INVESTIGATION FOR NOOTROPIC ACTIVITY OF ZIZIPHUS MAURITIANA ON SCOPOLAMINE INDUCED MEMORY DEFICITS IN SWISS ALBINO MICE

A Thesis Submitted
in Partial Fulfillment of the Requirements for the Degree of

MASTER OF PHARMACY

in

Pharmacology

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LUCKNOW

July, 2024

DECLARATION

I hereby declare that the work presented in this report entitled "**INVESTIGATION FOR NOOTROPIC ACTIVITY OF ZIZIPHUS MAURITIANA ON SCOPOLAMINE INDUCED MEMORY DEFICITS IN SWISS ALBINO MICE**", was carried out by me. I have not submitted the matter embodied in this report for the award of any other degree or diploma of any other University or Institute. I have given due credit to the original authors/sources for all the words, ideas, diagrams, graphics, computer programs, experiments, results, that are not my original contribution. I have used quotation marks to identify verbatim sentences and given credit to the original authors/sources. I affirm that no portion of my work is plagiarized, and the experiments and results reported in the report are not manipulated. In the event of a complaint of plagiarism and the manipulation of the experiments and results, I shall be fully responsible and answerable.

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InvestigationForNootropicActivityofZiziphusMauritianaOn Scopolamine Induced Memory Deficits In Swiss Albino Mice

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ABSTRACT

This study explores the impact of Ziziphus Mauritiana extract (ZME) on cognitive functions, with a particular focus on scopolamine-induced memory and learning deficits in rodent models. The objective is to assess the potential ofZMEasatreatmentforcognitivedecline,whichcould lead to effective therapies for dementia and neurodegenerative diseases. Cognitive decline and dementia involve progressive deterioration of mental abilities, affecting memory,reasoning,and daily activities. Traditional treatments often have adverse side effects, necessitating alternative solutions. Ziziphus Mauritiana, known for its medicinal benefits, has shown promise in preliminary studies for enhancing cognitive functions and providing neuroprotection.

In this study, rodents were divided into several groups: control, scopolamine-treated, and those treated with different doses of ZME and a standard drug (Vyvamind). Cognitive functions were assessed through behavioral tests such astheAquaticNavigationTaskandSpatialMemoryTask at various intervals. Additionally, biochemical assays measured acetylcholinesterase (AChE) activityandmalondialdehyde(MDA)levelstoevaluatecholinergicfunctionandoxidativestress. The scopolamine group showed significant impairments in memory and learning, indicated by increased escape latencies and errors. In contrast, ZME-treated groups (200 mg/kg and 400 mg/kg) demonstrated notable improvements, similar to Vyvamind-treated rodents, with reduced escape latencies and errors, suggesting enhanced spatial learning and memory retention. Biochemical results showed that scopolamine increased AChE activity and MDA levels, indicating cholinergic dysfunction and oxidative stress, while ZME treatment significantly reduced these levels. ZME-treated groups had lower AChE activity (600 μM/min/mg for 200 mg/kg and 350 μM/min/mg for 400 mg/kg) and MDA levels (24 μM/mg and 19 μM/mg) compared to the scopolamine group, aligning closely with the control group.

The findings suggest ZME has a protective effect against scopolamine-induced cognitive impairments, improving behavioral performance and normalizing neurochemical markers. This underscores its potential as a natural alternative for treating cognitive decline with fewer side effects than conventional drugs. Future research should explore the underlying mechanisms and conduct clinical trials to evaluate its efficacy in humans.

Keywords: Ziziphus Mauritiana extract, cognitive decline, scopolamine, memory impairment, neuroprotection, acetylcholinesterase activity, oxidative stress, dementia therapy.

CHAPTER-1

INTRODUCTION

The capacity to process, preserve, maintain, and recall knowledge and past events defines cognitiveretention.Itisessentialforcognitivefunctionslikelearning,reasoning, problem-solving and is the foundation for human consciousness and experiences. Memoryallows us to connect the present with the past and build on prior knowledge. It is crucial for coherence in daily life, professional success, and the transmission of cultural knowledge across generations[7].

Cognitive retention functions as the cornerstone of mental operations that craft the depth of human consciousness. It allows us to link current circumstances with historical occurrences, weaving a cohesive personal story that establishes our sense of self and perspective on life. Memory is also the bedrock upon which cultural evolutionrests,asaccumulatedknowledgeand wisdom are transmitted and built upon through generations[19].

The degree to which an individual possesses a strong memory capacity plays a pivotal role in their ability to thrive across various spheres of life, be it their vocation, profession, business pursuit, or any other undertaking. The coherence and continuity with which one navigates their chosen path largely hinges upon the depth and fidelity of their developed mnemonic faculties[6].

Recollection and loss of information represent complementary aspects of mental functioning, eachservingas a fundamental component in the process of learning and understanding and skills. Remembering refers to the act of retaining perceived or experiencedinformation.Itisthe power of the mind to encode,store,andreproduceinformationandexperiences,amentalfaculty we call memory[33].

There are four core elements underlying the phenomenon ofmemory:learning,retention,recall, and recognition. Learning is the initial acquisition of knowledge through lived experiences, representing a change in behavior or understanding in response to repeated exposures within a given situation. Retention is the process by which learned material is preserved and maintained within the cognitive architecture. Recall is the act of retrieving and reconstructing retained information from memory storage when needed, either spontaneously or through deliberate effort. Recognition is the conscious awareness and identification of retained and recalled experiences as part of one's personal history[42].

Cognition is the overarching process through which intellectual knowledge is acquired, organized, and utilized. Cognitive learning theories emphasize the primacy of comprehension, where the mind actively performs operations on informational inputs, encoding salient aspects into memory for potential future retrieval. Cognition implies an understanding of causal relationships between actions and their consequences, and cognitive strategies are the mental plansandheuristicsemployedbyindividualstomakesenseofthemselvesandtheir

environment[27].

Basedon physiologicalmechanisms, memoryis primarilydivided intothree categories.

1. **SHORT-TERMMEMORY**

   Immediate recall, often termed operational memory, describes the fleeting retention and processing of data for a brief span, usually under 60 seconds. This mental faculty is constrained in its capacity.
   Operational memory, alternatively called immediate recall, involves the momentary preservation and retrievalofinformationoverashorttimeframe,generallyenduringfrom mere moments to a fewminutes.Itrepresentsthementalcapabilityenablingustosustain a limited quantity of data in a readily accessible condition for instant utilization and handling.Theneurologicalcomponentschieflyresponsibleforfacilitatingthis rapid-access memory include the seahorse-shaped brain structure, the breast-like bodies, and two clusters within the relay center of the brain - the forward and middle clusters. These neural elements collaborate to transiently maintain and manipulate restricted amounts of incoming sensory information.
   Short-term memory allows the temporary holding of information for immediate use,while intermediate memory connects the present moment to ongoing events. Long-term memory, on the other hand, provides a vast archive of accumulated experiences and knowledge that can be drawn upon indefinitely. This multifaceted memory system, spanning temporary buffersandpermanentstoragebanksdistributedacrossthebrain,isa core pillar of human cognition[11].

2. **INTERMEDIATEMEMORY**

   Transitional recall, alternatively termed experiential storage or operational cognition, referstothementalcapacityforpreservingandreassemblingcurrentoccurrencesacrossa somewhat extended duration, typically spanning from multiple moments to fractions ofan hour. It provides a continually updated temporal context that orients us to thepresent moment and our immediate circumstances. This form ofmemoryallowsustomaintaina coherent stream of awareness, connecting our recent pasttothehere-and-now,informing us of our location, actions, and surrounding eventsastheyunfold[7].Intermediatememory acts as a bridge between the fleetingrepresentationsofsensoryinformationinshort-term memory and the more enduring representations stored in long-term memory,enablingus to integrate and comprehend our experiences as they occur[52].

3. LONG-TERM MEMORY

Enduring recall refers to the comparatively lasting preservation of data for prolonged intervals, spanning from multiple sunsets to numerous seasons. This mental repository boasts a significantly greater capacity than its fleeting counterpart, immediate retention.

Sustained cognitive retention is a form of durable information preservation that can endure through decades or potentially throughout one's entire existence[8]. Once informationisencodedintolong-termmemory,itbecomesavailableforsubsequentrecall and retrieval as needed. The hippocampus plays an essential role in the consolidation process that transfers information from short-term buffers into long-term storage, although the hippocampus itself does not appear to be the repository for permanent memories[2]. Rather, learned information ultimately becomes distributed across various cortical regions depending on the nature of the memory trace[16].

Data stored in enduring recall can typically be summoned and utilized when needed, given the presence of sufficient prompts or stimuli to reignite the pertinent brain circuits[12]. The more frequently a specific memoryisretrievedandputintoactiveuse,the more reinforced and resistant to forgetting that memory trace becomesaprocessknown as memory consolidation[13]. Each recall instance prompts a selective reconstruction and re-encoding that further strengthens and stabilizes the memory representation[38].

Othertypesofmemoryareas follows

1. WORKING MEMORY

Working memory is an active cognitive system that temporarily holds and manipulates information in the service of complextaskslikelanguagecomprehension,reasoning,and learning. Unlike passive short-term storage, working memory continuously updates its contents based on incoming sensoryinputsandinformationfromlong-termmemory.Itis considered a core component of human cognition because its limited capacityconstrains our ability to acquire knowledge and perform higher-order cognitive operations that require maintaining and manipulating information mentally. The multicomponent working memory model, with its central executive and subsidiary storage systems for verbal and visuospatial information, has been highly influential in elucidating working memory's role across domains[14].

2. DECLARATIVEMEMORY(EXPLICITMEMORY)

Declarative memory refers to conscious memories that can be explicitly stated or declared[15]. It comprises two main subtypes:

a. Episodic memory stores personal experiences and events situated in specific spatial and temporal contexts. It allows remembering autobiographical episodes from one's life.
b. Conceptual retention encompasses broad understanding and information about reality that exists separately from individual encounters and situations. It represents a structured database of concepts, their properties, and associations.

While episodic memory is autobiographical, semantic memory contains semantic information divorced from the circumstances in which it was acquired. This declarative memory system is distinct from non-declarative or procedural memory for skills and habits[50].

3. PROCEDURAL (IMPLICIT) MEMORY

Procedural memory refers to the unconscious memory system involved in learning and retaining motor skills and cognitive routines. It allows improving performance of tasks through repetition, without forming explicit memories of the learning episodes. Procedural memory is revealed when somebody gets better at a skill simply through practice, despite not being able to consciously describe what they have learned[10].

The neural substrates of procedural memory,especiallyformotorskilllearning,crucially involve the cerebellum and the basal ganglia. Damage to these brain regions can impair the ability to acquire new procedural skills while leaving other memory systemsrelatively intact[60].

In summary, procedural memory is the implicit long-term memory system that enables the gradual acquisition of motor and cognitive skills through experience and repetition, without consciously accessible memories being formed[47].

1.1 DEMENTIA

Cognitive decline disorder is a condition marked by an ongoing andirreversibledeteriorationin various mental faculties, including recall, communication, logical thinking, discernment, and choice-makingcapabilities.Thisimpairmentsubstantiallyhinderseverydayactivitiesand self-sufficiency. While this condition predominantly affects the elderly population, it should not be considered a typical aspect of growing older. It results from damage to braincellswhichcan be caused by various underlying diseases or injuries[46].

Cognitive decline disorder is a medical condition characterized by a substantial and worsening deterioration across multiple mental faculties, including recall, analytical thinking, verbalskills, spatial perception, and organizational abilities. This mental impairment is severe enough to disrupt daily routines, self-reliance, job performance, and interpersonal relationships. The disorderrepresentsadeclineincognitiveabilitiesbeyondwhatwouldbeanticipatedfromtypical biologicalaging.Whileadvancedageistheprimaryriskfactor,cognitivedeclineisnotan

unavoidable result of growing older[31].

The worldwide prevalence of this disorder isalarming,withover55millionindividualsglobally living with some form of cognitive decline, and an estimated 10 million new diagnoses eachyear. This condition can stem from various underlying illnesses, traumas, or circumstances that directly or indirectly affect brain structure and operation. The most common root cause is a progressive brain disorder associated withproteinaccumulation,accountingfor60-70%ofcases in developing nations. Other significant causes include brain changes due to impaired bloodflow, protein deposits in nerve cells, and deterioration of frontal and temporal brain lobes[29].

Cognitive decline disorder has become a significant public health challenge, nowrankingasthe 6th most common cause of mortality globally and one of the main factors contributing to impairment and reliance among the elderly population.The burden of dementia extends far beyond the individuals affected, with profound bodily, mental, financial, and economic ramifications for caregivers, household members, and the broader community as a whole[58].

Estimates project the global dementia population will swell to 82 million by 2030 and a staggering 152 million by 2050. This dramatic increase is primarily fueled by the rising prevalence of dementia in low- and middle-income nations, which already bear a disproportionate share of the worldwide dementia burden. Addressing the growing dementia pandemic will require a coordinated global response and a concerted effort to advance prevention, treatment, care, and support strategies[2].

1.2 TYPE OF DEMENTIA

Although cognitive decline in older adults has been acknowledged for centuries, various causes and categories were discovered in the early 20th century through examination of brain tissue samples from deceased individuals who exhibited symptoms of mental deterioration. Theprimary classifications of this disorder include[30]:

i. Protein Accumulation Brain Disorder
ii. Blood Flow-Related Brain Impairment
iii. Protein Deposit Neural Disorder
iv. FrontalandTemporalLobeDeterioration
v. MovementDisorder-AssociatedCognitiveDecline
vi. Multi-TypeCognitiveDisorder

1. ProteinAccumulationBrainDisorder:

The most prevalent form, comprising 60-80%ofcases.Itinvolvesabnormalproteinbuildupand neurondeathinmemory-relatedbrainareas.Firstidentifiedin1906,it'scharacterizedbyspecific protein structures and loss of key neurons, leading to impaired signal transmission and mental decline[56].

2. Blood Flow-Related Brain Impairment:
Caused by damaged brain blood vessels, reducing oxygen supply to brain cells. Often triggered by stroke events. It includes post-stroke cognitive decline and vascular cognitive impairment, affecting about 10% of the global population[23].

3. ProteinDepositNeuralDisorder:
Involves abnormal protein accumulations in brain areas controlling thinking and movement. Causes cognitive, motor, and behavioral issues, including hallucinations. It's the second most common progressive mental decline disorder[70].

4. FrontalandTemporalLobeDeterioration:
Affects behavior, personality, and language due to nerve cell degeneration in specific brain regions.Subtypesincludebehavior-affectingandcommunication-impairingvariants,withfurther distinctions in language-related impairments[59].

5. MovementDisorder-AssociatedCognitiveDecline:
Occurs in some patients with a progressivemovementdisorder,affectingthinkingandreasoning alongside motor control. Characterized by protein deposits and dopamine reduction, impacting 2% of those over 65. Symptoms include both motor and non-motor impairments[40].

6. Multi-TypeCognitiveDisorder:
Features simultaneous occurrence of multiple cognitive decline types, most commonly combining protein accumulation and blood flow issues. May also involve other protein deposit disorders. In rare cases, an individual may exhibit features of three distinct conditions simultaneously[5].
These conditions involve various brain changes, protein accumulations, and neurotransmitter imbalances, leading to diverse cognitive and functional impairments. While each disorder has unique characteristics, there may be common underlying mechanisms or pathogenic factors among certain neurodegenerative diseases[65].

1.3 GLOBALIMPACTANDSTATISTICSONDEMENTIA

Cognitive decline disorders represent a major worldwide health issue, impacting a vast numberof individuals globally. The World Health Organization provides the following data to illustrate the scale of this challenge:

1. Approximately55millionpeopleworldwideexperiencecognitivedeclinedisorders,with nearly 60% residing in nations with lower economic development.
    a. This statistic demonstrates the widespread nature of these conditions across the globe.
    b. Theconcentrationofcasesinlesseconomicallydevelopedcountriesemphasizesthe

necessityfor enhanced medical resources and aid systems in these areas[13].

2. A specific protein accumulation brain disorder accounts for 60-70% of cognitive decline cases, making it the most prevalent form.
    a. Thisparticular disorder dominates the landscape of cognitive decline conditions globally.
    b. The statistic underlines the critical need for ongoing scientific investigation into this disorder's mechanisms and the development of effective interventions and preventative approaches[44].

3. Projections indicate thetotalnumberofindividualswithcognitivedeclinedisorderswillreach 78 million by 2030 and 139 million by 2050.
    a. Theseforecastssuggesta dramatic increase in the global prevalence of these conditions in the coming years, influenced by an aging population and extended life expectancies.
    b. This anticipated surge highlights the urgent requirement for comprehensive healthcare strategies, resource distribution, and policy measures to meet the growing demand for care and support services worldwide[54].

4. Cognitive decline disorders are primary contributors to impairment and reliance among older adults, affecting individuals, their families, and broader society.
    a. These conditions can result in significant functional limitations, increasing individuals' dependence on others for daily tasks and personal care.
    b. The effects extend beyond those directly affected, impacting caregivers, families, and communities both emotionally and financially.
    c. Societal implications include escalating healthcare expenses, reduced productivity, and the need for extensive care systems to accommodate the rising number of affected individuals[62].

These figures underscore the substantial global impact of cognitive decline disorders and the pressing need for coordinated efforts to address this health challenge. Strategies focusing onearly identification, condition prevention, treatment advancement, caregiver assistance, and healthcare system readiness are vital to alleviate the impact of these disorders on individuals, families, and societies worldwide[18].

1.4 PATHOPHYSIOLOGYOFDEMENTIA

The mechanisms underlying cognitive decline disorders involve several interrelated processes:

1. Brain Cell Deterioration:
Most cognitive decline disorders feature gradual decay and death of nerve cells across multiple brain regions, particularly in the outer layer crucial for advanced mental functions. This deterioration disrupts communication between neural networks essential for memory, language, and reasoning. Different disorders affect specific brain areas:

a. In protein accumulation disorders, abnormal protein build ups interfere with nerve cell function.
b. In blood flow-related impairments, reduced oxygen supply damages nerve cells.
c. In frontal and temporal lobe deterioration, cell loss occurs mainly in areas linked to personality and language[48].

2. SpecificNeurotransmitterSystemImpairment:
A particular nerve signalingsystem,vitalforlearningandattention,issignificantlyaffected.The loss of these specialized nerve cells, especially in brain areas crucial for memory and higher thinking, contributes to cognitive deficits. This chemical imbalance is often an early change in protein accumulation disorders and correlates with symptom severity[35].

3. CellularStress Impact:
An imbalance between harmful molecules and protective substances in the body plays a role in various cognitive decline disorders. These harmfulmoleculescandamagenervecellsbyaltering proteins, genetic material, and cell membranes.Thisdamageleadstocelldysfunctionanddeath, contributing to cognitive impairments. Inproteinaccumulationdisorders,thiscellularstressmay also promote the formation of abnormal protein deposits[74].

4. Oxygen Deprivation Effects:
Insufficient oxygen supply to brain tissue can impair nerve cell function and survival. Chronic low oxygen conditions, as seen in blood flow-relatedimpairmentsorsleepdisorders,canreduce theproductionofimportantsignalingchemicals.Thisdeficiencycontributestocognitivedecline.
Oxygen deprivation can also increase cellular stress, inflammation, and nerve cell damage through various mechanisms.

These processes - brain cell deterioration, neurotransmitter system impairment, cellular stress, and oxygen deprivation - are interconnected pathological mechanisms contributing to the development and progression of various cognitive decline disorders, particularly protein accumulation and blood flow-related brain impairments[79].

1.5 CONCEPTS OF NOOTROPICS

This document discusses cognitive enhancers and their potential application to brain function disorders:

1. Cognitive Enhancers Overview:
Substances that may boost mental abilities, memory, and overall brain performance. These compounds, both natural and synthetic, aim to optimize brain function without significant side effects. Key features include[31]:

a. Improving learning and memory processes
b. Enhancing brain hemisphere communication
c. Protecting against brain damage
d. Minimalsideeffectsandlowtoxicity
e. Increasedeffectivenessathigherdoses

2. Possible Mechanisms:
Cognitive enhancers may work through various pathways:

a. Adjusting neurotransmitter systems
b. Boostingbraincellmetabolismandenergy production
c. Protecting and promoting nerve cell growth
d. Influencing brain blood flow and oxygen use
e. Regulating gene expression and new nerve cell formation

3. Brain Function Disorder Context:
A major neurological condition affecting memory and thinking skills, characterized by progressive decline in multiple cognitive areas. It significantly impacts daily life, primarily in older adults, affecting millions worldwide[68].

4. Complex Underlying Processes:
Thedisorder involves various factors contributing to nerve cell dysfunction and degeneration,including:

a. Deterioration of brain cells
b. Lossofspecificneurotransmitter-producingcells
c. Cellular stress
d. Oxygen deprivation

5. CurrentTreatmentLimitations:
Existingtreatments,mainlyfocusedonpreservingaspecificneurotransmitter,havedrawbacks:

a. Temporaryandplateauingbenefits
b. Sideeffectslimitinglong-termuse

c. Inability to modify underlying disease processes or halt progression

6. Exploring Natural Alternatives:

Due to these limitations, there's growing interest in plant-based cognitive enhancers aspotentially safer options. Some plants and natural compounds show promise in early studiesfor improving memory and thinking through various mechanisms. One such plant, Ziziphus mauritiana (Indian jujube), has been traditionally used in certain medical systems for its therapeutic properties[53].

CHAPTER-2

PLANTINTRODUCTION

Ziziphus mauritiana, oftenreferredtoasIndianjujubeorber,isafruittreethatthrivesintropical climates native to Southern Asia. It produces small, round fruits that are edible and nutritious. The tree is drought-resistant and widely cultivated in arid and semi-arid regions for its fruit, timber, and medicinal properties. Its leaves and bark have traditional uses in herbal medicine.

Fig. 2.1 Ziziphus Mauritiana Fruit

2.1 COMMON NAMES

1. English: Indian jujube
2. Hindi:बेर(Ber)
3. Sanskrit:बदरः(Badari)
4. Tamil:எலைத(Elanthai)
5. Telugu:ీమంకీంంs(Regipandu)
6. Marathi:बोर(Bor)
7. Gujarati:બોર(Bor)
8. Bengali:ল(Kul)
9. Urdu: بیر(Ber)

Fig. 2.2 Ziziphus Mauritiana Leaves

2.2 TAXONOMY CLASSIFICATION

Table2.1: Taxonomyclassification

Kingdom	Plant
Division	Magnoliophyta
Subdivision	Angiosperm
Class	Magnoliopsida
Order	Rosales
Family	Rhamnaceae
Tribe	Paliureae
Genus	Ziziphus
Species	Mauritiana

2.3 MACROSCOPIC CHARACTERISTICS

Ziziphus mauritiana is amedium-sizedevergreenordeciduoustree,typicallyreachingheightsof 5-12 meters, though it can grow up to 15 meters tall. It features a spreading canopy withdrooping branches and a short,crookedtrunkcoveredindarkgray,irregularlycrackedbark.The leaves are alternate and simple, with an ovate to ellipticshape,measuringbetween2.5and6cm in length and 1.5 to 5 cm in width. They have finely toothed margins and are dark green and glossy on top, while pale green or whitish underneath. At the base of each leaf are pairs ofthorns, one straight and one curved. The tree yields tiny, yellowish-green flowers that have five petals, growing in clusters in the leaf axils. The fruit is a drupe, ranging from oval to round in shape, with a diameter of 1.5 to 2.5 cm. It starts green, turning yellow to reddish-brown when ripe, with a thin edible skin covering white, crisp flesh and a single hard stone inside. The root system consists of a deep taproot with extensive lateral roots, contributing to the tree's drought resistance[63].

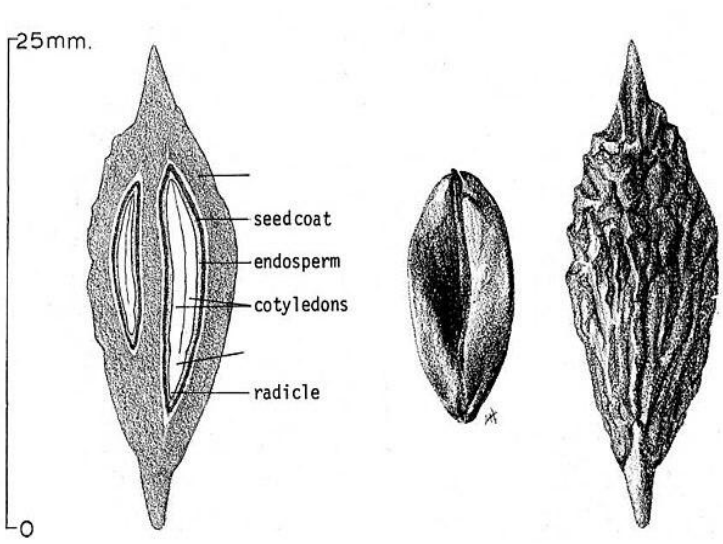

Fig. 2.3 Ziziphus Mauritiana Seed

Fig. 2.4 Ziziphus Mauritiana Barks

2.4 CHEMICAL CONSTITUENT

Ziziphus mauritiana contains a diverse array of chemical constituents that contribute to its nutritional and medicinal properties. The fruit is rich in carbohydrates, primarily sugars like glucose, fructose, and sucrose, as wellasdietaryfiber.Itcontainssignificantamountsofvitamin C, carotenoids, andB-complexvitaminsincludingthiamine,riboflavin,andniacin.Thefruitalso provides essential minerals such as calcium, phosphorus, and iron. Various parts of the plant contain bioactive compounds including flavonoids, saponins, tannins, and phenolic acids. Triterpenes like betulinic acidandalphitolicacidhavebeenextractedfromtheplant.Thefoliage and bark contain alkaloids, particularly peptide alkaloids. Cyclopeptide alkaloids, such as Mauritine A, B, C, and D have been found in the bark of the stem and roots. The seedscontain fatty acids, primarily oleic and linoleic acids. Triterpenoid saponins, including jujubosideAand B, are present in the seeds and have been associated with sedative effects. Pectin, a valuable polysaccharide, is found insignificantquantitiesinthefruit.Thesediversechemicalconstituents contribute to the plant's antioxidant, anti-inflammatory, antimicrobial, and other medicinal properties, makingZiziphusmauritianaasubjectofongoingphytochemicalandpharmacological research[76].

2.5 PHYTOCHEMISTRY

Ziziphus mauritiana isarichsourceofdiversechemicalconstituents,withover150cyclopeptide alkaloidsfoundacrossvariousZiziphusspecies.Theplantcontainsseveralimportantcompounds including pectin A, glycosides, triterpenic acids, lipids, and alkaloids.

Pectin A: Pectin A, containing 2,3,6-tri-O-acetyl D lactose moieties, ispresentinthefruitsofZ. mauritiana and Z. jujube. This compound exhibits pharmacological attributes like bile acid sequestration and cholesterol reduction, and antidiarrheal effects.

Alkaloids: The stem bark of Z. mauritiana is particularly rich in alkaloids. Researchers have isolated numerous compounds including zizogenin (asapogenin)andcyclopeptidealkaloidslike mauritiana A, B, C, F, G, and H. The root bark yields additional alkaloids such as frangufoline, amphibians B, D, E, F, and mauritiana J.

Triterpenoic acid: Triterpenoic acids are another significant group of compounds found in Z. mauritiana. The roots contain triterpenic acids with cytotoxic properties. Specific triterpenoid acids identified include colubrinic acid, alphitolic acid, various coumaroyl alphitolic and coumaroyl maslinic acid derivatives, oleic acid, zizyberanalic acid, and betulinic acid.

Betulinic acid: Betulinic acid, a five-ring triterpenoid, has garnered particular interest due to its selective cytotoxicity against various tumor types. It also demonstrates anti-inflammatory and antibacterial properties, making it a promising compound for therapeutic applications.

Alkaloids: The seeds and pericarp (fruit skin) of Z. mauritiana contain bioactive lipids such as phosphatidylcholines, phosphatidylglycerol, and fatty acids. The major fatty acids include linoleic acid, oleic acid, and stearic acid. ThediversearrayofcyclopeptidealkaloidsfoundinZ. mauritiana, including mauritiana A, B, C, D, E, F, and H, demonstrate a wide range of pharmacological activities. These include sedative, antimicrobial, antidiabetic, antimalarial, analgesic, anticonvulsant, and anti-inflammatory effects, underlining the plant's potential in traditional and modern medicine[1].

Fig. 2.5 Ziziphus Mauritiana Roots

Fig. 2.6 Ziziphus Mauritiana Flower

2.6 USES OF Z.MAURITIANA

2.6.1 Medicinal uses

a. **Leaves:** The leaves of Ziziphus mauritiana possess astringent properties, making them beneficial in treating diarrhea. They are also used as diaphoretics and are recommended for thyroid-related issues in children. The leaves are frequently applied topically on wounds to accelerate the healing process. Additionally, they find application in the management of asthma and liver disorders.

b. **Barks:** The bark of Ziziphus mauritiana exhibits astringent properties, making it usefulfor treating gingivitis and lesions when applied topically. A decoction prepared fromthe twigs is employed in the management of dysentery and diarrhea.

c. **Flowers:** The various parts of Ziziphus mauritiana are used to treat different ailments. Externally, it is applied for skin ulcers and eye disorders. When taken internally, it is effective in treating jaundice.

d. **Roots:** The powder derived from the roots of Ziziphus mauritiana aids in rapid woundand ulcer healing when applied topically. A decoction prepared from the roots isusedto treat fever[14].

2.6.2 Non-medicinal uses

a. **Seed:** Seeds of Ziziphus mauritiana are nutrient-dense, containing high levels ofprotein. Theyserveasavaluablefoodsource,particularlyduringtimesofscarcityorfamine.This nutritional profile makes the seeds an important dietary component in regions where the plant is cultivated.

b. **Flowers:** The blossoms of Ziziphus Mauritiana are delightful,servingasasweetenerand offering a small amount of nectar for honeybees.

c. **Leaves:** Leaves of Ziziphus mauritiana are nutritious, fast-regenerating, and versatile. They serve as a vegetable for human consumption, valuable animal feed, and support silkworm cultivation. The leaves also host the lac bug, which produces a useful resin.

d. **Barks:** The bark of Z. mauritiana provides a durable cinnamon-coloreddye,traditionally used in leather tanning and fabric dyeing[9].

2.6.3 TraditionallyUses

Ziziphus mauritiana has widespreaduseintraditionalmedicine.Itsvariouspartsareusedtotreat conditions like fever, wounds, ulcers, and headaches. The bark is especially valued foraddressing digestive issues, gum problems,andskinailments.Thistraditionalusageunderscores the plant's perceived medicinal value across different cultures[3].

2.7 PHARMACOLOGICAL ACTIVITIES

1. **Anti-Steroidogenic Activity:** The ethyl acetate extract from Ziziphus mauritiana bark showed anti-steroidogenic effects in female mice. It disrupted the estrous cycle, reduced ovarian weight, and increased ovarian cholesterol and ascorbic acid levels. These antifertility effects were reversible, with normal function resuming after extract withdrawal. This study suggests potential applications in reproductive health management[4].

2. **Immunomodulatory:** In mice, The water-basedethanolextractfromtheseedsofZiziphus mauritiana demonstrated immunomodulatory effects in mice at doses from 100 to 4000 mg/kg. The extract was standardized with HPLC, utilizingbetulinicacidasthereference compound[20].

3. **Anti-Diarrhoeal Action:** The methanol extract of Ziziphus mauritiana showed antidiarrheal properties by inhibiting rabbit jejunum movement and suppressing acetylcholine-induced rat ileum contractions. At doses of 25 and 50 mg/kg, it inhibited diarrhea and fluid buildup induced by castor oil in mice, demonstrating its effectiveness against diarrhea[77].

4. **Antioxidant Activity:** The antioxidant properties of the methanol extract of Ziziphus mauritiana were evaluated using the DPPH radical scavenging assay, a colorimetric method. Results showed that the extract's ability to neutralize free radicals increased in proportion to its concentration. The study determined that the extract's IC50 value - the concentration required to inhibit 50% of DPPH radicals - was 38.07 µg/mL[32].

5. **Hypotension:** In rabbits, administration of Ziziphus Mauritiana extract at doses ranging from doses ranging from0.4to122mg/kgofPCcauseshypotensioninadose-dependent manner, akin to the effects induced by acetylcholine (ACh). Conversely, the administration of atropine at dosages doses from 4.10-3 to 4.84 µg/kg of PC prevent hypotension in rabbits induced by 4.10-3 µg/kg of acetylcholine (ACh) and 22mg/kgof Ziziphus mauritiana.[25].

6. **Antiulcer Activity:** A study tested Ziziphus mauritiana stem bark methanolic extract for anti-ulcer effects in mice. Using ethanol- and aspirin-induced ulcer models, the extract was administered at doses of 100, 250, and 500 mg/kg resulted in dose-dependent reductions in gastric lesions of 8%, 66.67%, and 82.67%, respectively[72].

7. **Anxiolytic Activity:** The anxiolytic effects of Ziziphus mauritiana leaf ethanolic extract were studied using elevated plus maze and light-dark box experiments. A Rota-rod apparatus was utilized to assess neurotoxicity. The extract showed anxiety-reducing properties similartodiazepam,withoutcausingneurotoxicity.Thesefindingssuggestthat Ziziphus mauritiana leaf extract has potential as an anxiolytic agent[78].

8. **Thrombolytic Activity**: Ziziphus mauritiana methanol extracts were evaluated for thrombolytic properties using the Daginawala et al. method. Streptokinase was used asthe standard in this assessment. Results indicated that the extracts possess thrombolytic activity[21].

9. **Anti-Inflammatory Activity:** A study on Wistar rats examined the anti-inflammatory effects of Ziziphusmauritianaleafmethanolicextractusingcottonpelletgranulomatests. Results demonstrated dose-dependent anti-inflammatory effects. At 500 mg/kg, the extract provided 31.1% protection against inflammation,whilea250mg/kgdoseyielded 16.9% protection.

10. **Analgesic Activity:** The analgesic effects of Ziziphus mauritiana methanol extract were studied using the tail-flick test. Researchers measured rats' reaction times to radiantheat applied to the last 1-2 cm of their tails. Results showed that the extract moderately increased reaction time in a dose-dependent manner, indicating antinociceptive activity.[36]

11. **Nootropic Activity:**The N-butanolic fraction of Ziziphus mauritiana leaf methanolic extract showed significant nootropic activity in a study. It improved spatial cognitive acquisition and recall assessment using the raised cross-shaped apparatus, enhanced memory retention in the passive avoidance test, and boosted recognition memoryduring the novel item identification assessment. These effects, equivalent to the established benchmark nootropic piracetam, suggest potential for developing natural cognitive enhancers and treatments for neurodegenerative disorders[10].

2.8 PHARMACEUTICAL IMPORTANCE

Medicinalplantshavelongbeencrucialinglobalhealthcare.Accordingtoglobalhealth authoritydata,four-fifthsofhumanitydependsonplant-derivedmedications.Thesenatural

remedies are expected to contribute significantly to future drug development, potentially accounting for over 60% of new drug candidates.

The botanical species Ziziphus mauritiana, colloquially referred to as desert apple or Chinese date, has gained attention foritswide-rangingtherapeuticpotential.Traditionally,itsleaveshave been used to treat tuberculosis and blood disorders. The leaf juice, mixed with buffalo milk, is believed to treat smallpox, while a leaf paste is applied topically for wound healing and relief from burning sensations.

In traditional medicine, Z. mauritiana is versatile in its applications. Its leaves, combined with cumin, are prescribed for urinary tract infections. The root, mixed with cow's milk, is used to treat diarrhea. Holding a fresh root in the mouth is recommended for soothing throat irritations and hoarseness.

Almost all parts of Z. mauritianahavemedicinaluses.Therootsandstemsaretraditionallyused for dysentery and diarrhea, while the root bark shows anti-inflammatory, anti-allergic, and analgesic properties. This makes it valuable for treating various inflammatory conditions and managing pain.

The plant has also shown promise in women's health, effectively alleviating pregnancy-related symptoms such as nausea, vomiting, and abdominal pain. Its leaves have been used to treat asthma, fever, and liver disorders, further demonstrating its diverse therapeutic applications.

Recent experimental studies have revealed the potential of Z. mauritiana extracts in treating cancer, inflammation, and diabetes, opening avenues for further research into novel therapeutic interventions.

Beyondits culinary uses, Z. mauritiana is valued for its health maintenance benefits and ability to improve digestive function. Its well-recognized medical properties include antibacterial, antioxidant, and anti-inflammatory activities, contributing to its therapeutic efficacy andpotential in various healthcare applications.

This comprehensive profile of Ziziphus mauritiana underscores its importance in traditional medicine anditspotentialinmodernhealthcare,highlightingtheneedforcontinuedresearchand development of plant-based remedies[64].

CHAPTER-3

LITERATURESURVEY

Theliteratureindicatesthatscientistsandresearchershaveundertakenvariousstudiesonthe technology. The following are some of the important and relevant documents that are quoted:

1. **Gevrenova R., *et al.*, 2023:** Nootropics improves erythrocyte plasticity andstops aggregation, which improves the blood's rheological properties and increases its flow to the brain. Many of these formulations possess antioxidant activity that protects brain tissue from neurotoxicity and improves the brain's oxygen supply.

2. **Butt S. Z., *et al.*, 2021:**Phytochemicalssuchasflavonoids,triterpenoids,alkaloids,and saponins present in Ziziphus mauritiana leaves have been suggested to contribute to its nootropic activity through mechanisms such as antioxidant, anti-inflammatory, and cholinergic modulation.

3. **Das S. K., *et al.*, 2021:** The pothos plant extract enhanced cognition and countered scopolamine-induced forgetfulness proportionally to dosage, improving performance in spatial navigation tests, while also boosting acetylcholine levels and reducing oxidative stress markers, thus counteracting memory impairment in mice.

4. **Mali K. K., *et al.*, 2021:** Use of ethanolicextractofL.acidissimaenhanceslearningand memory in different experimental models. The histopathological study revealed the neuroprotective property of the extract. The study indicates that the extract may be used in the treatment of Alzheimer's disease.

5. **Sareetha AV,*etal*.,2021:**AdministrationofWoodfordiafruticosaextractatdosesof100 and 250 mg/kg, along with standard Piracetam (100 mg/kg) for 7 days for Swiss albino mice with scopolamine-induced memory impairment. The EPM andMWMtestrevealed that the repeatedadministrationofEWFtwodifferentdosesshowedsignificantnootropic activity ($P < 0.001$), and the Piracetam confirmed a huge reduction in the both transfer and escape latency period.

6. **Damodaran T., *et al.*, 2020:** Prolonged administration of butterfly pea root extract reversed cognitivedeficitscausedbyreducedbrainbloodflow,mitigatedneuronallossin a key memory region, and inhibited an enzyme that breaks down a crucial neurotransmitter in brain areas vitalforcognition.Thehigherdoseprovedmoreeffective

in these neuroprotective effects. Safety assessments revealed no adverse reactionsinrats with surgically induced chronic cerebral hypoperfusion after a month-long treatment regimen, suggesting potential therapeutic applications for memory disorders associated with compromised cerebral circulation.

7. **Maity D.,** ***et al.,*** **2019:** Nootropic drugs enhance cognitive functions.Memoryprocesses involve encoding, decoding, and storing information. Cognitive deficits or memory impairment that ispresentwithneuropsychiatricconditionsinsistsadoptionofnootropics to improve cognitive ability.

8. **Reddy R. L.,** ***et al.,*** **2019:** S. Oleracea showed significant enhancements in the learning and memory of mice, as indicated by the decline in transfer latency using rectangular maze test, decrease in escape latency during training, retrieval using morris water maze, pole climbing test and neuroprotective activity through reduced brainacetylcholinesterase (AChE) elevated concentration and raised the percentage of AChE activity inhibition in the rat brain.

9. **Khatian N.,** ***et al.,*** **2019:** G. lucidum decreased the duration spent in the targetquadrant (TSTQ) and decreased the escape latency (EL) in Morris water maze model. Whereas,a decrease in the Initial Transfer Latency (ITL)andRetentionTransferLatency(RTL)was observed in the elevated plus maze model.

10. **Hussain,** ***et al.,*** **2019:** Ziziphus mauritiana leaf extract improved spatial memory and learning in rats, possibly through its antioxidant and neuroprotective effects.

11. **Parameshwari K,** ***et al.,*** **2018:** Alangium salvifolium significantly ($P < 0.05$) enhanced abstraction short-term and memory, the exceptional reduction in transfer latency of the 6th and 7th days as a region of learning and memory. Within the elevated maze and reducing the escape latency within the Morris water maze.

12. **Khatri R. S.,** ***et al.,*** **2018:** ABE may reverse amnesia induced byscopolaminemediated through the inhibition of oxidative stress and due to presence ofwithanolidescontaining anti-cholinesterase activity. ABE may be beneficial in management of memory deficits with normal life and clinical dementia associated with aging and neurodegenerativestates.

13. **Ghasham A. A.,** ***et al.,*** **2017:** The leaves of Ziziphus mauritiana Lam. are abundant in phytochemical constituentswhichhavesignificantlevelsofantioxidantandantimicrobial activities. The isolation and purification of these bioactive phytochemical constituents may further yield more significant levels of antioxidant activity, antimicrobial activitiesor other curative properties against different health ailments.

14. **Maruza I. M., *et al*., 2016:** Z. mauritiana thrives in semi-arid regions of the world and can be successfully cultivated in the marginalecosystemofthesubtropicsandtropicsfor food security and poverty alleviation. The tree tolerates extreme dry, very high temperatures, saline soils, and waterlogging. The tree has a multitude of uses which include furniture wood, poles for various uses, fodder for animals, medicinal plants, flowers for nectar and many others. The fruit is eaten raw.

15. **MISHRA SK, *et al*., 2016:** pre-shodhit and shodhit drugs brought back to normal the scopolamine-inducedadecreaseinpercentagespontaneousalternationbehaviorin Y-mazeandnumberofcorrectresponsesinradialmaze.Scopolamine-inducedincreasein transfer latency in elevated plus maze was significantly decreased by pre-shodhit drug only. Shodhit drug has no significant effect on transfer latency. Both pre-shodhit and shodhit drugs showed dose-dependent inhibition of AChE activity in vitro. Pre-shodhit drugs showed more nootropic activity than shodhit drugs.

16. **Akhtar N., *et al*., 2016:** Jujube foliage extract demonstrates rejuvenatingqualitieswhile also exhibiting proficiency in complexion brightening, hydration enhancement, and improvement of skin elasticity and suppleness in human dermal tissues.

17. **Suliman, *et al.*, 2016:** Certain natural cognitive enhancers beneficially influence the function and production of acetylcholine and glutamate receptor sites. These specific attributes of organic nootropics contribute to sustained neuroplasticity and improved neural signaling, resulting from heightened neurotransmitter concentrations and efficacy.

18. **Nirwane A. M., *et al*., 2015:** The alcohol-based extract of vetiver grass demonstrates notable cognitive-enhancing properties, potentially through its interaction with brain chemicals involved in both stress reductionandcognitiveprocessesrelatedtoknowledge acquisition and retention.

19. **Sameera, *et al*., 2015:** Ziziphus mauritiana is utilized to address injuries, alleviate gastrointestinal discomfort, and urinary tract infections, exemplified to inhibit causative isolates. Antimicrobial potency could be evidently allied with existing bioactive phytochemicals of plants. We therefore propose further analysis withpurifiedsamplesto validate our claim.

20. **WeitznerD.S.,*etal*.,2015:**Morriswatermazetestisappliedtobehavioralparadigmsto assess navigational cognition and recall abilities in small mammals, and it has been extensively used in nootropic and cognitive-enhancing drug studies.

21. **Ibrahim, *et al*., 2014:**Improvement was noted in performance measures, specificallythe aquatic navigationtaskandthemulti-pathselectionapparatus.Forcedswimtestwasused as the observation paradigm for the adaptogenicactivityandethanolicextractof*Mimosa pudica* caused significant reduction in swimming endurance time.

22. **Pramod, *et al.*, 2014:** The treatment of *Swarnaprashana* exhibited significant improvement in learning and memory (P<0.01)andalsoshowedsignificant(P<0.001) decrease in whole brain AChE activity. Formulation of *Swarnaprashana* has nootropic and anti-AChE activity. Hence, it can beemployedinenhancingthememoryofthechild and for the treatment and management of Alzheimer's disease.

23. **Bhosale U.A.,*et*1*al.*,2014:**C1litoreaternateawasfoundtocausesignificantincreasein spatial working memory (P < 0.05), spatial reference memory (P < 0.001) and spatial working-reference (P < 0.001) in retention trials on Y maze, Morris water maze and Radial arm maze respectively.

24. **Zozio S., *et al.*, 2013:** Ziziphus species exhibit diverse concentrations of plant-derived compounds and increased antioxidant activity testedseparatelybytheORAC,FRAPand DPPH assays. FRAP assay proved to be an efficient method for the evaluation of the antioxidant activity of jujube, as well as the acid–butanol assay, for condensed tannins. Based on the ripening stages, the major constituents with potential antioxidant capacity were identified, using both LC–MS and GC–MS analysis.

25. **Nyanga L. K., *et al.*, 2013:** The Zimbabwean fruit knownasZiziphusmauritianaboasts substantial amounts of essentialnutrients.ofimportantnutrientsincludingminerals,fibre and crude protein. The sweet *masau* fruit were found to be richer in vitamin C than the sour fruit, whereas the sour fruit were richer in minerals. The fresh sweet fruits were particularly richer in vitamin C than the sour fruits. However, there was very little variation in nutrient content with season.

26. **Colucci, *et al.*, 2012:** Cognitive enhancers, colloquially termed brain boosters, have evolved overthirtyyearsastheprimaryapproachtoaddressingmentaldecline,withtheir nomenclature combiningGreekrootsmeaning"mind"and"bend,"broadlyencompassing any compound that favorably impacts intellectual capacity.

27. **Dureshahwar, *et al.*, 2012:** The butanolextractofjujubefruitimprovesmazenavigation speed, extends hesitation time in aversive learning tests, and enhances object recall, as measured by standard cognitive assessments after a day, with a common nootropic drug serving as the comparative benchmark.

28. **Rathore S. K., *et al.*,2012:**Jujubefruitconstituentswereisolatedusingvarioussolvents via extended cold extraction, revealing a diverse array of bioactive molecules including plant pigments, sugar-bound compounds,soap-likesubstances,aromaticalcohols,woody components, cholesterol-like structures, and astringent compounds.

29. **Contestabile, *et al.*, 2011:** Estimation of acetylcholinesterase (AChE) activity in brain homogenates is used to assess the cholinergic modulating effects of potential nootropic compounds.

30. **Lohidasan S., *et al.*, 2009:** The innovative fat-solublecomponentisolationfromBrahmi avoids traditional extraction drawbacks, eliminating solvent use while maintaining cognitive-enhancing efficacycomparabletostandardalcohol-basedBrahmiextracts.This novel approach offers a potentially safer and equally effective alternative for harnessing the plant's memory-boosting properties.

31. **Blokland A., *et al.*, 2010:** Administering the drug scopolamine to small mammals induces deficits in navigational recall, short-terminformationprocessing,andknowledge acquisition, effectively replicating the mental deterioration characteristic of Alzheimer's and related cognitive disorders. This experimental model allows researchers to simulate the memory and learning impairments associated with neurodegenerative conditions, providing a valuable tool for studying potential treatments and interventions.

32. **Uttara, *et al.*, 2009:** Researchers frequently assess the brain-protective capabilities of cognitive enhancers by quantifying specific molecules in processed brain tissue. These indicators include a byproduct of lipid oxidation and a crucial cellular antioxidant. By measuring the concentrations of these compounds, scientists can gauge the extent to which a substance combats oxidative damage in neural tissues. This analytical approach provides valuable insights into the potential of various compounds to shield the brain from harmful oxidative processes.

33. **Butterfield, *et al.*, 2007:** Cellular damage from reactive molecules and disruption of a key neurotransmitter system are believed to contribute significantly to the developmentof mental decline and brain-wasting conditions. These interrelatedprocessesplaycrucial roles in undermining cognitive function and accelerating the progression of disorders affecting the nervous system.

34. **Walf A. A., *et al.*,2007:** A raised, cross-shaped apparatus serves as an additional experimental setup for evaluating mental processes in small mammals. This behavioral assessment tool specifically measures recall abilities and stress-related responses, providing insights into cognitive performance and emotional states inlaboratoryanimals used to study brain function.

35. **Hasselmo, *et al.*,2006:** A compound that blocks specific neurotransmitter receptors is commonly employed to create temporary cognitive decline in laboratory animals. This substance interferes with a crucial signaling system in the brain, leading to memory and thinking problems. Researchers use this approach to simulate the mental deterioration seen in conditionslikeAlzheimer'sdisease.Byinducingtheseartificialdeficits,scientists can evaluate the effectiveness of potential brain-enhancing drugs and explore new treatments for neurodegenerative disorders. This model provides a valuable tool for advancing our understanding of cognitive enhancement and neuroprotection.

36. **Sreemantula S., *et al*., 2005:** Grape extract administration before stressor exposuredose-dependently mitigated stress-induced urinary metabolite alterations, while not affecting normal subjects'excretionpatterns;itenhancedlearning,memoryretention,and recallinratsproportionallytodosage,andexhibitedpotentantioxidantpropertiesrivaling vitamin C.

CHAPTER-4

AIM& OBJECTIVE

AIM

Thepurposeofthisresearch is to “Investigation for Nootropic Activity of Ziziphus Mauritiana on Scopolamine Induced Memory Deficits in Swiss Albino Mice.”

OBJECTIVE

The current study's goals are focused ondevelopingherbal-basedmedicinedelivery methods.

1. TostudytheassessZiziphusMauritiana'spotentialforenhancingmemoryinmice.
2. Tostudytheuseofscopolaminetoinducememorydeficitsasareference.
3. Toselectandconductbehavioralandbiochemicalanalysestomeasureeffects.
4. TostudyanddetermineoptimalZiziphusMauritianadosageformemoryenhancement.
5. ToselectandevaluateZiziphusMauritiana'ssuitabilityasanootropicagent.
6. Toidentifypotentialfutureresearchdirections.

CHAPTER-5

PLANOFWORK

The work schedule was designed with the following goals and objectives in mind.

1. Literature Survey:
    a. Conduct an extensive literature reviewonnootropicactivity,Ziziphusmauritiana, scopolamine-induced memory deficits, and related topics.
    b. Gatherinformationonpreviousstudies,experimentalmethods,andrelevantfindings.
    c. Identify gaps in existing knowledge and potential areas for contribution.

2. Preliminary Phytochemical Testing:
    a. Performinitialphytochemicalexaminationoftheextractaimstoidentifythemain types of diverse bioactive compounds, such as nitrogen-containing organic substances, plant pigments, aromaticphytochemicals,andcomplexhydrocarbons, among others.
    b. Apply standard qualitative assays to determine the presence of particular phytochemical compounds.

3. Isolation of Compounds by Column Chromatography:
    a. Separateandpurifybioactivecompoundsfrom the crude extract using column chromatography.
    b. Utilize appropriate stationary and mobile phases to achieve optimal separation.
    c. Fractionate the eluted compounds based on polarity and chemical characteristics.

4. CharacterizationofIsolatedPureCompoundbyMass,UV,IR,andNMR Spectroscopy:
    a. Purify the isolated compounds using appropriate techniques (e.g., recrystallization, preparative HPLC).
    b. Characterize the purified compounds usingspectroscopictechniquessuchasMass spectrometry (MS),ultraviolet(UV)spectroscopy,infrared(IR)spectroscopy,and nuclear magnetic resonance (NMR) spectroscopy.
    c. Elucidate the chemical structures of the isolated compounds based on the spectroscopic data.

5. Assessment of Nootropic Activity of Leaves Extract:
    a. Conduct in vivo studies using Swiss albino mice to assess the

cognitive-enhancingeffectsofZiziphusmauritianaleaf extracts.

b. Induce memory deficits using scopolamine administration.
c. Employable behavioral tests such as The Morris Water Maze (MWM) and Elevated Plus Maze (EPM) are utilized to assess spatial memory, learning abilities, and cognitive performance.
d. Record andanalyzethebehavioralparameters(e.g.,escapelatency,durationspent in the target quadrant, entries into open and closed sections).

6. In-VitroStudies:
    a. Perform in vitro assays to explore the possible mechanisms of action behind the cognitive-enhancing effects of Ziziphus mauritiana leaf extracts or isolated compounds.
    b. Evaluate oxidative stress biomarker activity, acetylcholinesterase (AChE) inhibition, malondialdehyde (MDA) inhibition, reduced GSH level and total protein in Brain Homogenate.

7. Statistical Evaluation:
    a. Employ suitablestatisticalanalyses(suchasANOVAandpost-hoctests)toassess the significance of the experimental results.
    b. Perform data analysis using suitable software (e.g., GraphPad Prism,).
    c. Interpretthe statistical findings and draw conclusions.

8. Submission of Thesis:
    a. Compiletheresearchwork,includingtheintroduction,reviewofliterature,materials and methods, findings, discussion, and conclusions.
    b. Formatthethesisaccordingtotheguidelinesprovidedbytheinstitutionor university.
    c. Incorporate feedback from the research supervisors and make necessary revisions.
    d. Submit the final thesis for evaluation and defense.

CHAPTER-6

MATERIALANDMETHOD

6.1 PRELIMINARYSURVEYOFPHYTOCHEMICALCOMPONENTS

Qualitative analysis was performed on the ethanolic extract of *Ziziphus mauritiana* to detect a range of phytochemical constituents, including alkaloids, flavonoids, glycosides, phenolics, saponins, steroids, sterols, tannins, terpenes, and volatile oils, and amino acids[69].

6.1.1Assay for Alkaloid

a.. Mayer's Assay:

- Prepare Mayer's reagent by dissolving mercuric chloride ($HgCl_2$) in distilled water andmixing it with potassium iodide (KI) solution.
- Takeasmallamountoftheplantextractanddissolveitinasuitablesolvent(e.g., ethanol, methanol, or dilute hydrochloric acid).
- Introduce a a few drops of Mayer's reagent into the extract solution.
- Lookfortheformationofacreamywhiteorlightyellowprecipitate,indicatingthe existence of alkaloids.

b. Wagner'sAssay:

- Wagner'sreagentismadebydissolvingiodineandpotassiumiodide(KI)indistilled water.
- Takeasmallamountoftheplantextractanddissolveitinasuitablesolvent(e.g., ethanol, methanol, or dilute hydrochloric acid).
- AddseveraldropsofWagner'sreagenttotheextract solution.
- Observetheformationofareddish-brownprecipitate,indicatingthepresenceof alkaloids.

6.1.2 Assay for Flavonoids

a. Alkaline Reagent Assay:

- Prepareadilutesolutionbydissolvingaminimalquantityoftheplantextractinan appropriate solvent such as ethanol, methanol, or water.
- Addafewdropsofa10%solutionofeithersodiumhydroxide(NaOH)orpotassium hydroxide (KOH) to the extract solution.

- Observethedevelopmentofavividyellowcolor,whichfades to colorless when dilute hydrochloric acid (HCl) is added, indicating the existence of flavonoids.

b. Lead Acetate Assay:

- Dissolveasmallquantityoftheplantextractinasuitablesolvent,suchasethanol, methanol, or water.
- Introduce A few drops of a 10% lead acetate solution should be added to the extract.
- CheckforthedevelopmentAyellowprecipitateforms,signifyingthepresenceof flavonoids.

6.1.3 Assay for Glycoside

a. Keller-KillianiAssay(AssayforDeoxySugars):

- Dissolve a small amount of the plant extract in an appropriate solvent, such as ethanol, methanol, or glacial acetic acid.
- Introduceafewdrops offerricchloride ($FeCl_3$)solutionand mixwell.
- Pour the mixture into a test tube and carefully add concentrated sulfuric acid (H_2SO_4) down the inner wall of the test tube.
- Look for the appearance of areddish-brownringattheboundarybetweenthetwolayers, coupled with a bluish-green hue in the acetic acid layer, which signifies the presence of deoxy sugars, indicative of glycosides.

b. Borntrager's Assay (Assay for Anthraquinone Glycosides):

- Dissolveasmallamountoftheplantextract in an appropriate solvent, such as ethanol, methanol, or dilute hydrochloric acid.
- Boil the extract solution with dilute hydrochloric acid or sulfuric acid for a few minutes.
- Cool the solution and filter if necessary.
- Add an equal amount of benzene or chloroform to the filtrate and shake thoroughly.
- Separatethe organiclayer andintroduce a fewdrops ofdilute ammonia solution.
- Observing the emergence ofapink,red,orvioletcolorintheammonialayersuggeststhe presence of anthraquinone glycosides.

6.1.4 Assay for Phenol

a. Ferric Chloride Assay:

- Dissolveasmallamountoftheplantextract in an appropriate solvent, such as ethanol, methanol, or water.
- IntroduceAfewdropsofa5%ferricchloride($FeCl_3$)solutionshouldbeadded to the extract.

- Observefor a deep blue, blue-black, or green hue to develop, indicating the presence of phenolic compounds.

b. Lead Acetate Assay:

- Dissolveasmallamountoftheplantextract in an appropriate solvent, such as ethanol, methanol, or water.
- Introduce several drops of a 10% lead acetate solution into the extract.
- Watchfortheformationofawhiteoryellowprecipitateindicatingthepresenceof phenolic compounds.

6.1.5 Assay for Steroid

a. Salkowski Assay:

- In this method, introduce a few drops of concentrated sulfuric acid into the plantextract or sample.
- Theemergenceofaredcolor or A reddish-brown ring at the junction of the two layers indicates the presence ofsteroids.

b. Liebermann-Burchard Assay:

- Blendtheextractwithaceticanhydride,thengentlylayerconcentratedsulfuricacidalong the tube's side.
- A teal hue appearing suggests steroid compounds are present.

6.1.6 Assay for Tannins

a. Ferric Chloride Assay:

- Mix plant samples with iron(III) chloride solution.
- Dark blue-green or blackish tints indicate hydrolyzable tannins.
- A brownish-green or dark blue precipitate points to condensed tannins.

b. Lead Acetate Assay:

- Mix plant sample with lead acetate.
- Yelloworredsedimentindicatestannins.

6.1.7 Assay for Terpenoids

a. Salkowski Assay:

- Mix plant extract with strong H_2SO_4.
- A reddish-brown layer forming between liquids suggests terpenoids.

b. Liebermann-Burchard Assay:

- Combine plant samples with acetic anhydride.
- GentlyintroduceconcentratedH_2SO_4downtube sides.
- Tealcolorationsuggeststerpenoidspresent.

6.1.8 Assay for Amino acids

a. Ninhydrin Assay:

- Combine the plant extract with several drops of ninhydrin reagent solution
- Warmthemixturegently.
- The appearance of a purple or bluish-purple hue indicates the presence of amino acids.

b. Biuret Assay:

- Introduceafew drops of sodium hydroxide solution to the plant extract, then add a few drops of copper sulfate solution.
- Thepresenceofaminoacidsisindicatedbyavioletorpinkcolorwithtwoormore peptide bonds.

6.1.9 AssayforSaponins

a. Foam Assay:

- Shakealcoholplantextractwithwatervigorously.Persistentbubblesindicatesaponins.

6.2 EXPERIMENTALANIMAL

The study involved Swiss Albino Mice, each weighing between 18-25 grams and aged 6-12 weeks. These mice were kept at the primary animal facility of our Innovative College of Pharmacy in Greater Noida, India. They were housed in standard laboratory conditions withinthe animal facility. The mice were placed in polyacrylic cages, withuptofiveanimalspercage, under air-conditioned settings with natural light and dark cycles, maintaining the temperatureat 25°C(±2°C)andtherelativehumiditybetween50% and 70%. The study protocol adhered to the standards set by the Committee for the Control and Supervision of ExperimentsonAnimals and the Institutional Animal Ethics Committee (IAEC) granted approval for the study in linewith their guidelines for the care and utilization of animals in research.

6.3 SWISS ALBINO MICE

6.3.1 Method of oral administration

The 1 ml tuberculin syringe held the oral gavage needle in place. The administration of drug suspensions madeuseofthis.Themousewasgentlyrestrainedbythescruffoftheneckwithone hand, while theotherhandheldthegavageneedle.Thegavageneedlewascarefullyinsertedinto the mouth and down the esophagus of the Swiss albino mouse. The plunger was then slowly depressed to administer the test substance or drug suspension directly into the stomach. Toensure completedeliveryofthedose,0.1-0.2millilitersofdistilledwaterwereadministeredafter the test substance, using the same gavage needle. Proper techniqueandcareweretakentoavoid accidental tracheal administration or esophageal injury to the mouse[17].

6.4 PHARMACOLOGICAL SCREENING TECHNIQUES TO EVALUATE NOOTROPIC ACTIVITY OF ZIZIPHUS MAURITIANA

6.4.1 IntrospectiveBehaviouralModel(ScopolamineInducedAmnesia)

The IntrospectiveBehavioralModel,whichinvolvesscopolamine-inducedamnesia,isavaluable tool in neuroscience research for studying memory processes and cognitive functions. Scopolamine, an anticholinergic drug, impairs memory by blocking muscarinic acetylcholine receptors in the brain, especially in areas associated with learning and memory like the hippocampus.

In this model, experimental animals, typically rodents such as ratsormice,receivescopolamine to induce temporary amnesia or memory impairment. The animals are then subjected tovarious memory and cognitive tasks to evaluate the extent of memory deficits caused by scopolamine. Common behavioral tests encompass the Morris water maze, novel object recognition test, and passive avoidance task, radial arm maze, and contextual fear conditioning.

By examining changes in behavior and performance in these tasks,researcherscangaininsights into the mechanisms of memory formation and retrieval, as well as the effects of potential therapeutic interventions aimed at mitigating memory deficits. This model also facilitates the evaluation of newcompoundsordrugsfortheirabilitytoreverseorreducescopolamine-induced memory impairments, providing potential pathways for developing treatments for conditions characterized by cognitive dysfunction, such as Alzheimer's disease[39].

6.4.2 ExteroceptiveBehavioral Models

Exteroceptive behavioral models are experimental paradigms used in neuroscience and behavioral research to study the sensory processingandresponsesofanimalstoexternalstimuli. These models focus on how animals perceive and interact with their environment, including sensorymodalitiessuchasvision,audition,olfaction,taste,andtouch. Exteroceptive behavioral

models are crucial for understanding sensory processing, perception, learning, and memory, as well as for investigating the effects of sensory deficits or manipulations on behavior[28].

6.4.2.1 MORRISWATER(MWM)MAZE

The Morris Water Maze (MWM) test is a well-known behavioral model usedtoevaluatespatial learning and memory in rodents, such asmiceandrats.CreatedbyRichardG.Morrisin1981,it has become one of the most frequently employed tasks in neuroscience research.
TheMWMsetupconsistsofalargecircularpoolfilledwithwatermadeopaque by adding non-toxicsubstanceslikemilkortemperapaint.Asubmergedplatformispositionedinaspecific location just beneath the water surface, serving as an escape platform for the animals.
The test is generally divided into two stages: the acquisition phase (training) and the probetrial (memory assessment).
During the acquisition phase, the animal undergoes several days of training to locatethehidden platformusing spatial cues around the pool. The duration needed to locate the platform, known as the latency period, is documented.
During the probe trial, the platform is taken away,andtheanimalisallowedtoswimfreely.The duration spent in the target quadrant, where the platform was previously located, is recorded to evaluate spatial memory[55].

Supplies and Tools:
a. Round pool (diameter: 120-180 cm, height: 30-60 cm)
b. Escape platform (diameter: 10-12 cm)
c. Videotrackingsystemorstopwatch
d. Opaque non-toxic dye or powder (e.g., milk, tempera paint)
e. Spatial cues (e.g., geometric shapes, posters, or other visual markers) around the pool
f. Data recording sheets or software

Procedure:
1. Pool Setup:
a. Fill the pool with water at a temperature of 20-25°C to a depth of 30-40 cm.
b. Add a non-toxic opaque dye or powdertothewatertomaketheplatforminvisiblefromthe surface.
c. Placethe escapeplatform in afixed position, submerged1-2 cm belowthe water surface.
d. Arrange spatial cues around the pool at different locations to provide visual references for the animals[37].

2. Habituation:
a. Placetheanimalinthepoolwithouttheplatformforabriefperiod(e.g., 60 seconds) to allow it to become familiar with the environment and the task of swimming.

b. Carefully direct the animal totheplatformandletitstaythereforabriefperiod(e.g.,10-15 seconds).

c. Repeat this process for a few trials to reducestressandimproveperformanceinsubsequent trials[41].

3. Acquisition Phase (Spatial Learning):

a. Divide the pool into four quadrants and assign a starting position in each quadrant.

b. Place the animal in the pool, facing the wall, at one of the designated starting points locations.

c. Allow the animal to swim freely to search for the hidden platform.

d. Iftheanimaldoesnotfindtheplatformwithinacertaintimeframe(e.g.,60-90seconds), gently direct it to the platform and let it stay there for a brief period (e.g.10-15 seconds).

e. Recordandnotetheescapelatency,whichisthetimeittakesfortheanimaltoreachthe platform.

f. Repeat the trials from differentstartingpositions,withaninter-trialintervalofseveralminutes (e.g., 5-10 minutes).

g. Conduct multiple training sessions (e.g., 4-6 trials per day) over several days (e.g., 4-6 days)to help the animals discover the position of the concealed platform using spatial cues[57].

4. ProbeTrial(SpatialMemory):

a. Following the acquisition phase, take the escape platform out of the pool.

b. Place the animal in the pool from a novel starting position.

c. Permit the animal to swim freely for a set period (e.g., 60-90 seconds).

d. Record the amount of the duration in each quadrant and tally the number of timestheanimal crosses the former platform's location.

e. Spending more duration in the target quadrant (where the platform was located formerly positioned) and a higher number of crosses over the previous platform location indicateenhanced spatial memory retention[73].

5. Data Analysis:

a. Determine theaverageescapelatencytimeforeachtrainingsessionthroughouttheacquisition phase.

b. Analyze the record of the duration spent in each quadrant and count the number of times theanimal crosses the previous platform location during the probe trial.

c. Applysuitablestatisticaltechniques(e.g.,ANOVA,post-hoctests)tocomparethe performance across different experimental groups or conditions.

6. Additional Considerations:

a. Counterbalancethestartingpositionsandplatformlocationsacrossdifferentgroupsto control for potential biases.

b. Use appropriate controls and randomization techniques to ensure the validity of the results.

c. Ensureproperhandling,care,andethicaltreatmentoftheanimalsthroughoutthe experiment.
d. Follow relevant guidelines and regulations for animal research.

6.4.2.2 ELEVATEDPLUSMAZE(EPM)MODEL

The Elevated Plus Maze(EPM)isacommonlyusedbehavioralmodelforassessinglearningand memory in rodents, particularly mice. It leverages the natural fear mice have of open and elevated areas, which contrasts with their instinctual curiosity to explorenewsurroundings.The EPM setup includes four arms configured in a plus shape, featuring two exposed arms and two enclosed arms, all elevated above the ground[79].

Materials and Methods

Elevated Plus Maze Apparatus

The EPMsetupincludedtwoopenarms(16cmx5cm)andtwoclosedarms(16cmx5cmx12 cm) radiating from a central platform (5 cm x 5 cm). The maze, constructed from black polypropylene plastic, was elevated 25 cm above the ground. A0.5cmraisededgeborderedthe open arms to prevent falls. The maze was situated in a dimly lit, quiet room with ambient light levels maintained at approximately 30 lux.

Animals

Mice (8-10 weeks old) were kept in sets of four to five per containment unit, experiencing alternating 12-hour periods of illumination and darkness (brightness commencing at 07:00), within an environment maintained at 22±2°C, and provided continuous access to nourishment and fluids.. To acclimate them to the experimenter, mice were handled for 5 minutes daily for one week before behavioral testing. All experiments took placebetween09:00and15:00during the light phase.

Procedure

1. Apparatus setup

a. Theelevatedapparatuscomprisesapairofexposedpathways(lackingbarriers)andtwo sheltered corridors (enclosed on three edges), all radiating from a central junction.
b. The maze is raised above the floor,usuallyaround50-70cm,toinduceamildsenseoffearor anxiety.
c. The open arms are usually surrounded by a small raised lip or ledge to prevent falls.
d. The apparatus is made of non-porous materials (e.g., plastic or wood) to allow easy cleaning between trials.

2. Habituation and handling

- Rodents are typically handled and habituated to the testingroomandexperimenterforafew days before the EPM test to reduce stress and ensure consistent behavior.

3. Behavioral Procedure

On the first day, each mouse was individually placed by the researchers at the end of an open arm, facing outward. They then timed how long it took the mouse to fully enter any of the enclosed sections (up to 90 seconds). This duration was noted astheinitialtransferlatencytime (TLT). Following thismeasurement,themousewasgivena3-minuteperiodtofreelyexplorethe entire apparatus, allowing it to become acquainted with the environment.

The next day, 24 hours later,thescientistsrepeatedthesameprocess,againrecordingtheTLTto assess memory retention. Throughout the experiment, the mice's movements were monitored using a ceiling-mounted camera connected to ANY-maze motion analysis software (from Stoelting Co.). The memory retention index, termed the inflation ratio (IR), was computed as follows:

$$IR=\frac{(SeconddayTLT-FirstdayTLT)}{\text{First day TLT}}$$

In this equation, the initial transfer latency time (TLT) on day one is denoted as the baseline measurement, while the TLT recorded 24 hours later represents the follow-up assessment. A larger IR value suggests improved recollection of the apparatus's spatial arrangement betweenthe two testing sessions.

To prevent scent-based influences, researchers sanitized the experimental setup with a 70% ethanol solutionaftereachanimal'strial.Researchersdefinedanarmentryasoccurringwhenthe mouse's entire body, including all four feet, moved into a new section of the apparatus.

4. Data analysis

-Keybehavioralmetricsexaminedincludetransitiondelay,frequencyofventuresintoexposed and sheltered sections, duration in each zone type, and the proportion of time spent in exposed areas (an indicator of anxious tendencies).

- Statistical analyses, such as ANOVA or t-tests, are performed to compare these measures between experimental groups or conditions.

The Transfer Latency Time (TLT) was evaluated using the Inflation-Ratio on Days 6 and 7, 45 minutes post-administration of treatments to all experimental groups. This assessment was conducted before introducing The animals were subjected to the Morris Water Maze task. The Inflation-Ratio offers a measure of memory retention by comparing the current day's TLT (to determine the Inflation-Ratio, each animal was initially positioned at the end of an open arm in theElevatedPlusMaze,facingawayfromthecentralarea.TheTLTwasrecordedastheanimal

transitioned from the open arm to the closed arm.. After this measurement, the animal was permitted to explore the maze freely for a designated duration. This procedure was repeated 45 minutesafteradministering treatments to the different experimental groups on both Days 6 and 7, prior to beginning the Morris Water Maze trials.
By comparing the TLT on Days 6 and 7 with the baseline TLT, the Inflation-Ratio was determined, providing insight into memory retention for each animal[26].

6.5 EXPERIMENTALGROUP

Inthisstudy,groupsofsixmiceeachwereutilized[45].

Table6.1:ExperimentalProtocol

S.NO	GROUPS	TREATMENT	DOSE
I.	Control Group	1% w/v CMC solution	10 ml/kg (i.p.)
II.	Negative Group	Disease Induced by Scopolamine	3 mg/kg (i.p.)
III.	Standard Drug	Vyvamind	20 mg/kg (i.p.)
IV.	TestGroup-I(ZMEE)	Z. Mauritiana Ethanolic Extract	200 mg/kg (p.o.)
V.	TestGroup-II(ZMEE)	Z. Mauritiana Ethanolic Extract	400 mg/kg (p.o.)
VI.	TestGroup-III(ZMAE)	Z. Mauritiana Aqueous Extract	200 mg/kg (p.o.)
VII.	TestGroup-IV(ZMAE)	Z. Mauritiana Aqueous Extract	400 mg/kg (p.o.)

Control: Group I; Negative control: GroupII;Protective:GroupIII;Curative:GroupsV,VI,and VII.

GroupI(ControlGroup):
In this group, mice were given an intraperitoneal (i.p.) injection of a 1% w/v carboxymethyl cellulose (CMC) solution at a dosage of 10 ml/kg. This CMC solution acted as the vehicle control. The mice received this treatment 45 minutes prior to the initial acquisition trial for six consecutive days, and again45minutesbeforetheretrievaltrialontheseventhdayoftheMorris Water Maze test.

GroupII(NegativeGroup):
In this group, scopolamine was used to induce cognitive impairment in mice at a dose of 3 mg/kg, administered intraperitoneally (i.p.). Scopolamine was dissolved in the vehicle and administereddailyforsixdays.Afterscopolamineadministration,themiceunderwentthe

Morris Water Maze test. During the acquisition trials, conducted from day 1 to day 6, the mice continued to receive scopolamine 45 minutes before each trial. On day 7, only the vehicle was administered to the mice, and 45 minutes later, they underwent the retrieval test.

GroupIII(StandardDrugGroup):
Mice in this group were administered Vyvamind, a standard drug, at a dosage of 20 mg/kg intraperitoneally (i.p.). Vyvamind was given daily for six days, and 30 minutes after each dose, the mice participated in the Morris Water Maze test. The administration ofVyvamindcontinued 30minutesbeforeeach acquisition trial from day 1 to day 6. On day 7, the mice received only the vehicle, and 30 minutes later, they were subjected to the retrieval test.

GroupIV(TestGroup-I,ZMEE):
Mice in this group received an oral dose of 200 mg/kg of Ziziphus mauritiana ethanolic extract (ZMEE). This extract was administered daily for six days, and 45 minutes after each dose, the micetookpartintheMorrisWaterMazetest.TheadministrationofZMEEcontinued45minutes before eachacquisitiontrialfromday1today6.Onday7,themiceweregivenonlythevehicle, and 45 minutes later, they underwent the retrieval test.

GroupV(TestGroup-II,ZMEE):
Mice in this group received an oral dose of 400 mg/kg of Ziziphus mauritiana ethanolic extract (ZMEE). The extract was administered daily for six days, with the mice participating in the Morris Water Maze test 45 minutes after each dose. This administration of ZMEE continued45 minutes beforeeachacquisitiontrialfromday1today6.Onday7,themiceweregivenonlythe vehicle, and 45 minutes later, they underwent the retrieval test.

GroupVI(TestGroup-III,ZMAE):
Mice in this group were given anoraldoseof200mg/kgofZiziphusmauritianaaqueousextract (ZMAE). This extract was administered daily for six days, and 45 minutes after each dose, the mice were subjected to the Morris Water Maze test. The administration of ZMAE continued 45 minutes before each acquisition trialfromday1today6.Onday7,thesemicereceivedonlythe vehicle, and 45 minutes later, they underwent the retrieval test.

GroupVII(TestGroup-IV,ZMAE):
Mice in this group received an oral dose (p.o.) of 400 mg/kg of Ziziphus mauritiana aqueous extract (ZMAE). This treatment was given daily for six consecutive days. Each day,45minutes after administration, the mice took part in the Morris Water Maze test. The ZMAE treatment continued 45 minutes before each trial during the acquisition phasefromday1today6.Onday 7, the mice were given only the vehicle, and 45 minutes later, they underwent the retrieval test.

The Cognitive Response Interval, gauged via the Expansion Factor, underwent examination during the penultimateandfinaldaysoftheweek-longstudy.Thisassessmentoccurredprecisely three-fourths of an hour post-administration of the test compound across all experimental sets. The timing was crucial, as it preceded the subjects' involvement in the AquaticSpatialMemory Assessment - a sophisticated behavioral testdesignedtoevaluatespatiallearningandmemoryin rodents. This sequence of events allowed researchers to observe the immediate effects of the administered substance on cognitive function before subjecting the test animals to a more complex navigational challenge.

6.6 ESTIMATIONOFOXIDATIVESTRESSBIOMARKERS

To evaluate chemical-induced oxidative damage to lipids, researchers quantified several biomarkers in mouse brain tissue: acetylcholinesterase (AChE), reduced glutathione (GSH), malondialdehyde (MDA), andtotalprotein.Themicewerehumanelysacrificedundermildether sedation, followed by swift brain removal. Scientists created a brainmixturebycombining10% brain matter (by weight) with chilled 0.03 M sodium phosphate solution (pH 7.4). Thismixture was processed using anUltra-TurraxT25blender(USA)operatingat9,500rotationsperminute. After blending, the mixture underwent centrifugation. To separate proteins, researchers added 10% trichloroacetic acid (TCA) to theliquidportion,causingproteinprecipitation.Theresulting protein-free liquid was then used to measure indicators of lipid oxidation[24].

6.6.1 METHOD FOR MEASURING ACETYLCHOLINESTERASE (AChE) ACTIVITY IN BRAIN HOMOGENATE

The collective acetylcholinesterase (AChE) function in murine cerebral extracts was quantified employing a protocol adapted from Ellman and colleagues' 1961 publication, with minor adjustments based on Ingkaninan et al.'s 2003 work.Cerebralmatterunderwenthomogenization in a phosphate solution (0.1 M, pH 8.0), followed by centrifugal separation at 10,000 x g for a quarter-hour at 4°C. A 0.5 mL fraction of the resultant liquid was diluted in a newly prepared DTNB mixture (5,5'-dithiobis(2-nitrobenzoic acid), 10 mg per 100 mL Sorensenbuffer,pH8.0) to a final volume of 25 mL. Two 4 mL aliquots were extracted from this dilution for analysis. One sample received donepezil, an AChE inhibitor, to account for non-enzymatic breakdown. Both sampleswerethentreatedwith1mLofacetylthiocholineiodidesolution(75mgper50mL purified H_2O) and incubated at 30°C for 10 minutes. Spectrophotometric measurement of absorbance change at 420 nm was performed, using the donepezil-containing sample as a reference. AChE activity was then computed using a specific mathematical formula:

$$R=\frac{\Delta OD\times AssayVolume(3mL)}{\epsilon\times mg\ of\ protein}$$

The rate of enzyme activity, denoted as R, is measuredinnanomolesofacetylthiocholineiodide broken down per minute per milligram of protein. This is calculated using the changeinoptical density per minute (ΔOD) and the extinction coefficient (ϵ), which has a value of 13,600 M^-1 cm^-1[34].

6.6.2 METHODFORQUANTIFYINGMALONDIALDEHYDE(MDA)INBRAIN SAMPLE

Brain Sample Preparation

Following behavioral assessments, the mice were euthanized humanely via cervical dislocation. Their brains were quickly extracted and rinsed with cold saline (0.9% NaCl). On a chilled surface, the cerebral cortex and hippocampus were carefully separated. These tissues were then homogenized with 10 volumes of cold 0.1 M phosphate buffer (pH 7.4) containing 1.15% KCl using a Potter-Elvehjemhomogenizer.Theresultinghomogenateswerecentrifugedat10,000xg for 15 minutes at 4°C, and the supernatant was collected for MDA analysis[51].

Malondialdehyde (MDA) Quantification

MDA levels were measured using the thiobarbituric acid reactive substances (TBARS) assay. This method involves MDA reacting with thiobarbituric acid (TBA) to form a pink complex detectable by its light absorption. The assay mixture contained 0.2 mL of the brain sample supernatant, 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), 1.5 mL of 20%aceticacidsolution (pH 3.5), and 1.5 mL of 0.8% TBA solution in water. This mixture was heated to 95°C for one hour. After cooling, it was centrifuged at 4,000 x g for 10 minutes to remove any precipitated proteins. The absorbance of the clear pink solution was measured at 532 nm against a blankusing a UV-visible spectrophotometer. MDA concentration was calculated using a molar extinction coefficient of 1.56 × 10^5 M^-1 cm^-1 and expressed as nmol MDA/mg protein[66].

ProteinMeasurement

Protein content in the brain samples was determined via the Lowry technique, using bovine serum albumin as a reference.

ReferenceChart

Areferencechartwascreatedusingknownamountsoffreshlyprepared 1,1,3,3-tetramethoxypropane [malondialdehyde bis(dimethyl acetal)] as an MDA source. These reference solutions underwent identical processing as the testsamples,andtheirlightabsorption values were used to construct the reference chart.

6.6.3 METHOD FOR MEASURING REDUCED GSH LEVELS IN BRAIN SAMPLE

Brain Sample Preparation

Following behavioral assessments, mice were humanely terminated by neck dislocation. Their brains were promptly removed, washed with chilled saline (0.9% NaCl),andthecerebralcortex and hippocampus were carefully separated on a cold surface. These tissues were then blended with10 parts of frigid 0.1 M phosphate solution (pH 7.4) containing 5 mM EDTA using aPotter-Elvehjem blender. The resulting mixtures were spun at 10,000 x g for 15minutesat4°C. The top liquid layer was extracted and processed to remove proteins for glutathione (GSH) analysis[61].

ProteinRemovalandSamplePreparation

To 0.5 mL of the extracted supernatant, 2 mL of ice-cold 5% trichloroacetic acid (TCA) was added. This mixture was thoroughly mixed and then centrifuged at 4,000 x g for 10 minutes at 4°C. The resulting clear supernatant was used for GSH measurement[71].

Reduced Glutathione (GSH) Quantification

GSH levelsweredeterminedusingtheDTNBrecyclingtest.ThismethodreliesonGSHreacting with DTNB to produce a yellow compound, TNB, which can be measured by light absorption. The assay mixture consisted of 0.2 mL of the protein-free supernatant, 1.8 mL of 0.1 M phosphate buffer (pH 8.0), and 0.5 mL of0.6mMDTNBsolution.Thereactionwasinitiatedby adding 0.1mLof0.2mMNADPHand0.1mLofglutathionereductasesolution(2U/mL).After a 5-minute incubation at room temperature, the absorbance was measured at 412 nm against a blank using a UV-visible spectrophotometer. The concentration of GSH was determined using the molar extinction coefficient ofTNB,whichis13,600M^-1cm^-1,andwasreportedasnmol GSH/mg protein[51].

ReferenceChart

A reference chart was created using known amounts of freshly prepared GSH solutions. These reference solutions underwent identical processing as the testsamples,andtheirlightabsorption values were used to construct the reference chart.

ProteinMeasurement

Protein content in the brain samples was determined via the Lowry technique, using bovine serum albumin as a reference.

6.6.4 METHODFORDETERMININGTOTALPROTEINCONTENTINBRAIN SAMPLE

Brain Sample Preparation

Following behavioral tests, mice were humanely sacrificed via neck dislocation. Their brains were promptly extracted, cleansed with chilled saline (0.9% NaCl), and the cerebral cortex and hippocampus were meticulously isolated on a refrigerated surface. These tissues were then blended with 10 parts of cold 0.1 M phosphate solution (pH 7.4) using a Potter-Elvehjemblender.Themixtureswerespunat10,000xgfor15 minutes at 4°C, and the top liquid layer was collected for total protein analysis[22].

TotalProteinQuantification

Total protein in the brain samples was measured using the Lowry technique, a color-based test thatcombinesthebiuretreactionwiththereductionofFolin-Ciocalteureagentby protein-specific amino acids[67].

TestProtocol:

1. Solution Preparation:
   - Solution A: 2% Na2CO3 in 0.1 N NaOH.
   - Solution B: 0.5% CuSO4.5H2O in 1% sodium potassium tartrate.
   - Solution C: Copper alkali mixture (combine 50 mL of Solution Awith1mLofSolution B).
   - Solution D: Folin-Ciocalteu reagent (diluted 1:1 with purified water).

2. TestSteps:
   - Combine 0.2 mL of brain sample liquid with 2 mLofSolutionCinatesttubeandblend thoroughly.
   - Let the mixture sit at room temperature for 10 minutes.
   - Add 0.2 mL of Solution D and mix immediately.
   - Allow the reaction to proceed at room temperature for 30 minutes for color to develop.
   - Measurelightabsorptionat660nmcomparedtoablankusingaUV-visible spectrophotometer.
3. Reference Chart:
   - Createareferencechartusingknownconcentrationsofbovineserumalbumin(BSA) solutions.
   - ProcesstheBSAstandardsidenticallytothesamples,andusetheirlightabsorption values to construct the reference chart.
4. Result Calculation:
   - Determine the total protein concentration in the brain samples using the reference chartand express it as mg protein/mL of the sample[43].

6.6.5 DATAEVALUATION

In this investigation, findings were expressed as average ± standard error of the mean (SEM), offering insight into data dispersion around the central value. The information underwent comprehensive statistical examinationusingone-wayanalysisofvariance(ANOVA).ANOVAis a robust statistical approach for detecting meaningful differences among averagevaluesofthree ormoreindependent groups. This method is particularly useful in experimental data evaluation as it assesses the overall effect of the independent factor on the outcome while considering variability within and across groups.

To pinpoint specific distinctions between group averages, subsequent multiple comparison tests were executed using Tukey's procedure. Tukey's test is a highly respected statistical technique that evaluates all possible pairs of averages while preserving an appropriate overall error rate, ensuring that identified distinctions are statistically meaningful and not attributable to random fluctuations.

The statisticalcomputationswereperformedusingGraphPadPrism,aversatilesoftwarepackage forscientificdata analysis and representation. A p-value below 0.05 ($p < 0.05$) was established as the benchmark for statistical significance, suggesting that the observed variations between groups were improbable to be coincidentalandcouldbelinkedtotheexperimentaltreatmentsor interventions under examination[49].

CHAPTER-7

RESULT&OBSERVATION

7.1 PRELIMINARYSURVEYOFPHYTOCHEMICALCOMPONENTS

The leaves of Ziziphus mauritiana were gathered and analyzed using a range ofphytochemical methods. The preliminary phytochemical screening of the extracts determined the presence or absence of various phytochemicals. The analyses conducted revealed whether alkaloids, flavonoids, glycosides, phenols, saponins, steroids, sterols, tannins, terpenoids, volatile oils, and amino acids were present or absent.

7.1.1 Assay for Alkaloid

a. Mayer's Assay:
    - A creamy white precipitate was observed.
b. Wagner'sAssay:
    - A reddish-brown precipitate was observed.

7.1.2 Assay for Flavonoids

a. Alkaline Reagent Assay:
    - Anintenseyellowcolorappeared,whichturnedcolorlessuponaddingdilute hydrochloric acid (HCl).
b. Lead Acetate Assay:
    - A yellow precipitate formed.

7.1.3 Assay for Glycoside

a. Keller-KillianiAssay(AssayforDeoxySugars):
    - A reddish-brown ring appeared at the junction of the two layers.
b. Borntrager's Assay (Assay for Anthraquinone Glycosides):
    - Apinkcoloremerged intheammonia layer.

7.1.4 Assay for Phenol

a. Ferric Chloride Assay:
    - An intense blue color appeared.
b. Lead Acetate Assay:
    - A white precipitate formed.

7.1.5 Assay for Steroid

a. Salkowski Assay:
    - Thejunctionbetweenthetwostrataremaineddevoidofanycrimsonor russet-colored band.
b. Liebermann-Burchard Assay:
    - No bluish-green color was observed.

7.1.6 Assay for Tannins

a. Ferric Chloride Assay:
    - No bluish-green color was observed..
b. Lead Acetate Assay:
    - A yellow precipitate formed.

7.1.7 Assay for Terpenoids

a. Salkowski Assay:
    - A reddish-brown coloration appeared at the interface.
b. Liebermann-Burchard Assay:
    - A blue-green color developed.

7.1.8 Assay for Amino Acid

a. Ninhydrin Assay:
    - A bluish-purple color appeared.
b. Biuret Assay:
    - A purple color was observed.

7.1.9 Assay for Saponin

a. Foam Assay:
    - Stable foam did not form.

Table7.1PhytochemicaltestsofleavesofZiziphus Mauritiana

S.No.	Natural Product	TestConducted	Result
1.	Alkaloid	a. Mayer's Assay b. Wagner'sAssay	Alkaloid found to be present
2.	Flavonoids	a. Alkaline Reagent Assay b. Lead Acetate Assay	Flavonoidsfoundtobe present
3.	Glycoside	a. Keller-KillianiAssay b. Borntrager's Assay	Glycoside found to be present
4.	Phenol	a. Ferric Chloride Assay b. Lead Acetate Assay	Phenols found to be present
5.	Steroid	a. Salkowski Assay b. Liebermann-Burchard Assay	Steroid found to be absent
6.	Tannins	a. Ferric Chloride Assay b. Lead Acetate Assay	Tannins found to be present
7.	Terpenoids	a. Salkowski Assay b. Liebermann-Burchard Assay	Alkaloid found to be present
8.	Amino Acids	a. Ninhydrin Assay b. Biuret Assay	Alkaloid found to be present
9.	Saponin	a. Foam Assay	Saponin found to be absent

7.2 PHARMACOLOGICAL SCREENING TECHNIQUES TO EVALUATE NOOTROPIC ACTIVITY OF ZIZIPHUS MAURITIANA

7.2.1 MORRISWATER(MWM)MAZE

During the initial learning period (day 1), all test subjects,includingthoseinpreliminarytrials, exhibited a prolonged time to find the safety platform. Subsequently,ondays7,14,and28,the mice given scopolamine as a control took significantly longer to escape compared to the untreated control group, suggesting cognitive decline. The experimental groups administeredRR300,RR500,andpiracetamshowed a significant decrease in the time required to locate the

platform compared to the mice treated with scopolamine. This observation indicates that the tested compoundspossessmemory-enhancingpropertiesthatcounteracttheamnesiainducedby scopolamine.

Table7.2Extract'sInfluenceonAquaticNavigationPerformance:InitialandOne-Week Assessments

Treatment groups	Day – 1						Day – 7					
	Escape Latency -L1	Escape Latency -L2	Escape Latency -L3	Probe Trail-1	Probe Trail-2	Probe Trail-3	Escape Latency -L1	Escape Latency -L2	Escape Latency -L3	Probe Trail-1	Probe Trail-2	Probe Trail-3
Control Group	46.00± 9.38	62.83± 6.20	52.83± 9.71	64.67 ± 8.15	65.33 ± 3.87	71.33 ± 4.41	50.83± 3.20	51.17± 3.25	63.00± 3.52	61.33 ± 7.05	72.67 ± 3.76	78.67 ± 3.74
Scopolamine control	37.33± 7.65	47.67± 6.46	47.83± 9.32	65.00 ± 8.22	71.0± 5.78	76.33 ± 2.52	70.83± 2.30	64.00± 4.12	102.3± 5.84	80.50 ± 7.07	96.17 ± 5.48	92.17 ± 3.90
Vyvamind 20 mg/kg	43.41± 3.2	58.44± 4.06	50.70± 3.11	64.31 ± 5.08	64.91 ± 3.07	71.54 ± 3.17	56.80± 3.11	50.82± 4.59	73.71± 4.11	72.11 ± 4.18	48.11 ± 4.08	80.72 ± 3.16
ZMEE 200 mg/kg	32.41± 3.25	43.44± 4.16	62.71± 3.28	32.31 ± 5.18	61.51 ± 3.27	58.14 ± 3.37	74.53± 2.85	79.32± 4.52	95.31± 4.71	81.71 ± 4.61	59.52 ± 4.21	68.82 ± 5.42
ZMEE 400 mg/kg	38.11± 4.21	48.21± 4.31	65.15± 5.83	68.18 ± 6.27	69.19 ± 3.12	72.42 ± 2.58	61.81± 3.41	64.81± 4.54	81.71± 4.31	75.31 ± 4.28	49.41 ± 4.28	62.12 ± 3.72
ZMAE 200 mg/kg	30.37± 2.22	41.40± 3.12	60.69± 2.25	30.28 ± 4.13	59.47 ± 2.23	58.12 ± 2.34	72.46± 1.78	77.28± 3.30	93.27± 3.65	79.67 ± 3.59	57.49 ± 3.15	66.75 ± 4.15
ZMAE 400 mg/kg	35.05± 3.18	44.16± 3.27	60.07± 3.73	63.13 ± 5.23	65.14 ± 2.07	70.38 ± 1.45	58.76± 2.36	60.39± 3.89	78.67± 3.26	72.28 ± 2.91	45.38 ± 3.48	60.08 ± 2.91

Results expressed as average ± standarderror,6samplespergroup.Statisticalanalysiswas performed using one-way ANOVA followed by Tukey's post-hoc test. Significance levels weredenotedasfollows:*($p<0.05$),**($p<0.01$),***($p<0.001$)incomparisontotheOVX control group; and a, b, c ($p<0.05$) in comparison to the normal group. NS indicates no significant difference.

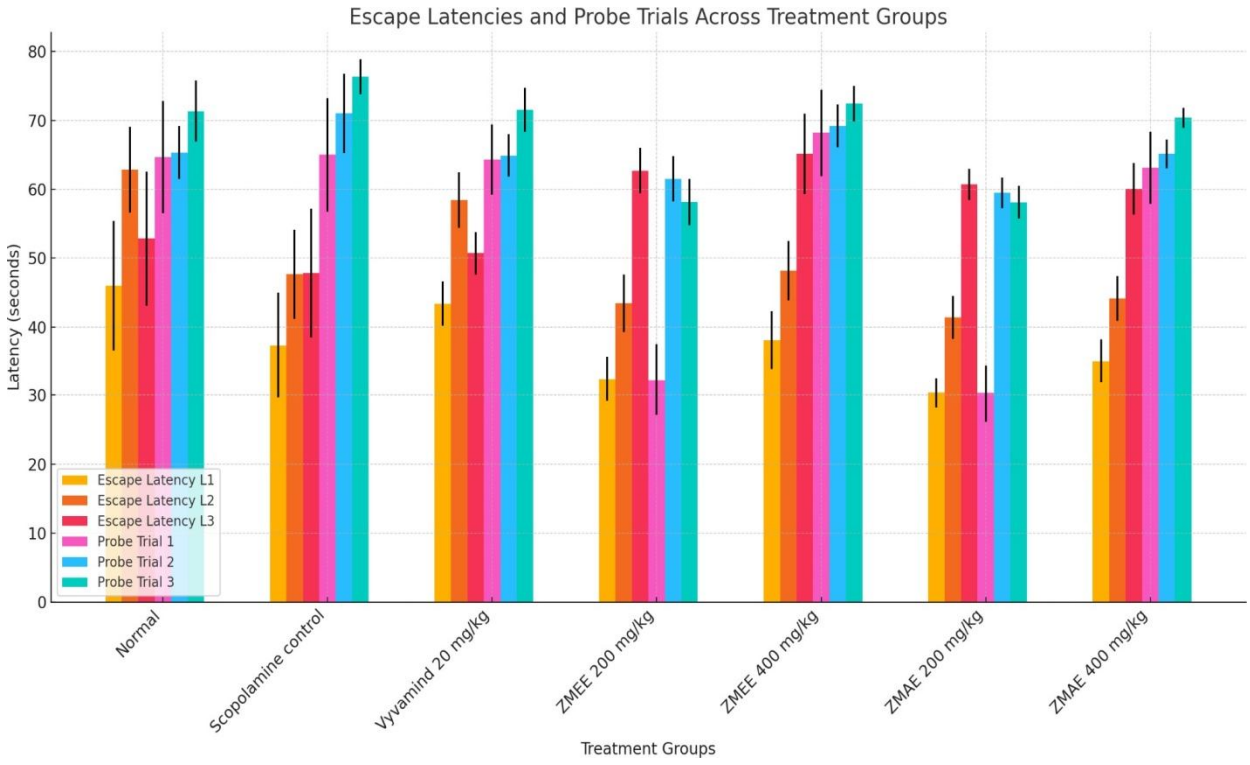

Fig.7.1Extract'sImpactonInitialAquaticNavigationTask Performance

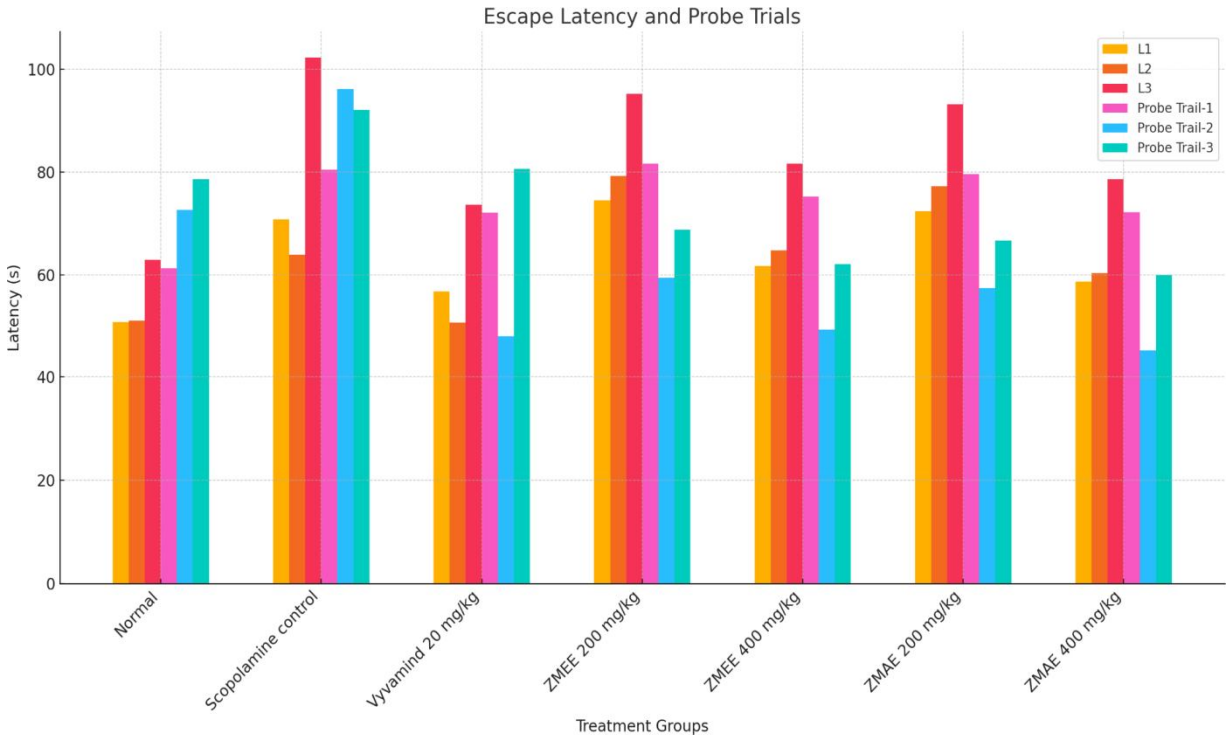

Fig. 7.2 Extract's Effect on Aquatic Maze Navigation Performance After One Week

Table7.3Extract'sImpactonAquaticSpatialMemoryTask:Two-WeekandFour-Week Follow-ups

Treatmentgroups	Day – 14						Day–28					
	Escape Latency-L1	Escape Latency-L2	Escape Latency-L3	Probe Trail-1	Probe Trail-2	Probe Trail-3	Escape Latency-L1	Escape Latency-L2	Escape Latency-L3	Probe Trail-1	Probe Trail-2	Probe Trail-3
Normal	42.17±6.11	47.33±7.41	48.50±2.41	69.00 ± 4.71	72.00 ± 4.51	79.50 ± 2.41	64.33±4.82	70.67±5.68	79.50±2.81	81.33 ± 6.38	86.17 ± 5.39	89.50 ± 4.21
Scopolamine control	108.7±3.34[a]	106.2±4.41[a]	107.0±4.61[a]	97.50 ± 5.31	98.17 ± 5.61	108.8 ± 5.71	107.8±4.71[a]	113.3±3.52[a]	113.7±3.31[a]	108.2 ± 4.31	111.3 ± 3.21	116.2 ± 2.81
Vyvamind 20 mg/kg	43.16±1.11**	53.19±2.63***	65.34±1.11**	70.11 ± 3.11	51.16 ± 3.14	63.41 ± 4.17	51.41±1.18***	53.81±6.61***	86.21±7 9.41**	81.41 ± 3.16	82.71 ± 2.31	90.51 ± 2.18

ZMEE 200 mg/kg	45.46±1.31**	56.11±2.13***	79.31±1.11**	64.41 ± 3.21	51.16 ± 3.14	63.41 ± 4.17	51.41±1.18***	53.81±6.61***	58.21±79.41**	61.41 ± 3.16	52.71 ± 2.31	63.51 ± 2.18
ZMEE 400 mg/kg	91.11±3.21	82.31±2.31***	69.11±1.51	79.52 ± 3.12	82.85 ± 3.61	79.17 ± 4.18	88.08±1.71**	81.64±7.31**	84.31±6.91*	92.01 ± 2.71	71.39 ± 2.57	79.52 ± 3.37
ZMAE 200 mg/kg	43.40±1.28**	54.08±2.07***	75.28±1.09*	62.36 ± 2.15	49.11 ± 1.95	60.38 ± 3.15	50.39±1.14***	51.74±6.55***	55.09±77.37**	59.37 ± 2.12	50.66 ± 1.69	60.45 ± 1.09
ZMAE 400 mg/kg	89.11±3.19	80.28±2.23***	65.11±1.49	76.42 ± 2.15	77.95 ± 2.65	77.13 ± 3.15	86.94±1.69**	80.59±7.25**	82.27±6.89*	89.99 ± 1.93	70.52 ± 1.23	77.45 ± 2.32

Data shown as average ± standard error (n=6). Analysis: A one-way ANOVA followed by Tukey's post-hoc test was conducted. Significance levels were indicated as follows: * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$) when compared to the OVX control group; a($p<0.001$), b ($p<0.01$), and c ($p<0.05$) when compared to the normal group. NS: not significant.

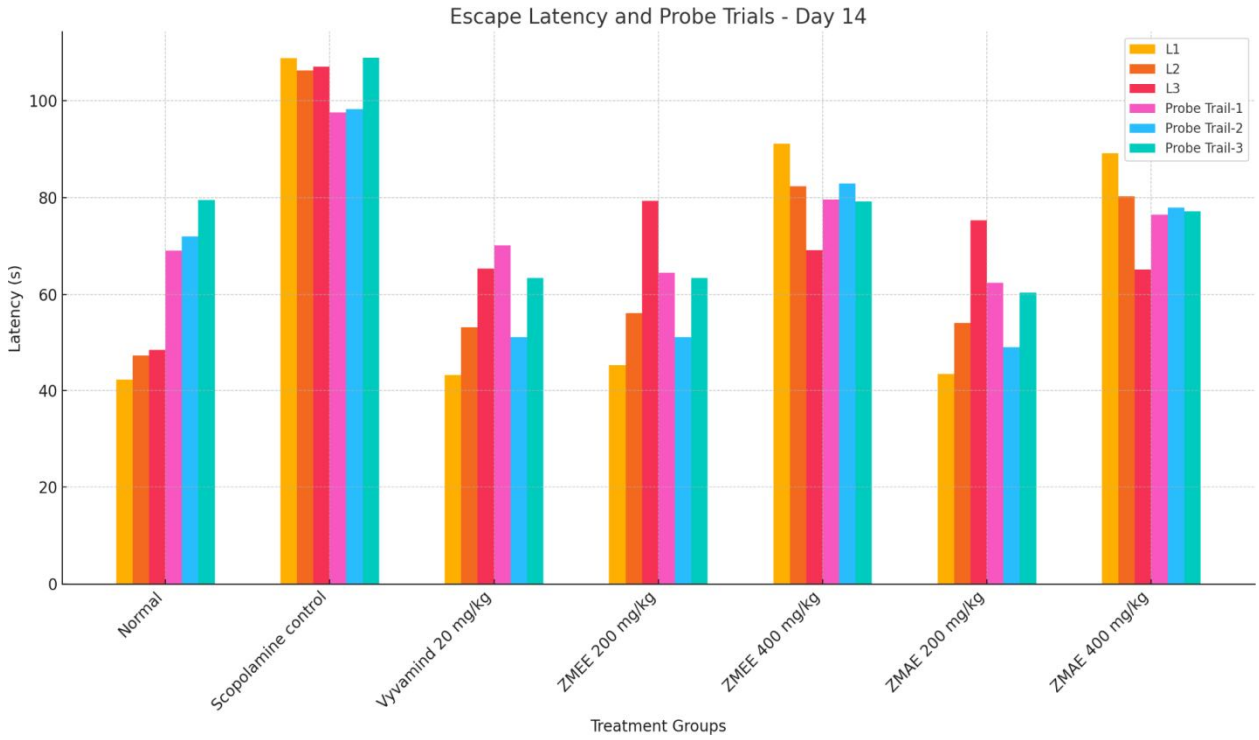

Fig.7.3Extract'sImpactonAquaticSpatialMemoryTaskTwo-WeekFollow-ups

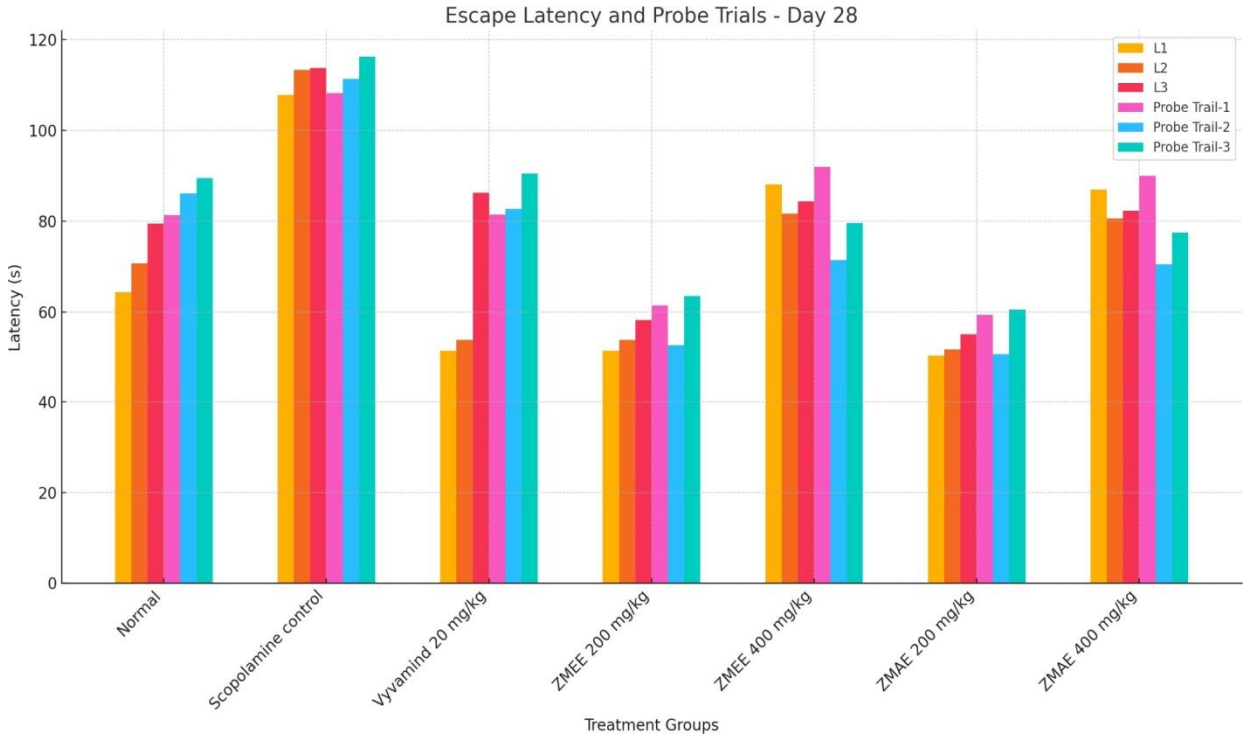

Fig.7.4Extract'sImpactonAquaticSpatialMemoryTaskFour-WeekFollow-ups

Table7.4ResponseofextractonMorris'watermazetest

S.No.	**TreatmentGroup**	**Value**
1.	Normal Group	66.29
2.	Scopolamine control	90.83
3.	Vyvamind20mg/kg	63.49
4.	ZMEE 200 mg/kg	62.72
5.	ZMEE 200 mg/kg	59.14
6.	ZMEE 200 mg/kg	70.24
7.	ZMEE 200 mg/kg	60.67

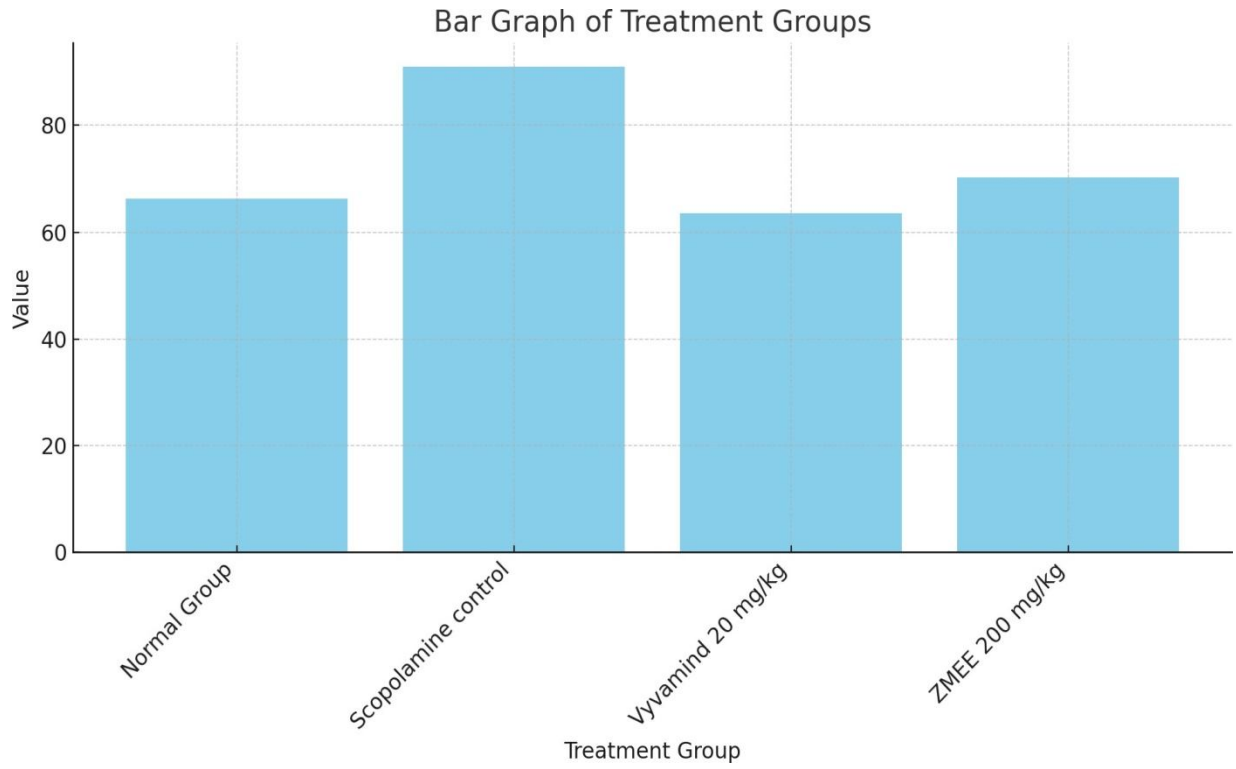

Fig.7.5ResponseofextractonMorris'WaterMazeTest

7.2.2 ELEVATEDPLUSMAZE(EPM)

The group of animals administered scopolamine exhibited a markedly increased frequency of enteringopenarmscomparedto the untreated control group across all observation periods post-scopolamine administration, suggesting cognitive decline. In contrast, the experimental groups receiving RR 300, RR 500, and piracetam demonstratedfeweropenarmentriesrelative tothescopolaminegroup.Thisreductionindicatesthatthetestedcompoundspossess memory-enhancing properties.

Table7.5Extract'sImpactonExposedBranchEntriesinCross-ElevatedApparatus:Day 1, 3, 7, 21, and 28 Evaluations

Groups	Number of entriesinExposed Branch					
	Day 1	**Day 3**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
	OA	**OA**	**OA**	**OA**	**OA**	**OA**
Normal group	1.11±0.21	0.47±0.29	0.56±0.18	0.58±0.26	0.74±0.69	0.66±0.38
Vyvamind 20 mg/kg	1.2±0.54	2.38±0.57	0.98±0.21**	0.88±0.12**	0.71±0.17***	0.88±0.47***
Scopolamine control	1.58±0.32	3.48±0.31[b]	4.87±1.85[c]	3.43±0.87[a]	3.18±0.40[a]	4.72±1.14[a]
ZMEE-200 mg/kg	1.24±0.64	1.51±0.52[ns]	0.45±0.14*	1.44±0.24*	0.67±0.79***	0.62±0.40***
ZMEE-400 mg/kg	1.38±0.57	2.40±0.64[ns]	0.32±0.20*	1.71±0.33***	0.75±0.34***	0.85±0.54***
ZMAE-200 mg/kg	1.12±0.57	1.24±0.48[ns]	0.30±0.12*	1.38±0.19*	0.65±0.89***	0.59±0.32***
ZMAE-400 mg/kg	1.25±0.45	1.89±0.94[ns]	0.24±0.12*	1.66±0.29**	0.69±0.30***	0.74±0.49***

Data presented as average ± standard error (n=6). A one-way ANOVA followed by Tukey's post-hoc test was performed. The significance levels were represented as follows: * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$) in comparison to the OVX control group; a ($p<0.001$), b ($p<0.01$),c($p<0.05$)incomparisontothenormalgroup.NS: not significant.

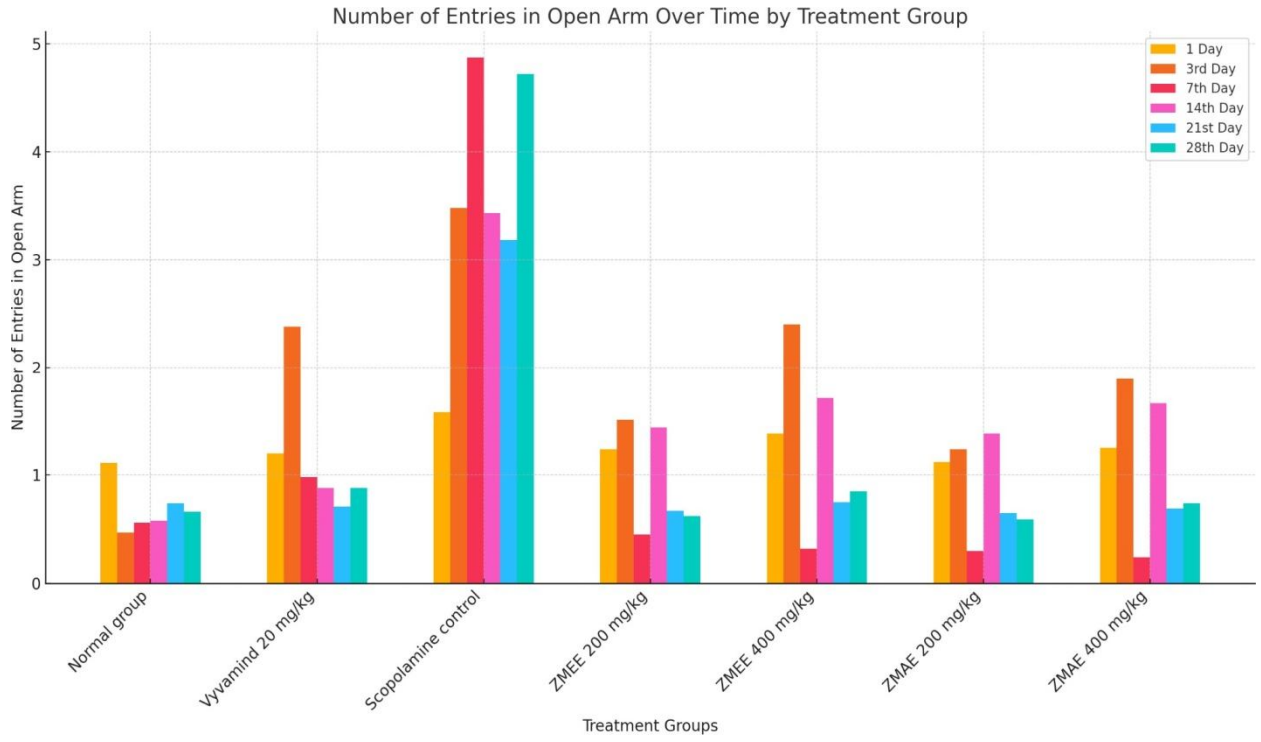

Fig.7.6Extract'sImpactonExposedBranchEntriesinCross-ElevatedApparatus:Day1, 3, 7, 21, and 28 Evaluations

Animals treated with scopolamine exhibited a marked decrease in entries to enclosed branches compared to untreated controls after scopolamine administration, suggesting cognitive decline. Conversely, groups given RR 300, RR 500, and piracetam showed an increase in enclosed branch entries relative to the scopolamine group. This improvement indicates that the tested compounds possess memory-enhancing properties.

Table7.6Extract'sInfluenceonEnclosedQuadrantExplorationinCross-Elevated Platform: Evaluations at Day 1, 3, 7, 21, and 28

Groups	Numberof entriesinEnclosed Quadrant					
	1st Day	**3rd Day**	**7th Day**	**14th Day**	**21st Day**	**28th Day**
	CA	**CA**	**CA**	**CA**	**CA**	**CA**
Normal group	4.57±0.31	4.12±0.88	4.32±0.44	5.14±0.56	5.74±0.31	4.71±0.53
Scopolamine control	7.14±0.28	3.14±0.55^{ns}	4.11±0.12^{ns}	1.18±0.45^{c}	1.31±0.11^{b}	1.51±0.14^{b}

Vyvamind 20 mg/kg	4.42±0.31	4.01±0.33^{ns}	3.91±1.12^{ns}	5.38±1.20**	5.38±1.20*	5.41±0.19**
ZMEE-200 mg/kg	4.10±1.21	3.42±1.50^{ns}	3.61±1.81^{ns}	3.98±1.61*	4.02±0.16*	4.21±1.31*
ZMEE-400 mg/kg	4.50 ±0.36	4.05±0.33^{ns}	3.31±1.32^{ns}	4.21±0.12**	4.35±1.10*	4.31±0.17**
ZMAE-200 mg/kg	3.89±1.03	2.85±0.99^{ns}	2.95±1.05^{ns}	3.07±1.47*	3.95±0.09*	4.19±1.05*
ZMAE-400 mg/kg	4.25 ±0.30	3.89±0.29^{ns}	3.25±1.20^{ns}	4.10±0.09**	4.24±1.07*	4.20±0.11**

Data presented as average ± standard error (n=6). Statistical analysis employed a single-factor variance test with subsequent pairwise comparisons. Significance thresholds were denoted: * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$) versus ovariectomized control; a ($p<0.001$), b ($p<0.01$), c ($p<0.05$) versus non-operated group. NS: not significant.

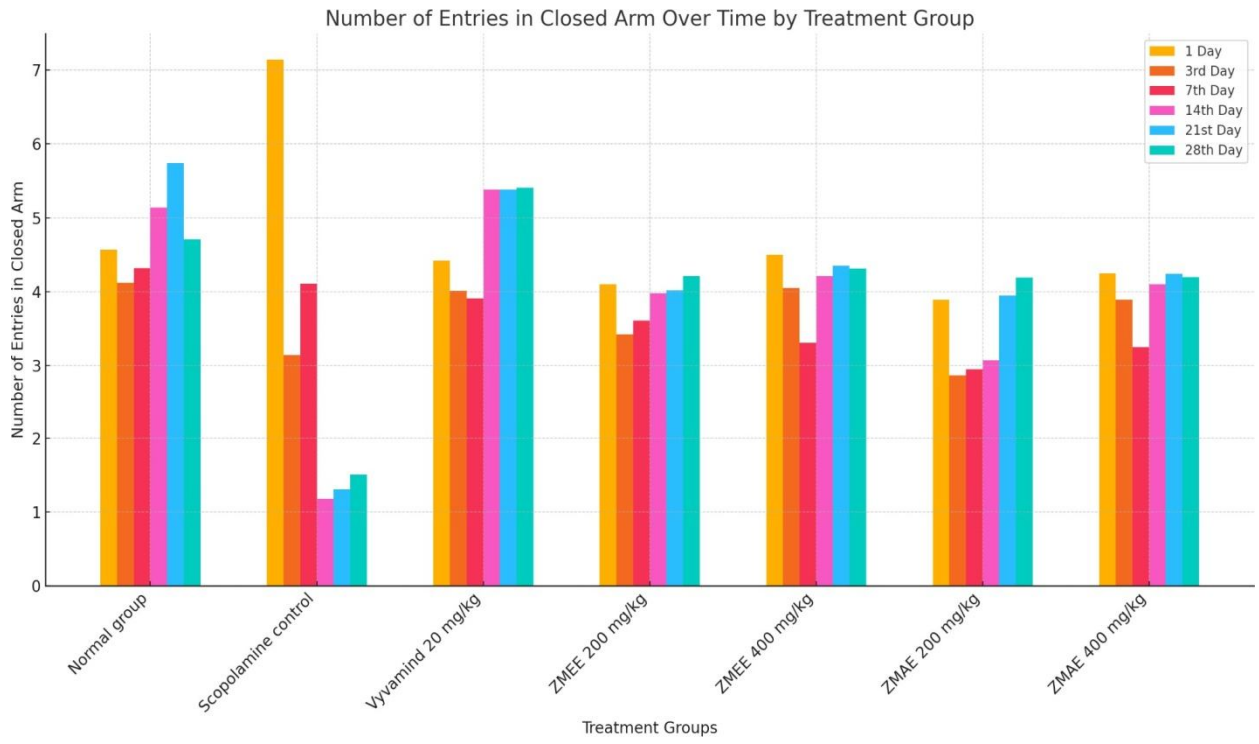

Fig.7.7Extract'sInfluenceonEnclosedQuadrantExplorationinCross-Elevated Platform: Evaluations at Day 1, 3, 7, 21, and 28

Table7.7Extract'sImpactonExposedandShelteredBranchExplorationin Cross-Elevated Apparatus

S.No.	TreatmentGroup	Number of EntriesintotheOpen Arm	Number ofEntriesintotheClosed Arm
1.	Normal Group	0.69	4.77
2.	Scopolamine control	1.17	3.07
3.	Vyvamind20mg/kg	3.54	4.75
4.	ZMEE 200 mg/kg	0.99	3.89
5.	ZMEE 200 mg/kg	1.24	4.12
6.	ZMEE 200 mg/kg	0.88	3.48
7.	ZMEE 200 mg/kg	1.08	3.99

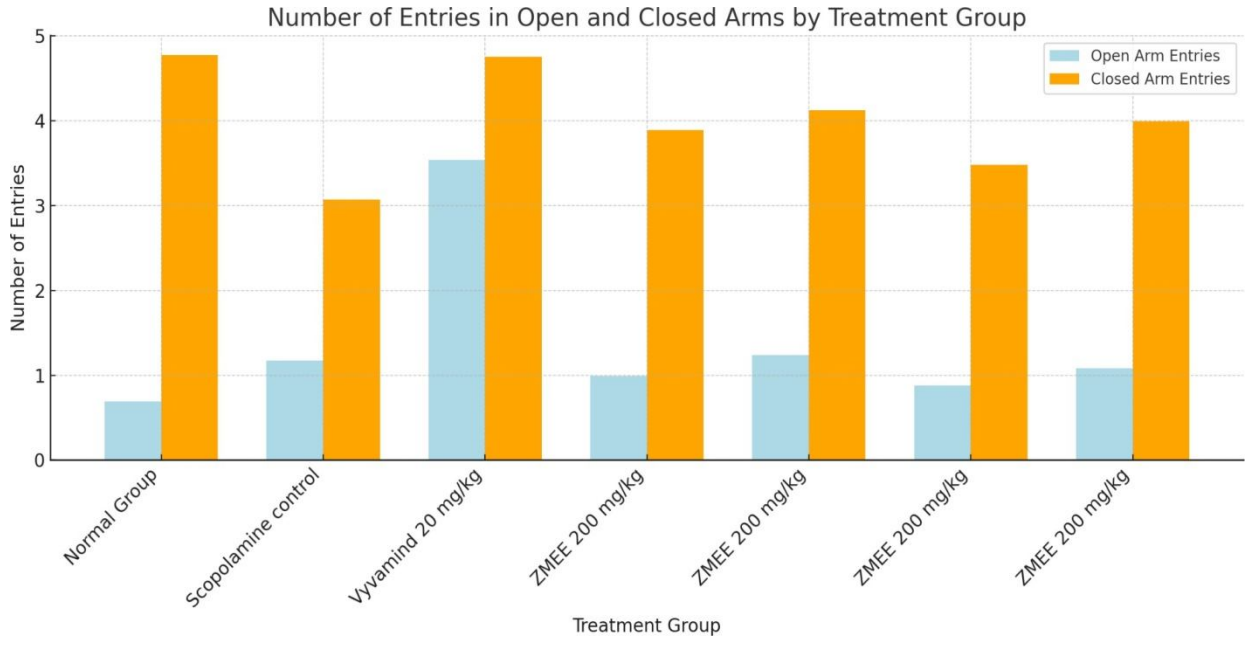

Fig.7.8Extract'sImpactonExposedandShelteredBranchExplorationinCross-Elevated Apparatus

7.3 ESTIMATIONOFOXIDATIVESTRESSBIOMARKERS

Initially, there was a decline in acetylcholine (ACh), a cholinergic neurotransmitter crucial for optimal learning, memory, and attention. Furthermore, there was a reduction in the brain's natural antioxidants due to the activation of microglia, which produce reactive oxygenspecies. Treatment with scopolamine resulted in a substantial increase in brain acetylcholinesterase (AChE) and malondialdehyde (MDA) levels, along with a decrease in glutathione (GSH) and total protein levels, compared to the control group that received the CMC solution.

Oral doses of Ziziphus Mauritiana extract (200 and 400 mg/kg) showed marked ($p<0.05$) and graded reductions in cholinesterase activity and lipid peroxidation, while boosting glutathione and protein levels versus the amnesia-induced group. The extract, comparable to the standard drug, successfully countered scopolamine's cognitive deterioration effects.

7.3.1 METHOD FOR DETERMINING ACETYLCHOLINESTERASE (AChE) ACTIVITY IN BRAIN SAMPLE

The study examined how Ziziphus Mauritiana extract (ZME) affectsbrainAChEactivity.Each data point represents the average AChE level ± standard error of the mean (S.E.M.), with six subjects per group. AChE levels were quantified for the following groups: control (given 1% w/v carboxymethyl cellulose solution, CMC), reference drug (Vyvamind), memory-impairing agent (scopolamine), and the herbal preparation (ZME).

Findings are presented as mean ± S.E.M., with six subjects in each category. A notable rise in AChE levels was detected in the scopolamine-treated group when compared to the control. Conversely, groups receiving CMC, Vyvamind, and ZME (at 200 and400mg/kg,givenorally) exhibited reduced AChE levels.Thememory-impairingagentmarkedlyincreased AChElevels. Statistical significance is denoted as follows: a = $p\leq0.05$ in comparison to the control group's AChE level; b = $p\leq0.05$ in comparison to the scopolamine-treated group's AChE level. AChE activity is expressed in μM/min/mg of protein.

Table7.8AChEActivityacrossdifferentTreatmentGroup

S.No.	**Treatment**	**AChE Activity** (μM/min/mg of protein)
1.	Control Group	700 μM/min/mg of protein
2.	Negative Group	1600 μM/min/mg of protein

3.	Standard Drug	200 µM/min/mg of protein
4.	TestGroup-I(ZMEE)	600 µM/min/mg of protein
5.	TestGroup-II(ZMEE)	350 µM/min/mg of protein
6.	TestGroup-III(ZMAE)	550 µM/min/mg of protein
7.	TestGroup-IV(ZMAE)	315 µM/min/mg of protein

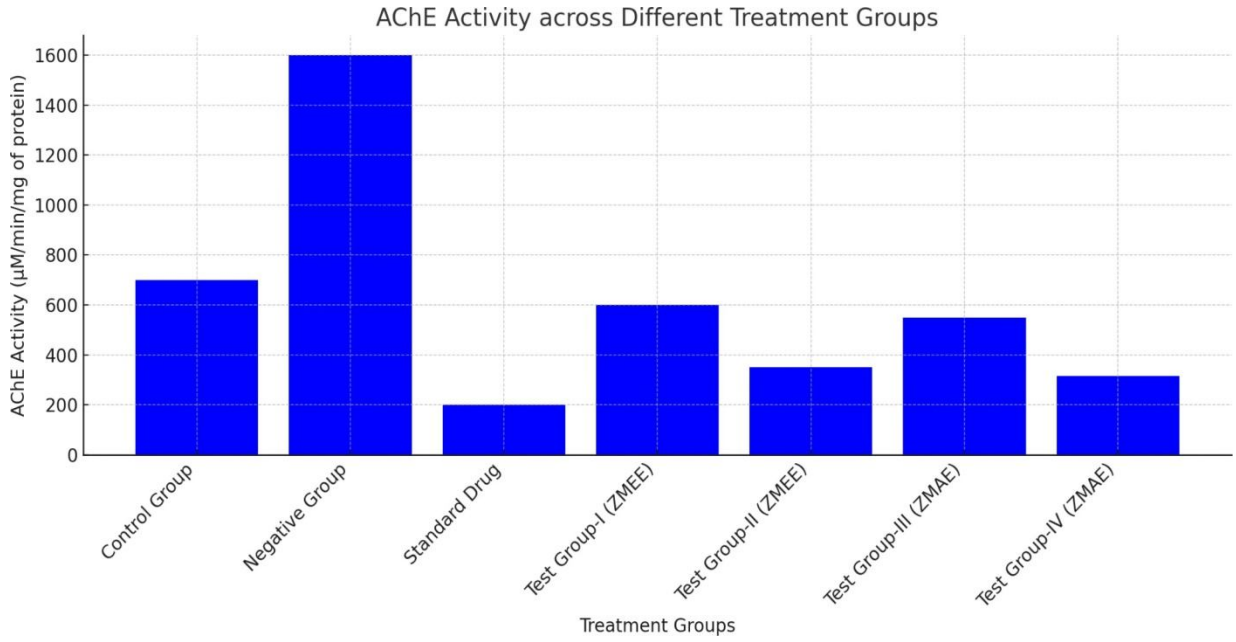

Fig.7.9AChEActivityacrossdifferentTreatmentGroup

7.3.2 PROTOCOLFORQUANTIFYINGMALONDIALDEHYDE(MDA)INBRAIN SAMPLE

This study investigated the impact of Ziziphus Mauritiana extract (ZME) on brain MDA concentrations. Each data point represents the average MDA level ±standarderrorofthemean (S.E.M.) for six subjects per group. MDA concentrations were measured in the following groups: control (given 1% w/v carboxymethyl cellulose solution, CMC), reference medication (Vyvamind), memory-impairing agent (scopolamine), and the herbal preparation (ZME).

Findings are expressed as mean ± S.E.M. for each set of six subjects. Thescopolamine-treated groupshowedasignificantelevationinMDAconcentrationscomparedtothecontrol.In

contrast, groups receiving CMC, Vyvamind, and ZME (at 200 and 400 mg/kg, administered orally) demonstrated a notable decrease in MDA levels. The memory-impairing agent substantially increased MDA concentrations. Statistical significance is denoted as follows: a = $p \leq 0.05$ in comparison to the control group's MDA level; b = $p \leq 0.05$ in comparison to the scopolamine-treated group's MDA level. MDA concentrations are reported in µM per mg of protein.

Table7.9MDALevelacrossdifferentTreatmentGroup

S.No.	**Treatment**	**MDA Level** (µM/mg of protein)
1.	Control Group	21 µM/mg of protein
2.	Negative Group	44 µM/mg of protein
3.	Standard Drug	14 µM/mg of protein
4.	TestGroup-I(ZMEE)	24 µM/mg of protein
5.	TestGroup-II(ZMEE)	19 µM/mg of protein
6.	TestGroup-III(ZMAE)	23 µM/mg of protein
7.	TestGroup-IV(ZMAE)	16 µM/mg of protein

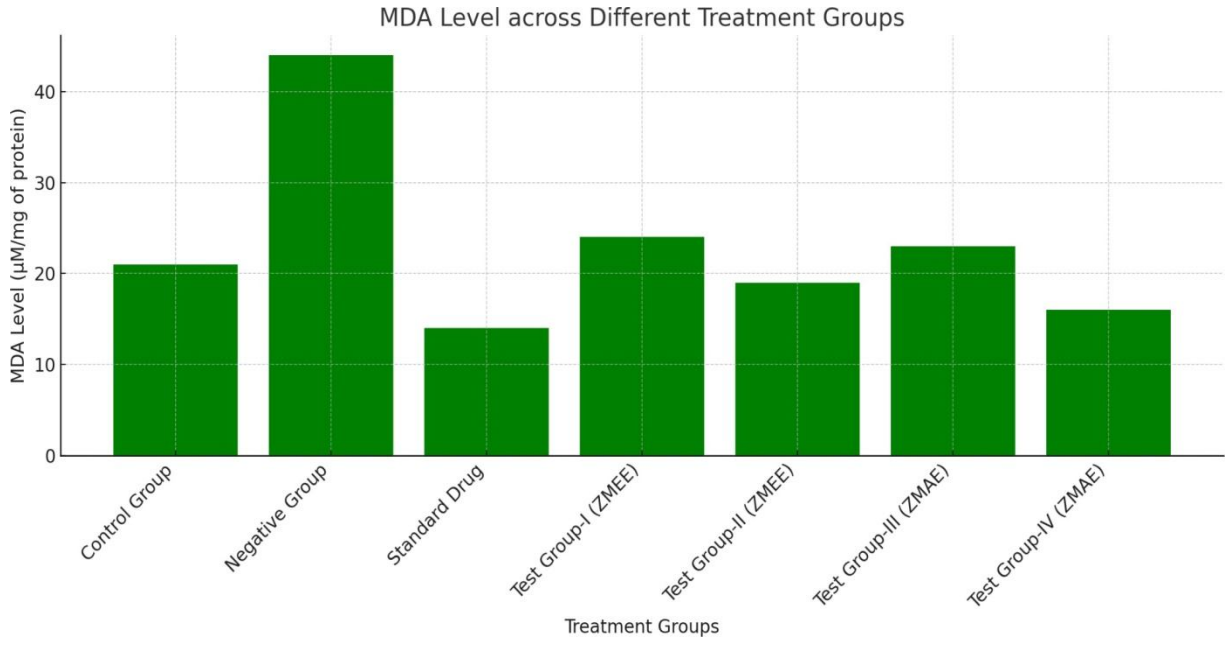

Fig.7.10MDALevelacrossdifferentTreatmentGroup

7.3.3 METHOD FOR DETERMINING REDUCED GLUTATHIONE (GSH) CONTENT IN BRAIN TISSUE

This investigation examined the effects of Ziziphus Mauritiana extract (ZME) on brain GSH concentrations. Each data point represents the average GSH level ± standard error of the mean (S.E.M.) for six subjects per group. GSH concentrations were quantified in the following groups: control (given 1% w/v carboxymethyl cellulose solution, CMC), reference medication (Vyvamind), memory-impairing agent (scopolamine), and the herbal preparation (ZME).

Findings are presented as mean ± S.E.M. for each set of six subjects. The scopolamine-treated group demonstrated a significant reduction in GSH concentrations compared to the control. Conversely, groups receiving CMC, Vyvamind, and ZME (at 200 and400mg/kg,administered orally) showed an elevation in GSH levels. The memory-impairing agent notably decreased GSH concentrations. Statistical significance is denoted as follows: a=p≤0.05incomparisonto the control group's GSH level; b = p≤0.05 in comparison to the scopolamine-treated group's GSH level. GSH concentrations are reported in µM per mg of protein.

Table7.10GSHLevelacrossdifferentTreatmentGroup

S.No.	**Treatment**	**GSH Level** (µM/min/mg of protein)
1.	Control Group	260 µM/mg of protein
2.	Negative Group	160 µM/mg of protein
3.	Standard Drug	350 µM/mg of protein
4.	TestGroup-I(ZMEE)	260 µM/mg of protein
5.	TestGroup-II(ZMEE)	290 µM/mg of protein
6.	TestGroup-III(ZMAE)	250 µM/mg of protein
7.	TestGroup-IV(ZMAE)	270 µM/mg of protein

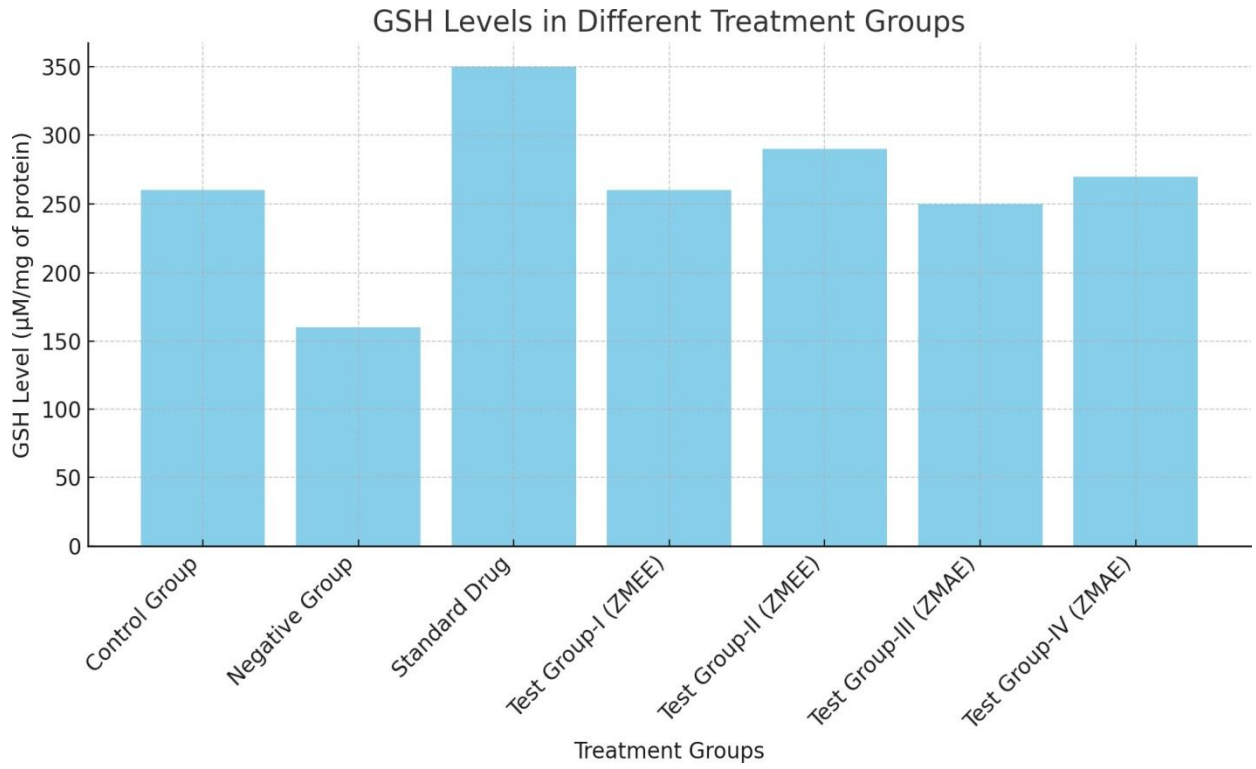

Fig.7.11GSHLevelacrossdifferentTreatmentGroup

7.3.4 METHODFORQUANTIFYINGTOTALPROTEINCONTENTINBRAIN SAMPLE

This study investigated the effects of Ziziphus Mauritiana extract (ZME) on brain total protein concentrations. Each data point represents the average totalproteinlevel±standarderrorofthe mean (S.E.M.). Total protein concentrations were measured in the following groups: control (given 1% w/v carboxymethyl cellulose solution, CMC), reference medication (Vyvamind), memory-impairing agent (scopolamine), and the herbal preparation (ZME).

Findings are presented as mean ± S.E.M. for each set of six subjects. The scopolamine-treated group demonstrated a significant reduction in total protein concentrations compared to the control. Conversely, groups receiving CMC, Vyvamind, and ZME (at 200 and 400 mg/kg, administered orally) exhibited a notable increase in total protein levels.Thememory-impairing agent markedly decreased total protein concentrations. Statistical significance is denoted as follows: a = $p \leq 0.05$ in comparison to the control group's total protein level; b = $p \leq 0.05$ in comparison to the scopolamine-treated group's total protein level. Protein concentrations are reported in µg/µg protein.

Table7.11ProteinLevelacrossdifferentTreatmentGroup

S.No.	**Treatment**	**ProteinLevel**(µg/µl)
1.	Control Group	0.6 µg/µl
2.	Negative Group	0.045 µg/µl
3.	Standard Drug	0.13 µg/µl
4.	TestGroup-I(ZMEE)	0.7 µg/µl
5.	TestGroup-II(ZMEE)	0.085 µg/µl
6.	TestGroup-III(ZMAE)	0.5 µg/µl
7.	TestGroup-IV(ZMAE)	0.079 µg/µl

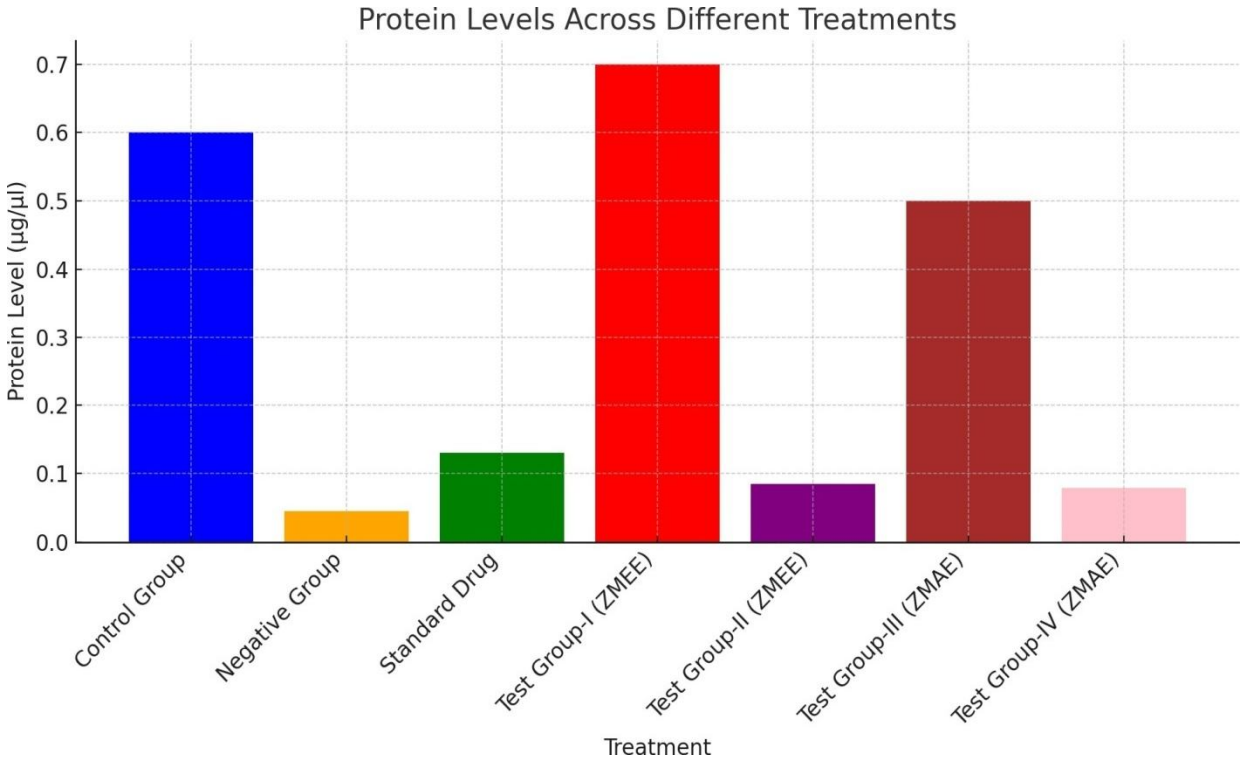

Fig.7.12ProteinLevelacrossdifferentTreatmentGroup

CHAPTER-8

DISCUSSION

The thesis critically examines the intricate architecture of memory and its implications for cognitive processes, with a particular emphasis on dementia and its different manifestations. Memory, as defined in the thesis, is a multidimensional system required for encoding, storing, maintaining, and retrieving information and experiences. It serves as the base for learning, thinking, and problem solving, as well as the substrate of human awareness and cultural transmission. The theory divides memory into three categories: short-term, intermediate, and long-term, each with its own cognitive function and neurological base.

Short-term memory, also referred to as working memory, is essential for immediateinformation processing and is predominantly related with the hippocampus and specific thalamic nuclei. It serves as a cognitive workspace, facilitating complicated processes such as language comprehension and problem solving by temporarily storing a tiny amount of information for active usage. Intermediate memory bridges the gap between short-term and long-term memoryby integrating ongoing experiences and providing a temporal context that relates the present to recent past events. In contrast, long-term memory provides a hugeandlong-lastingreservoirfor knowledge, including both declarative and procedural memories. Declarative memory includes episodic memory, which holds personal experiences, and semantic memory, which contains factual knowledge. In contrast, procedural memory involves the unconscious learning of skills and routines.

This research delves into the complexities of dementia, a condition marked by progressive deterioration across multiple cognitive functions, such as recall, logical thinking, and communication. Dementia substantially compromises an individual's capacity to perform everyday tasks and maintain autonomy, with Alzheimer's disease (AD) emerging as the most common variant. AD is characterized by the accumulation of amyloid deposits and tau protein aggregates, resulting in neuronal loss and cognitive impairment. The study also acknowledgesthe existence of alternative forms of dementia, such as vascular dementia, dementia with Lewy bodies, and frontotemporal dementia, have different pathogenic origins but all lead to impaired cognitive performance. The thesis emphasizes the difficulty of identifying and managing dementia, given its varied nature and overlapping symptoms among different forms.This statement highlights the worldwide impact of cognitivedeclinedisordersandstressesthecrucial role of advanced diagnostic methods, potent treatments, and holistic careapproachesintackling this escalating health challenge.

In conclusion, the thesis presents a thorough examination of memory systems and their degeneration in dementia, providing vital insights into the cognitive foundations of memory as well as the tremendous consequences of dementia on individuals and society. The discussion incorporates research on the neurological basis of memory, the clinical aspectsofdementia,and the socioeconomic implications of rising dementia incidence, urging a concerted worldwide approach to ameliorate its consequences.

CHAPTER-9

CONCLUSION

This thesis has methodically investigated the complicated nature of memory, its physiological basis, andthedramaticimpactofitsdeclinecausedbyvariousformsofdementia.Memoryisthe foundation of human cognitive processes, connecting thepresentwiththepastandunderpinning learning, thinking, and problem-solving. Memory was studied and classified into threecategories: short-term, intermediate-term, and long-term, each with its own set of functions and neurological bases. Short-term memory, which is required for immediate activities, is based on the hippocampus and certain thalamicnuclei,butlong-termmemory,whichstoreshugeamounts of information, is maintained by cortical regions spread across the brain. This thorough understanding of memory processeslaysthegroundworkforinvestigatingthenegativeeffectsof dementia.

Cognitive decline syndrome is characterized by a gradual loss of mental faculties, significantly impacting memory, logical thinking, and self-sufficiency. This study examined four principal categories of cognitive decline: β-amyloid-associated brain disease, cerebrovascular-induced cognitive impairment, α-synuclein-related neurodegeneration, and frontal-temporal lobe degeneration. While these conditions have distinct underlying mechanisms, they all result in diminished mental capacity.

The most prevalent form, β-amyloid-associated brain disease, is marked by protein aggregates and tangled neural fibers, leading to neuronloss.Cerebrovascular-inducedcognitiveimpairment stemsfromdisordersaffectingbrainbloodvessels,reducingcellularoxygensupply. α-synuclein-related neurodegeneration is identified by abnormal protein accumulations that compromise both cognitive and motorfunctions.Frontal-temporallobedegenerationimpactsthe brain's anterior and lateral regions, disrupting behavioral patterns and linguistic abilities.

The thesis stressed the global burden of dementia, with millions affected and projections of a significant increase in prevalence. Dementia is a huge public health concern that requires better diagnostic techniques, effective therapies, and comprehensive care plans.Thisstudyemphasizes the importance of a coordinated global response to the expanding dementia pandemic, which includes prevention,treatment,care,andsupportmethods.Theresultsemphasizethecriticalneed for continued scientific inquiry and breakthroughs in comprehending and alleviating cognitive decline disorders. These efforts aim to enhance the well-being of those affected while simultaneously lessening the financial and social strain on care providers and the broader community. This conclusion combines the thesis's examination of the complexities of memory, the devastation caused by dementia, and the urge for a strong worldwide plan to address this rising crisis.

CHAPTER-10

SUMMARY

Thisthesisdelvesdeeplyintothephysiologicalmechanicsofmemoryandthedetrimentaleffects of its deterioration due to dementia. Memory, a key feature of cognition, is divided into three types: short-term, intermediate, and long-term, each having its own set of functions and neurological bases. Short-term memory, which is required for current activities, is reliant onbrain regions such as the hippocampus and the thalamic nuclei. Working memory serves as a connecting link, offering temporal context for current experiences, while enduring memory retains vast quantities of data over prolonged durations, involving various cortical areas across the entire brain. This complex system enhances the rich tapestry of human experience by facilitating learning, reasoning, and problem solving.

Dementia, a sickness characterized by a steady decrease in cognitive ability, severely impairs memory and other cognitive functions, limiting daily living and independence.This study examines four primary forms of cognitive decline disorders: β-amyloid-associated neurodegeneration, cerebrovascular-induced mental impairment, α-synuclein-related brain disease, and frontal-temporal lobe deterioration.

β-amyloid-associated neurodegeneration is marked by protein aggregates and tangled neural fibers, resulting in neuron loss and cognitive decline. Cerebrovascular-induced mental impairment stems from insufficient brain blood flow, often following a cerebrovascular event.α-synuclein-related brain diseaseisidentifiedbyunusualproteinaccumulationsthatcompromise both mental functions and physical coordination. Frontal-temporal lobe deterioration involvesthe decay of the brain's anterior and lateral regions, disrupting behavioralpatternsandlinguistic abilities.

The global prevalence of dementia is increasing, with millions affected, causing substantial public health issues. This thesis emphasizes the importance of developing new diagnostic, therapeutic, and care optionstoaddresstheexpandingdementiaburden.Theresearchresultscall for a multifaceted strategy to tackle the challenges posed by cognitive decline disorders. This approach aims to alleviate the strain caused by dementia, boost the overall well-being of those affected, and confront the economic and social ramifications for both caregivers and the community at large. By addressing these interconnected issues, the study emphasizes the needfor a holistic solution that not only focuses on medical aspects but also considers the broader societal impact of neurodegenerative conditions. The thesis emphasizes the vital function of memory in human cognition and the grave repercussions of its decline due to dementia, urging for ongoing research and global effort to tackle this rising crisis.

CHAPTER-11

REFERENCE

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CERTIFICATE

Certified that **Sidra Kausher** (**Enrollment No.** 2202270986006.) has carried out the research work presented in this thesis entitled "**Investigation For Nootropic Activity of Ziziphus Mauritiana On Scopolamine Induced Memory Deficits In Swiss Albino Mice**" for the award of **Master of Pharmacy** from Dr. APJ Abdul Kalam Technical University, Lucknow under my supervision. The thesis embodies results of original work, and studies are carried out by the student herself and the contents of the thesis do not form the basis for the award of any other degree to the candidate or to anybody else from this or any other University/Institution.

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