Cognitive-Enhancing Effect of Ethyl acetate fraction of *Erythrophleum ivorense* stems bark against Ketamine-induced Memory Impairment in Mice

Abstract

Background: Conventional treatments for managing Alzheimer's disease (AD) and related cognitive deficits are not said to be curative; they relieve symptoms but can result in adverse side effects. Additionally, the use of herbal therapies to manage cognitive illnesses has increased significantly. *Erythrophylum ivorense* is commonly used in herbal medicine to manage neurological disorders. The present study evaluated the cognitive-enhancing potential of the ethyl acetate fraction of *Erythrophlem ivorensis* (EAFEI) against ketamine-induced memory impairment in mice.

Materials and Methods: Ketamine (15 mg/kg) was administered to induce memory impairment. Donepezil (1 mg/kg) served as the standard reference compound. An ethyl acetate fraction of *Erythrophleum ivorense* (20, 40, 80 mg/kg) was evaluated for its cognitive-enhancing effects, as measured by step-through latency in a passive avoidance test. Additionally, the anti-acetylcholinesterase (anti-AChE) activity and antioxidant potential of the extract were assessed using brain tissues from the test animals

Results: The ethyl acetate fraction of *Erythrophylum ivorense* demonstrated significant effectiveness in combating cognitive deficits in the test animals. This was evidenced by increased step-through latencies significantly ($p < 0.05$) in the extract-treated mice compared to the untreated cognitively impaired mice. Moreover, the cognitively impaired mice that received the extract exhibited significantly lower levels of malondialdehyde and AChE activity ($p < 0.05$) compared to the negative control mice. The antioxidant and anti-AChE properties of the extract were confirmed in this study, therefore indicating its potential to reduce oxidative stress in the brain and enhance cholinergic transmission.

Conclusion: This study highlights the ethyl acetate fraction of *Erythrophleum ivorensis* as a promising candidate for Alzheimer's disease (AD) and related disorders.

Keywords: *Erythrophylum ivorense*, cognitive impairment,ketamine,passive avoidance, acethylcholinesterase

INTRODUCTION

Cognitive impairment greatly increases the risk of neurological disorders, especially Alzheimer's disease (AD), the most common form of dementia. Alzheimer's disease is a complex condition. It is characterized by gradual memory decline, cognitive impairment, and changes in personality. These symptoms come from neuronal damage in the frontal cortex and hippocampus. Major neurochemical disruptions seen in Alzheimer's disease (AD) include a decline in cholinergic function in the central nervous system. An imbalance in redox homeostasis is also observed. These changes are closely related to AD's hallmark pathological features. Accumulation of amyloid-β plaques and neurofibrillary tangles in the brain marks the pathogenesis of Alzheimer's disease (Kwon et al., 2017; Shin et al., 2019). Some research suggests that amyloid buildup in the brain may be the primary cause of AD. Other studies argue that hyperphosphorylation is the key pathogenic mechanism. It is widely accepted that all these factors together contribute to the neuropathogenesis seen in AD. Various approaches have been used to develop agents for managing Alzheimer's disease. These efforts have led to several drugs that can alleviate symptoms to some extent. Common treatments include memantine, an N-Methyl-D-aspartate antagonist, and acetylcholinesterase inhibitors such as donepezil, galantamine, rivastigmine, and tacrine. However, these drugs often cause side effects like cardiovascular issues, muscle cramps, and urinary incontinence. Current pharmacotherapy for Alzheimer's mainly aims to relieve symptoms and does not cure the disease. Therefore, there is strong interest in researching new, safe agents that could potentially cure Alzheimer's.

Forests play a vital role in our atmosphere. According to WHO (2001), the need to study forest plant species stems from their extensive use in folk medicine, forming a basis for daily life. Many African indigenous trees have recognized health-protective properties **(**Okeno et al., 2003; Einosho and Ayorinde, 2008). *Erythrophleum ivorense*, of the family Caesalpiniaceae (Leguminosae-Caesalpinioideae). Its timber in Nigeria is known as ‘Iyin’ (Edo), ‘erun’ (Yoruba), and ‘ihi’ (Igbo) (Aigbokhan, 2014), and as ‘Ordeal tree’ or ‘Sasswood tree’ (English), among other names in various regions (Burkill, 1995). Some Erythrophleum species are venomous and toxic to livestock. The bark, traded as ‘sassy-bark’ or ‘man cona bark’, is used medicinally (ITTO, 2004), for example as an emetic, purgative, and pain reliever (Richter and Dallwitz, 2000; Betti, 2004). The bark is also used as fish poison, for tanning, and to enhance palm wine (PROTA, 2008;Voorhoeve, 1979). The wood is hard, making it suitable for construction and charcoal. In Cameroon, it is the fourth most important timber, also exported to China (PROTA, 2008). E. ivorense is an evergreen tree reaching up to 40 meters, with alternate leaves and bisexual flowers. Previous research reported its toxicology and phytochemical components (Amoah et al., 2014; Sima et al., 2016). However, these studies did not quantify the phytochemicals. Therefore, this research specifically aims to determine the cognitive-enhancing potential of the ethylacetate fraction of *Erythrophleum ivorense* in mice with ketamine-induced Alzheimer's disease-like cognitive deficits.

MATERIALS AND METHODS

Plant material

Plant material Identification, collection, and authentication of plant materials. Fresh stem bark of Erythrophleum ivorense was identified and collected from trees in Iwo, Iwo Local Government Area, Osun State, Nigeria, between April and October 2018. The plant was authenticated by a Botanist in the Department of Botany, Obafemi Awolowo University, Ile-Ife. A voucher specimen was deposited (voucher number 16878) (Wakeel et al., 2018).

Extraction of plant material

Extraction of plant material followed the methods of Wadood et al. (2013). Erythrophleum ivorense stem bark (2.5 kg) was air-dried for eight weeks. The material was reduced to coarse powder using an electric blender (Christy and Norris – 47362, England). Extraction was performed by adding the stem bark powder to 5 liters of absolute methanol in a sterile, stoppered flask. This prevented loss of volatile liquid. The mixture was agitated for 24 hours, then decanted and filtered (filter paper No. 1, Whatmann London, UK). The filtrate was evaporated to dryness using a rotary evaporator (Buchi Rota Vapour R110) and freeze-dried to yield a solid mass. The dried residue (85.6 g) was sealed in glass vials and stored in a refrigerator. The stored crude methanol extract (85 g) was suspended in distilled water and partitioned between ethyl acetate and water with a separating funnel. The organic (ethyl acetate) phase was pooled and concentrated using a rotary evaporator to yield a dark brown fraction (EAF; 27.0 g; 31.8% w/w).

Animal materials

Healthy male Swiss mice (20-30 g) were obtained from the Animal house of Ladoke Akintola University of Technology, Ogbomosho, Oyo State, Nigeria. Six animals were housed per standard cage. Mice were kept in temperature-controlled quarters (22.5°C ±2.5°C) with lights on/off at 7 o'clock. They had free access to food and water except during behavioural tests. Mice were fed commercial standard rodent chow (29% protein, 13% fat, 58% carbohydrate) throughout the experiment. All rules for animal safety and care were observed.

Passive avoidance test

The passive avoidance test (PAT) was conducted for 6 days using a box (25cm x 20cm x 20cm) with a brightly lit and a dark compartment, separated by a wall with a sliding door. An electric circuit (0.5 mA) was installed in the dark chamber (Kim et al., 2020).

The test comprised three phases: habituation, training, and testing. In habituation, mice explored the lit chamber for 20 seconds before the door opened, then could move freely between compartments for 300 seconds with the electric circuit off.

During training, mice began in the lit chamber for 20 seconds. After opening the sliding door, they roamed for 300 seconds. Once fully inside the dark chamber, they received a 0.5mA shock for 10 seconds and stayed for 30 seconds to associate discomfort with the area.

After training, memory retention was tested over three days. Each mouse received treatment, entered the lit chamber for 20 seconds, and, after the door was opened, was observed in the box for 5 minutes. Step-through latency to the dark chamber was recorded.

Between each mouse's habituation, testing, and training session, the box was wiped with 70% ethanol to remove any lingering olfactory stimuli (Eagle et al., 2016). All experiments were conducted at 08:00 am throughout the study period, with 24-hour intervals.

Cholinesterase inhibitory activities

Acetylcholinesterase (AChE) inhibition was assessed using Ellman's colorimetric method (1961) (Nan & Atirlar, 2015). Crude AChE was prepared from mouse brain as described previously. Acetylthiocholine iodide served as the substrate for AChE assays, and its hydrolysis was measured spectrophotometrically. Plant extract or reference compound was added to the enzyme solution, which was then incubated at 37 °C for 15 minutes. Next, 50 mM sodium phosphate buffer (pH 8.0) containing 0.5 mM acetylthiocholine was added, and absorbance was immediately recorded against a blank. All experiments were performed in triplicate. Donepezil served as the reference compound for AChE activity. The percent inhibition was calculated using the following equation: **(**Edwards et al., 2019) : : $a-b/a \times 100/1$ Where a= change in absorbance per min of control ($\wedge$A/min), b= Change in absorbance per minute of test sample.

Biochemical assays

On the 6th day of the PAB test, mice were humanely euthanized, and their brains were promptly harvested. Each brain was then homogenized in 0.6 mL of sodium phosphate buffer (pH 7.4, 0.1 M). The homogenate obtained was then centrifuged at 14,000 rpm for 20 minutes at 4°C. The resulting supernatant was used to measure malondialdehyde (MDA) levels, as previously described (Jilani et al., 2018), and to assess acetylcholinesterase (AChE) activity, following established protocols (Chen et al., 2021)

Statistical analyses

Results of parametric tests were expressed in terms of mean±SEM. In the assays involving comparison of more than two means, one-way ANOVA was used, followed by the Student

Dunnett's post-hoc test. test when statistical difference was detected among the groups. P-values less than 0.05 were considered statistically significant

RESULTS

Effects of EAFEI extract on passive avoidance

Effects of EAFEI was investigated in memory-impaired mice, tested for three consecutive days after habituation and training. The negative control group consistently showed significantly reduced step-through latency (Table 1) compared to all treatments. In contrast, the extract improved cognition, especially at 20 mg/kg bw, which resulted in the highest step-through latency throughout testing. On first and second days, 20 mg/kg bw nearly restored cognition to normal, matching normal controls. Additionally, on day 1, similar latency was seen for the 20 and 80 mg/kg bw groups; however, across days 2 and 3, 20 mg/kg bw maintained the highest latency, while 40 mg/kg bw produced the lowest.

Table 1: Effects of EAFEI extract on passive avoidance

Treatment	**Doses (mg/kg)**	**Time (second)**		
		Ist day	**2nd day**	**3rd day**
Normal saline	0	221.01±7.32	295.21±9.01	281.05±7.21
Ketamine	15	141.21±6.51	139.16±4.42	75.22±3.31
MEEI	20	221.69±5.11	261.98±5.97	167.04±4.72
MEEI	40	175.19±5.07	132.07±3.97	239.12±6.35
MEEI	80	164.34±5.35	158.09±4.31	158.74±6.04
Donepezi	1	137.36±4.43	129.07±5.35	205.5±7.74

**Values are mean ± SEM (n=5).

*Values are statistically significant (P<0.05) compared with control using one-way ANOVA followed by Dunnett's post-hoc test.

Effects of EAFEI on acetylcholinesterase activity

The present study investigated effects of EAFEI on AChE activity in brain homogenates of ketamine-treated mice. All tested extract concentrations (20, 40, 80, and 200 mg/kg bw) and donepezil produced significant inhibition of AChE activity compared to the negative control. Notably, the 80 mg/kg bw extract group showed the highest anti-AChE efficacy, but this was not significantly different from the 40 mg/kg bw group (p>0.001; Figure 1). Both the 40 mg/kg bw extract and donepezil groups exhibited similar degrees of anti-AChE activity. In contrast, the 20 mg/kg bw extract group demonstrated the lowest anti-AChE activity among the tested doses (Table 2).

Table 2: Effects of EAFEI on acetylcholinesterase activity

Treatments	Doses (mg/kg)	Change in absorbance**	% inhibition
Control	0	0.085±0.000	0
Donepezi	1	0.003±0.000*	96.5
EAFEI	20	0.021±0.001*	75.4
EAFEI	40	0.006±0.000*	92.9
EAFEI	80	0.005±0.000*	94.1

**Values are mean ± SEM (n=5).

*Values are statistically significant (P<0.05) compared with control using one-way ANOVA followed by Dunnett's post-hoc test.

Effects of EAFEI on MDA profile

The mean and standard deviation of MDA serum levels of all groups are summarized in Table 3. Notably, the EAFEI-treated groups exhibited a significant reduction in MDA levels compared to ketamine -treated group, highlighting the potential effectiveness of EAFEI in mitigating oxidative stress. A comparison between the control group and ketamine group revealed that significantly higher MDA levels were observed in ketamine group. These results suggest increased oxidative stress or lipid peroxidation in this group. Further comparisons between ketamine group and ketamine plus EAFEI at a dosage of 20 or 40, or 80 mg/kg, showed that a dose-dependent reduction in MDA levels.

Table 3: Effects of EAFEI on MDA profile

Treatments	**Doses (mg/kg)**	**MDA(nmol/mL)**
Control	0	3.78±0.05
Ketamine	15	31.79 ± 3.07
Donepezi	1	7.85±2.67
EAFEI	20	20.21±1.64
EAFEI	40	13.34±2.31
EAFEI	80	8.41±1.01

**Values are mean ± SEM (n=5).

*Values are statistically significant ($P<0.05$) compared with control using one-way ANOVA followed by Dunnett's post-hoc test.

DISCUSSION

This study examined how a part of *Erythrophleum ivorense* improved memory in mice treated with ketamine. Ketamine causes memory problems similar to those in Alzheimer's disease, such as disruptions in brain chemicals and increased oxidative stress.

Ketamine has been reported to disrupt metabolism of key neurochemicals, especially acetylcholine. This leads to a decline in cholinergic function (Aalikhani et al., 2022). It also causes significant oxidative brain damage. These effects contribute to hippocampal memory deficits seen in Alzheimer's disease (AD) (Ben-Azu et al., 2016). After clinical administration, ketamine increases hyperphosphorylation of proteins. This can induce cognitive dysfunction similar to Alzheimer's disease. Negative control animals showed decreased step-through latency, elevated malondialdehyde (MDA) levels, and increased acetylcholinesterase activity (Imran et al., 2021). Extracts can have a significant impact on the malondialdehyde (MDA) profile, which is a biomarker of oxidative stress. Specifically, many extracts, particularly those rich in antioxidants like phenolic compounds and flavonoids, have been shown to reduce MDA levels in various tissues. This reduction indicates a decrease in oxidative damage, as MDA is a byproduct of lipid peroxidation, a process where free radicals attack cell membranes.

A passive avoidance test—a fear-driven avoidance method (Lee et al., 2016)—was used to evaluate memory recovery after extract intervention. This technique assesses an animal's ability to learn and remember aversive stimuli such as electrical foot shocks in the dark chamber of a passive avoidance box (PAB). Rodents naturally prefer darkness over light (Collins et al., 2018), so they usually occupy the PAB's dark chamber. However, this chamber contains hostile stimuli like electroshocks. Mice with good cognition recall aversive experiences from training and hesitate to re-enter the dark chamber. This method is widely used to assess cognitive function in rodents [eight]. Treating cognitively impaired mice with the ethyl acetate fraction of *Erythrophleum ivorense* significantly reversed ketamine-induced memory decline. This was indicated by increased step-through latency. Memory formation involves acquiring, retaining, and retrieving information. All these processes occur in the hippocampus (Abdel-Salam et al., 2023). Longer step-through latency suggests extract-treated mice recalled avoiding aversive stimuli in the dark chamber where they experienced electric shocks [Abdel-Salam et al., 2023). The extract improves cognition by enhancing the recall of information learned during training.

Biochemical evaluations revealed how the ethylacetate fraction of *Erythrophleum ivorense may improve cognition*. Acetylcholinesterase (AChE) is a recognized cognitive biomarker (Han et al., 2018). The extract showed anti-acetylcholinesterase effects in the brains of impaired mice. Measuring AChE activity is common for finding cognitive enhancers (Butterfield & Boyd-Kimball). More AChE activity is linked to cognitive decline. AChE breaks down acetylcholine (ACh), which is crucial for cholinergic receptors and synaptic transmission (Bakhtiari et al., 2017). Low ACh levels weaken cholinergic neurotransmission. This leads to memory issues often seen in Alzheimer's patients (Marucci et al., 2021). Modulating the cholinergic system is important because of its direct tie to Alzheimer's disease (Schuster et al., 2010). Inhibiting AChE prevents acetylcholine breakdown, restoring cholinergic transmission and improving cognition (Dani et al., 2017). Researchers are seeking new AChE inhibitors for Alzheimer's disease (AD) (Maghsoud-Nia et al., 2021). This study shows that the ethyl acetate fraction of *Erythrophleum ivorense inhibits AChE, indicating its potential as a* treatment for neurological disorders like AD.

The ethylacetate fraction of *Erythrophleum ivorense* reversed ketamine-induced oxidative damage in this study. Low malondialdehyde (MDA) levels in brain homogenates show this effect. MDA is a biomarker for oxidative cell imbalance (Ghani et al 2017). Researchers measure MDA in brain homogenates to assess oxidative stress (Maghsoud-Nia et al 2021; Rao et al., 2021). Higher MDA means more oxidative stress. This stress advances neurological illnesses, including Alzheimer's disease (AD) (Chen & Zhong, 2014). The brain is very vulnerable to oxidative stress. Causes include its high oxygen use, intense metabolism, many polyunsaturated fatty acids, and low antioxidants (Imran et al., 2021). Oxidative stress damages the molecular components of brain cells, such as lipids, DNA, and RNA. This damage triggers apoptosis and ultimately impairs learning and memory processes (Chen & Zhong, 2014). Antioxidants help limit the development and progression of neurological disorders. Research links higher antioxidant intake to less dementia (Bohouth & Tahrir, 2015). Thus, antioxidants are promising for reducing cognitive disorder onset and progression (Bohouth & Tahrir, 2015). This study supports the ethyl acetate fraction of *Erythrophleum ivorense* as a potential antioxidant therapy for Alzheimer's disease (AD).

The cognitive-enhancing effects of the ethyl acetate fraction may come from its phytochemicals. Many studies show their therapeutic and neuroprotective properties. Most identified compounds have both anti-acetylcholinesterase and antioxidant effects. E. ivorensis contain tannins, terpenoids, flavonoids, polyphenols, anthracenosids, alkaloids, polyphenols, flavonoids, tannins gallic, and triterpenoids sa previously reported (Cédric et al., 2016) Phenolic compounds and flavonoids reportedly reduce oxidative stress, their hydroxyl groups directly scavenge, or neutralize, free radicals (Kumar & Pandey, 2013). Erythrophleum ivorensis also reported to have a very strong antioxidant activity which would enable them to play a beneficial role in terms of very significant preventive actions for human and animal health (Cédric et al., 2016). Antioxidant activity of the plant should be at least partially justified by the presence of phenolic and the flavonoids highlighted by the phytochemical study (Andzi et al., 2015).

Cognitive disorders such as Alzheimer's Disease (AD) are complex and caused by many factors (Kumar & Murleedharan, 2016). This makes it harder to find the best treatment targets. Recent strategies aim to develop combinations or agents that affect several disease pathways **(**Kametani & Hasegawa, 2018)**.** Researchers are now investigating multimodal therapies for cognitive disorders (Simone et al., 2014). In this study, the ethyl acetate fraction of *Erythrophleum ivorense* had dual effects. It showed both anti-acetylcholinesterase (AChE) and antioxidant activity. These effects reduced cognitive dysfunction.

This suggests that the ethyl acetate fraction of *Erythrophleum ivorense* can enhance cognition through multiple pathways. The study supports its potential for multi-functional therapies targeting cognitive disorders.

CONCLUSION

The ethyl acetate fraction of *Erythrophleum ivorense* reversed ketamine-induced cognitive problems in mice. It improves cognition partly by activating the cholinergic system through AChE inhibition. The extract also prevents decline with antioxidant action that limits brain damage from ketamine. Its effects likely come from phytochemicals that fight oxidative stress and boost cholinergic neurotransmission. This study highlights the ethyl acetate fraction of *Erythrophleum ivorensis* as a promising candidate for Alzheimer's disease (AD) and related disorders.

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