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Neuroprotective effects of *Aframomum melegueta* on lithium–pilocarpine-induced prefrontal cortical damage in *Wistar rats*

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Abstract

Epileptic seizures are linked to significant neurochemical, oxidative, inflammatory, and structural changes in the brain, especially within the prefrontal cortex, a region essential for cognition and behaviour. Oxidative stress and neuroinflammation are essential factors in seizure-induced neurodegeneration. *Aframomum melegueta*, a medicinal plant abundant in bioactive phytochemicals, has exhibited antioxidant and neuroprotective activities; nevertheless, its impact on prefrontal cortical damage subsequent to epilepsy is little defined. This study assessed the impact of *Aframomum melegueta* on behavioural, biochemical, neurochemical, histological, and inflammatory alterations in the prefrontal cortex of Wistar rats with epilepsy caused by lithium chloride and pilocarpine.

Twenty-four male Wistar rats were randomly allocated into four groups: control, lithium-pilocarpine (LP), LP treated with *Aframomum melegueta* (400 mg/kg), and LP treated with carbamazepine (100 mg/kg). Seizures were elicited with the administration of lithium chloride, succeeded by pilocarpine. Behavioural evaluations were performed via the open field and Y-maze assessments. Prefrontal cortical tissues were examined for neurotransmitters (GABA and glutamate), oxidative stress indicators (malondialdehyde and superoxide dismutase), and the inflammatory cytokine (IL-6). Histological and immunohistochemical assessments were conducted utilising haematoxylin and eosin, Cresyl fast violet, Luxol fast blue, and GFAP staining techniques.

Lithium-pilocarpine induction resulted in considerable behavioural abnormalities, an imbalance of excitatory and inhibitory neurotransmitters, heightened lipid peroxidation, diminished antioxidant activity, raised IL-6 levels, and pronounced neuronal degeneration, demyelination, and astrogliosis in the prefrontal cortex. Treatment with *Aframomum melegueta* markedly improved behavioural deficits, decreased glutamate and IL-6 concentrations, bolstered antioxidant defences, maintained neuronal and myelin integrity, and mitigated astrocytic activation, exhibiting results akin to carbamazepine.

Aframomum melegueta demonstrates considerable neuroprotective properties against lithium-pilocarpine-induced damage in the prefrontal cortex, presumably via antioxidant, anti-inflammatory, and neuromodulatory pathways. The results endorse its potential as an adjunctive treatment agent in the management of epilepsy.

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Keywords: Lithium Chloride–Pilocarpine; Epilepsy; Oxidative Stress; Neuroinflammation

1. Introduction

Epilepsy is a chronic neurological disorder characterised by repeated, unprovoked seizures resulting from aberrant and excessive electrical activity in the brain[1]. It impacts more over 50 million individuals globally, with a disproportionately greater burden in low- and middle-income nations where infectious, metabolic, and environmental risk factors are prevalent [2]. Seizures are intricately linked to neuronal hyperexcitability, oxidative stress, neuroinflammation, and structural damage in susceptible brain areas [3]. The prefrontal cortex is notably significant among these regions due to its involvement in cognition, behaviour, and executive function [4]. Damage to the prefrontal cortex during epileptogenesis leads to memory problems, affective disturbances, and enduring neurocognitive impairments frequently seen in persons with chronic epilepsy [5].

The lithium chloride pilocarpine paradigm is a well-established experimental framework for investigating limbic seizure disorders [6,7]. Pilocarpine, an agonist of muscarinic M1 receptors, precipitates status epilepticus through the overactivation of cholinergic pathways, leading to heightened glutamate release, excitotoxicity, and prolonged neuronal depolarisation [8]. Pretreatment with lithium chloride augments neuronal vulnerability to pilocarpine by inhibiting inositol monophosphatase, which enhances intracellular inositol triphosphate (IP_3) signalling and reduces the seizure threshold. This model closely mimics human temporal lobe epilepsy, replicating essential characteristics including hippocampal sclerosis, cortical neurodegeneration, spontaneous recurring seizures, oxidative imbalance, mitochondrial dysfunction, and compromised neural plasticity [9]. The excitotoxic and oxidative processes elicited by this model significantly impact the prefrontal cortex, resulting in structural damage and functional impairment [10].

Oxidative stress is widely acknowledged as a significant factor in neuronal damage during epileptogenesis [3]. Seizure activity induces the overproduction of reactive oxygen species (ROS), lipid peroxidation, and a reduction in endogenous antioxidant defences, such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) [11]. These metabolic changes undermine membrane integrity, impair synaptic transmission, and induce neuronal death [12]. Moreover, neuroinflammatory responses, driven by pro-inflammatory cytokines and microglial activation, exacerbate neuronal injury [3]. Despite antiepileptic drugs (AEDs) being the cornerstone of epilepsy treatment, a significant proportion of patients exhibit resistance to therapy, and prolonged administration of AEDs frequently correlates with adverse effects, including cognitive impairment, hepatotoxicity, and metabolic disturbances [13]. These constraints underscore the necessity for safer, plant-based neuroprotective medicines capable of addressing oxidative stress, inflammation, and neurotransmitter dysregulation.

Aframomum melegueta, known as grains of paradise, is a medicinal plant from the Zingiberaceae family, extensively utilised in traditional medicine in West Africa. The seeds have historically been utilised for treating inflammatory illnesses, gastrointestinal issues, and neurological ailments [14]. Phytochemical investigations indicate that A. melegueta comprises several bioactive compounds, such as gingerols, paradols, shogaols, flavonoids, tannins, and essential oils, which exhibit potent antioxidant, anti-inflammatory, antibacterial, and analgesic properties [14]. Experimental studies have shown its capacity to scavenge free radicals, augment endogenous antioxidant enzyme activity, stabilise neuronal membranes, and modulate neurotransmitter systems, indicating potential advantages in neurological disorders linked to oxidative damage and neuronal hyper-excitability [15,16].

Despite its recognized medicinal importance, there is limited experimental evidence on the neuroprotective role of *Aframomum melegueta* in seizure disorders, particularly with respect to the prefrontal cortex. Given the central involvement of oxidative stress and inflammation in LiCl–pilocarpine-induced epileptogenesis, investigating the protective effects of *A. melegueta* on cortical integrity is both relevant and necessary. Therefore, this study evaluates the effects of *Aframomum melegueta* on oxidative stress markers, neuronal morphology, and biochemical alterations in the prefrontal cortex of Wistar rats subjected to lithium chloride–pilocarpine-induced seizures. Findings from this study may provide valuable insights into the potential of this plant as a complementary or alternative therapeutic approach for epilepsy and for reducing seizure-associated neurodegeneration.

2. Materials and methods

2.1. Collection and Identification of Plant Material

Fresh seeds of *Aframomum melegueta* were acquired from the Flora Reserve for Agriculture Masters and Education, FRAME firm, located in Ibadan, Oyo State. The seeds were washed, air-dried at ambient temperature for ten days, and then pulverised using a mechanical grinder.

2.2. Preparation of Plant Extracts

Plant extraction was conducted with the ethanolic cold maceration technique [17]. The seeds were meticulously rinsed under running water to eliminate dirt and impurities, thereafter air-dried for two weeks at 25°C. The desiccated seeds were subsequently ground into a fine powder utilising a commercial grinding machine. Five hundred grams of powdered substance were macerated with 2.5 litres of 80% ethanol for 72 hours and subsequently filtered through a thin cotton cloth. The filtrate was concentrated with a rotary evaporator at 45°C to gently eliminate ethanol. The semi-solid extract was placed in a glass petri dish and air-dried for 48 to 72 hours to eliminate residual moisture. Consequently producing a viscous brownish resinous crude extract.

2.3. Experimental Animals

Twenty-four (24) Wistar rats were obtained from the Babcock University Animal Holding in Ilishan Remo, Ogun State. The rats were maintained in well-ventilated plastic cages at the animal holding facility during the research period.

2.4. Drugs

Pilocarpine and Lithium Chloride were obtained from Sigma Aldrich, USA. Carbamazepine was acquired from the pharmacy department at Babcock University Teaching Hospital (BUTH).

2.5. Animal groupings

Experimental rats were grouped into four groups with each group containing six (6) animals each and the experiment was carried out for 35days. Acclamitization was done for two weeks before the commencement of the experiment.

2.6. Seizure Induction

Seizures were generated using the protocol used by Olatunji *et al*., [18]. Initially, rats received an intraperitoneal injection of lithium chloride at a dosage of 127 mg/kg body weight, followed by pilocarpine at 30 mg/kg body weight 24 hours later. The rats were examined for around twenty minutes to assess the epileptic symptoms utilising the Racine scale. Upon observation of the symptoms, a 1ml dosage of Diazepam was delivered intramuscularly by syringe and needle to mitigate the effects of LiCl-P and induce muscle relaxation in the rats.

2.7. Experimental Design

Twenty-four (24) male Wistar rats, weighing between 120 and 150 grams, were randomly allocated into four groups (A-D) of six animals each. All groups were administered a regular diet throughout the 21-day testing period. Group A acted as the control and was supplied distilled water, whereas Group B received LP only. Group C was administered LP and treated with AM at a dosage of 400 mg/kg body weight. Group D was administered LP and treated with carbamazepine at a dosage of 100 mg/kg body weight. At the conclusion of the administration phase, on day 22, the animals were subjected to behavioural paradigms (Open Field and Y Maze). On day 23, animals were euthanised with diethyl ether, their brains were dissected, and the cerebral cortex was removed, processed, sectioned at 5 µm, and stained with haematoxylin/eosin, luxol fast blue, and cresyl fast violet. Homogenates of the cerebral cortex were utilised for the evaluation of GABA, glutamate, MDA, SOD, and IL-6.

2.8. Behavioural Testing

Behavioural assessments were performed in the subsequent order: Open field (line crossing, rearing, grooming, freezing, center square frequency) and Y-maze. All neurobehavioral tests were videotaped using a webcam connected via cable from a laptop to the behavioural apparatus and subsequently assessed manually.

2.8.1. Open Field Test

The open field test was performed following the methodology established by Hall [19] to assess locomotor activity, anxiety-related behaviours, and exploratory inclinations in rats. The apparatus was a square plywood arena of 72 x 72 cm, including 36 cm high walls, with the inner surfaces painted white. The floor was delineated with red lines to form 18 x 18 cm squares, with a centrally located green square (18 × 18 cm) illustrated at the center. A translucent Plexiglas sheet measuring 72 × 72 cm was positioned over the floor surface. Each Wistar rat was individually positioned in the center of the arena and permitted to wander freely for five minutes. The device was sanitised with ethanol between trials to remove smell cues from prior individuals. The recorded behavioural parameters encompassed: (i) line crossing – frequency of the animal crossing a grid line with all four paws; (ii) rearing – frequency of the animal standing upright on its hind limbs; (iii) center square entry – frequency of entries into the central square with all four paws; (iv) freezing – frequency of episodes in which the animal remained entirely motionless.

2.8.2. Y-Maze Test

The Y-maze test was utilised to assess spatial working memory in rats, predicated on the inherent inclination of rodents to investigate a novel arm instead of revisiting a previously explored one. The apparatus comprised three white-painted wooden arms (labelled A, B, and C) positioned at 120° angles relative to one another. Each rat was positioned at the center of the maze and let to navigate all three arms freely. one arm entrance was documented when all four paws of the animal were situated within one arm. The sequence and quantity of arm entries were documented to compute the proportion of spontaneous alternation, characterised as consecutive entries into three distinct arms (a triad). The brain regions implicated in this task encompass the hippocampus, septum, basal forebrain, and prefrontal cortex.

2.9. Sacrifice and Histological Analysis

At the end of the studies, the animals were euthanised using diethyl ether. The rat brains were meticulously removed, wiped dry, and weighed using an AB204 Mettler Toledo scale. The brains were immersed in 10% neutral buffered formalin for fixation. Coronal brain slices of the prefrontal cortex, each measuring one millimetre in thickness, were acquired and subjected to standard paraffin embedding procedures. Sections were stained with haematoxylin and eosin to illustrate the general histoarchitecture of the PFC, while Cresyl fast Violet and Luxol fast Blue were employed to assess the condition of Nissl substance and the integrity of the myelin sheath.

2.10. Immunohistochemistry

Immunohistochemical examination was conducted utilising a modified methodology derived from Tascos et al., [20]. Serial slices (5 μm) of the prefrontal cortex were extracted from paraffin-embedded blocks, affixed to glass slides, and incubated on a hot plate at 70 °C for 1 hour. The sections were deparaffinized using two changes of xylene, rehydrated using decreasing concentrations of ethanol, and washed with distilled water. Antigen retrieval was conducted by heating the sections in 0.01 M citric acid buffer (pH 6.0) for 25 minutes, followed by progressive cooling under running tap water for a minimum of 5 minutes. Endogenous peroxidase activity was inhibited using 3% hydrogen peroxide for 15 minutes, followed by rinsing the sections in phosphate-buffered saline (PBS). Non-specific binding was inhibited with avidin for 15 minutes, followed by the obstruction of endogenous biotin with a biotin solution for 15 minutes, with PBS washes between each procedure.

The sections were subsequently treated with the primary antibody (GFAP, 1:100 dilution) for the specified duration, rinsed in PBS, and then incubated with the secondary antibody (LINK) for 15 minutes. Subsequent to an additional PBS wash, horseradish peroxidase (HRP) was administered for 15 minutes, followed by visualisation with 3,3′-diaminobenzidine (DAB) for 5 minutes. Excess DAB was eliminated through PBS washes, and the sections were counterstained with haematoxylin for 2 minutes, dehydrated in graded alcohols, cleaned in xylene, and mounted with DPX. Photographs were obtained with a Leica DM750 microscope paired with a Leica ICC50 camera, and digital photomicrographs were stored for subsequent study.

2.11. Biochemical Test

2.11.1. Evaluation of Lipid peroxidation

Lipid peroxidation levels were evaluated by measuring malondialdehyde concentration, which quantifies thiobarbituric acid reactive substances in biological samples. Coloured complexes are generated when free malondialdehyde interacts with thiobarbituric acid reactive substances. The coloured complexes are subsequently quantified spectrophotometrically and reported in micromoles (μmol).

2.11.2. Evaluation of Superoxide Dismutase (SOD)

Superoxide Dismutase activity was measured following the directions of the kit manufacturer.

2.11.3. Interleukin (IL) -6 and Tumour Necrosis Factor-α

Inflammatory cytokine levels (Interleukin (IL)-6 and Tumour Necrosis Factor-α) were evaluated using enzyme-linked immunosorbent assay techniques with commercially available kits (Biovision Inc., Milpitas, CA, USA).

2.11.4. Neurotransmitters

Prefrontal cortical slices were homogenised. The homogenates were subsequently centrifuged, and the supernatant was utilised to evaluate the concentrations of acetylcholine, gamma-aminobutyric acid (GABA), and glutamate using conventional test kits.

2.12. Statistical Analysis

Data were processed via Graph Pad Prism 6. Data analysis was conducted using One-way analysis of variance (ANOVA), followed by a post-hoc test (Tukey HSD) for comparisons. All findings were shown as mean ± S.E.M., with $p < 0.05$ being the threshold for significant deviation from control.

3. Result

3.1. Effects of *Aframomum melegueta* on horizontal locomotion

Figure 1 shows the effects of AM on open field horizontal locomotion measured as number of lines crossed. In lithium pilocarpine group, there is a reduction of horizontal locomotion in contrast to the control and AM and carbamazepine treated groups.

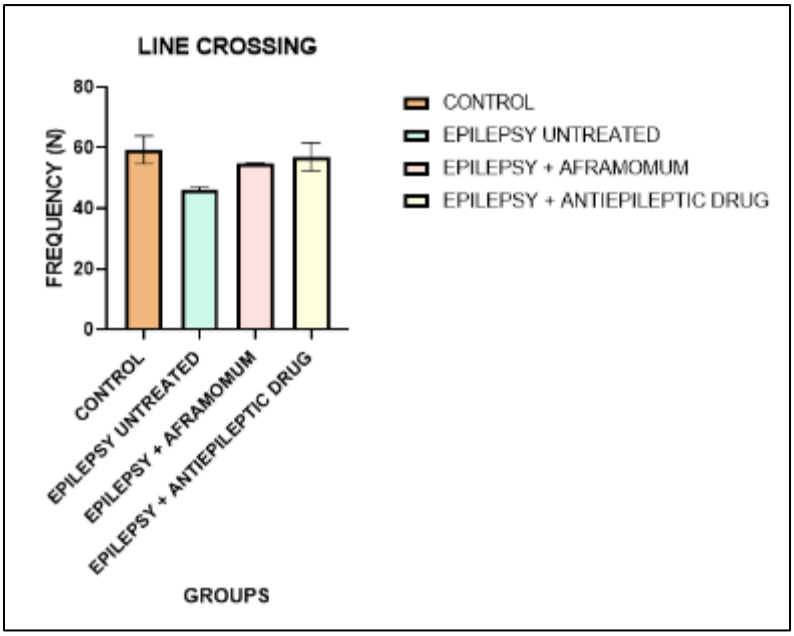

Figure 1 The frequency of the lines crossed by animals across all groups

3.2. Effects of *Aframomum melegueta* on vertical locomotion

Figure 2 shows the effect of AM on open-field vertical locomotion measured as number of rears which shows a reduction in the untreated epilepsy and aframomum treated epileptic groups.

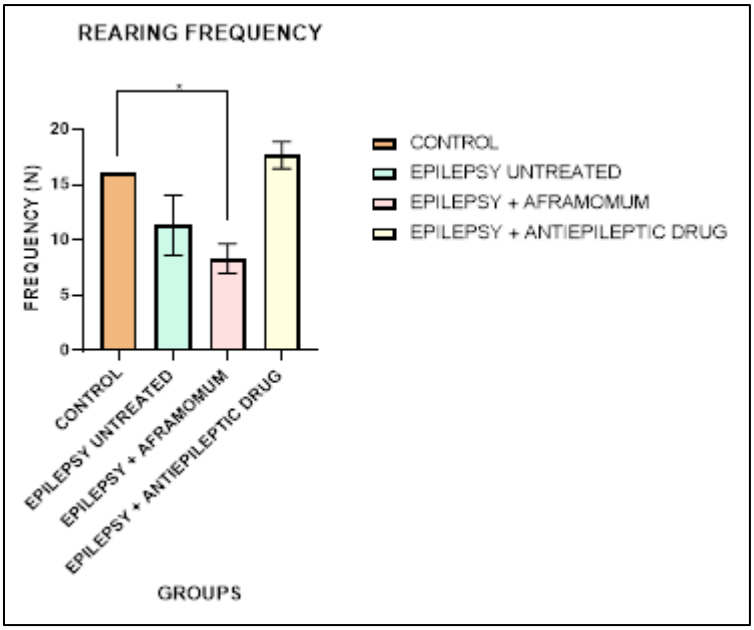

Figure 2 The rearing frequency across the groups

3.3. Effects of *Aframomum melegueta* on centre square entry

Figure 3 shows the effects of AM on open field test centre square frequency which is a measure inversely related to anxiety. This shows a significant reduction in the untreated epileptic groups.

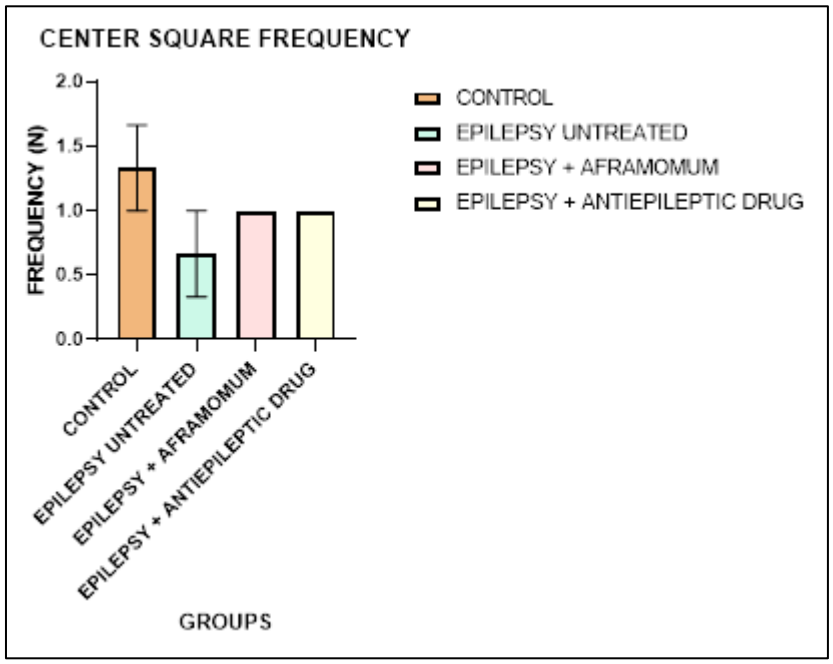

Figure 3 The center square frequency across the groups

3.4. Effect of *Aframomum melegueta* on Freezing frequency

Figure 4 showing the effects of BP on open field test freezing frequency. This is significantly increased in the untreated epileptic groups.

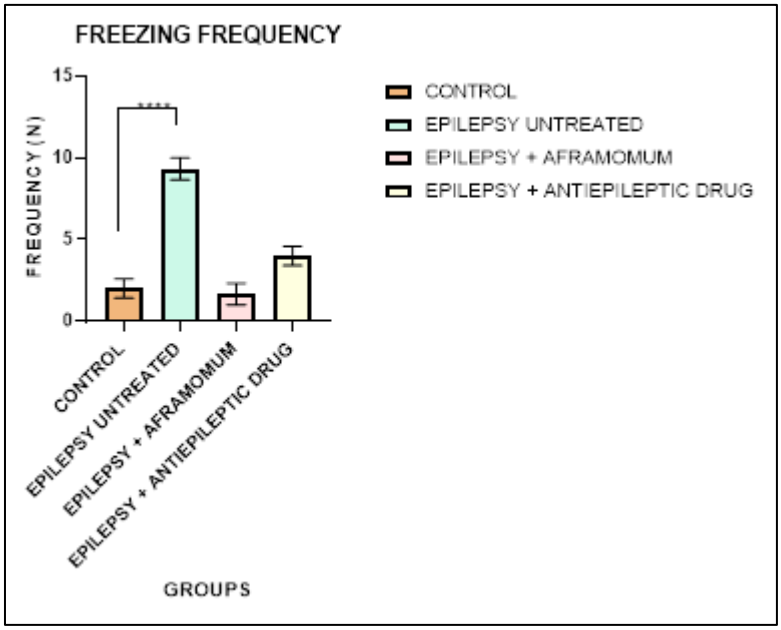

Figure 4 The Freezing frequency across the groups

3.5. Effect of *Aframomum melegueta* on Glutamate Levels in the Prefrontal Cortex

Figure 5 illustrates the effect of *Aframomum melegueta* on glutamate levels in the prefrontal cortex of pilocarpine-induced epileptic rats. Pilocarpine administration resulted in a significant elevation of glutamate levels compared with the control group, indicating enhanced excitatory neurotransmission. Treatment with *Aframomum melegueta* significantly reduced glutamate levels relative to the control, while carbamazepine treatment produced a marked alteration in glutamate concentration. These findings suggest that *Aframomum melegueta* modulates glutamatergic activity following seizure induction.

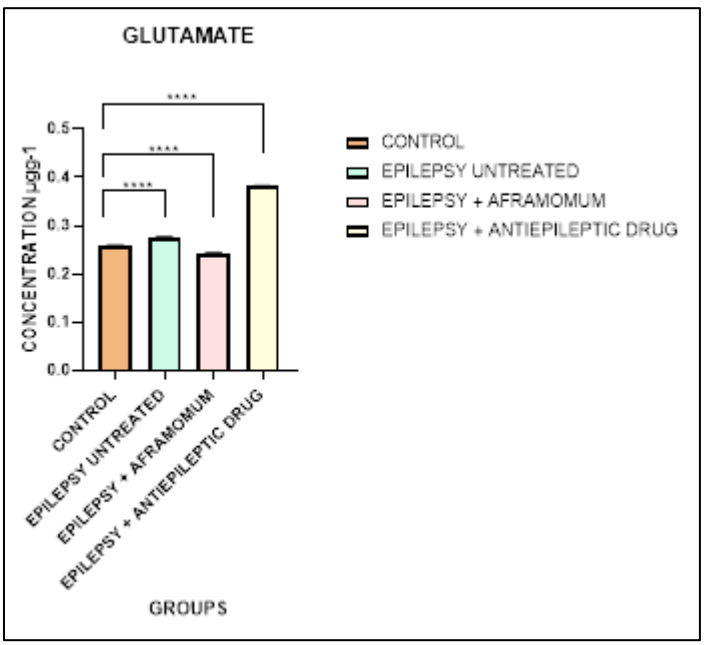

Figure 5 The glutamate level across the groups

3.6. Effect of *Aframomum melegueta* on GABA Levels in the Prefrontal Cortex

Figure 6 shows the effect of *Aframomum melegueta* on gamma-aminobutyric acid (GABA) levels in the prefrontal cortex. Pilocarpine-induced epilepsy caused a significant reduction in GABA levels compared with the control group, reflecting impaired inhibitory neurotransmission. Treatment with carbamazepine significantly increased GABA levels, whereas *Aframomum melegueta* maintained GABA concentrations comparable to control values. This indicates a stabilizing effect of *Aframomum melegueta* on inhibitory signaling in the prefrontal cortex.

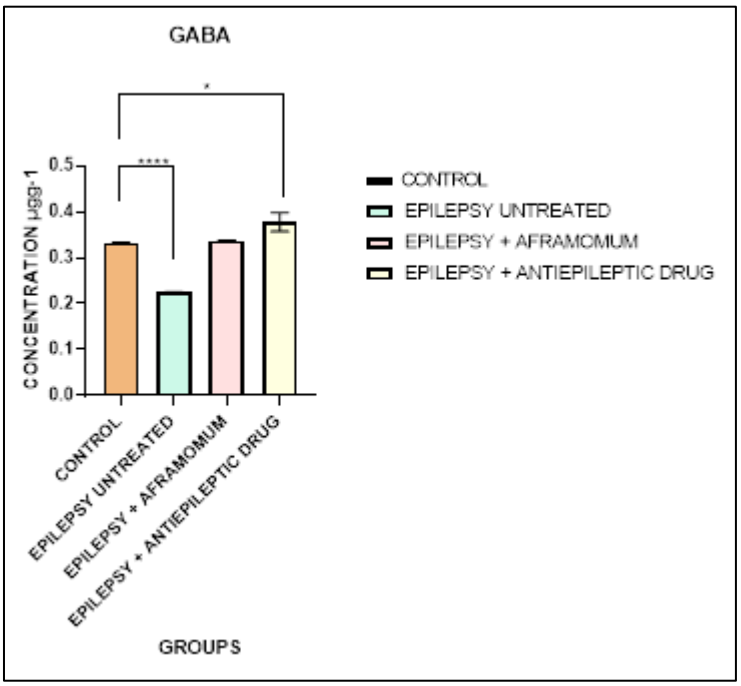

Figure 6 The GABA level across the groups

3.7. Effect of Aframomum melegueta on Malondialdehyde (MDA) Levels

Figure 7 presents the effect of Aframomum melegueta on malondialdehyde (MDA) levels in the prefrontal cortex. Pilocarpine treatment significantly increased MDA levels, indicating elevated lipid peroxidation and oxidative stress. Administration of Aframomum melegueta significantly reduced MDA levels compared with the control group, suggesting strong antioxidative activity and protection against seizure-induced oxidative damage.

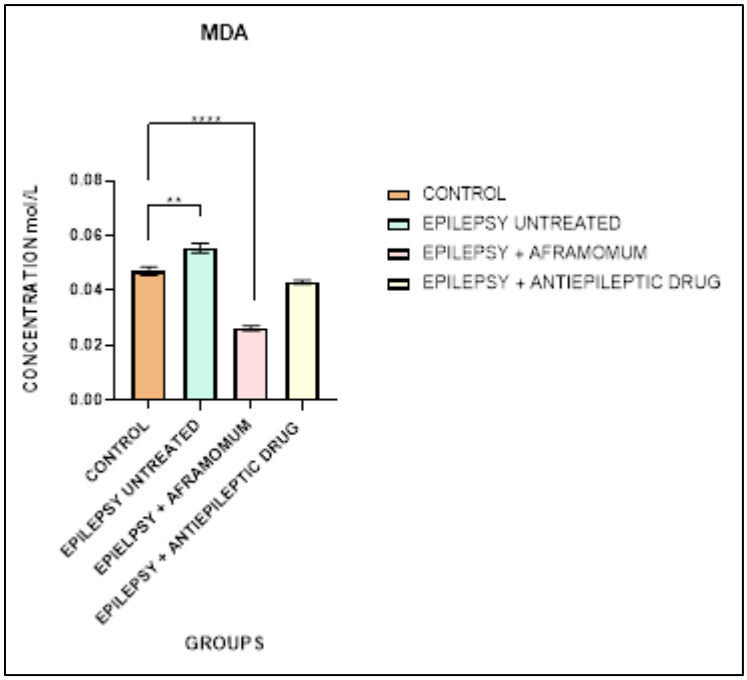

Figure 7 The MDA level across the groups

3.8. Effect of *Aframomum melegueta* on Superoxide Dismutase (SOD) Activity

Figure 8 depicts the effect of *Aframomum melegueta* on superoxide dismutase (SOD) activity in the prefrontal cortex. Treatment with *Aframomum melegueta* significantly increased SOD activity compared with the control group. A similar increase was observed with carbamazepine treatment. These findings indicate that *Aframomum melegueta* enhances endogenous antioxidant defense mechanisms in the prefrontal cortex following epileptic insult.

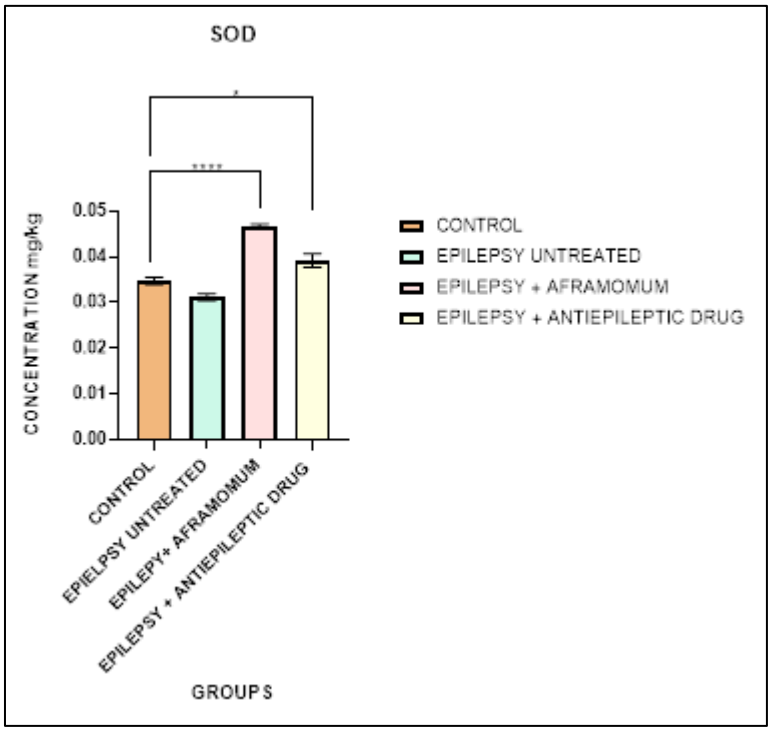

Figure 8 The SOD level across the groups

3.9. Effect of *Aframomum melegueta* on Interleukin-6 (IL-6) Levels

Figure 9 shows the effect of *Aframomum melegueta* on interleukin-6 (IL-6) levels in the prefrontal cortex. Pilocarpine-induced epilepsy resulted in a significant elevation of IL-6 levels, reflecting neuroinflammatory activation. Treatment with *Aframomum melegueta* significantly reduced IL-6 levels compared with the control group, while carbamazepine also produced a significant modulatory effect. This suggests that *Aframomum melegueta* exerts anti-inflammatory effects in the prefrontal cortex following seizure induction.

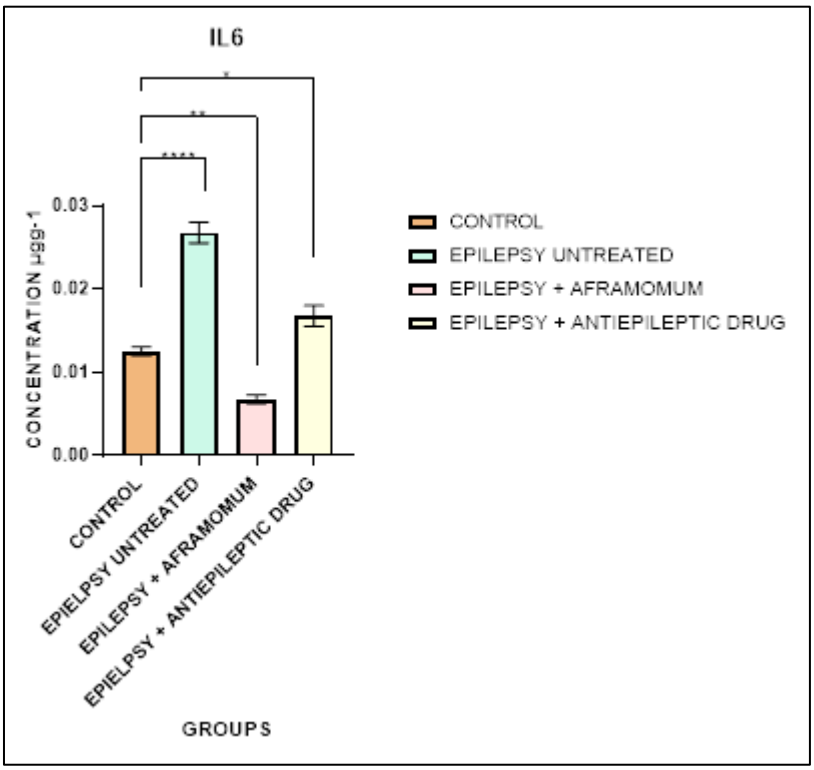

Figure 9 The IL-6 Level across the groups

3.10. Effect of *Aframomum melegueta* on histological assessment

3.10.1. Haematoxylin and Eosin (H&E) Staining of the Prefrontal Cortex

Figure 10 shows the histological architecture of the prefrontal cortex following H&E staining. The control group (Group A) displayed normal cortical cytoarchitecture with well-preserved neurons, intact nuclei, and clearly defined blood vessels.

The epilepsy-untreated group (Group B) showed marked histopathological alterations characterized by neuronal degeneration, pyknotic nuclei, vacuolated neurons, and disrupted cortical organization. These changes indicate severe neuronal damage following pilocarpine-induced epilepsy.

In the *Aframomum melegueta*-treated group (Group C), the prefrontal cortex exhibited improved histological appearance with a predominance of normal neurons and reduced neuronal degeneration compared with the untreated epileptic group. The carbamazepine-treated group (Group D) also showed preserved neuronal morphology with fewer degenerative changes.

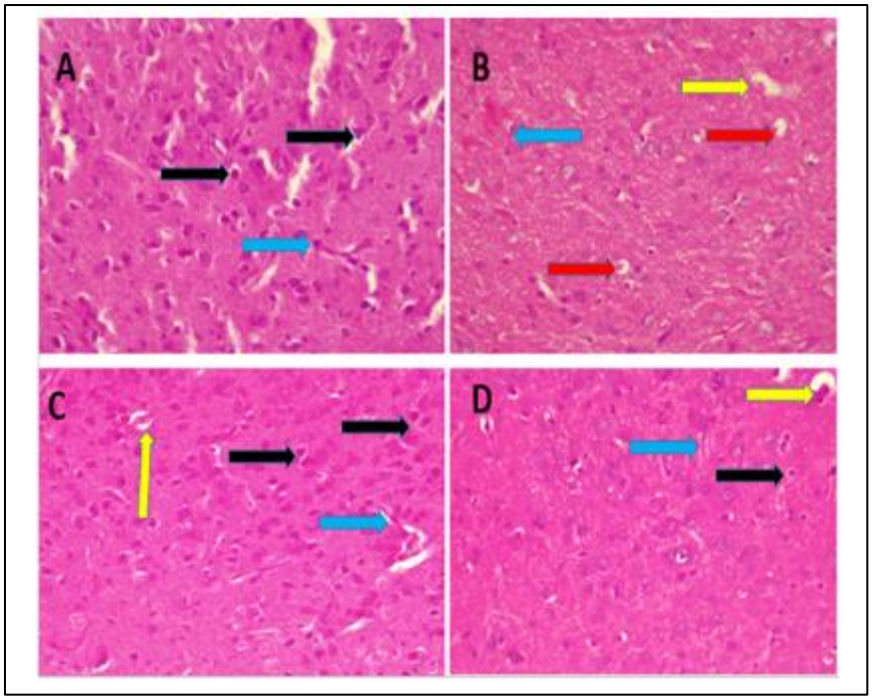

Figure 10 Photomicrograph of Prefrontal Cortex stained with H&E at Mag.X400 where Group A = control group, Group B = Epilepesy untreated, Group C = Epilepsy + *Aframomum melegueta* and Group D = epilepsy + carbamazepine. Black arrows= normal neurons, Yellow arrow = vacuolated neurons, Red arrows= pyknotic and degenerated neurons and Blue arrow= Blood vessel

3.10.2. Cresyl Fast Violet (Nissl) Staining of the Prefrontal Cortex

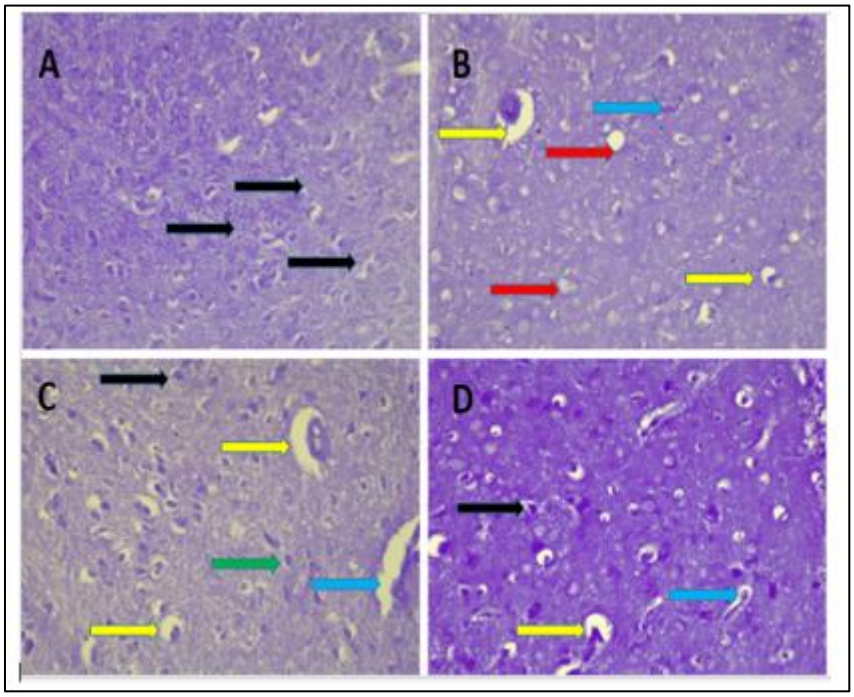

Figure 11 Photomicrograph of Prefrontal Cortex stained with Cresyl fast violet at Mag.X400 where Group A = control group, Group B = Epilepsy untreated, Group C = Epilepsy + *Aframomum melegueta* and Group D = epilepsy + carbamazepine. Black arrows= normal neurons, Yellow arrow = vacuolated neurons, Red arrows= pyknotic and degenerated neurons and Blue arrow= Blood vessel

Figure 11 presents the Cresyl fast violet-stained sections of the prefrontal cortex. The control group demonstrated normal Nissl substance distribution, indicating intact neuronal cell bodies and protein synthesis machinery.

The epilepsy-untreated group showed disrupted Nissl staining with reduced neuronal density, vacuolation, and the presence of pyknotic and degenerated neurons, reflecting impaired neuronal integrity.

Sections from the *Aframomum melegueta*-treated group revealed improved neuronal preservation with enhanced Nissl substance staining and reduced cellular degeneration relative to the untreated epileptic group. Similar preservation of neuronal cell bodies was observed in the carbamazepine-treated group.

3.10.3. Luxol Fast Blue Staining of the Prefrontal Cortex

Figure 12 shows Luxol fast blue-stained sections of the prefrontal cortex. The control group exhibited intact and well-defined myelin sheaths, indicating normal white matter integrity.

The epilepsy-untreated group demonstrated noticeable disruption and reduction of myelin staining, suggesting seizure-induced demyelination and white matter damage in the prefrontal cortex.

In contrast, the *Aframomum melegueta*-treated group showed improved myelin integrity with more prominent and continuous myelin sheaths compared with the untreated epileptic group. The carbamazepine-treated group also displayed preservation of myelin structure.

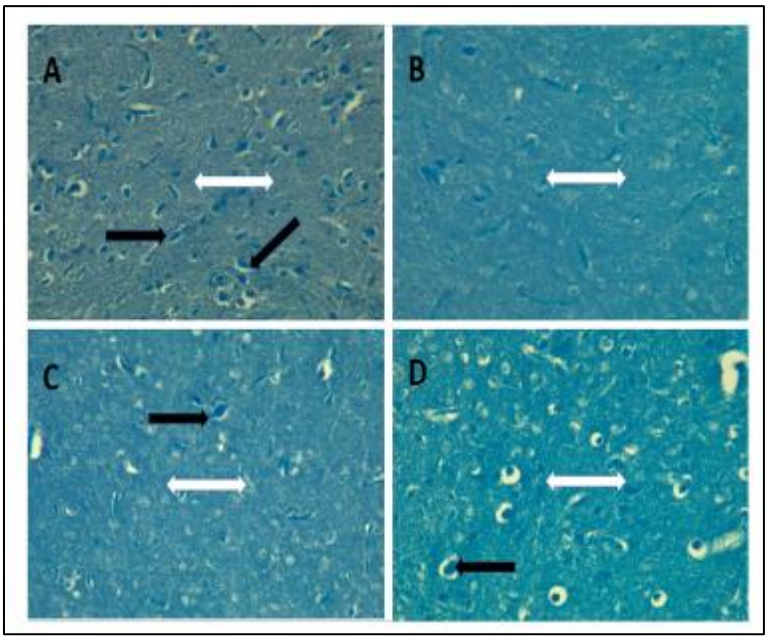

Figure 12 Photomicrograph of Prefrontal Cortex stained with H&E at Mag.X400 where Group A = control group, Group B = Epilepsy untreated, Group C = Epilepsy + *Aframomum melegueta* and Group D = epilepsy + carbamazepine. Black arrows= normal neurons and White arrows= myelin sheath

3.10.4. Immunohistochemical Analysis of GFAP Expression

Figure 13 shows immunohistochemical staining of the prefrontal cortex for glial fibrillary acidic protein (GFAP), a marker of astrocyte activation and astrogliosis.

Where Figure 13A is the control group; Figure 13B is epilepsy untreated group; Figure 13C is induced epilepsy group treated with *Aframomum melegueta* and Figure 13D is induced epilepsy group treated with Anti-epileptic drug (Carbamazepine).

Red arrow= astrogliosis/presence of active astrocytes, Blue arrow= normal neuron, and Black arrow= affected neurons (ranging from pyknosis to vacuolation and to degenerated neurons).

The control group (Figure 13A) exhibited minimal GFAP expression, indicating normal astrocytic distribution. The epilepsy-untreated group (Figure 13B) showed intense GFAP immunoreactivity with increased astrocyte activation, indicative of pronounced astrogliosis following seizure induction.

Treatment with *Aframomum melegueta* (Figure 13C) resulted in reduced GFAP expression and fewer reactive astrocytes compared with the untreated epileptic group. The carbamazepine-treated group (Figure 13D) similarly showed attenuation of astrogliosis.

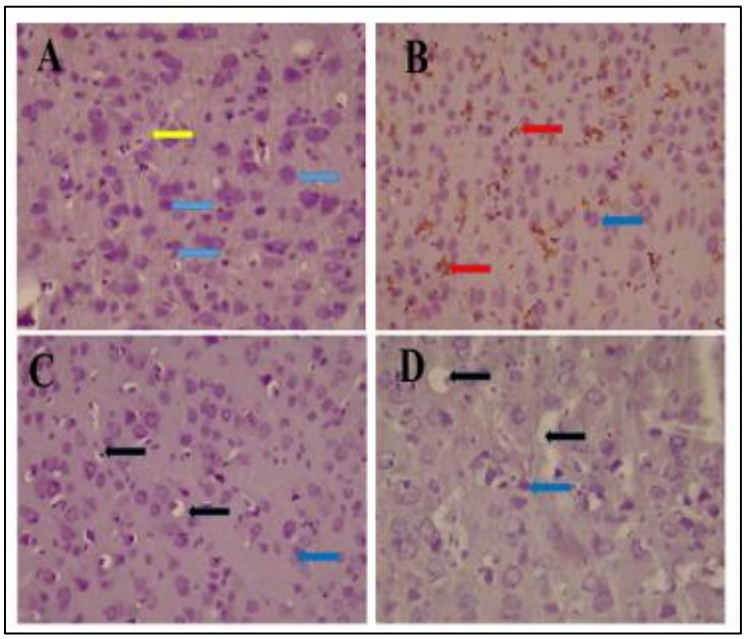

Figure 13 Immunohistochemistry photomicrographs of Prefrontal cortex (PFC) stain with Glial Fibrillary Acidic Protein (GFAP) for astrogliosis

4. Discussion

This study examined the neuroprotective effects of *Aframomum melegueta* on behavioural, biochemical, neurochemical, histological, and neuro-inflammatory changes in the prefrontal cortex of Wistar rats with epilepsy caused by lithium chloride and pilocarpine. The results indicate that pilocarpine-induced seizures caused significant behavioural deficits, oxidative stress, neurotransmitter imbalance, neuroinflammation, and structural damage in the prefrontal cortex, whereas treatment with *Aframomum melegueta* notably mitigated these pathological alterations.

The open field test for behavioural assessment indicated that lithium pilocarpine induction markedly diminished horizontal and vertical locomotor activity, center square entry, and heightened frozen behaviour. These alterations indicate compromised locomotion, increased anxiety-like behaviour, and diminished exploratory motivation, which are frequently documented outcomes of seizure-induced cortical impairment [21]. The prefrontal cortex is essential for motor planning, affective regulation, and behavioural flexibility [22]; therefore, injury to this area is likely accountable for the noted behavioural anomalies. Treatment with *Aframomum melegueta* enhanced locomotor activity and decreased anxiety-related behaviours, indicating a protective influence on prefrontal cortex function. The observed behavioural enhancements were similar, albeit not identical, to those associated with carbamazepine, suggesting that the plant extract may have neuromodulatory effects rather than solely sedative properties.

At the neurochemical level, pilocarpine-induced epilepsy caused a notable increase in glutamate and a decrease in GABA levels in the prefrontal cortex, indicating a transition towards excitatory dominance. Excessive glutamatergic transmission is a primary mechanism in the development and propagation of seizures, resulting in excitotoxic neuronal damage via prolonged depolarisation, calcium influx, and mitochondrial dysfunction [23]. The decrease in inhibitory GABAergic activity further intensifies neuronal hyperexcitability. Treatment with *Aframomum melegueta* markedly lowered glutamate levels while preserving GABA concentrations near control values, indicating a restoration of excitatory-inhibitory equilibrium. The modulatory influence on neurotransmitter systems may account for the noted behavioural and histological enhancements, hence substantiating the extract's possible anticonvulsant capabilities.

Markers of oxidative stress further validated neuronal damage mediated by seizures. Increased malondialdehyde levels in the untreated epileptic cohort signify heightened lipid peroxidation and membrane impairment [24], aligning with the overproduction of reactive oxygen species during extended seizures. Simultaneously, modifications in antioxidant defence systems jeopardise neuronal viability. Treatment with *Aframomum melegueta* markedly diminished MDA levels and augmented superoxide dismutase activity, signifying potent antioxidant actions. These findings align with other studies that detail the extensive antioxidant properties of *A. melegueta*, ascribed to its flavonoids, gingerols, paradols, and various other phenolic substances. The extract likely safeguarded neuronal membranes and synaptic integrity in the prefrontal cortex by augmenting endogenous antioxidant defences and mitigating lipid peroxidation.

Neuroinflammatory responses significantly contributed to seizure-induced pathology, as seen by the marked increase in interleukin-6 levels in the untreated epileptic cohort. Interleukin-6 (IL-6) is a pivotal pro-inflammatory cytokine associated with epileptogenesis, astrocyte activation, disruption of the blood-brain barrier, and neuronal degeneration [25]. The significant decrease in IL-6 levels after *Aframomum melegueta* treatment indicates an anti-inflammatory impact, potentially aiding in the mitigation of secondary neuronal injury. The anti-inflammatory effect is corroborated by immunohistochemistry evidence indicating decreased GFAP expression and reduced astrogliosis in the treated subjects.

Histological investigation provide structural validation of the biochemical and behavioural observations. The untreated epileptic cohort demonstrated significant neuronal degeneration, pyknotic nuclei, vacuolation, disorganised cortical structure, depletion of Nissl substance, and myelin disruption in the prefrontal cortex. These alterations are indicative of excitotoxic and oxidative neuronal damage subsequent to status epilepticus [26,27]. *Aframomum melegueta*-treated rats exhibited improved neuronal preservation, enhanced Nissl staining, and superior myelin integrity, signifying protection of both grey and white matter components. The diminished astrogliosis evident in GFAP-stained sections indicates a decrease in reactive glial responses and a more conducive milieu for neuronal survival.

The results of this investigation demonstrate that *Aframomum melegueta* provides substantial neuroprotection against epileptic damage generated by lithium pilocarpine in the prefrontal cortex. The protective effects seem to be facilitated by a combination of antioxidant activity, regulation of excitatory and inhibitory neurotransmission, suppression of neuroinflammation, and maintenance of cortical cytoarchitecture. Although carbamazepine is an established antiepileptic medication, the similar protective effects demonstrated by *Aframomum melegueta* underscore its potential as a complementary or alternative therapeutic agent, possibly offering reduced toxicity and enhanced neuroprotective properties.

Nevertheless, additional research is necessary to identify the exact bioactive components responsible for these effects, elucidate dose-response interactions, and investigate long-term results and the molecular signalling pathways involved. This work offers experimental evidence that corroborates the traditional use of *Aframomum melegueta* and highlights its potential in alleviating seizure-related prefrontal cortical damage.

5. Conclusion

Lithium chloride pilocarpine induced epilepsy resulted in notable behavioural impairments, neurotransmitter dysregulation, oxidative stress, neuroinflammation, and histomorphological damage in the prefrontal cortex of Wistar rats. Treatment with *Aframomum melegueta* mitigated these changes by boosting locomotor and exploratory behaviours, lowering glutamate levels, stabilising GABA activity, inhibiting lipid peroxidation, augmenting antioxidant defences, and reducing inflammatory markers and astrogliosis. Histological findings further validated the preservation of neuronal architecture and myelin integrity.

Aframomum melegueta exhibited significant neuroprotective effects akin to carbamazepine, indicating its potential as an adjunctive treatment drug in the management of epilepsy. Additional molecular and translational investigations are necessary to confirm its therapeutic relevance.

Compliance with ethical standard

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of Ethical Approval

All procedures were executed in compliance with the standard protocols of the Babcock University Health Research Ethics Committee, bearing approval number BU1073/24.

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