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Anti-Proliferative Effect Of *Khaya Senegalensis* And *Tinospora Cordifolia* Alkaloids On The Prefrontal Cortex Of Ischemic Stroke Rat Model

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ABSTRACT

Cortical cells proliferation has been one of the underlying ischemic cascades after a stroke. The study aimed to investigate the therapeutic combination of *Khaya senegalensis* and *Tinospora cordifolia* alkaloid on the frontal cortex of ischemic stroke rat model. A total of eighty adult male Wistar rats (weighing 144–341g) were randomly divided into five groups. Group I (CTL) served as the control group, received feed and water only. Groups II–V were induced with ischemic stroke orally using 1.4 mg/kg of amitriptyline hydrochloride for a period of 1 week. Group II ischemic stroke rats (AMT) were left untreated, while Groups III, IV, and V were treated with low (200 mg/kg), medium (300 mg/kg), and high (400 mg/kg) doses of a combined therapy of *K. senegalensis* and *T. cordifolia* alkaloid for 14 days. normal histoarchitecture of frontal cortex observed in Group I (CTL) while AMT (Group II) showed cortical degeneration and upregulation of reactive astroglia. Upon treatment, there was reduction in the cortical degeneration and down regulation of reactive astroglia cells across the treated groups. Group V (High dose) demonstrated significant improvement in the neurovascular unit, with near-normal orientation of cortical cells and down regulation of reactive astroglia cells These findings suggest that *Khaya senegalensis* alkaloid in combination with *Tinospora cordifolia* alkaloid has protective effects on the prefrontal ischemic brain injury induced by amitriptyline hydrochloride

Keywords: Amitriptyline, prefrontal cortex, *Khaya senegalenesis, Tinospora cardiofolia,* Alkaloid.

INTRODUCTION

Antidepressant medications are important in treating mood disorders by providing essential support for individuals grappling with anxiety and depression. These drugs exert a broad range of effects on overall health, encompassing both beneficial and adverse outcomes, through complex neurobiological mechanisms (Abeyaratne *et al*., 2016). The positive effects, including significant mood improvements, alleviation of distressing symptoms, and enhanced quality of life, are vital in restoring emotional well-being and enabling individuals to re-engage with their environment (Güloğlu *et al*., 2011). However, antidepressants also present common side effects such as nausea, weight gain, sexual dysfunction, and sleep disturbances, which may cause physical and emotional distress, occasionally leading to the cessation of treatment (Mandour., 2012).

Amitriptyline, a widely used tricyclic antidepressant, exhibits both therapeutic and detrimental effects on human health and animal models (Abeyaratne *et al*., 2016). Striking a balance between the therapeutic benefits and the potential side effects of these drugs is crucial in both clinical practice and experimental settings. Amitriptyline, in particular, has been associated with conditions such as nausea, weight gain, and sexual dysfunction, and its use has been linked to heart failure and cerebral ischemia (Moore *et al*., 2015; Schmieget *et al.,* 2022). As a commonly used tool in laboratory settings, amitriptyline effectively simulates ischemic stroke conditions in rodents, providing a reliable and reproducible model for testing neuroprotective interventions (Davis *et al*., 2018). This model offers a strong foundation to assess the efficacy of neuroprotective treatments aimed at mitigating the damage induced by ischemia (Abeyaratne *et al.,* 2016). Stroke, a silent and devastating public health crisis, strikes without warning, leaving cognitive and sensory impairments in its wake. This underlines the urgent need for novel approaches to combat this pervasive health challenge (Shadman *et al.,* 2019). Ischemic stroke is a major cause of long-term disability and death worldwide (WHO, 2019). It results from a sudden interruption of blood flow to a specific region of the brain, initiating a complex cascade of cellular events that culminate in extensive neuronal damage and subsequent deficits in cognitive and motor functions (Johnson *et al*., 2017; Shadman *et al*., 2019).

Natural compounds have long been discovered for their bioactive potential, offering diverse mechanisms of action (Abdullahi *et al.,* 2016). *Khaya senegalensis* and *Tinospora cordifolia* alkaloid, in particular, have attracted attention for their promising pharmacological properties, including antioxidant, anti-inflammatory, and neuroprotective effects. These compounds may interact with neural pathways and potentially mitigate the damage caused by ischemic strokes (Gupta *et al*., 2015; Abdullahi *et al.,* 2016).

Khaya senegalensis is a medicinal plant used in traditional African medicine. Its leaf, stem bark, seeds, and roots are used to treat various conditions, including inflammation, arthritis, infections, ulcers, malaria, fever, and dermatosis. Studies have shown that reactive oxygen species play a key role in the pathogenesis of inflammation, arthritis, ulcers, and malaria (Abdullahi *et al.,* 2016; Delfanet *et al*., 2014). The anti-inflammatory, analgesic, and antipyretic effects of extracts from the stem bark of *Khaya senegalensis* have also been documented (Elagibet *et al.,* 2016).

Tinospora cordifolia Miers (Menispermaceae) is a widely used plant in Indian medicine, recognized for its therapeutic properties. Classified as a "Rasayana" in Ayurveda, it is utilized for its adaptogenic and immunomodulatory activities, aiding in the fight against infections. Chemical investigations have revealed several active compounds in *T. cordifolia*, including protoberberine and aporphine alkaloids, with tetrahydropalmatine and jatrorrhizine identified in the roots (Sarma *et al.*, 2019; Sarma *et al.*, 2009).

Saponins are naturally occurring glycosides found in higher plants and some marine organisms, and they are known for their ability to produce soap-like frothing in aqueous solutions (Sparg, Light, & van Staden, 2004). Saponin-rich plants have been reported used in traditional medicine because of their therapeutic properties in anti-inflammatory, Immunomodulatory, cardioprotective activities and as Antioxidant (Sun *et al.*, 2010; Liu *et al.*, 2019). Naturally occurring compounds from plants have attracted scientific interest due to their multiple pharmacological effects, potential role in drug development, and importance in plant defense mechanisms (Shoaib *et al.*, 2016). They occur in various parts of plants such as roots, leaves, bark, and seeds, and have been documented in families including *Sapindaceae*, *Fabaceae*, *Araliaceae*, and *Meliaceae*, the latter to which *Khaya senegalensis* belongs (Akinmoladun *et al.*, 2020). Their broad pharmacological spectrum makes them relevant to both human health and industrial applications.

The prefrontal cortex, a modified part of the brain responsible for higher-order cognitive processes such as decision-making, planning, and personality expression, plays a critical role in cognitive and sensory functions. Damage to this region in stroke patients often leads to cognitive deficits, motor impairments, and sensory disturbances (Badree *et al.*, 2010; Bisley *et al.*, 2010).

In Nigeria and Africa at large, the use of traditional plants in medicine is rapidly gaining popularity, often rivaling conventional drug therapies. Increasingly, individuals are turning to plants for treating a variety of illnesses (Ahmed *et al.*, 2015). The limited effectiveness of conventional medications in stroke treatment, as highlighted by the WHO's 2007 study, calls for more innovative solutions to address the growing global incidence of stroke-related mortality.
Therefore, investigating the anti-proliferative activity of *khaya senegalensis* and *tinospora cordifolia* alkaloids on the frontal cortex of ischemic stroke rat model may serve a novel intervention approach to alleviate cognitive and sensory impairments after a stroke.

MATERIALS AND METHODS

Plant Material

Fresh bark of *Khaya senegalensis* (KS) was collected from a forest in Ukelle-Yala, Cross River State, Nigeria, while *Tinospora cordifolia* (TC) leaves were obtained from a well-maintained garden in Okpoma-Yala, Cross River State, Nigeria. Both plant materials were identified and authenticated at the Department of Botany, University of Lagos, Nigeria. The respective voucher IDs were assigned as follows: *Tinospora cordifolia* (ID No. 8003) and *Khaya senegalensis* (ID No. 8004), and the specimens were deposited in the university herbarium for reference.

Animals

Eighty adult male Wistar rats, weighing between 149–341g, were obtained from the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences, University of Cross River State (UNICROSS). The rats were housed in standard plastic cages under controlled conditions in the Department's animal facility. They were allowed a two-week acclimatization period before the experiment, during which they were fed with water and standard animal feed (growers mash). The animals were then randomly divided into eight groups, with ten animals per group (n = 10).

Materials and Chemicals Used

Materials and chemicals used during the experiment are cages, surgical gloves, cotton wool, face masks, spatula, oral cannula, feeding plates, distilled water, sensitive weighing balance, and web cam, syringe (1ml, 2ml, 5ml, and 10ml), feed, towel, mortar and pestle, normal saline, 10% formal-saline solution, phosphate-buffered saline (PBS), sample bottles, dissecting set.

Drug and Phytochemical Used

Amitriptyline hydrochloride, the drug used in this study, was purchased from a reputable pharmacy in Lagos, Nigeria. Phytochemical screening of both the *Khaya senegalensis* stem bark and *Tinospora cordifolia* leaf extracts was conducted according to the methods outlined by Sofowora (1993) and Trease and Evans (2002).

Extracts Preparation

To accelerate the drying process, the fresh bark of *Khaya senegalensis* was thoroughly cleaned and sliced into smaller pieces using a sterile knife. The pieces were air-dried at room temperature for four weeks. The stem bark was then oven-dried at 50°C for 3 hours before being crushed into a semi-powder using a grinding machine. A total of 244g of coarse powder was packed into a thimble and placed into a Soxhlet extractor. The extraction process was performed with an appropriate water solvent, and the solution was concentrated using a rotary evaporator, yielded 144g which was then collected and stored at 4°C for future use. Similarly, the fresh leaves of *Tinospora cordifolia* were cleaned, sliced into smaller pieces, and air-dried at room temperature for three weeks. The leaves were then oven-dried at 50°C for 3 hours and crushed into a semi-powder. A total of 200g of the coarse powder was packed into a Soxhlet extractor, where it was subjected to solvent extraction. The solution was concentrated using a rotary evaporator, yielding 122g of dried extract, which was collected stored at 4°C for future use.

Extraction of Alkaloid

Extraction of alkaloid was done accordily to Ushie *et al*., 2022; Manjunath *et al*., 2012, method of extraction

Experimental Design

The study was conducted in two phases: Phase I involved induction of experimental stroke, and Phase II focused on treatment.

Phase I: Animals were divided into seven groups. One normal control group received only food and distilled water, while the remaining groups underwent stroke induction via a single dose of 1.4 mg/kg amitriptyline hydrochloride, administered for 3 consecutive days.

Phase II: The animals were further divided into nine groups, including the amitriptyline-treated stroke group (AMT), control group, and seven treatment groups. The treatment groups were administered combined *Khaya senegalensis* and *Tinospora cordifolia* extracts at doses of 200, 300, and 400 mg/kg body weight orally for a period of two weeks.
All experimental protocols and treatment procedures were approved by the Research Ethics Committee of the Faculty of Basic Medical Sciences, UNICROSS, Okuku Campus, Cross River State, Nigeria.

Induction of Experimental Stroke
Amitriptyline hydrochloride was administered at a dose of 1.4 mg/100g body weight as a single daily dose for 3 consecutive days. The drug was dissolved in water, and the dose was calculated based on the animal's weight. Administration was carried out via an oropharyngeal cannula, ensuring accurate delivery. Food and water intake were closely monitored following drug administration.

Drug Treatments
Treatment commenced 24 hours after confirming stroke induction and continued for two weeks. The treatment groups were as follows:

Group I: (CTR): Non-stroke animals, receiving distilled water only (normal control).

Group II: (AMT): Stroke-induced animals, receiving distilled water.

Group III: AMT + $COM_{ALK+ALK\ (LD)}$; ischemic stroke rats, receiving 1.4 mg/kg amitriptyline hydrochloride + 200 mg/kg body weight combined *Khaya senegalensis* and *Tinospora cordifolia* alkaloid

Group IV: AMT + $COM_{ALK+ALK\ ((MD)}$; ischemic stroke rats, receiving 300 mg/kg body weight combined *Khaya senegalensis* and *Tinospora cordifolia* alkaloid

Group V: AMT + $COM_{ALK+ALK\ (HD)}$; ischemic stroke rats, receiving 400 mg/kg body weight combined *Khaya senegalensis* and *Tinospora cordifolia* alkaloid

Animal Sacrifice and Tissue Processing
Twenty-four hours after the final administration of treatments, the animals were humanely sacrificed via cervical dislocation. The brains were carefully harvested and fixed in 10% neutral buffered formalin. The fixed brains were subsequently processed for histological analysis.

Histological analysis

Histological analysis included fixation, trimming, dehydration, embedding, cutting, staining, mounting, and observation, were processed for routine paraffin embedding using standard methods, and subjected to routine Haematoxylin and Eosin staining protocols. Stained slides were observed under a Digital Microscope (OMAX 40-2000X 3MP Digital Compound Microscope, USA) and photomicrographs obtained.

Immunohistochemistry

Sections of 5 µm thickness obtained from routine paraffin were deparaffinized, and subjected to antigen retrieval by heating in a citrate-based antigen unmasking solution, pH 6.0 (Vector Labs, CA, USA) for 30 mins in a steamer and allowed to cool on the bench at room temperature for 30 mins. Endogenous peroxidase blocking was performed in 0.3 % hydrogen peroxide in Phosphate Buffered Saline (PBS, pH 7.4) for 10 mins. Sections were blocked in animal free serum and blocker (Vector Labs, USA) for 20 mins. Sections were then incubated at room temperature for 2 hours in primary rabbit antibodies: GFAP (ThermoFisher, USA; #16825-1-AP) at 1:10000 dilution and IBA1 (Cell Signaling, USA; #17198) at 1:1250 dilution. Sections were washed in PBS and incubated in ImmPRESS™ HRP Anti-Rabbit IgG (Peroxidase) Polymer Reagent, made in horse (Vector Labs, USA). Colour was developed with DAB Peroxidase (HRP) Substrate Kit (Vector Labs, USA), and sections were counter-stained in hematoxylin (Erukainure et al. 2019; Ijomone et al. 2018).

Statistical analysis

Data were analyzed for descriptive statistics and expressed as mean ± standard error. Values were compared using the one-way or two-way analysis of variance (ANOVA). Differences were performed using Tukey's multiple comparison test. From $p < 0.05$, the differences were considered significant

RESULTS

The biochemical analysis of the prefrontal cortex revealed significant alterations across all measured parameters, reflecting the oxidative and excitotoxic impact of ischemic stroke and the modulatory effects of treatments. In the control group, baseline values of SOD, catalase, GSH, NO, MDA, and glutamate were within normal physiological ranges, reflecting intact antioxidant defense and balanced neurotransmission.

In contrast, the amitriptyline (AMT) group demonstrated severe oxidative stress and excitotoxicity, characterized by a profound decline in SOD, catalase, and GSH levels, coupled with marked elevations in NO, MDA, and glutamate.

Administration of the combination of Khaya senegalensis flavonoid and Tinospora cordifolia cardiac glycoside produced dose-dependent improvements. At low dose, there was partial restoration of SOD and GSH, with reductions in NO and MDA compared to AMT, but glutamate levels paradoxically increased, suggesting inadequate suppression of excitatory neurotransmission. The medium dose (MD) of the combination yielded the most balanced effects across parameters, as SOD and catalase were moderately restored, NO and MDA significantly reduced toward control values, and glutamate markedly decreased compared to AMT. GSH was also improved relative to AMT, though not as high as in the low dose group. At high dose (HD), the combination was highly effective in suppressing oxidative stress, as evidenced by near-normalization of NO and MDA, alongside modest improvements in SOD

and catalase. However, glutamate levels remained relatively high compared to the standard dose, suggesting that while antioxidant restoration was strong, excitotoxic regulation was less effective at higher concentrations. This agrees with Shan *et al*. (2023), who noted that dose optimization is critical to balance antioxidant defenses and excitatory neurotransmission in stroke management.

Table 1: *Khaya Senegalensis* bark and *Tinospora Cordifolia* alkaloid restores neurotoxicity in the prefrontal cortex of ischemic stroke rat model.

Groups	*SOD(U/ml)*	*CATALASE (U/mg)*	*NITRIC OXIDE (µM)*	*MDA (µM)*	*GSH (mM)*	*GLUTAMATE (mM)*
Control	4.11±0.12[a]	198.9±47.09[a]	1.74±0.20[a]	0.86±0.12[a]	1.79±0.25[a]	18.14±2.98[a]
AMT	0.27±0.03*	56.92±7.43*	15.02±0.84*	5.01±0.26*	0.18±0.04*	131.54±0.00*
AMT + COM$_{ALK+ALK}$ (LD)	1.23±0.01*[a]	0.33±0.01*	8.11±0.02*[a]	2.59±0.03*[a]	1.32 ±0.00*[a]	277.33±2.03*[a]
AMT + COM ALK+ALK (MD)	1.02±0.01*[a]	3.30±0.06*	5.21±0.05*[a]	1.06±0.01*[a]	0.67±0.02*[a]	91.67±4.06*[a]
AMT + COM ALK+ALK (HD)	1.23±0.15*[a]	3.82±0.01*	3.43±0.01*[a]	0.96±0.02[a]	0.43±0.01*	130.53±0.57*

n=10; mean ± SEM, one-way ANOVA, *= $p<0.05$; significance difference when compared to control; a=$p<0.05$ when compared to Amitriptyline (3days).

Histological Observations: In fig Figure 1, Photomicrographs of the prefrontal cortex cross-sections from the control group (CTL), administered water, revealed an intact neurovascular unit (NVU), normal neurons, blood vessels, and astrocytes (Section A). This normal histoarchitecture of the cortex is shown clearly, with well-organized cellular structures. In contrast, photomicrographs of the AMT-treated group exhibited various degrees of cortical cell degeneration. These included significant neuronal loss, gliosis, and evidence of neuronal unit damage (NVU), characterized by mild lymphocytic infiltrates, vascular congestion, moderate to severe neuronal degeneration, capillary proliferation, cellular hypertrophy, perivascular edema, and astrocytosis. These pathological changes were observed compared to the control group, where the cortex maintained a normal neuroarchitecture. The photomicrograph of the COM$_{ALK+ALK}$ $_{LD}$ group, which was administered 200 mg/kg of *Khaya senegalensis* and *Tinospora cordifolia* alkaloid, showed intact meninges and a moderate degree of degeneration in cortical cells. This included moderately degenerated neurons with nuclear loss, glial cells with dense nuclei, mild tissue edema, and mild capillary proliferation with perivascular edema. These changes were less severe compared to the AMT group.

In the COM **ALK+ALK**; MD group (300 mg/kg), mild degeneration of cortical cells was observed. This group showed some preservation of the neurovascular unit, with less extensive lymphocytic infiltrates and minimal neuronal degeneration, indicating a moderate degree of neuroprotection.

A marked improvement was observed in the COM **ALK+ALK;** (HD) group (400 mg/kg), where normal orientation of cortical cells was noted. This group exhibited near-normal neuronal morphology, with minimal lymphocytic infiltrates, negligible neuronal degeneration, and mild tissue edema and gliosis, as compared to the AMT group.

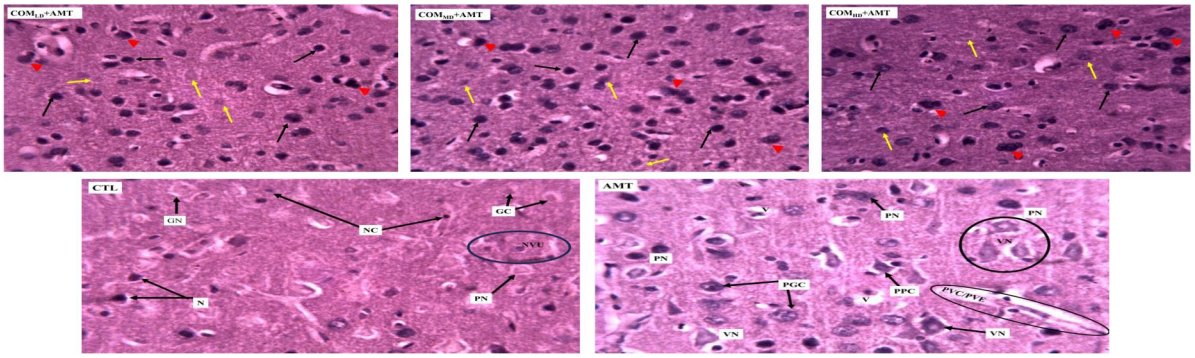

Figure 1: Photomicrographs of the prefrontal cortex from different treatment groups.

CTL group: Showing normal histoarchitecture of the prefrontal cortex with well-defined pyramidal cells (PC), neurovascular unit (NVU), neurons (N), blood vessels (BV), neuroglial cells (NC) with dense nuclei, and granule cells (GC) with pale open-face nuclei (H&E, X400). **AMT group**: Showing degeneration in the prefrontal cortex with evidence of marked astrocytosis (A), severe lymphocytic infiltrates (LI), vacuolated neutrophils (VN), vascular congestion, pyramidal cells with irregular shapes (IPC), surrounded by pericellular halos, perivascular edema (PVE), perivascular cuffing (PVC), and shrunken granule cells (GC), deeply stained (H&E, X400). COM**ALK+ALK (LD) group (200 mg/kg)**: Showing intact meninges, minimal degeneration in the prefrontal cortex, neurovascular unit (NVU) with reduced lymphocytic infiltrates, minimal degenerated neurons (N), mild tissue edema, and gliosis (H&E, X400). COM**ALK+ALK (LD) (MD) group (300 mg/kg)**: Showing intact meninges, moderate degeneration in the prefrontal cortex, neurovascular unit (NVU) with fewer lymphocytic infiltrates, minimal degenerated neurons (N), mild tissue edema, and gliosis (H&E, X400). COM**ALK+ALK (LD) (HD) group (400 mg/kg)**: Showing near-normal orientation of cortical cells, with fewer lymphocytic infiltrates, minimal degenerated neurons (N), mild tissue edema, and gliosis (H&E, X400).

Demonstrate the morphology of GFAP immunoreactivity, a marker of astroglia, in the prefrontal cortex, across various different treatments groups associated with amitriptyline induce stroke**.** There was immunoreactivity of astroglia, a observed in prefrontal cortex, showing strong immunoreactivity of astroglia with thick, dense processes and darkly stained cell body (red arrow) as compared to normal control that appeared with thin processes and lightly stained cell body. *Khaya senegalenesis* bark and *Tinospora cordifolia* leave alkaloid

treat groups (**Fig. 2&3;** $COM_{ALK+ALK\ (LD)\ (LD)}$ **,** $COM_{ALK+ALK\ (LD)\ (MD)}$ **and** $COM_{ALK+ALK\ (LD)\ (HD)}$) showed a significant reduction in GFAP immunostaining compared to the AMT group.

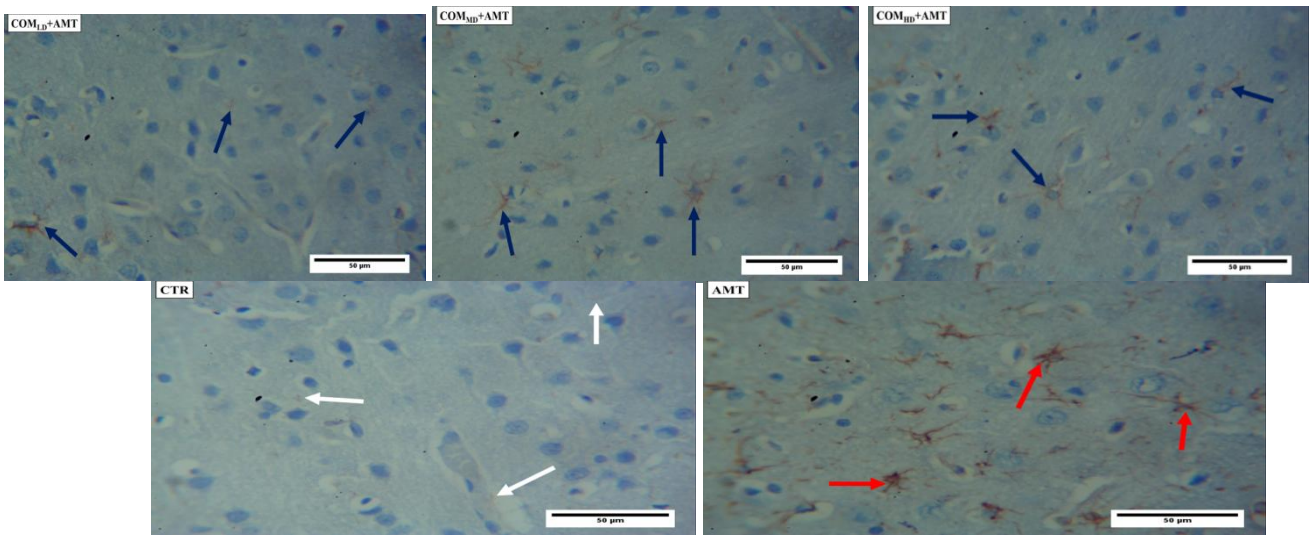

Fig. 2: Demonstrate the morphology of GFAP immunoreactivity, a marker of astroglia, in the prefrontal cortex (PFC) across various different treatments groups associated with amitriptyline induce stroke.
AMT (4.2 mg/kg of AMT) group showed strong immunoreactivity of astroglia with thick, dense processes and darkly stained cell body (red arrow) as compared to normal control. Thin immunostaining of astroglia cells (dark blue arrow) was observed in COM_{LD} + AMT (200 mg/kg + AMT) treated group as compared to AMT group. There was a moderate immunoreactivity of astroglia in COM_{MD} + AMT (300 mg/kg + AMT) treated group as compared to AMT group while a marked reduction of the reactive astrocytes was seen in the (400 mg/kg + AMT) COM_{HD} + AMT (Fig. 2).

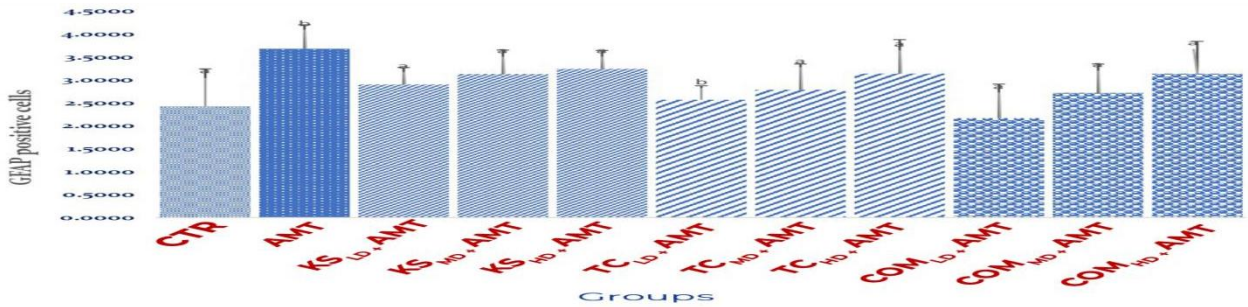

Fig. 2: Demonstrate the morphology of GFAP immunoreactivity, a marker of astroglia, in the prefrontal cortex (PFC) across various different treatments groups associated with amitriptyline induce stroke. Fig. 3: GFAP immunolocalization densitometrical analysis of the Prefrontal cortex. The various groups were key as CTR group, AMT (4.2 mg/kg of AMT), *Khaya senegalenesis* (KS) and *Tinospora cordifolia* (TC) combined + amitriptyline group ($COM_{KS\ +\ TC}$ + AMT); COM_{LD} + AMT (200 mg/kg + AMT), COM_{MD} + AMT (300 mg/kg + AMT) and COM_{HD} + AMT (400 mg/kg + AMT). In normal control rats (CTR group), few

astrocytes with long processes were seen between cells. *LEGEND*: Control (CTR) group, AMT (4.2 mg/kg of AMT), *Khaya senegalenesis* (KS) and *Tinospora cordifolia* (TC) combined + amitriptyline group (COM_{KS+TC} + AMT); COM_{LD} + AMT (200 mg/kg + AMT), COM_{MD} + AMT (300 mg/kg + AMT) and COM_{HD} + AMT (400 mg/kg + AMT).

DICUSSIONS

Ischemic stroke in the prefrontal cortex is a multifaceted condition that involves complex inflammatory processes and the disruption of essential cortical functions. Amitriptyline, a tricyclic antidepressant, has been associated with several neurological and cardiovascular side effects, including arrhythmias, myocardial infarction, and stroke, primarily due to its anti-adrenergic and anti-muscarinic properties (Umaharan *et al*., 2021; Gupta *et al.,* 2015; Bansal *et al*., 2009).

This study aimed to investigate the neuroprotective and ameliorative effects of *Khaya senegalensis* and *Tinospora cordifolia alkaloid* on the prefrontal cortex in an ischemic stroke rat model.

Our present study demonstrates that ischemic stroke in the prefrontal cortex is associated with oxidative stress, lipid peroxidation, glutathione depletion, and glutamate excitotoxicity. Amitriptyline treatment exacerbated these disturbances, as evidenced by the profound reductions in SOD, catalase, and GSH, alongside elevations in NO, MDA, and glutamate. This suggests that under ischemic conditions, amitriptyline may potentiate oxidative and excitotoxic neuronal injury, which agrees with the report of Chen and Yoshioka (2011) that oxidative imbalance is a hallmark of ischemic brain injury, and is further supported by Bautista-Ferrufino *et al*. (2011) who demonstrated that amitriptyline increases oxidative stress through mitochondrial dysfunction.

Conversely, treatment with a combination of *Khaya senegalensis* and *Tinospora cordifolia alkaloid* significantly attenuated oxidative stress in a dose-dependent manner. Both the medium and high doses effectively reduced NO and MDA levels, indicating strong free radical scavenging and lipid peroxidation inhibitory effects. This finding is consistent with Hasan et al. (2023), who described alkaoid as potent antioxidants that neutralize reactive oxygen species and inhibit lipid peroxidation. Similarly, Gupta *et al*. (2024) reported that *Tinospora cordifolia* enhances enzymatic and non-enzymatic antioxidant defenses in experimental models, supporting the observed restoration of SOD and catalase. Furthermore, the GSH recovery seen particularly at the low dose is similar to the findings of Kandeda *et al.,* (2022), who demonstrated that *Khaya senegalensis* extract exhibits CNS protective effects via redox homeostasis.

Glutamate modulation was also dose-dependent, with the medium dose demonstrating the most effective reduction, thereby protecting against neurotoxicity. This agrees with Shen et al. (2022), who highlighted glutamate excitotoxicity as a critical therapeutic target in ischemic stroke. The paradoxical rise in glutamate at the low dose contrasts with its antioxidant improvements, suggesting that incomplete dose optimization may fail to suppress excitatory neurotransmission. The high dose, while beneficial against oxidative markers, showed less efficacy against glutamate when compared to the standard dose. This trend is consistent with Shan *et al*. (2023), who noted that optimal modulation of extra

synaptic NMDA receptor activity is necessary to balance excitotoxicity control. Wu *et al.* (2018) also emphasized that targeting glutamatergic excitotoxicity alongside oxidative stress provides better neuroprotection in stroke, which supports the superiority of the standard dose in this study.

Our results also demonstrated normal histoarchitecture in the control group, with clear features such as pyramidal cells, neuroglial cells with dense nuclei, and granule cells with pale open-face nuclei, consistent with the work of Berlacchie *et al.* (2018). When compared with amitriptyline treated (100 mg/kg) group which showed series of pathological changes, with of astrocytosis, lymphocytic infiltration, vacuolated neutrophils, and irregularly shaped pyramidal cells. Perivascular halos, edema, cuffing, and deeply stained granular cells were also observed, which are typical responses to brain injury. These findings are consistent with the work of Lukpata *et al.* (2020) and Zhe *et al* (2021), who noted astrocytosis and glial scar formation following amitriptyline administration. This confirms that amitriptyline induces ischemic changes in the prefrontal cortex, in line with the findings of Jane *et al.* (2009), who reported that amitriptyline induced neurodegeneration when administered at over dose.

At a lower dose of 200 mg/kg, the combined therapy of *Khaya senegalensis* and *Tinospora cordifolia alkaloid* showed promising ameliorative and neurorestorative effects. The prefrontal cortex displayed reduced neurovascular damage, with fewer vacuoles, irregular pyramidal cells, and minimal perivascular halos, as well as a reduction in lymphocytic infiltration and edema. This suggests that the combined extracts at this low dose have potential therapeutic benefits for amitriptyline-induced ischemic stroke. These findings are in line with those of Onu *et al.* (2013), who observed that *Khaya senegalensis* and *Tinospora cordifolia alkaloid* could effectively treat ischemic stroke when the dosage or duration was increased. Additionally, Srivastava *et al.* (2011) also reported that a combined therapy of *Khaya senegalensis* and *Tinospora cordifolia alkaloid* significantly improved histological features of brain tissue damage, even in small quantities.

At a dose of 300 mg/kg, the combined therapy further enhanced neuroprotection, showing prominent neurovascular units, blood vessels, pyramidal cells, and neuroglial cells with distinct nucleoli. The presence of vacuolated neutrophils and granule cells was also evident, suggesting that the combined extracts were effective in mitigating the effects of oxidative stress and reducing ischemic damage. This result supports the notion that the antioxidant components of *Khaya senegalensis* and *Tinospora cordifolia* contributed to these neuroprotective effects, as previously indicated by Lukpata *et al.* (2020).
Finally, at a higher dose of 400 mg/kg, the prefrontal cortex showed near-normal orientation of blood vessels and cortical cells, with clearly defined pyramidal cells and granule cells. The neurovascular unit appeared intact, with minimal vacuolated neutrophils and reduced granule cell degeneration. This restoration of cortical architecture suggests that the combined therapy of *Khaya senegalensis* and *Tinospora cordifolia alkaloid* at 400 mg/kg is highly effective in reversing amitriptyline-induced ischemic stroke damage, which aligns with findings from Gill *et al.* (1992) and Onu *et al.* (2013).

Our findings in this present study showed that amitriptyline-induced ischemic stroke, presenting markedly increase immunoreactivity GFAP expression of astroglial in the

prefrontal cortex, in AMT treated animals compared to the normal control. The findings in this present is in agreement with reports of Pekny *et al*., 2019; Li *et al*., 2014, whose studies reported that there were increases in GFAP-positive astrocytes focal cerebral ischemia in experimental models. This present study showed that upregulated GFAP immune expression astroglia was downregulated by *Khaya senegalensis* and *Tinospora cordifolia* alkaloids across the treatment groups especially the combine therapy treatment. The findings of this present study is consistent the studies of Zhao *et al*., 2023; Chen *et al*., 2018;

CONCLUSION

Khaya senegalensis and *Tinospora cordifolia alkaloid* have long been used in various parts of Africa and Asia for their medicinal properties. These plants are known to exhibit significant anti-inflammatory effects and have been shown to inhibit pro-inflammatory processes, such as macrophage activation and glial cell proliferation. In this present study, we found out that the combined administration of *Khaya senegalensis* and *Tinospora cordifolia alkaloid* at a dose of 200 and 300 mg/kg significantly reduced ischemic stroke, suggesting the therapeutic potential of combined therapy in the treatment of ischemic stroke, as a promising approach to mitigating the damage caused by this debilitating condition.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest

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