinical observations to be important in the treatment of isoniazid overdose in tuberculosis patients, while preclinical studies indicate that, it reverses the isoniazid- induced seizure in experimental rodents [18]. This significant presence of Vitamin B6 in the extract could impact similar therapeutic intervention.

To explore the anticonvulsant role-delete] of *Dennettia tripetala* fruit extract was explored against convulsion induced chemically using isoniazid in experimental rats. Isoniazid a well-known anti-tuberculosis drug, but the drug is also known for its neurotoxicity when its overdose is administered often result in seizure episode. Hence, is one of the seizure induced models in rodents of its own class [8]. Convulsions induced by Isoniazid (INH) involved disruption of GABAergic neurotransmission in the central nervous system leading to deficiency of pyridoxine (vitamin B6) by inhibition of pyridoxine phosphokinase. The enzyme converts pyridoxine to it active B6 which is required by glutamic acid decarboxylase to convert glutamic acid to GABA [13] Thus, decreased levels of GABA are believed to potentiate seizures [13].The present study revealed that ethanolic extract of *Dennettia tripetala* fruit exhibited a significant anticonvulsant activity against Isoniazid (INH) induced seizures in rats. Total anticonvulsant activity was observed at a dose of 250 mg/kg and 500 mg/kg respectively as same as the pyridoxine treated group which provided 100% protection against seizure. It is evident that, it has an antagonistic effect on isoniazid induced seizure [17]. However, the extract exhibited an onset boost of seizure at the administration of 750 mg/kg dosage of *Dennettia tripetala* with a significant increase ($p<0.005$) in meantime of latency to seizure when compared with the group treated with Isoniazid only.It can be deduced from the result in the low and medium dose of administration of extract and corroborate with the work of Okogun and Ekong [19] which had earlierisolated 1-Nitro-2-phenylethane from the essential oils of dry fruits of *Dennettia tripetala* reported to exhibit dose dependent significant hypnotic, anticonvulsant and anxiolytic effects.The seizure observed on administration of high dose (750 mg/kg) of *Dennettia tripetala* which resulted in 25% of the rats in the group not being fully protected may be attributed to pharmacological properties as seen; for example in pyridoxine which as a potent anticonvulsant to isoniazid induced seizure [17] has been recorded by Drug bank (2018); Dalton and Dalton (1987) to exhibit several neuropathic side effects including convulsion when toxic or excess.

Histologically, the photomicrographs of the processed brain tissues under the light microscope showed that the prefrontal lobe of group I which received normal saline (negative control) showed a normal brain tissue while the prefrontal lobe of the positive control (group II) given isoniazid only shows severe degeneration with well-developed vacuolated cytoplasm (VC) which is an adaptive survival response to plethora of environmental changes that has the potential to lead to a particular and distinctive cell death [20] shrinking of the neurons (SN) and dilated perivascular spaces (DPS); these changes could be as a result of the neurotoxic

effect resulting from isoniazid dosing. The micrograph of the extract groups when compared with group I (positive control) presented with no microcystic spaces, shrinking of neuron or vacuolated cytoplasm which was almost the same effect as with the standard control group but for the presences of microcytic spaces; except in extract groups V and VI (500 and 750 mg/kg) that had mild shrinking of neurons, vacuolated cytoplasm and microcystic spaces which were severe in the high dosed group (750 mg/kg) but still minimal when compared with the positive control group. It can be inferred from the result of the micrograph above that the extract exhibited higher protective or prophlactic effect in the lower dosage administered group (250 mg/kg) than the medium dosed group (500 mg/kg) and high dose (750 mg/kg), and have almost the same histological architecture when compared with the standard control section (pyridoxine group) with its potency decreases as the dosage increases.

5. CONCLUSION

It is evident that, the ethanolic fruit extracts of *Dennettia tripetrala* has anticonvulsant potencies, and exhibits varying degree of anticonvulsant activities, having a dose dependent effect with its effect decreases as the dosage increaseson isoniazid-induced seizure on the cerebral cortex of wistar rats. This was presented with significant changes on the histology of cerebral cortex (prefrontal lobe) when examined under a light microscope with a profound histo-architectural maintenance when compared with a standard anticonvulsant drug (pyridoxine).

CONSENT

It is not applicable.

ETHICAL APPROVAL

Approval for the study was sought from the ESUT College of Medicine and Animals were cared for in accordance with institutional and international guidelines.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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