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Therapeutic Effects of *Ziziphus mauritiana* Leaves Extract on Aluminium Chloride and D-Galactose Induced Cognitive Impairment on the Brain of Wistar Rats: Histological and Biochemical Approach

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Abstract

Neurodegenerative disorders represent a significant and escalating global healthcare challenge, characterized by the progressive degeneration of neurons and a consequent decline in cognitive and motor functions. Among older adults, Alzheimer's disease (AD) is the most prevalent neurodegenerative condition, marked by hallmark neuropathological features such as intracellular hyperphosphorylated tau (p-tau) forming neurofibrillary tangles, synaptic dysfunction, neuronal loss, and extracellular amyloid beta (Aβ) plaque accumulation. Aluminium (Al), a known neurotoxic metal, can cross the blood-brain barrier and accumulate in brain tissue, inducing changes associated with neurodegeneration. Additionally, D-galactose (D-gal) is frequently used in animal models to accelerate aging by inducing oxidative stress and inflammation, thus serving as a valuable tool in anti-aging and

neurodegenerative research. This study investigates the therapeutic potential of *Ziziphus mauritiana* extract (ZME), a traditional medicinal plant with recognized nutritional and pharmacological value, in mitigating cognitive dysfunction induced by Aluminium Chloride ($AlCl_3$) and D-galactose in Wistar rats. Thirty-five healthy adult male rats (80–120 g) were administered D-gal (60 mg/kg, intraperitoneally) and $AlCl_3$ (200 mg/kg, orally) to induce neurodegeneration. Two experimental groups received ZME at doses of 100 mg/kg and 50 mg/kg, respectively, while the positive control group was treated with donepezil (1 mg/kg). Cognitive function was assessed using the Novel Object Recognition (NOR) test, oxidative stress was evaluated via Superoxide Dismutase (SOD) activity, and histological examination of the prefrontal cortex was conducted to assess neuronal integrity. Results revealed that exposure to $AlCl_3$ and D-gal significantly impaired cognitive performance, reduced SOD levels ($p < 0.05$), and induced morphological alterations in the prefrontal cortex. Treatment with ZME significantly improved cognitive performance, elevated SOD activity, and restored cortical architecture, indicating neuroprotective effects. These findings support the potential of *Ziziphus mauritiana* as a therapeutic agent for mitigating AD-related cognitive impairments through its antioxidative and neuroprotective mechanisms.

Keywords: *Ziziphus mauritiana*; Cognitive Function; Aluminium Chloride; D-galactose; Alzheimer's Disease; Oxidative Stress; Neurodegeneration

INTRODUCTION

Neurological health conditions including cognitive impairments has been reportedly associated with neurodegenerative diseases like Alzheimer's disease (AD). AD is one of the most common neurodegenerative disorders that affect about 46 million people worldwide and estimated to rise to about 131.5 million by 2050. AD patients suffer from memory loss and related cognitive deficits due to degenerating cholinergic neurons or loss of neurons in the hippocampus and cortical regions (Majeed and Abdulrahman, 2023).

AD is marked by a continuous degenerative process, initially manifests as memory impairment and progressively worsening to cognitive decline, behavioral changes, language difficulties, visuospatial disorientation, and ultimately affects the motor function (Gao *et al.,* 2024).

Morphologically, the key histopathological characteristics of AD are the presence of neurofibrillary tangles (NFTs) formed by hyperphosphorylated *tau* protein (p-*tau*), senile

plaques (SPs) due deposition of beta amyloid (Aβ) and the loss of cholinergic neurons in the basal forebrain in humans. Additionally, oxidative stress and mitochondrial dysfunction are implicated in brain ageing, thus, playing a vital role in early neuronal changes in AD patient resulting in memory decline (Xue *et al.,* 2024).

Two main types of AD have been established; these include the early-onset AD, which affects individuals less than 65 years old, while the other type of AD is known as the late-onset AD, which affects people older than 65 years (Kumar *et al.*, 2022). The disease is progressive by nature, wherein the symptoms continue to worsen and the quality of life declines over the years. Furthermore, AD is classified clinically based on its severity and develops via three stages: mild, moderate and severe. In its early stages, AD patients suffer mild memory loss, but as the disease advances, individuals with the disease lose their ability to perform routine activities (Mahdi *et al.,* 2021).

The brain has been established as the most vulnerable to the toxic manifestation of Aluminium and is further linked to other brain disorders such as Alzheimer's and Parkinson's diseases (Usen and Enogieru, 2023).

Aluminium is one of the third most common elements on the planet which has been found to be associated with AD and nervous system impairments (Yang *et al.,* 2019; Bryliński *et al.*, 2023). Aluminum Chloride ($AlCl_3$) is used as a constituent in manufactured of many products such as antacids, foil paper, toothpaste, and for the sedimentation of drinking water (Colomina, & Peris-Sampedro, 2017). It has been suggested that Aluminium is a major neurotoxic agent that can pass through the blood-brain barrier into the brain. Once gained access into the brain, it accumulates in various areas including the cerebral cortex and hippocampus and has been documented to contribute induce neurodegenerative disease (Alam and Bansal, 2020).

D-galactose is a physiological nutrient and a normal reducing sugar in the body. It is a monosaccharide sugar contained in some products such as dairy products, avocados, sugar beets, and mucilage, and can be used as a senescence agent in age-related neurodegenerative diseases (Nguedia *et al.,* 2021). Based on research D-galactose is a commonly used experimental aging inducer and consumption of D-galactose can contribute to the development of aging markers, including advanced glycation end-products products (AGEs), the receptor for advanced glycation end-products (RAGEs), telomere shortening, amyloid-β (Aβ), and aging-related pathways (Liu *et al.,* 2024).

Furthermore, research has shown that long term exposure to D-galactose leads to a reduction in antioxidant enzymes, such as catalase (CAT), hemeoxygenase 1 (HO-1), superoxide dismutase (SOD), glutathione peroxidase (GPx), and nitric oxide synthase (NOS), resulting in a decrease in overall antioxidant capacity (Wang *et al.,* 2024). Therefore several studies have shown that combined treatment of D-galactose and $AlCl_3$ lead to production of AD-like symptoms, such as cognitive and memory impairments, oxidative damage, and inflammation (Luo *et al.,* 2024).

Medicinal plants have been widely studied due to their exceptional nutritional and pharmaceutical value. These plants contain essential macronutrients such as carbohydrate protein and fats which enable them to provide the human body nutritional requirement. Additionally these components play a vital role in various metabolic activities, morphological and physiological process (Butt *et al.,* 2021)

Ziziphus mauritiana, the subject of the present study, is one of the known traditional medicinal plants been reported to possess nutritional and therapeutic potential (Ghobadi *et al*., 201). *Ziziphus mauritiana* commonly known as the Indian Jujube tree and Magarya in Hausa-Northern Nigeria, is a medium-sized tropical fruit that belongs to the *Rhamnaceae* family (Ramli *et al.,* 2025). It is a rich source of various essential minerals such as iron, zinc, copper, potassium, magnesium, and calcium; however, the edible part of this plant is also rich in ascorbic acid and vitamins, such as, vitamin A and vitamin B complex. Numerous scientific studies have shown that Z. *mauritiana* is a potent antioxidant and antimicrobial agent; notably, it has exhibited hydrogen peroxide scavenging activity, indicating its ability to neutralize reactive oxygen species (Hwang *et al*., 2011). These unique properties are due to their complex chemical composition, components such as flavonoids, alkaloids, and terpenoids,however various phenolic compounds have been isolated and extracted from different parts of this plant. Several studies have shown that various part of this plant possess anticancer, anti-inflammatory, and anti-allergy properties (Biyanzi, and Doumta, 2024; Kumar et al., 2022; Nilofar *et al*., 2024).

Yet, there is paucity of studies on the therapeutic effect of *Ziziphus mauritiana* in mitigating neurodegenerative disorders, like AD, in rodent species. Thus, this study assessed the therapeutic effect of *Ziziphus mauritiana* on $AlCl_3$ and D-gal- induced cognitive impairment in rats.

MATERIALS AND METHODS

Animals

Animals (Wistar rats) were obtained from the Department of Human Anatomy, University of Jos, Nigeria and acclimatized for seven (7) days at Animal House, Physiology Department Gombe State University, Gombe, Nigeria. A total of thirty-five (35) healthy male Wistar rats of 8-10 Weeks- old and weight between 80 g - 120 g were used for the study.

The rats were kept seven (7) per cage and were randomly allocated into five (5) groups in a clean plastic cage under optimal room temperature and natural light /day circle. During the experiment, the rats had free access to food and water. The male rats were used to minimize the influence of hormonal imbalance. Ethical approval was obtained from the Ethical Committee for the Care and Handling of Laboratory Animals of the College of Basic Medical Sciences, Gombe State University (GSU-FBAMS 010/2024), and the rats were handled in humane manner according to the approved animal experimental procedures.

Plant Extract and Chemicals

Dry fruits of desert apple (Ziziphus mauritiana) were procured from a local herbal shop in North-Eastern Nigeria. The specimen was identified and authenticated by a Botanist at the Department of Biological Sciences Herbarium, Gombe State University and a voucher specimen number GSUH245 was assigned for future reference.

The extract was prepared in aqueous form using a method described by (Buniyamin *et al.*, 2023). The dried seeds were pounded and sieved through a mesh to acquire a fine powder (400 g). The fine powder was mixed in a beaker containing 2000 ml of water. The mixture was thoroughly stirred intermittently for 12 hours and afterwards, filtered with a filter rag. Excess water was evaporated out by heating at a temperature of between 60℃ – 80℃ for about 30 minutes. It was then allowed to cool down at room temperature and stored in a refrigerator at 4℃ for subsequent usage.

Aluminium Chloride, which is a whitish powdered solute was acquired and utilised as a neurotoxicant for the experiment. The product is manufactured by Sigma Adrich Sigma Aldrich (237051-100G Aluminum Chloride 99%, ReagentPlus®, 100g).

D-galactose-anhydrous (brain sugar), D-gal (Biosynth) was a generous donation from the departmen tof Biochemistry, Gombe State University, Gombe.

Similarly, *Ziziphus mauritiana* seeds were purchased from commercial sellers in Gombe main market (Nigeria) with a Voucher NO –GSUH245 and was used for the extraction. The seeds were prepared into aqueous extract using standard method as described by Bitwell *et al.*, (2023) in which 400 g of Ziziphus powdered was dissolved in 2 liter of water and administered orally.

Experimental Design

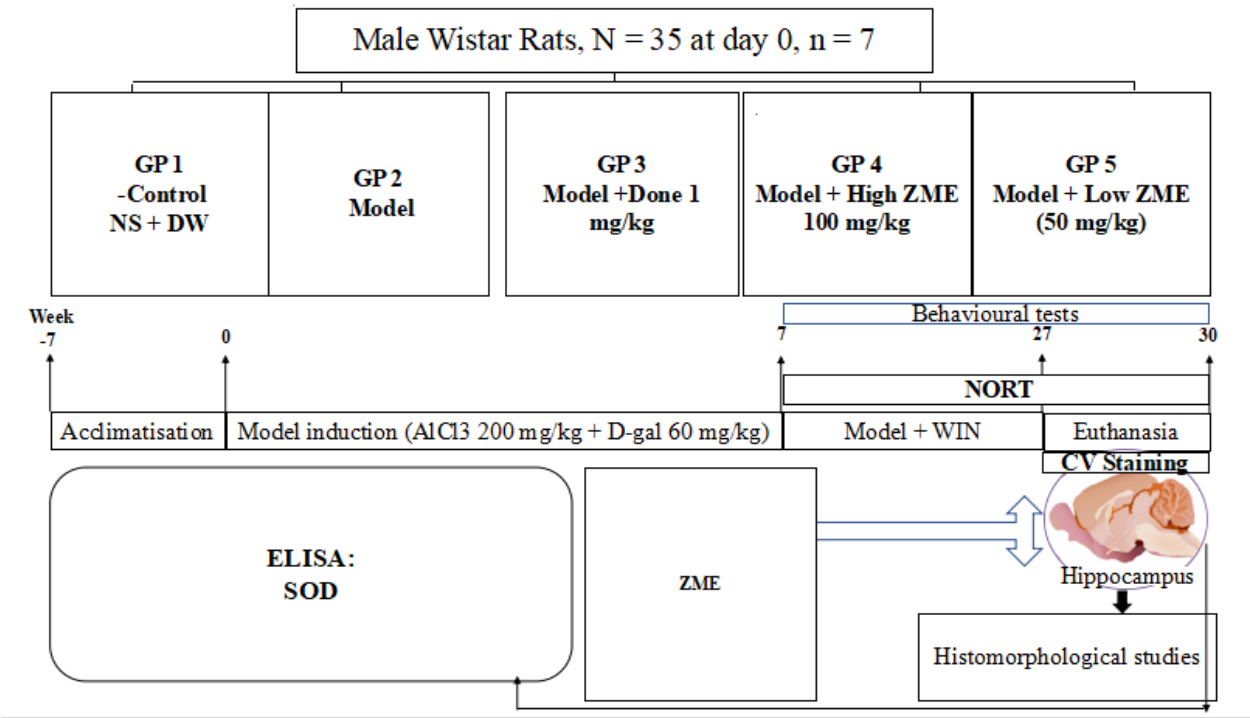


Figure 1: Experimental design; indicating the treatment regimen. Cognitive impairment was induced using $AlCl_3$= Aluminum Chloride (orally), D-gal = D-galactose (intraperitoneally), Donepezil (orally), ZME = *Ziziphus mauritiana* extract (orally) extract was used as a therapeutic agent, DW = distil water, Model = $AlCl_3$ + D-gal, SOD = Superoxide Dismutase

Treatment Protocol

The rats in group 1 served as control group and received only feed and water, while rats in groups 2, 3, 4 and 5 served as treatment groups. These rats received a combination of $AlCl_3$ at 200 mg/kg orally daily and D- gal at 60 mg/kg intraperitoneally (IP). In addition

to $AlCl_3$ and D-galactose, the rats in group 3 were given donepezil (1 mg/kg), (commonly prescribed/standard AD medication (Mahdi *et al.*, 2021). The rats in group 4 were administered ZME at high dose of 100 mg/kg while group 5 was given ZME at a low dose (50 mg/kg) orally/daily. Thus, the donepezil administered group serves as a positive control.. Body weight of the rats was measured in first week of the experiment, before starting administration and in the last week of the experiment.

Novel Object Recognition Test (NORT)

The NOR test was conducted as previously described (Denninger *et al.*, 2018; Giorgetti *et al.*, 2010), with slight modifications. The NOR test device was comprised of Plexiglas box with dimensions of 75 cm x 75 cm base x 40 cm height (Lueptow, 2017). Two objects with different shaped (cylindrical and square) made of plastic were maximised for object discrimination.

Novel object recognition is a commonly used behavioral assay for the investigation of various aspects of learning and memory in rodents; it's developed on the concept that naturally, rodents have the tendencies to preferentially explore novel object rather than familiar ones (Adam *et al.*, 2025).

Briefly, the test has three phases: habituation, acquisition, and retention. The first phase is called habituation, in this phase; rats were allowed to familiarize with the open field for 5 minutes, to reduce stress due to neophobia. The second phase is called the acquisition phase during this phase, the rats were allowed to explore 2 identical objects, which also lasted for 5 minutes and termed as (A1and A2) respectively. The last phase is called retention phase, in this phase one of the familiar object (A2) was replaced with a new object called novel object and termed as (B), the rats were allowed to freely explore the two objects for a duration of 5 minutes each.

The time taken for each rat to explore each object (exploration time) during the acquisition and retention phases was recorded manually by using a stopwatch. The ability of the rat to distinguish the previously (familiar) object from the new object (novel) is termed as discrimination index (DI) and was therefore evaluated for the retention phase as DI = B - A1 / B + A1(A1 = familiar object and B = Novel object). A rat is exploring a particular object when it's sniffing the object or points its muzzle at the object at least 2 cm away from the object. This is due to the fact that rodents have an innate preference for

novelty, if the animal recognizes the familiar object, it will spend most of its time on the novel object. This inherent bias drive in the rodent's explanatory behavior to explore novel object rather than a familiar one reflects underlying recognition and memory discrimination.

Sample Collection and Preparation

At the end of the experimentation, rats were euthanazied by decapitation. After euthanasia, the brain tissue was collected and processed for histomorphological studies. The samples collected were fixed in 10% buffered formalin for the duration of 24hrs,this step is followed by dehydration to remove water and unbound fixatives, during this step the tissues were placed in a series of alcohol 70% for 1hr,80% for 1hr, 90% for 1hr ,100% for 1hr, Absolute for 1hr and another absolute for 1hr. this step is followed by clearing ,the tissue were then placed in xylene for 30min to remove the dehydrating agent and make the tissue receptive to infiltrating medium (paraffin wax). The tissue was then infiltrate in paraffin wax for 2hrs and then embedded using tissue mold and cassette to create a tissue block. The tissues were then sectioned in Federal Teaching Hospital Gombe and then stained using Hematoxylin and Eosin (H&E) stain and Cresyl Violet (Nissl's stain) for the brain tissue. The tissues were then viewed under a light microscope and micrographs were acquired in Histology Laboratory, Department of Human Anatomy, Gombe State University.

Measurement of Superoxide Dismutase

The level of Superoxide Dismutase **(**SOD) was measured in the blood sample because oxidative stress and antioxidant defense system are crucial in the pathophysiology of AD. These tests were carried out in the laboratory of Biochemistry Department, Gombe State University. Sample of blood was collected from the jugular vein using plain EDTA container for the biochemical analysis. The blood sample taken was centrifuged at 4°C and the serum was used to evaluate the MDA level in the rats.

Data Analysis

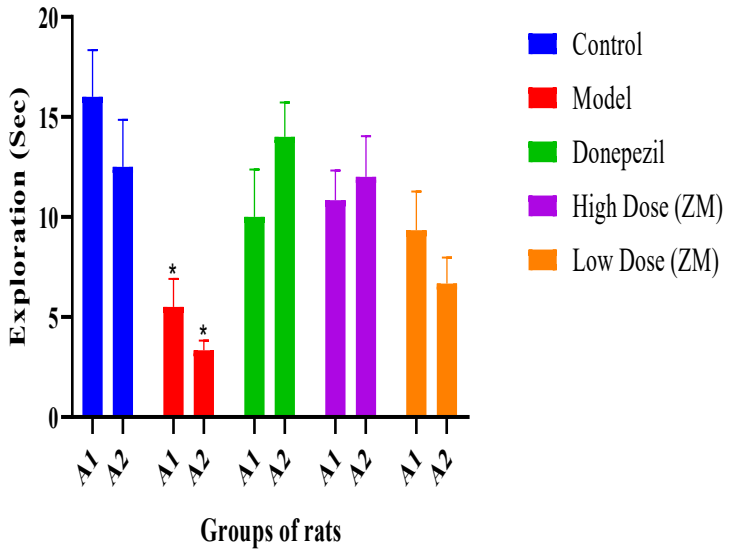
The collected data were computed and statistical analysis performed using GraphPadPrism version 10.4.1 for Windows. One-way Analysis of Variance (ANOVA)

and Dunnett's *Post-hoc* tests were carried out to compare means between groups. A *p*-value less than 0.05 ($p < 0.05$) was established as the benchmark for statistical significance.

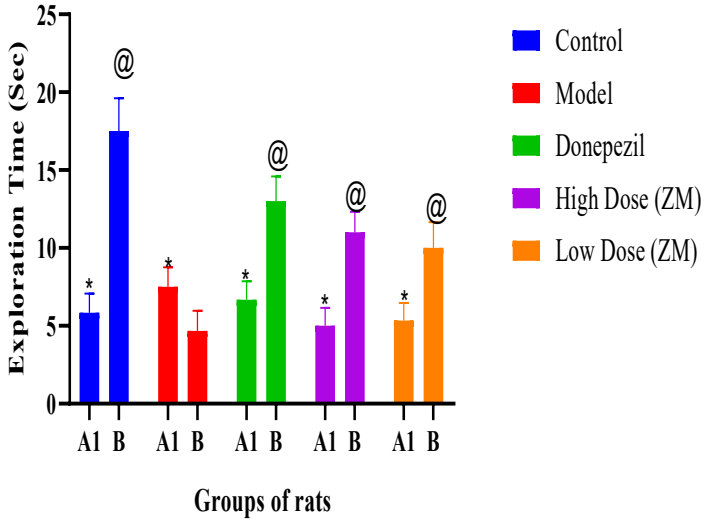
RESULTS

Behavioural Assessment

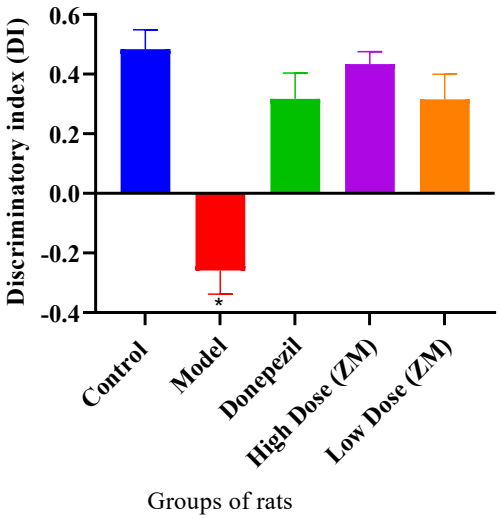
Cognitive function was assessed using novel object recognition test, which is a test performed to evaluate different phase of memory and learning in rodents.



A



B



C

A: Familiar object

B: Novel object (new object)

Figure 2: Assessment of memory and learning in rats using novel object recognition test

The result evaluated the therapeutic potential of *Ziziphus mauritiana* extract in rats induced with $AlCl_3$ and D-galactose to model Alzheimer's like Disease**. Figure 2A** showed the result for the novel object recognition test among various rat's group, the acquisition test; exploration of two items that are alike. Two-way ANOVA shows that there is no significant statistical difference between the groups [$F(4,40)=1.615$, $p<0.1893$]. However, Sidak's multiple comparisons test showed a significant statistical difference in the control group when compared with the model group and low dose group of rats. Also, the test further suggests that there is no significant statistical difference in the control group when compared with the donepezil and high dose (ZME) of group of rats.

Figure 2B Showed the results of the novel object recognition test among various rat's groups. The retentiveness test exploration of new object plus previously encountered object. Two-way ANOVA indicated significant statistical difference between the groups [$F(4,40)=6.413$, $p<0.0004$)]. Dunnett's multiple comparison test in the time taken by the rats in the control group to explore familiar object (A1) indicated that there is no significant difference when compared with the other treatment groups; Model, Donepezil, High and Low Doses (ZME). Furthermore, multiple comparisons in the time taken by the rats in the control to explore the novel object (B) showed a significant statistical difference when compared with the model group at $p=0.0119$ while there is no significant difference

when compared with the rest of the treatment group; donepezil at p=0.574, High Dose (ZME) at p=0.1544 and Low Dose (ZME) at p=0.0534.

Figure 2C showed the result for discrimination index (DI),which is the ability of the rats to distinguish the previously encountered object (familiar) from the new object novel) and is evaluated for the retentiveness trial. 1way ANOVA shows a very significant statistical difference between the groups [F(2,36 11.8)=17.7, p<0.0002]. However, multiple comparisons test show a significant difference in control groups (0.0654 ± 0.6515, p=0.0002) when compare to the model group (0.0798 ± 0.50018, p=0.0018). Again, the test further suggest that there is no significant statistical difference in control group (0.0654 ± 0.6515, p=0.0002) when compared with the donepezil (0.0872 ± 0.5409, p=0.1455), High Dose (ZME) (0.64216 ± 0.5417, p=0.7891) and Low Dose (ZME) (0.08421± 0.5315, p=0.6028) respectively. Values are shown as mean ± SEM, n=6 and p<0.05

Biochemical analysis of oxidative stress biomarker (SOD)

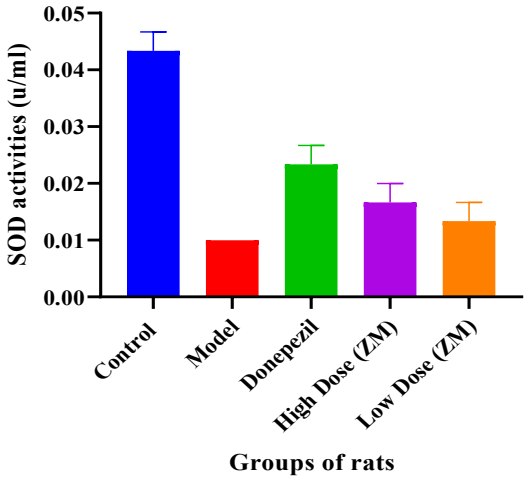


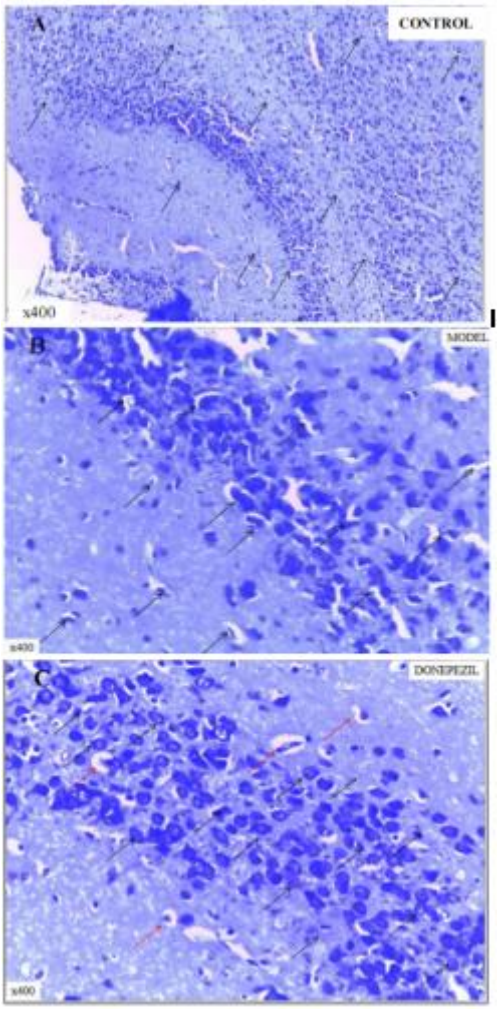
Figure 3: Effects of Ziziphusmauritiana on superoxide dismutase (SOD) activities

To evaluate antioxidant effect of *Ziziphus mauritiana* SOD activities were asses in the brain of $AlCl_3$ and D-gal induced rat. One-way ANOVA indicated a significant statistical difference of SOD activities in the prefrontal cortex of various groups [F (1.542, 3.084) =19.75, p<0.0183]. Multiple comparisons test show significant statistical difference in the reduction of SOD activities in the model group (0.00± 0.01, p=0.0183) when compared with the control group (0.0033± 0.0576, p<0.0218). However, the test further indicates that there is no significant statistical difference between the model group (0.00 ±

0.01, p=0.0183) when compare to donepezil group (0.0033±0.0376, p=0.1237), high dose group (0.0033 ± 0.0310, p=0.3734) and low dose group (0.0033 ± 0.0296, p=0.7434) respectively. Values are expressed as mean ± SEM,n = 6.

Histological Findings

Figure 4 showed histomorphological changes in the prefrontal cortex. The brain morphology was observed using cresyl violet, the pictomicrograph of the model group showed neuronal loss and degenerating cells, whereas the control group's brain structure remained intact. The image of the donepezil group showed vast number of normal cells. Similarly, the brain image of the ZME high dose showed vast number of normal cells of the prefrontal cortex while that of low dose ZME showed degenerating cells and and normal cells.



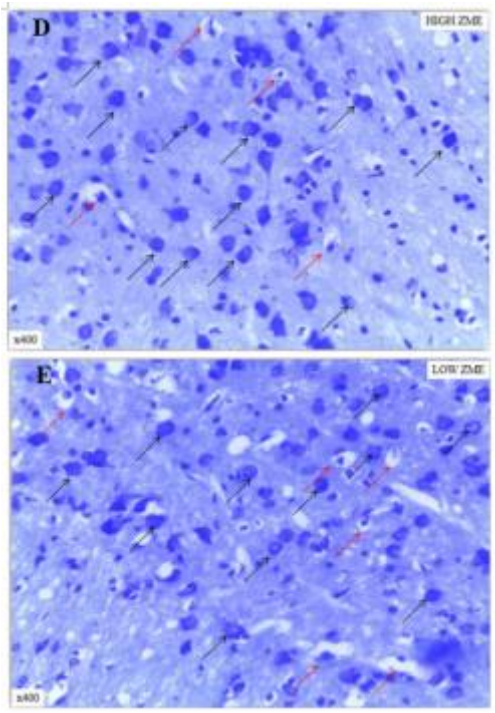


Figure 4 Histology of the brain of rats treated with $AlCl_3$ and D-gal: Showed full section of the prefrontal cortex. Histomorphological changes were assessed using cresyl violet stain. The photomicrograph of the control group (**A**) showed normal histology of the prefrontal cortex with normal cells typically pyramidal and neuroglia cells (black arrow). The section of the model group (**B**) showed multiple neuronal vacoulation and degenerating pyramidal cells (black arrow) with few numbers of viable cell. On the other hand, the section of donepezil group (**C**) showed minute number of degenerating cells (red arrow) and vast amount of viable cell (black arrow) having nearly similar morphology to the control group. The section of the high dose group (**D**) also showed minute number of degenerating cells (red arrow) and vast number of viable cells (black arrow) having similar morphology to the control group. The low dose group (**E**) also showed degenerating cells (red arrow) and of viable cells (black arrow).

DISCUSSION

The findings of this study revealed important insight into the effectiveness of different treatments doses of *Ziziphus mauritiana* extract in improving cognitive function of rats following experimentally-induced cognitive impairments. The model group which received $AlCl_3$ and D-gal, only exhibited a significant cognitive impairment when compared to the control group and other treatments. This finding is in line with the previous research

on the development of AD-like symptoms following co-administration of $AlCl_3$ and D-gal in rats (Liaquat *et al*., 2017).

The group treated with donepezil showed notable improvement in cognitive function. Likewise, the group that received both high and low doses of ZME showed cognitive improvement. The high dose group revealed significant improvement in cognitive function when compared to the donepezil group, this indicates the potent therapeutic effect of ZME against cognitive impairment induce by $AlCl_3$and D-gal. This finding aligns with the previous research of Kandeda *et al.,* 2021 where similar species *Ziziphus jujube* was used to treat the rats induced with D-gal.

The NORT in the current study found that the rats administered with high dose (ZME) showed improvements in retention as the rats were able to recognize the familiar object (A) (figure 2B) after treatment with$AlCl_3$ + D-gal. In addition, the rats treated with high dose of ZME showed increase in time taken to explore the novel object over the familiar object and also increase in discrimination index, as shown in (figure 2C). This result is in line with the previous finding of Kandeda *et al*., (2021 where a similar result was observed when rats were treated with *Ziziphus jujube* extract following administration of D-gal.

The outcome of this research showed a significant increase in SOD activities in the rats treated with *Ziziphus mauritiana* (figure 3). This result is in line with the previous finding of Mahdi *et a*l., (2021), where a similar result was observed when rats were treated with WIN55, 212-2 following $AlCl_3$ and D-gal induction of cognitive impairment.

Furthermore, histomorphological analysis showed that the rats treated with a high dose of ZME have similar tissue morphology to the control group. This result is in line with the previous finding of Usen, and Enogieru, 2023 where similar result was observed when the rats were treated with P*isidium Guajava* leaf extract following cognitive damage induced by $AlCl_3$ and D-gal.

CONCLUSION

In conclusion, treatment with the aqueous extract of *Ziziphus mauritiana* protected the rats from neurotoxic effects of $AlCl_3$ and D-galactose. Indeed, the extract significantly restored cognitive functions in the object recognition test and increase SOD activities in

biochemical analysis. Thus, suggesting that ZME could be a therapeutic candidate for the treatment of cognitive impairments such as Alzheimer's. In addition, the findings of the current study further showed thatthe rats treated with *Ziziphus mauritiana* extract had histomorphological appearances similar to those of the control group. In essence, the study uncovered that the extract has the potential to attenuate neurotoxicity induced by $AlCl_3$ and D-gal.

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Declaration of Conflicts of Interests

Authors have declared that there is no conflict of interest.

Abbreviations

Aβ	Amyloid beta
AD	Alzheimer's disease
AGES	Advanced glycation end products
$AlCl_3$	Aluminium chloride
ANOVA	Analysis of variance
BBB	Blood brain barrier
D-gal	D-galactose
IP	Intraperitoneal
MDA	Malonaldehyde
N	Total number of rats
n	Number of rats per group
NFT	Neurofibrillary tau tangles
NOR	Novel object recognition
SEM	Standard error of mean
SOD	Superoxide dismutase
ZME	Ziziphus mauritiana

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