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Research article

# *Antiaris africana* aqueous extract inhibits chronic demyelination and seizures in mice

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ABSTRACT

Demyelinating diseases are commonly associated with epileptic seizures and have limited management options. Hence, the need to investigate potential options for management of such seizures. *Antiaris Africana* extract (AE) was investigated for effect in chronic demyelinating seizures. Cuprizone treatment induced short but frequent spike discharges in mice. *Antiaris Africana* extract (300 mg/kg) treatment abolished epileptiform discharges. Cuprizone administration caused severe demyelination in the corpus callosum. After the demyelination phase, myelin content decreased to 22.86 ± 1.92 % in the cuprizone-only group. However, there was an increase to 52.14 ± 3.91 % in cuprizone-only group and 62.00 ± 2.78 % in the *Antiaris africana* extract group respectively, after a 4-week cuprizone cessation period. Treatment with AE and LEV visibly altered myelin growth. *Antiaris africana* extract treatment produced significant ($P < 0.001$, $F(3, 16) = 698.4$) increase in locomotor activity similar to LEV ($P < 0.001$,$F(2, 12) = 678.7$) and DZP ($P < 0.001$, $F(2, 12) = 620.4$) and improved beam traversal time (18.71 ± 2.244 s; 95 % CI: 13.22–24.20) while causing significantly ($P < 0.05$, $F(2, 15) = 6.667$) fewer stepping errors. *Antiaris africana* extract inhibits seizures induced by chronic demyelination and has beneficial effects on motor coordination.

## 1. Introduction

Demyelinating diseases like multiple sclerosis (MS), pose significant challenges in clinical management due to their unpredictability and harmful impact on patients. These diseases present with varied symptoms of which epileptic seizures are common [1]. In multiple sclerosis, a 6-fold prevalence of seizures has been recorded [2,3]. Despite the well-documented association between MS and seizures, the mechanisms that mediate this association are still under debate. These mechanisms have been linked to axonal damage [4,5]. Over the years, animal models have played a pivotal role in understanding the intricacies of seizures associated with demyelination, to shed light on disease mechanisms and potential therapeutic targets. Consequently, demyelination of the corpus callosum using cuprizone (CPZ) has been used widely to study demyelination conditions of the CNS in rodents [6]. By feeding mice with cuprizone, a copper chelator, researchers have induced reproducible demyelination of vital neurons, proposing mechanisms underlying the pathophysiology of demyelinating disorders. This model has been shown to induce epileptic seizures in mice by causing consistent demyelination of the corpus callosum after weeks of cuprizone administration [7]. The mechanism by which cuprizone achieves demyelination is still unknown. However, it is believed to result in an energy disruption through the decline of mitochondrial enzymes

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such as cytochrome oxidase in the CNS [8].

Further to the complex etiology, managing the seizures induced by demyelination disease conditions presents a complicated challenge [9]. This requires a comprehensive understanding of the underlying etiology of these seizures. Antiseizure drugs (ASDs) remain the cornerstone of seizure management in these conditions. These drugs aim to suppress neuronal hyperexcitability and mitigate seizure frequency and severity [10]. However, selecting appropriate ASDs can be complicated by factors such as comorbidities, drug interactions, and potential adverse effects, requiring careful consideration and close monitoring [9]. In addition to pharmacotherapy, there has been growing interest in exploring natural remedies for the treatment of seizures and epilepsy. These natural remedies offer potential alternatives to conventional pharmaceutical drugs which may be more efficacious [11]. One such herbal remedy that has shown promise is *Antiaris africana*. *Antiaris africana* (Moraceae) is a medicinal plant which serves as a source of wood and bark cloth. It contains several pharmacologically active substances [12]. It is commonly referred to as the bark cloth tree in English, and 'Kyenkyen' in the local Akan dialect in Ghana. It is employed in the treatment of various conditions in Ghanaian traditional medicine, namely skin irritation, throat infection, cancer and rheumatism [12,13]. There have also been studies which show that extracts from *Antiaris africana* possess antineuropathic and anticonvulsant properties [14,15].

In the present study, authors investigate the potential activity of *Antiaris africana* aqueous extract against chronic demyelination and the consequent seizures in C57BL/6 mice that have been subjected to cuprizone-induced demyelination. By assessing the seizure phenotype and histology within the corpus callosum, authors aim to explain the therapeutic potential of *Antiaris africana* in alleviating seizures associated with demyelination. Hence, advancing our understanding of the complex relationship between demyelination and epileptogenesis.

## 2. Materials and methods

### 2.1. Chemicals

Cuprizone (CPZ) and Levetiracetam (LEV) were obtained from Sigma Aldrich, St. Louis, MO; Diazepam (DZP) injection was obtained from Oson's Chemist Ltd, Accra, Ghana; Levetiracetam (10 mg/kg) and Diazepam (1 and 3 mg/kg) were dissolved physiological normal saline (NaCl, 0.9 %, NS) and administered intraperitoneally.

### 2.2. Plant material

Stem bark of *Antiaris africana* was harvested from the Kwame Nkrumah University of Science and Technology (KNUST) campus, Kumasi, and authenticated at the Herbal Medicine Department of the Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana. A voucher specimen (KNUST/HM1/011/S008) has been retained in the herbarium.

### 2.3. Preparation of Antiaris africana aqueous extract

The harvested stem bark was air dried under shade and subsequently milled into powder using a commercial grinder. Extraction of the coarse powder (505 g) was achieved by cold maceration with distilled water as solvent at room temperature for five days. Filtrate was concentrated in a rotary evaporator at 60 °C and the concentrate oven-dried at 65 °C to obtain a yield of 26.60 % (w/w) of *Antiaris africana* aqueous extract (AE).

### 2.4. Phytochemistry analysis of Antiaris africana aqueous extract using liquid chromatography-mass spectrometry (LC-MS)

*Antiaris africana* aqueous extract underwent analysis using Liquid Chromatography-Mass Spectrometry (LC-MS) on a UHPLC system. The system was equipped with an autosampler with a Waters nanoAcquityHSS T3 column, measuring 1.8 μm × 100 mm. The mobile phases consisted of 0.1 % formic acid in water (A) and 90 % acetonitrile in water with 0.1 % formic acid (B), running at a flow rate of 500 μL/min. LC conditions involved an initial 5 % B concentration from 0 to 3 min, followed by a linear increase from 5 to 20 % B over 55–63 min, maintaining 95 % B for 15 min. Mass spectrometry analysis involved a Thermo Electron LTQ-Orbitrap XL mass spectrometer equipped with a nano electrospray ion source (ThermoFisher Scientific, Bremen, Germany). The instrument operated under Xcalibur 2.1 version software, utilizing positive ionization mode. Data-dependent automatic switching between MS and MS/MS acquisition modes was utilized for the analysis.

### 2.5. Animals

C57BL/6 mice (male; 8 weeks old; 25–30 g) were obtained from the Noguchi Memorial Institute for Medical Research, Accra, Ghana. Animals were kept under standard conditions (room temperature 23–27 °C, humidity 50–60 %, light-dark cycle (6:00 a.m. - 6:00 p.m.). All experiments were carried out according to established principles for laboratory animal care and utilization [16]. Significant effort was made to keep animal suffering to the minimum and reduce number of animals used.

### 2.6. Treatment groups

Prior to experiments, animals were allowed seven (7) days to acclimatize to the laboratory conditions. All treatment groups

consisted of seven (7) mice each. For cuprizone-induced demyelination tests, the control group received normal saline (10 ml/kg) only; the cuprizone group (CPZ) received (0.2 % cuprizone and normal saline (10 ml/kg); the antiaris groups (AE) received 0.2 % cuprizone and either 100 or 300 mg/kg AE while the levetiracetam group (LEV) received 0.2 % cuprizone and LEV 10 mg/kg. All treatments were administered daily (9:00 a.m.) for 12 weeks. Two additional groups of cuprizone-naïve mice (n = 7) were added to the behavioral test groups. These animals received diazepam (1 mg/kg, i.p.) in the activity meter test and diazepam (3 mg/kg, i.p.) in the beam walking tests, 30 min before behavioral testing. These groups served as reference drugs for only the behavioral tests. A total of 84 animals were used in this study. Scheme 1 summarizes the experimental procedures carried out in this study.

### 2.7. Cuprizone treatment

In the demyelination phase, cuprizone 0.2 % (w/w), milled into standard mouse chow was fed to C57BL/6 mice for 12 weeks ad libitum. Control mice received normal mouse chow. Mice were observed daily for all behavioral changes. Mice were allowed 4 weeks after cuprizone treatment for remyelination to occur. The cuprizone-containing diet was prepared in large, homogenous batches to ensure uniform distribution of cuprizone. Animals were housed individually to monitor and control food intake, ensuring each mouse received the intended dose.

### 2.8. Behavioral testing

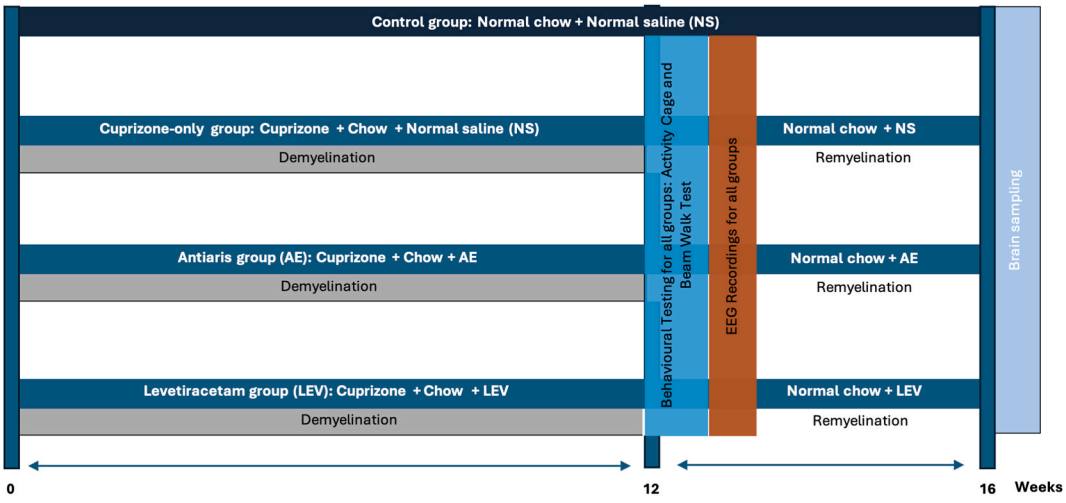
Behavioral testing was done using the activity meter and the beam walking tests. Activity Meter Test was carried out after 12 weeks of cuprizone and daily drug treatment with AE and LEV. The mice activity cage (Ugo Basile model 7401, Comerio, *VA*, Italy) was employed in the study. Mice were placed individually into the activity cage. Activity was observed every 5 min for 30 min.

In the beam walking test, animals were individually placed at the starting end of the beam and given 60 s to navigate across the beam to the goal box. Sessions were recorded and videos analyzed for time to cross the beam and stepping errors (defined as complete forepaw slips from the beam). A score of 60 s was given to animals that were unable to cross the beam or fell off. Animals were allowed two consecutive sessions. A training session was not conducted to eliminate any potential differences arising from the learning abilities of the mice.

### 2.9. EEG recordings

Following behavioral testing, mice were positioned in a stereotaxic frame after receiving anesthesia (ketamine, 80 mg/kg and xylazine, 10 mg/kg; i.p.). Under sterile conditions, two screw electrodes (1.6 mm each; stainless steel) were fixed into the exposed skull superior to the parietal cortices with stereotaxic coordinates: 2 mm lateral; 2 mm posterior, relative to bregma. A third screw, serving as indifferent reference-electrode was placed 1 mm both laterally and anteriorly relative to bregma. A stainless-steel wire (0.1 mm) with teflon-insulation and a standard microelectronic connector was joined to the screw heads forming screw electrodes. A female connector was fixed to the skull with dental acrylic cement. A recovery period of 1 week after surgery was allowed for mice to recover.

Spontaneous seizures were monitored using both video and EEG recordings. A bendable cable linked to an amplifier (8-channel; AD Instruments Ltd., East Sussex, UK) and an analogue–digital converter (AD Instruments Ltd., East Sussex, UK) was used. Chart4 software for windows (AD Instruments Ltd., East Sussex, UK) was used for data recording and analyses. A high pass filter (0.1 Hz) and a low pass filter (60 Hz) were used with a sampling rate of 200 Hz. Animals were also simultaneously video recorded to assess behavioral




**Scheme 1.** Experimental scheme. Eight-week-old male C57BL/6 mice were fed with chow either supplemented with or without 0.2 % (w/w) cuprizone for 12 weeks whiles receiving normal saline (10 ml/kg), AE (100 and 300 mg/kg), LEV (10 mg/kg).

correlates to epileptiform activity observed in the EEG. Control and treated animals (n = 7) were monitored for 3 days, 24 h a day. Recordings started on the day of termination of a 12-week period of treatment with cuprizone. Additionally, resultant overt seizures were scored using the racine scale as follows: Stage 0 (no response); Stage 1 (hyperactivity, restlessness and vibrissae twitching); Stage 2 (head nodding, head clonus, myoclonic jerks and freezing); Stage 3 (unilateral or bilateral limb clonus); Stage 4 (forelimb clonic seizures); Stage 5 (generalized clonic seizures with loss of postural control).

### 2.10. Brain sampling

Anaesthetized mice were opened up and perfused transcardially with cold 5 ml phosphate buffered saline and then with 5 ml of neutral formalin (4 %). After perfusion, the brain was removed. Isolated brains were postfixed in 4 % paraformaldehyde (PFA) and embedded in paraffin. Four sections (7 μm) were stained with luxol fast blue (LFB; Sigma, USA) overnight at 56 °C (bregma −1.7, −1.94, −2.18, −2.46 as described in mouse atlas by Ref. [17].

### 2.11. Myelin quantification

Brain slice samples were stained with LFB. To remove excess blue stain, slices were washed with distilled water and 95 % alcohol. Samples were then exposed to lithium carbonate for 20 s to trap the blue color within the myelinated white matter. Alcohol (75 %) was used to wash slides thrice and background stained using Cresyl violet over a 1 min period. Leica DM750 Digital Microscope (Leica, Germany) with a 4 × objective magnification was used to capture the image of each slide. The corpus callosum was analyzed with Image J software (National Institute of Health, Bethesda, Maryland, USA) to estimate myelin density. Briefly, captured images were imported into ImageJ software for analysis. A micrometer slide was used to calibrate the software to ensure accurate measurement scales. RGB images were split into their constituent colors, isolating the blue channel that corresponds to LFB-stained myelin. An appropriate threshold was set to differentiate myelin from the background. The particle analysis function in ImageJ was used to measure the area of the regions containing stained myelin. Myelin density was subsequently calculated by dividing the myelin-stained

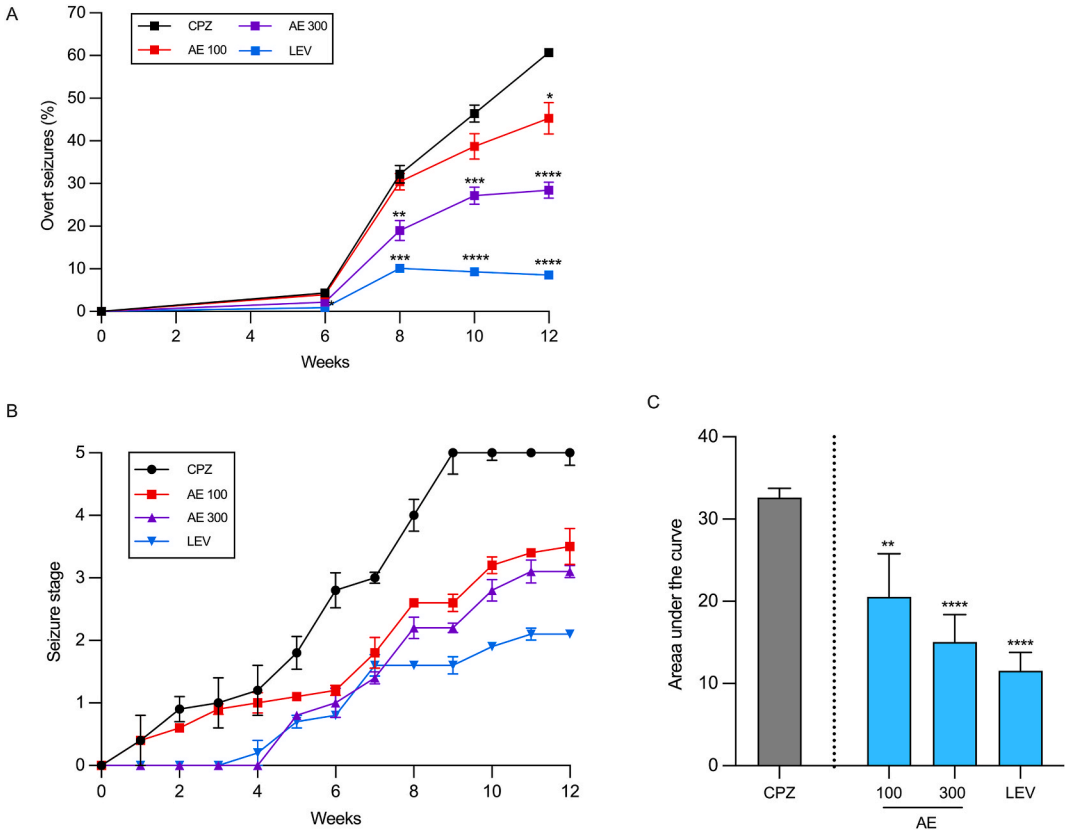



**Fig. 1.** Occurrence of overt seizures during demyelination phase. A) Effects of chronic normal saline (10 ml/kg), AE (100 and 300 mg/kg, i.p.) and Levetiracetam (10 mg/kg, i.p.) treatment in chronic cuprizone treated animals over time. B) and C) Effects of chronic normal saline (10 ml/kg), AE (100 and 300 mg/kg, i.p.) and Levetiracetam (10 mg/kg, i.p.) treatment in chronic cuprizone treated animals over time on development of the stages of seizures. Data represent mean ± S.E.M. (n = 7). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$ ****$P \leq 0.0001$ indicates significant interaction between time and treatment (Two-way ANOVA followed by Bonferroni's *post hoc* test).

area by the total area of the corpus callosum in each image. Myelin density values from seven sections of each sample were averaged to obtain a representative value for each slide.

### 2.12. Statistical analysis

Data was analyzed for statistical differences by one-way analysis of variance (ANOVA) followed by Tukey post hoc test and two-way ANOVA followed by Bonferroni's test using GraphPad Prism software v 8.0 (San Diego, CA). $P \leq 0.05$ was described as statistically significant.

## 3. Results

### 3.1. Behavioral observations

Seizures lasting between 5 and 20 s that persisted throughout the demyelination phase were observed during disturbances e.g.

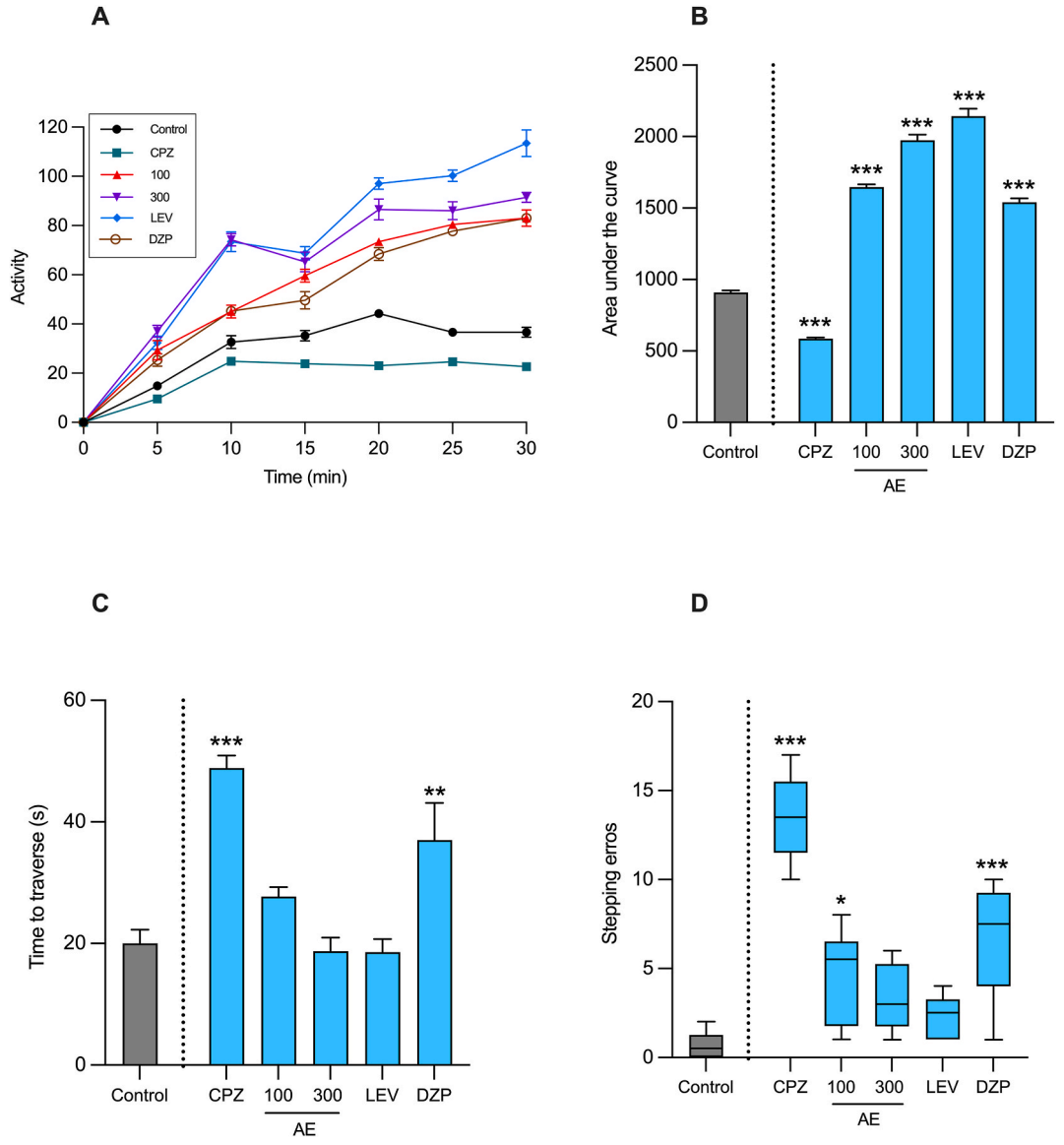



**Fig. 2.** Effects of normal saline (10 ml/kg), AE (100 and 300 mg/kg, i.p.), Levetiracetam (10 mg/kg, i.p.) and Diazepam (1 and 3 mg/kg, i.p.) treatment in the activity meter (A and B) and beam walking (C and D) tests in cuprizone treated animals. Control group received only NaCl 0.9 %. Data represent mean ± S.E.M. (n = 7). **P ≤ 0.01, ***P ≤ 0.001* compared with vehicle-treated group (One-way ANOVA followed by Tukey *post hoc* test).

whenever the cage is opened, or mice are lifted by their tails. Mice exhibited seizures characterized by jerking, freezing, falling and loss of righting reflex. Nearly 40 % of mice on CPZ diet exhibited overt seizure during handling. In week 12, seizure activity increased in 60.71 ± 0.42 % of animals in the cuprizone and normal saline group. Chronic treatment with AE (100 mg/kg) significantly ($P < 0.05$; $F(12, 96) = 38.11$) reduced seizures to 45.29 ± 3.7 % while AE (300 mg/kg) significantly ($P < 0.0001$; $F(12, 96) = 38.11$) reduced overt seizures to 28.43 ± 1.88 %. Levetiracetam (10 mg/kg) similarly reduced seizure occurrence significantly ($P < 0.0001$; $F(3, 24) = 161.2$) to 8.571 ± 0.84 % (Fig. 1A). Chronic treatment with AE (100 mg/kg; $P < 0.05$; $F(3, 48) = 3.928$) and 300 mg/kg ($P < 0.0001$; $F(3, 48) = 3.928$) significantly suppressed the development of stage 5 seizures in cuprizone treated mice (Fig. 1B and C).

Cuprizone treatment significantly ($P < 0.001$; $F(2, 12) = 620.4$; Fig. 2B) reduced spontaneous locomotor activity compared to all other treatment groups (Fig. 2A). Animals receiving AE showed significant ($P < 0.001$; $F(3, 16) = 698.4$; Fig. 2B) increase in locomotor activity similar to LEV ($P < 0.001$; $F(2, 12) = 678.7$; Fig. 2B) and DZP ($P < 0.001$; $F(2, 12) = 620.4$; Fig. 2B).

Cuprizone significantly ($P < 0.001$; $F(2, 18) = 13.51$; Fig. 2C) increased average time required to traverse the beam to 48.86 ± 2.075 s (95 % CI: 43.78–53.93) compared to control (20.00 ± 2.257 s; 95 % CI: 14.48–25.52). Animals receiving AE and LEV traversed the beam in averagely 18.71 ± 2.244 s (95 % CI: 13.22–24.20) and 18.57 ± 2.170 s (95 % CI: 13.26–23.88) respectively; not significantly different from naïve control. DZP treated animals traversed the beam in averagely 37 ± 6.110 s (95 % CI: 22.05–51.95).

Stepping errors, defined as total misses of the forelimb, significantly ($P < 0.001$; $F(2, 15) = 43.07$; Fig. 2D) increased after CPZ treatment. Animals receiving AE at 100 mg/kg also exhibited significant ($P < 0.05$; $F(2, 15) = 6.667$; Fig. 2D) stepping errors, similar to animals pretreated with DZP ($P < 0.001$; $F(2, 15) = 43.07$; Fig. 2D).

### 3.2. EEG recordings

Mice treated with cuprizone and assessed with EEG presented with spike discharges which were short but frequent (Fig. 3A2) compared to naïve controls (Fig. 3A1). *Antiaris Africana* extract (100 mg/kg; Fig. 3A3) treated mice exhibited partially shorter spike discharges whiles *Antiaris africana* extract (300 mg/kg; Fig. 3A4) treatment abolished epileptiform discharges similar to levetiracetam (Fig. 3A5).

### 3.3. Brain sampling

CPZ administration caused severe demyelination of the corpus callosum (Fig. 4B) compared with naïve control (Fig. 4A). After 4 weeks of cuprizone cessation, CPZ-only group still showed some demyelination in the corpus callosum compared to naïve control (Fig. 4A). Levetiracetam and *Antiaris africana* extract treated groups enhanced remyelination (Fig. 4C and D).

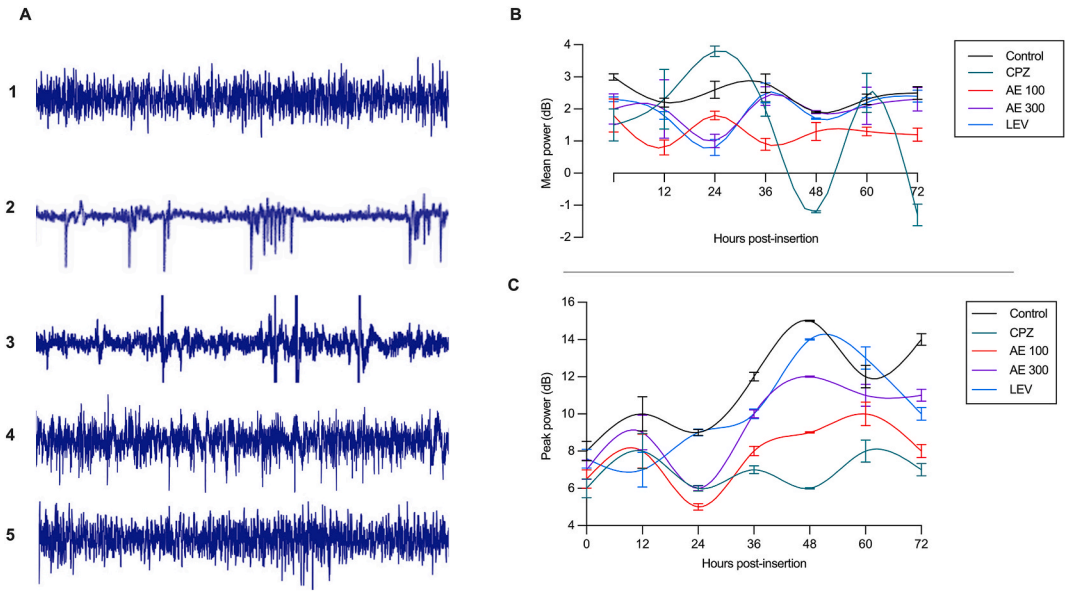



**Fig. 3.** Cortical EEG-recordings from A) 1. Naïve control mouse; 2. Cuprizone and normal saline treated mice; 3. Cuprizone and AE (100 mg/kg) treated mice; 4. Cuprizone and AE (300 mg/kg); 5. Cuprizone and levetiracetam (10 mg/kg) treated mice treated mice. Cuprizone and normal saline treated mice presented with short, frequent spike discharges (A2) and were not associated with any observed behavioral defects compared with naive control mice. B) Cuprizone treatment induces oscillations in mean power over entire frequency spectrum for 3 days compared to control and drug-treated mice C) Cuprizone treatment reduces peaks power compared to control and drug-treated mice.

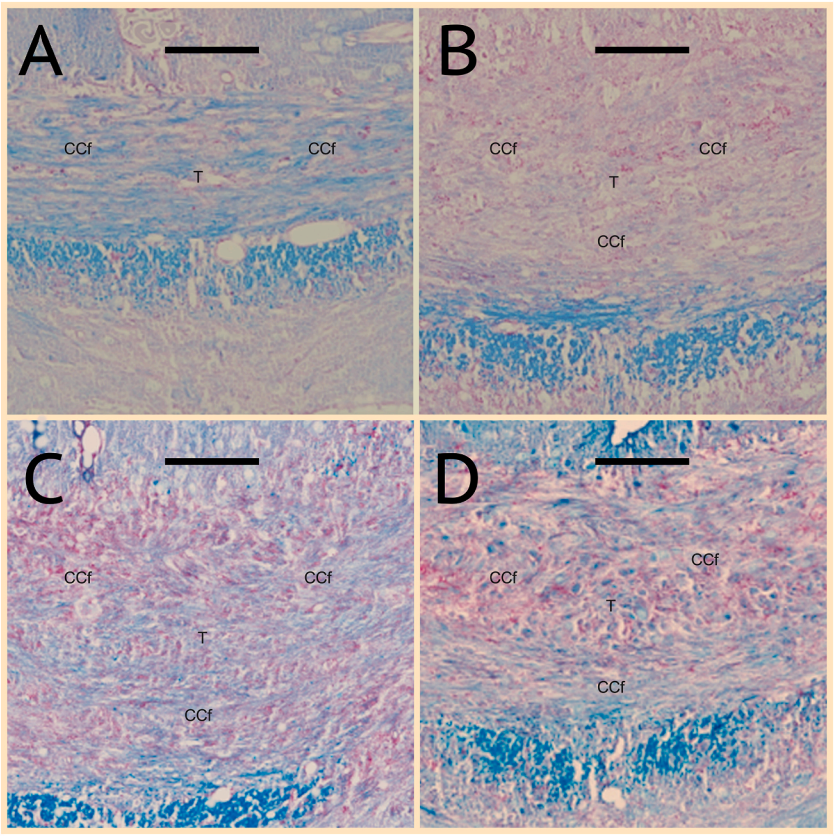



**Fig. 4.** Representative sections showing myelin content in the corpus callosum after 4 weeks of remyelination of LFB - stained sections after CPZ treatment cessation. Micrograph A from control group shows normal myelin (blue colour). Micrograph B from CPZ and normal saline group still shows demyelination (loss of blue colour) in the corpus callosum. Micrographs C and D from CPZ and levetiracetam (10 mg/kg; i.p.) and CPZ and AE (300 mg/kg; i.p.) treated groups respectively show levels of remyelination (traces of blue colour). Scale bar 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

### *3.4. Myelin quantification*

The average myelin density in corpus callosum of the cuprizone-free mice was estimated to be 100 % and all treatment groups then compared to it. Evaluations were done at the end of the 12-week cuprizone treatment and 4-week remyelination periods. All groups

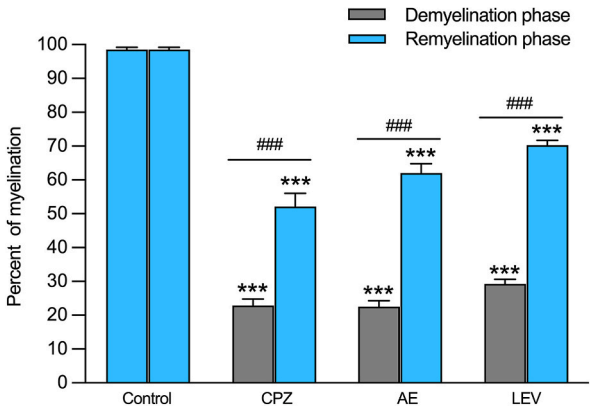



**Fig. 5.** Effect of normal saline (10 ml/kg), AE (300 mg/kg, i.p.) and Levetiracetam (10 mg/kg, i.p.) on cuprizone-induced demyelination after 12 weeks of demyelination and 4 weeks of remyelination. Data represents mean ± S.E.M (n = 7). Significant differences in comparison with the control group, ***P ≤ 0.001 (One-Way ANOVA followed by Tukey test). $^{###}$P ≤ 0.001 (Two-Way ANOVA followed by Bonferroni's test) indicates statistical differences in myelin density for treatment groups during both phases. Control group received only NaCl 0.9 %.

showed significantly ($P < 0.001$; Fig. 5) lower myelin content compared to the control group in the demyelination phase. After the demyelination phase, myelin content decreased to 22.86 ± 1.92 % in the cuprizone-only group. After a 4-week cuprizone cessation period, myelin content increased to 52.14 ± 3.91 % in cuprizone-only group. In AE and LEV treated groups, myelin content increased to 62.00 ± 2.78 % and 70.29 ± 1.43 % ($p < 0.0001$) respectively. Treatment with AE and LEV significantly altered myelin growth in comparison to the untreated cuprizone-only mice.

### 3.5. LC-MS analysis of *Antiaris africana* aqueous extract

LC-MS chromatogram the *Antiaris africana* aqueous extract showed fifteen (15) peaks which indicates the presence of fifteen phytochemical constituents (Fig. 6). Mass spectra was compared with the MassBank library, and the fifteen phytocompounds were characterized and identified (Table 1).

## 4. Discussion

Demyelination as occurs in conditions such as multiple sclerosis presents with numerous clinical symptoms including epileptic seizures, which may sometimes be the first observable symptom [18]. Epileptic seizure prevalence in these patients is roughly 2.3 % [19]. Partial seizures with secondary generalized tonic–clonic seizures have been observed to be frequently associated with demyelination conditions [19].

Similarly, following 12 weeks of 0.2 % cuprizone treatment, all mice that were examined for the presence of seizures using EEG-recordings presented with frequent epileptiform activity. The seizure activity observed was also accompanied with observed extensive demyelination of the corpus callosum as previously described by Ref. [7]. The periodic, brief spikes were somewhat similar to those observed in patients with partial epilepsy and have been widely accepted as being representative of epilepsy [7]. Furthermore, seizures were mostly triggered by disturbances in the environment underscoring the susceptibility of the demyelinated circuits to external stimuli [20]. The observed association between demyelination and epileptic seizures in these mice further highlights the complex relationship between the neural pathology and seizure susceptibility.

*Antiaris africana* has been widely studied for its neuroprotective activity [21–23]. Building on this foundation, preliminary data from our lab demonstrates efficacy against pentylenetetrazole and picrotoxin-induced seizures similar to the main specie *Antiaris toxicaria*. This anticonvulsant activity is postulated to be mediated by enhancement of GABA-mediated neurotransmission. Current research shows that GABA-mediated signaling plays a pertinent role in oligodendrocyte progenitor cells (OPCs) and Schwann cells (SC) precursor development and function [24,25]. Notably, GABA regulates differentiation and myelination of OPCs in the white matter of the cerebellum [26]. OPCs and SC precursor differentiation is essential for remyelination in conditions characterized by demyelination [24]. *Antiaris africana* extract's activity against demyelination-induced seizures may be linked to its proposed GABAergic activity warranting further investigation into these mechanisms.

Further to this, investigation into the effects of the extract on myelination further clarifies its mechanisms of action on key molecular pathways involved in neuroprotection. *Antiaris africana* extract treatment visibly affected myelination compared to the cuprizone group, suggesting a potential role in promoting remyelination processes. It is a possibility that *Antiaris africana* extract may possess direct neurotrophic effects on neurons. Atractylenolides isolated from *Antiaris africana* [27] have been previously shown to enhance BDNF signalling and suppressed the A1 differentiation of astrocyte, preventing inflammation [28]. Additionally, neuroprotective effects of *Antiaris africana* extract treatment has been hitherto demonstrated to be mediated through modulation of $Na^+/K^+$ ATPase activity, xanthine oxidase (XO) activity and the mitochondrial electron transport system [21,22,29]. Of particular relevance is

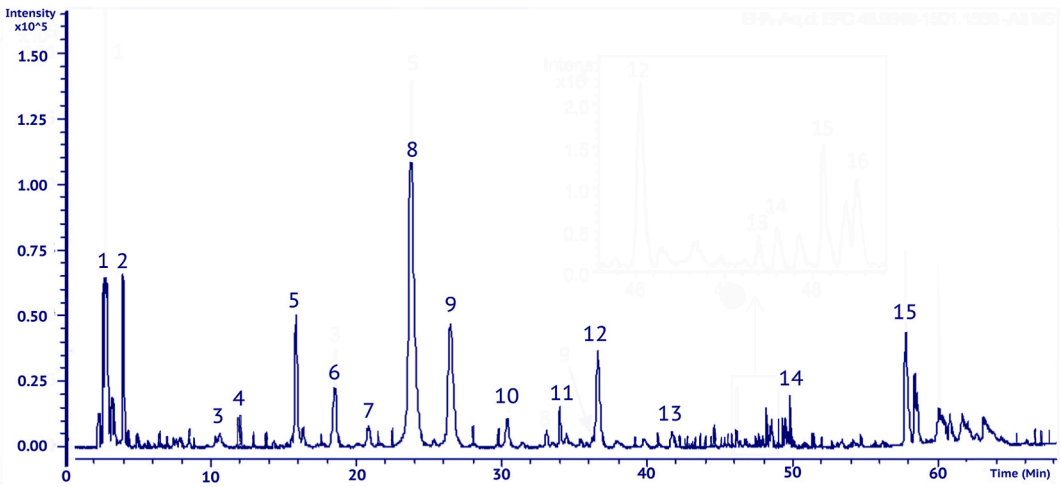



**Fig. 6.** LC-MS chromatogram of *Antiaris africana* aqueous extract.

**Table 1**
Phytocomponents in *Antiaris africana* stem bark aqueous extract.

| Peak N° | $R_t$ (min) | Formula | Identification |
|---|---|---|---|
| 1 | 2.66 | $C_7H_{11}O_6$ | Quinic acid |
| 2 | 4.2 | $C_{18}H_{15}NO_4$ | Piperolactam C |
| 3 | 11.02 | $C_{28}H_{34}O_{14}$ | Didymin |
| 4 | 12.14 | $C_{15}H_{12}N_5$ | Naringenin |
| 5 | 15.67 | $C_{16}H_{17}O_9$ | Chlorogenic acid isomer |
| 6 | 18.79 | $C_{32}H_{33}O_{18}$ | Caffeoylquinic acid derivative |
| 7 | 20.89 | $C_{32}H_{33}O_{18}$ | Caffeoylquinic acid derivative |
| 8 | 23.65 | $C_{16}H_{17}O_9$ | Chlorogenic acid |
| 9 | 25.90 | $C_{16}H_{17}O_9$ | Chlorogenic acid isomer |
| 10 | 30.45 | $C_{16}H_{17}O_9$ | Chlorogenic acid isomer |
| 11 | 34.02 | $C_{16}H_{17}O_8$ | Coumaroylquinic acid isomer |
| 12 | 37.10 | $C_{17}H_{19}O_9$ | Feruoylquinic acid |
| 13 | 41.33 | $C_{30}H_{45}O_{13}$ $[M + HCOO]^-$ | Toxicarioside K |
| 14 | 48.44 | $C_{25}H_{23}O_{12}$ | Caffeoylquinic acid derivative |
| 15 | 58 | $C_{25}H_{45}NO$ | 1-(Piperidinyl)-2,4-eicosadien-1-one |

the role of xanthine oxidase. XO has been implicated in the pathogenesis of experimental autoimmune encephalomyelitis and suggested as a target for MS treatment [30]. Additionally, studies suggest that energy changes associated with demyelination requires increases in mitochondria. Demyelinated axons greatly rely on mitochondria to fulfil both energy demands and oxidative stress protection. Hence, any energy deficit will most likely lead to an accumulation of sodium in axoplasm, reversal of sodium/calcium exchange, and hence axonal degeneration [31,32]. In this context, the extract's inhibitory actions against xanthine oxidase (XO) activity and enhancement of mitochondrial function may be critical in reversal of demyelination.

Furthermore, the observed enhancement of motor coordination and balance in extract-treated animals underscores its broader neuroprotective effects, potentially involving anxiolytic activity, which warrants further exploration.

The beam walking test measures skilled motor coordination, walking and balance [33]. Inability or difficulty in completing the task is therefore an indication of impaired motor coordination [33]. Cuprizone treatment resulted in significant adverse effect on motor coordination and balance. Demyelinated animals tend to display a markedly impaired capacity for fine motor balance and coordination associated with corticospinal tract injury [34,35]. Demyelination of the corpus callosum by cuprizone administration may have contributed to the motor incoordination, as seen with other studies [35–37]. *Antiaris africana* extract treated animals exhibited finer motor skill and coordination. This may be attributed to the previously established neuroprotective effects of the extract. The increase in spontaneous locomotor activity observed in *Antiaris Africana* extract treated mice may be attributed to the potential presence of anxiolytic-like activity. Previous studies have reported that Antiaris species possess anxiolytic-like activity in rodents by acting like benzodiazepines and enhancing GABAergic mechanisms [38,39]. Spontaneous locomotor activity may be increased due to increased exploratory behavior as evident with anxiolytic compounds such as benzodiazepines [40]. Additionally, modulation of neurotransmitters such serotonin and norepinephrine which are known to affect locomotor activity, may play a role. Again, Antiaris species have been previously shown to enhance adrenergic and serotoninergic mechanisms. These combined effects may contribute to the increase in activity observed. Treatment with benzodiazepines at high doses has been shown frequently to produce muscle relaxant actions [41]. Hence, the observed effect of diazepam on motor coordination.

*Antiaris africana* extract was shown to contain several secondary metabolites which may be contributing to the activity observed in this study. Bioactive constituents such as didymin, naringin, naringenin, chlorogenic acid and quinic acid, have been previously studied. Didymin demonstrates antioxidant activity by scavenging free radicals and reducing oxidative stress, crucial for preventing damage to myelin sheaths, as well as providing neuroprotection by activating antioxidant defense enzymes and inhibiting apoptotic pathways [42]. Chlorogenic acid, and its metabolite caffeic acid, exhibit anti-inflammatory effects by inhibiting pro-inflammatory cytokines like interleukin-8 and macrophage inflammatory protein 2, reducing inflammation that leads to demyelination, and scavenging reactive oxygen species (ROS) to maintain cellular health and protect myelin [43,44].

Naringin and its metabolite naringenin play a significant role in seizure suppression by reducing spontaneous recurrent seizures, inhibiting kainic acid-induced granule cell dispersion and neuronal cell death in the hippocampus [45]. Didymin, furthermore, contributes to antiseizure effects by reducing oxidative stress and neuronal damage in addition to enhancing GABAergic mechanisms [46]. Overall, the combined effects of these compounds, through their antioxidant, anti-inflammatory, antiseizure and neuroprotective activities, may potentially enhance the therapeutic efficacy of *Antiaris africana* extract, making it a promising natural remedy for conditions involving demyelination and seizures.

Taken together, our findings show that *Antiaris africana* extract improves seizures induced by chronic demyelination and has beneficial effects on motor coordination. It may be inferred that *Antiaris Africana* extract treatment may play a vital role in neuronal cell growth. However, further studies will, therefore, be essential to establish its effect further firmly on myelination.

In conclusion, *Antiaris africana* extract could be considered as a therapeutic option for managing seizures in chronic demyelination that occurs in conditions such as multiple sclerosis. There currently is a lack of effective therapeutic options targeting remyelination for patients with demyelinating diseases.

## Ethics statement

Ethical clearance for the study was given by the Animal Ethics Committee, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana (KNUST 0055). All experiments were conducted in accordance with the National Institutes of Health Office of Laboratory Animal Welfare policies and laws. All experiments complied with the ARRIVE guidelines.

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## Data availability statement

The data will be provided on request to the authors.

## CRediT authorship contribution statement

**Ransford Amoah:** Writing – review & editing, Writing – original draft, Investigation. **John Danquah:** Writing – original draft, Visualization, Software, Methodology. **Priscilla Kolibea Mante:** Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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