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THE EFFECT OF ALZHEIMER'S TREATMENT STRATEGIES ON SPATIAL MEMORY PERFORMANCE IN SCOPOLAMINE-INDUCED ALZHEIMER'S DISEASE MODEL IN MICE

MASTER THESIS

Shaima Shaweesh Almahameed

İstanbul-2023

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APPROVAL

This thesis has been deemed by the jury following the relevant articles of Yeditepe University Graduate Education and Examinations Regulation and has been approved by the Administrative Board of the Institute with a decision dated 26.01.2023 and numbered .....

(Signature)

Prof. Dr. Bayram YILMAZ
Director of the Institute of Health Sciences

DECLARATION

I hereby declare that this thesis is my work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgement has been made in the text.

Shaima Shaweesh Almahameed

DEDICATION

I would like to dedicate this thesis to my family, who have been a constant source of support and encouragement throughout my academic journey. Without their unwavering belief in me and their guidance, this achievement would not have been possible.

I am so grateful to my friends, who have provided me with their love and understanding, even when I had to sacrifice time to complete this thesis. Their understanding and patience have been invaluable to me.

Finally, I would like to dedicate this thesis to all the individuals who have inspired me with their knowledge and insights and who have paved the way for future generations of scholars. I hope my work can contribute to their legacy and inspire others to continue their important work.

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Special thanks to my father's **soul**, who always believed in my ability to be successful in the academic arena. You are gone, but your belief in me has made this journey possible.

To my beloved, loving, and supportive mother, **Muneera**: my deepest gratitude. Your encouragement when the times got rough is much appreciated and duly noted.

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LIST OF SYMBOLS AND ABBREVIATIONS

AD: Alzheimer's Disease

BDNF: Brain-derived neurotrophic factor

CREB: cAMP response element binding protein

NLRP3: NOD-like receptor pyrin domain containing 3

MWM: Morris water maze

ACh: Acetylcholine

AChI: Acetylcholinesterase inhibitor

AChE: Acetylcholinesterase

mAChR: Muscarinic acetylcholine receptor

NMDA: N-methyl-d-aspartate

NMDAR: N-methyl-d-aspartate receptor

Aβ: Amyloid β-peptide

NFTs: Neurofibrillary tangles

APP: Amyloid Precursor Protein

FDA: Food and Drug Administration

STM: Short-term memory

LTM: Long-term memory

WM: Working memory

LTP: Long-term potentiation

i.p: Intraperitoneal injection

s.c: Subcutaneous

ABSTRACT

ALMAHAMEED.SH.SH.H (2022). The Effect of Alzheimer Treatment Strategies on Spatial Memory Performance in Scopolamine Induced Alzheimer Disease Model in Mice.

Alzheimer's disease (AD) is one of the most progressive common neurodegenerative disease. Its statistics continue to rise year after year. The degeneration of the brain cell that makes dementia lead to a decrease in thinking and memory. Many hypotheses explain the cause of AD. Also, many risk factors play an important role in this disease but the exact cause is still unknown. Drugs reduce the symptoms of the disease not cure it. N-methyl d-aspartate (NMDA) antagonists and acetylcholinesterase enzyme inhibitors (naturally derived, synthetic, and hybrid analogues) are the two categories of drugs that are readily available for use. The mice were givin scopolamine (1 mg/kg i. p.) for 15 days to cause anti-amnesic Alzheimer-like disease. Donepezil (5mg/kg s.c.) was administered for 15 days, and Memantine (1mg/kg i.p.) was administered for 15 days, both were administered as a treatment for amnesia. The evaluation of the mice's spatial memory and learning was done using Morris Water Maze test. MWM test shows that there is no change in memory performance in the memantine and donepezil group that compered with control and scopolamine group. Also, brain samples was studied by measurement of NLRP3 protein in mice, and there is no change of NLRP3 concentration in memantine, donepezil groups that compared with control group. And the studied of AchE showed that the Donepezil group was significantly associated with elevated AchE activity in comparison to control group and scopolamine group. Values were expressed as the mean ± standard deviation (Stdev). A value of $p<0.05$ was considered statistically significant.

Key words: AD, Mice, Scopolamine, Donepezil, Memantine, MWM.

ÖZET

ALMAHAMEED.SH.SH.H (2022). Farelerde Skopolamin Kaynaklı Alzheimer Hastalığı Modelinde Alzheimer Tedavi Stratejilerinin Uzamsal Bellek Performansına Etkisi.

Alzheimer hastalığı (AD), en ilerleyici yaygın nörodejeneratif hastalıklardan biridir. İstatistikler yıldan yıla bu hastalığın arttığını göstermektedir. Demansa neden olan beyin hücresinin dejenerasyonu, düşünme ve hafızada azalmaya yol açar. AD'nin nedenini açıklayan birçok hipotez vardır. Ayrıca bir çok risk faktörü bu hastalıkta önemli rol oynamaktadır ancak kesin nedeni hala bilinmemektedir. İlaçlar hastalığı iyileştirmez, sadece semptomlarını azaltır. N-metil d-aspartat (NMDA) antagonistleri ve asetilkolinesteraz enzim inhibitörleri (doğal olarak türetilmiş, sentetik ve hibrit analoglar), kullanıma hazır iki ilaç kategorisidir. Farelere, anti-amnezik Alzheimer benzeri hastalık durumunu oluşturmak için 15 gün boyunca skopolamin (1 mg/kg i.p.) verildi. Donepezil (5mg/kg s.c.) 15 gün, Memantine (1mg/kg i.p.) 15 gün uygulandı, her iki ilaç da skopolamin'in oluşturduğu amneziye tedavi olarak uygulandı. Farelerin uzamsal hafızasının ve öğrenmesinin değerlendirilmesi, Morris Water Maze testi kullanılarak yapıldı. Kontrol ve skopolamin grubunun MWM testi sonuçları, memantin ve donepezil grupları ile karşılaştırıldığında, gruplar arasında bellek performansında değişiklik olmadığı gözlendi. Değerler ortalama ± Standart sapma (SS) olarak ifade edildi. $p<0,05$ değeri istatistiksel olarak anlamlı kabul edildi.

Anahtar kelimeler: Alzheimer, fare, Scopolamine, Donepezil, Memantine, MWM.

1. INTRODUCTION and PURPOSE

Alzheimer's disease (AD) is a neurodegenerative disease that progressively worsens. Alois Alzheimer described it for the first time in 1906[1]. It is a neurodegenerative disease associated with aging that causes memory loss and declines in cognitive abilities [2]. It is considered to be a top research priority because it is a significant public health issue [3].

AD affects vulnerable brain areas, especially the hippocampus. İt is the first and the most severely affected brain areas. It is also one of the marker areas for cognitive decline and diagnosis of AD [2]. Research demonstrated the crucial role of the hippocampus for cognitive functions, memory and learning, in which enhancement of neurogenesis in this area can compensate development of AD [2].

Scopolamine is a muscarinic acetylcholine receptor (mAChR) antagonist which inhibits rodent learning and memory acquisition. To evaluate anti-amnesic effects on animals, the scopolamine-induced amnesia modal has been widely used [4]. Numerous neurobehavioral investigations have demonstrated that scopolamine can impair rodent memory function, particularly short-term memory, and learning. Traditional methods for evaluating these cognitive abilities in rodents include the water maze test, the passive avoidance test, and the radial arm maze test. Numerous studies have been existing on scopolamine to identify AD and treatment strategies. It is a great behavioral model to research dementia-related diseases. Scopolamine inhibits the cholinergic neurotransmitter system, which is a powerful pharmacological tool for studying the cellular and molecular alterations associated with AD pathogenesis [5]. Also, both general activity and licking will change massively when scopolamine is used also Scopolamine considerably decreases the ability to complete a reversal-learning test [6].

The cause of the pathological changes in AD (neurofibrillary tangles (NFTs), Amyloid βeta plaques (Aβ), and synaptic loss) is still unclear [7]. Currently, five approved anti-Alzheimer's disease drugs are existing. Tacrine, donepezil, rivastigmine, galantamine, and memantine. The first four medications work by inhibiting acetylcholinesterase, while memantine act by inhibiting N-methyl-d-aspartate (NMDA) receptors. The medications that mentioned above, do not cure AD, they offer symptomatic therapy. Although the

specific causation of the disease is unknown, some research suggests that Aβ aggregation is the main cause. Because beta-amyloid aggregation produces neurotoxicity. Therefore, the development of the next anti-AD strategy mostly is relies on the Aβ theory [8].

The cholinergic hypothesis states that AD is caused by a loss of acetylcholine (ACh) synthesis. One method of therapy options for improving cognitive and neural cell performance is Inhibiting acetylcholinesterase (AChE) that cause increase cholinergic levels. Acetylcholinesterase inhibitors (AChEIs) stoping the acetylcholine degradation in the synapses, As a result, ACh accumulates and cholinergic receptors are activated [7].

Donepezil is a second-generation AChEI and an indanone-benzyl piperidine derivative that is widely used for symptomatic treatment of AD. Donepezil inhibits Ach hydrolysis by binding to AchE reversibly, resulting in a greater concentration of ACh in synapses. This medication is acceptable, among only with temporary and minor cholinergic adverse effects affecting the gastrointestinal and nervous systems. AD symptoms are managed with donepezil without slowing the disease's progression [7].

Memantine a low-affinity amd noncompetitive antagonist of N-methyl-d-aspartate receptor (NMDAR), a glutamate receptor subtype that inhibits the overactivation of the glutaminergic system. Memantine is used alone or in combination with AChEI to treat mild to severe AD symptoms. Because it inhibits the excitatory receptors without interfering with normal synaptic transmission, the medication is both well tolerated and safe. This is because memantine has a low affinity and is quickly replaced from NMDAR by higher glutamate concentrations, preventing a long-term blockage. The latter is associated with a slew of negative side effects, most notably in terms of memory and learning [7].

The aim of this research is to identify the pathology AD and highlight it to determine the AD treatment strategies in the scopolamine-induced AD model in mice.

2. LITERATURE REVIEW

2.1.Alzheimer's disease overview

2.1.1 Background

The number of persons living with dementia grows as the world's population ages, and this number is expected to keep growing, especially in low- and middle-income nations [9].

In 2015, there were around 47 million dementia sufferers worldwide. Dementia affects both the sufferers, who progressively lose their skills, and their family, friends, and other supports, who must deal with watching a family member or friend get sick and decline while performing their necessities, such as strong dependence and behavioral changes. Additionally, because dementia patients also need medical and social care, it has an impact on a larger portion of society [9].

The most predomenant form of dementia is AD, which is a slowly progressive neurodegenerative disease [10]. The AD pathological features are Neurofibrillary tangles and neuropathic plaques (shown in figure 1), which are related to the amyloid-beta peptide (Aβ) deposition in brain tissues and cytoskeletal alterations caused by hyperphosphorylation of microtubule-associated Tau protein in neurons, respectively [11]. A variety of conditions, including cancers, intoxication, infections, problems in the respiratory and circulatory systems, nutritional deficiencies, vitamin B12 insufficiency, and other conditions, can all progressively impair cognitive function. One such condition is the brain disease AD [10].

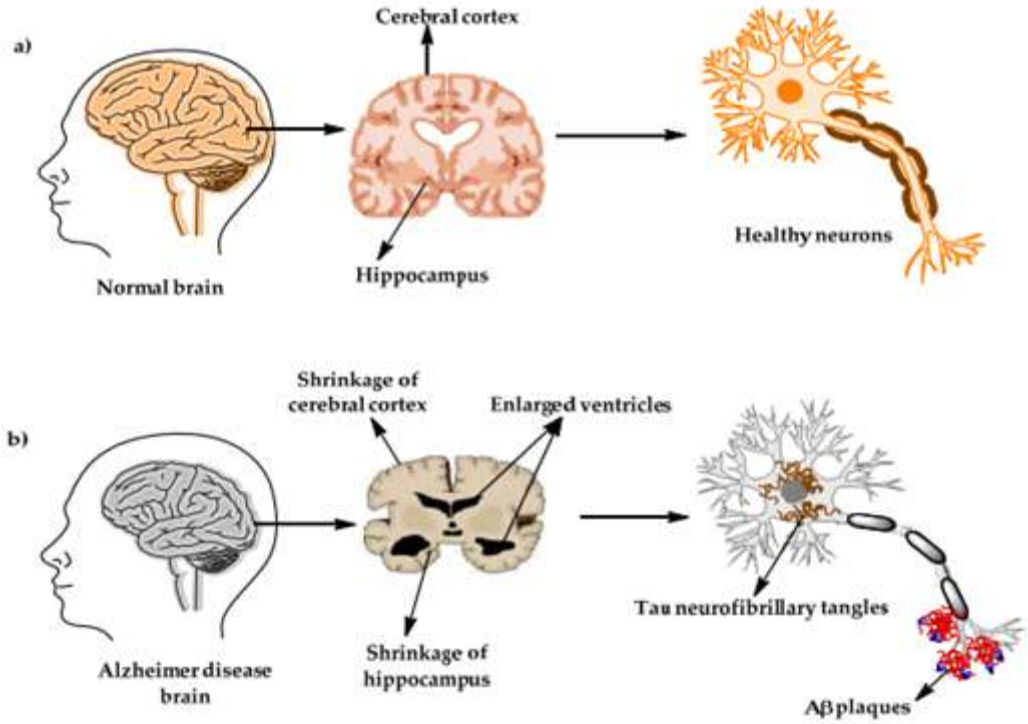

Figure 1: Comparision of normal brain and AD brain [10]

2.1.2 Epidemiology

AD is a rapidly growing public health and healthcare system threat, with far-reaching implications on societal and individual levels . The development and progression of dementia and AD are significantly influenced by vascular risk factors in middle-aged and older adults; A strong social network and active participation in mental, social, and physical activities, on the other hand, may help to delay the onset of the dementing disorder [12]. To evaluate the effectiveness of preventive measures for the best treatment of different disorder and vascular factors , along with active life style maintaining , against dementia and AD, three - dimensional (3d) organized community trials are required [12].

AD affects more than 35% of people aged 85 and older. It is the sixth most common cause of death in the United States and affects more than 5 million people. Slowly progressing memory loss and changes in higher intellectual function and cognitive ability are the clinical markers of AD. Amyloid buildup in senile plaques and cerebral blood vessels, together with neurofibrillary tangles in the neurons of the hippocampus and cerebral cortex are pathological indicators of AD in the brain. AD can only be accurately diagnosed through direct examination of the brain after death [13].

Individuals suffering from AD who are in the intermediate or terminal phases of the disease need round-the-clock care, which increases the social and financial load on family members and caregivers. The AD prevalence is predicted to threefold by 2050, with annual costs exceeding $50 billion, this burden will only grow. It is impossible to accurately predict the disease before symptoms appear, and preventive measures won't be available until the mechanism of AD is thoroughly understood [13].

Most of the fundamental causes of AD are still unclear. The environmental risk factors contain lower educational attainment, physical trauma (usually a head injury), and dietary and lifestyle choices that frequently lead to cardiovascular disease, type II diabetes, obesity, and high-fat diets. Although AD affects people of all races and ethnicities, it is still unclear if there are substantial differences in incidence and prevalence. In the experiencing of AD women's rates are higher than men's. The largest risk factor of them all is likely age, with prevalence rising quickly between 65 and 85. Up to 35% of people after the age of 85 will have AD [13].

2.1.3. Etiology

Dominantly inherited familial AD (FAD) can be a result by mutations in the APP genes, presenilin 1 (PSEN1), or presenilin 2 (PSEN2). These uncommon familial variants account for less than 1% of all cases of Alzheimer's disease. FAD typically appears at the age of 46.2 years, but it can appear as early as 20 years old. Individuals under the age of 65 are said to have early-onset Alzheimer's disease (EOAD), Although slightly more common than FAD cases, it accounts for less than 5% of all AD cases with a pathology diagnosis. The course and presentation of EOAD are frequently aggressive. By the age of 65, the majority of these patients have dementia. Despite the discovery of genetic risk factors, most notably the apolipoprotein E gene (APOE), The other more common late-onset Alzheimer's disease (LOAD), is still thought to be sporadic. The highest risk factors for developing AD are age, a first-degree relative's family history, and the APOE4 genotype [14].

The specific causes of AD have not yet been discovered. The disease's most common occurrence is LOAD, typically affects people more than 65. Several risk factors have been identified, including female gender, age, previous head injury, sleep disorders, low educational and occupational attainment, estrogen replacement therapy, and hypertension. Furthermore, studies suggest that the apolipoprotein E 4 gene allele raises

the risk of developing LOAD. The other type, EOAD, a much more uncommon disease, affects people between the ages of thirty and sixty-five. what causes the problem is the genetically mutated Amyloid Precursor Protein (APP), (PS1), and (PS2), which are all γ-secretase subunits. Besides the risk factors mentioned above, cardiovascular diseases, cerebrovascular diseases, diabetes mellitus, overweight and obesity, elevated cholesterol levels, smoking, excessive alcohol consumption, a diet high in saturated fats, and depression have been identified as potential risk factors for AD [15].

Senile plaques and neurofibrillary tangles appear to be the predominant neuropathological signs of AD [16]. As the disease advances, the senile plaques appear to start to form in brain regions connected to cognition before spreading to other cortical regions. The Aβ, a portion of the amyloid precursor protein, is one of the substances that makes up the senile plaques (APP) [16]. Aβ peptide is produced from APP by two simultaneous cleavage events: one end of the peptide is produced by the secretase's proteolytic activity, and the other end is produced by the secretase's proteolytic activity as well. There seem to be two different types of Aβ: Aβ40, which is shorter, and Aβ42, which is longer. Aβ42 appears to be deposited first and may play a part in sparking the processes that eventually result in amyloid deposition. Even while there is growing evidence that the malfunction in the metabolism of APP, which leads to a rise in the insoluble Aβ, is what causes AD, it is still unclear whether senile plaques are the disease's cause or a byproduct. Aβ seems to be toxic to neurons, either directly or by indirect effects such as inflammation or an increase in free radical generation. Another characteristic of AD is the buildup of neurofibrillary tangles in neurons. Tau protein, a protein involved in the production of microtubules, is chemically changed (abnormally folded and phosphorylated) and is the main component of neurofibrillary tangles. The amount of tau tangles in the brain increases with the severity of the disease; the more advanced the stage of the disease, the more tangles there are. No cases of AD caused by mutations in the tau gene on chromosome 174 have been recorded, even though neurofibrillary tangles are present in AD; nonetheless, frontotemporal dementias with parkinsonism have been discovered in some families with that mutation. Recent studies confirm the finding that the tau change follows A buildup in patients with AD [16].

2.1.4. AD Pathogenesis

AD is a very complicated and advanced neurodegenerative disease [17]. In the brain the accumulation of the aggregated proteins tau and Aβ causes intracellular NFTs, and extracellular neuritic plaques which are associated with AD. Neuronal loss, synaptic abnormalities, cognitive decline, and mental problems are all symptoms of AD that worsen as the condition progresses. This is in addition to Aβ and tau pathology. Aβ, again a proteolytic byproduct of the amyloid precursor protein (APP), is the main component of neuritic plaques. Tau, a microtubule-associated protein that has been hyperphosphorylated, is converted into NFTs. Neuroinflammation and neuronal death caused by Aβ and tau have a role in the causes of AD. AD is a complex, multifactorial disorder. A cholinergic theory, a tau hypothesis, a glutamate dysfunction hypothesis, an amyloid cascade hypothesis, an inflammatory hypothesis, and a mitochondrial cascade hypothesis have all been proposed to explain the pathologic process of AD. These theories can only explain a section of the disease, and the pathogenesis of AD remains a mystery. Because neurodegeneration is the fundamental cause of cognitive impairment in AD, neurotrophic factors such as brain-derived neurotrophic factor (BDNF) has been shown to slow the progression of neurodegeneration and could be a viable option for AD intervention [18].

AD pathogenesis is a complicated process involving neither environmental and genetic factors . Present treatments may ease symptoms by reducing the rate of cognitive decline and temporarily improving symptoms. According to the results of clinical trials, cholinesterase inhibitors do not lower the risk of or delay the onset of AD in people with mild cognitive impairment. The physiological and pathophysiological conditions connected to abnormal tau protein hyperphosphorylation are still incompletely understood. Therefore, various efforts are made to develop effective treatment strategies for reducing Aβ production or increasing Aβ clearance [19].

2.1.5. AD risk factors

Numerous risk factors (fig 2), such as those relating to ageing, genes, metals, traumatic brain injury (TBI), nutrition, vascular factors, the immunological system, and exposure to infectious agents, have been associated with AD [20]. Except for a small number of extremely uncommon EO-FAD cases, AD is a multifactorial disorder in which the interaction of internal and external risk factors affects a variety of common processes, including oxygen free radical production, gene regulation, and allostasis. The main cause

of AD is an acceleration of ageing as a result of these processes. To slow the rate of ageing and the risk of AD, lifestyle changes that reduce the influence of these processes are a viable method [20].

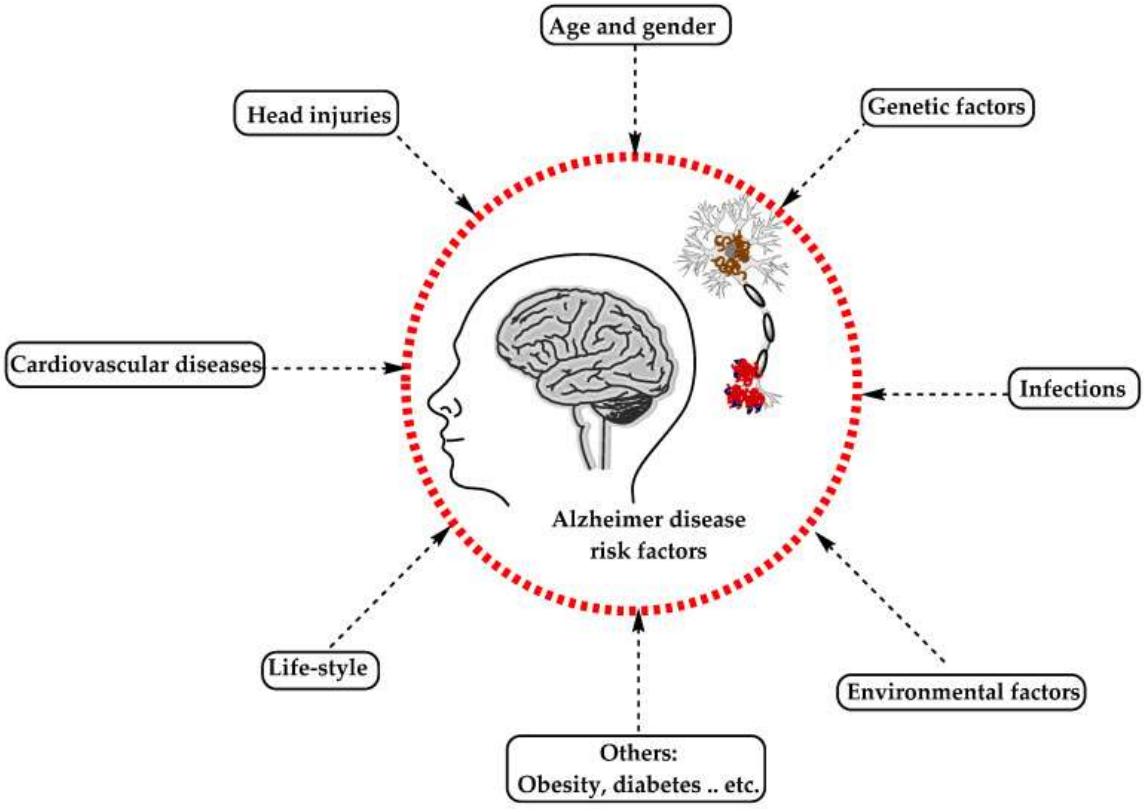

Figure 2: AD risk factor [10]

2.1.6. AD hypothesis

Many hypotheses on the pathophysiology of AD have been introduced during the past few decades [19]. It is thought that two of these are the primary cause: Some people think that cholinergic dysfunction is a significant AD risk factor. whereas some believe that changes in the synthesis and processing of amyloid β-protein are the primary cause of the disease. However, there is currently no approved explanation for describing the pathophysiology of AD [10].

2.1.6.1. Cholinergic hypothesis

According to cholinergic dysfunction, it is the first theory [19]. The enzyme choline acetyltransferase (ChAT), which is responsible for acetylcholine (ACh) production, was

linked with neocortical and presynaptic cholinergic deficiencies in the 1970s . Because ACh plays an important role in brain performance, the cholinergic hypothesis of AD was proposed. The vesicular acetylcholine transporter (VAChT) and the ChAT enzyme produce ACh in the cholinergic neurons cytoplasm and transport it to the synaptic vesicles, respectively. ACh plays a number of physiological roles in the brain, including attention, memory, sensory information, learning, and more essential functions. It was discovered that degeneration of the cholinergic neurons occurs in AD and causes changes in memory loss and cognitive function. It is believed that Aβ reduces choline absorption, impairs cholinergic neurotransmission, and releases Ach. [10].

consequently, the cholinergic hypothesis is defined by three ideas: decreased levels of presynaptic cholinergic markers in the cerebral cortex, as well as severe neurodegeneration of the nucleus basalis of Meynert (NBM)the source of cortical cholinergic innervation, and the role of cholinergic antagonists in memory decline as opposed to agonists, which have the opposite effect [10].

2.1.6.2. Amyloid cascade hypothesis

Discussions of the pathogenesis of AD have been dominated for more than 25 years by the amyloid hypothesis, which also known as the amyloid cascade hypothesis, the Aβ hypothesis, and other names. In healthy people, Aβ is excised from APP by β - γ and secretase and released outside the cell, where it is rapidly degraded or removed. Moreover, in elderly people or those suffering from a disease, the metabolic ability to degrade Aβ is reduced, and Aβ peptides may accumulate. The following are significant components of the accumulated Aβ: Aβ40 and Aβ42 (more hydrophobic than Aβ40), which contain 40 and 42 amino acid residues, respectively. An increase in the level of Aβ42 or the ratio of Aβ42 causes the formation of Aβ amyloid fibrils, and the accumulated Aβ amyloid fibrils create senile plaque, which causes neurotoxicity and the induction of tau pathology, which results in the degeneration of neurons. The APP gene is on chromosome 21, and the identification of APP genetic mutations in EOAD appeared to support the amyloid hypothesis. Near β-secretase or γ-secretase cleavage sites, these pathogenic APP mutations are associated with an increase in Aβ 42 production and/or a change in the ratio of Aβ 42 formation [21].

In a summary, according to the amyloid cascade theory, neurodegeneration in AD is due to the abnormal accumulation of Aβ plaques in different regions of the brain [19]. According to the amyloid hypothesis, the formation of neurofibrillary tangles through the use of tau protein results in neuronal dysfunction and death of cells in AD, and the aggregation of Aβ plaques serves as a pathogenic trigger for a cascade involving neuritic damage [19].

2.1.6.3. Tau protein hypothesis

Tau is a protein associated with microtubules that regulates the stability of tubulin assemblies. In humans, the tau gene is located on chromosome 17. As a result of mRNA alternative splicing, six tau isoforms are expressed in the adult human brain, Including or excluding exons 2, 3, and 10. Exon 10 contains the microtubule-binding region. Exon 10 insertion produces 4-repeat (4R) tau isoforms, while exon 10 deletion produces 3-repeat (3R) tau isoforms. The human brain reflects both 3R and 4R tau isoforms, which are primarily found in the axons of neurons under normal physiological conditions. The tau hypothesis states that tau is the main AD-causing substance. Hyperphosphorylated 3R and 4R tau is found in the pathological inclusions of AD brains. According to the findings, tau abnormalities cause tau accumulation and neurodegeneration. In additional sporadic tauopathies, such as AD [21].

2.2. AD treatment:

Finding new treatments for AD has become a top priority [22]. Drug therapies are now approved only for these types of dementia: AD, Parkinson's disease dementia, and dementia with Lewy bodies. They plan to treat biochemical abnormalities caused by neuronal loss, but they do not affect the underlying neuropathology or its progression [9].

2.2.1. Psychosocial treatment

Environmental manipulation, family support, and the prevention of other medical comorbidities can all help AD patients improve their function. A patient's environment needs to be adjusted to keep AD patients in their homes for the longest amount of time possible. Written daily reminders might be useful in carrying out everyday tasks. It's crucial to have prominent clocks, calendars, and windows. Activities performed by patients should not alter much. Maintaining proper nutrition, exercise, hydration, and sanitation is

crucial. Family support is essential because members are predisposed to depression, anxiety disorders, and insomnia [16].

2.2.2. Treatment of cognitive disturbance

The drugs were implemented according to the cholinergic and glutamatergic hypotheses. The cholinergic hypothesis is the first theory put forth to explain the etiology and mechanisms behind neuropsychiatric symptoms connected to AD [23], and it assumes that the loss of ACh neurons and enzymatic functions in the amygdala, basal forebrain, cortex, and hippocampal regions of the central nervous system are determining factors [23]. Unfortunately, these drugs have been linked to a number of side effects, including nausea, vomiting, anorexia, and insomnia, due to their nonselective action on a variety of organ tissues both centrally and peripherally [24].

Also the initial and most popular class of drugs approved by the US FDA (Food and Drug Administration) for the treatment of cognitive disturbances caused by AD is reversible acetylcholinesterase inhibitors [25].

Donepezil, rivastigmine, galantamine, and tacrine are examples of drugs in this class. AchEIs are believed to enhance brain's overall cognitive functioning by elevating the concentration of neurotransmitters at cholinergic synapses [25].

Memantine, a non-competitive, low-affinity NMDA receptor antagonist, is the only other medication approved by the FDA for treating the cognitive symptoms of Alzheimer's disease. Memantine is said to work by preventing excitotoxicity, despite the fact that this putative mechanism has not been demonstrated in humans (the death of neurons caused by excessive glutamate stimulation). Memantine has the potential to improve cognitive function by modifying NMDA receptors, which hone neural signals and decrease background noise. Nevertheless, research suggests that the therapeutic consequences of memantine could be due, at minimum in part, to its role as a dopaminergic agonist. Although acetylcholinesterase inhibitors are frequently prescribed in conjunction with memantine, which is only approved for the treatment of moderate-to-severe AD [25].

Drug name and molecular formula	Absorption	Distribution	Metabolism	Excretion
Donepezil [57] ($C_{24}H_{29}NO_3.HCl$)	good gastrointestinal absorption	about 95% plasma protein bound	partial metabolism through the cytochrome p450 superfamily of enzymes	mainly excreted via urine and stools [58]
Galantamine [59] ($C_{17}H_{21}NO_3.HBr$)	well absorbed from gastrointestinal tract	about 20% plasma protein bound[60]	partially metabolized by Cytochrome p450 isoenzymes	mainly excreted by urine [61]
Rivastigmine [62] ($C_{14}H_{22}N_2O_2$)	high GI absorption	about 20% plasma protein bound	primarily metabolized by cholinesterase	mainly excreted by urine [63]
Memantine [64] ($C_{12}H_{21}N.HCl$)	high GI absorption	about 45% bound to plasma proteins	about 45% bound to plasma proteins	mainly excreted by kidneys unchanged [1]
Namzaric® (available as combinations of 7 mg, 14 mg, 21 mg or 28 mg memantine with 10 mg of donepezil) [65]	combination of pharmacokinetic properties shown by memantine and donepezil			

Figure 3: The drugs used to treat AD currently [15].

Cholinesterase inhibitors

The cholinergic hypothesis supposes that AD results from a decrease in ACh production. An example of the treatment strategies which improve neural cell and cognitive performance is the increase cholinergic levels by inhibiting AChE. ACh degradation in synapses is prevented by AChEIs, leading to continuous ACh accumulation and cholinergic receptor activation. Tacrine (tetrahydroaminoacridine), which works by increasing ACh in muscarinic neurons, was the first FDA-approved cholinesterase inhibitor drug for the treatment of AD, but it was removed from the market immediately because of a high prevalence of side effects such as hepatotoxicity and a lack of benefits observed in many trials, the drug should be discontinued [10].

Galantamine, donepezil, and rivastigmine are the three cholinesterase inhibitors that are frequently used to treat AD. Donepezil is given as a tablet or dispersible tablet, Rivastigmine is offered in the form of a transdermal patch, a capsule, or a liquid, while galantamine comes in the form of a capsule. In comparison to the placebo, all cholinesterase inhibitors show a little improvement in cognition when used at safe doses [9].

Another potential treatment for AD involves raising choline reuptake and, consequently, the generation of ACh at presynaptic terminals. This can be managed to

accomplish by concentrating on the choline transporter (CHT1), which provides choline for the creation of ACh. The future of AD treatment may lie in creating medications that can increase CHT1 at the plasma membrane [10].

Galantamine: An inhibitor that is both competitive and reversible could bind to the allosteric site of nAChRs. Numerous systematic reviews have speculated on galantamine's clinical relevance in the treatment of mild-to-moderate AD [23].

Rivastigmine: It is a slow-reversible carbamate, and inhibits butyrylcholinesterase (BuChE) and acetylcholinesterase (AChE), which are predominantly prominent in the cerebrospinal fluid (CSF) of the AD brains [23].

Donepezil: A noncompetitive and reversible AChE inhibitor also known as donepezil hydrochloride, it has been approved since 1996 for the symptomatic treatment of mild-to-moderate AD [23].

Is considered the most suitable medication for treating AD. It is a second-generation AChEI and a derivative of indanone benzyl piperidine. A larger concentration of ACh is present at the synapses as a result of donepezil's reversible binding to acetylcholinesterase and inhibition of ACh hydrolysis. The medication is very well, with only minor and transient cholinergic side effects affecting the nervous and gastrointestinal systems. It is noteworthy that donepezil is used to treat AD symptoms such as improving cognition and behavior while not affecting AD progression [10].

Memantine: In comparison to other cholinesterase inhibitors, memantine received FDA approval in 2003 to primarily target moderate-to-severe Alzheimer's disease. By preferentially preventing the activation of NMDA ion channels, memantine, a type of uncompetitive NMDA receptor antagonist, produces antagonistic effects that can be achieved by controlling dosage intake [23].

The release of apoptosis-inducing substances may be blocked by decreasing the NMDA ion channels' permeability to Ca2+, which could mediate normal neuronal activity [23].

Unexpectedly, memantine clinical studies to treat moderate to severe AD did not live up to expectations because the tested medicine just postponed the onset of cognitive

decline. The majority of research methods compared memantine's therapeutic response as a monotherapy vs as part of a combination therapy with other ChEIs. For example, the results of assessments of cognitive function, behavioral disturbances, activities of daily living, and overall function showed therapeutic benefits when memantine was used alone [23].

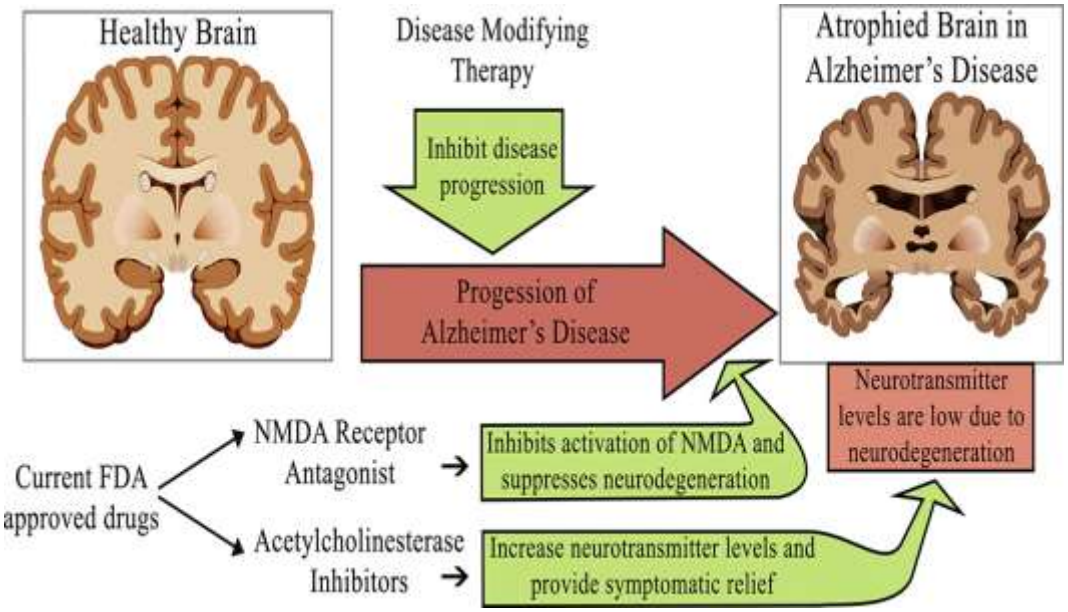

Figure 4: The effect of the current approved drugs on AD brains [15]

2.3.Mice as an animal model of AD

One of the most important research methods for developing new AD treatments in mouse models. Despite this, many possible disease-modifying therapies that have failed in human trials in recent years claimed to show some promise in mice models. So this is the best time to examine the recent AD mice models and how they fit into the translational process [26].

Mice are a common type of animal that is used as a model for studying pathogenesis and developing treatment methods for AD [27]. Several transgenic mice models developed over the years to express various pathological changes associated with AD, such as amyloid pathology, cerebral amyloid angiopathy, tau pathologies, synaptic loss, dystrophic neurites, reactive gliosis, as well as deficits in synaptic plasticity, memory and learning [27]. These mouse models vary in their degree of exhibiting the mentioned traits,

and numerous studies that are currently accessible have independently demonstrated a considerable variance in the pathophysiology of AD in different mice models [27].

2.4. Scopolamine induced amnesia-like AD in mice

The model of scopolamine has been widely employed to to understand the cholinergic system and how it affects cognition in aging and AD as well as to assess potential memory loss treatments [28].

Scopolamine easily crosses the blood-brain barrier, and blocking muscarinic receptors in the central nervous system is thought to cause a cholinergic deficit that affects memory. Scopolamine administration has frequently been employed as a dementia model since it is believed that an age-related reduction in cognitive function is mostly attributable to a decline in cholinergic neurotransmission. As a result, scopolamine has been widely employed in preclinical and clinical trials of therapies for cognitive impairment [29].

Scopolamine, a general muscarinic cholinergic blocker, is well recognized to cause AD-like amnesia in animal models by causing significant behavioral and cognitive impairments and metabolic abnormalities such as oxidative stress and neuro-inflammation [30].

2.5.Memory tasks, assessment of Mice memory

2.5.1. Background

Many tasks have been created to evaluate problems associated with memory and learning impairments in mice models. These tasks are based on the spontaneous tendency of an animal to spend more time examining new objects than familiar ones. As a result, tasks can be used to evaluate the performance of learning and recognition memory, with each task designed to replicate a specific memory trace; WM, reference memory, spatial memory, and so on [31].

Several behavioral test models for assessing spatial learning and memory have been created, and several mouse strains have been examined in different places. However, the Morris water maze (MWM) is commonly and primarily employed as a single paradigm [32].

2.5.2. Morris water maze

The MWM has been investigated and used in neuropharmacology, neuropathological, and physiological research, including ageing, to test spatial learning and memory.

MWM performance varied between mouse strains, highlighting the need of using more than one mouse strain for MWM testing [32].

The (MWM) is a well-known test of spatial memory that is now considered to be a classic, and it has long been understood that the success of this test depends on the health of the hippocampal neuronal cell [33].

2.6. MEMORY DEFINITION and TYPES

Memory has been defined as our brain's capability to store, retain, and retrieve information [34].

2.6.1. Physiology of memory

There are two types of memory. Explicit memory which associated with awareness and which dependent on the hippocampus and other medial temporal lobe in the brain area. It is mostly used to learn information about places, people, and things. It is also subdivided into semantic and episodic memory. Semantic memory focuses on words, rules, and language, while episodic memory focuses on events. Implicit memory, also known as non-declarative memory, is a type of memory that does not require conscious processing and is not typically processed in the hippocampus. When some explicit memories are learned frequently and become a reflex, they will be transformed into implicit memories. Explicit memory and many forms of implicit memory involve short-term and long-term memory. Planning actions requires the use of working memory, a type of short-term memory that stores information for incredibly brief periods of time. Memory is preserved by varying the force of specific synaptic connections in the brain. In AD, the damage of hippocampal neurons impairs the formation of new memories. Semantic memory is the most severely affected by Alzheimer's disease, with defects occurring years before diagnosis. Language problems and poor item naming ability become more popular in Alzheimer's patients as the disease progresses [15].

2.6.2. Memory Formation

Mammals' memories can be divided into at least two phases: a short-term memory (STM) phase, which lasts for between a few minutes and up to three hours (a protein- and mRNA synthesis-independent phase), and a longer-term memory phase long-term memory (LTM), which can last for days, weeks, or even longer periods (a protein- and mRNA synthesis-dependent phase) [35].

The ability of the brain to collect information from the environment and store it in order to change behaviour is its most remarkable feature. Memory is a process that occurs in real time. It is divided into at least four stages or phases: acquisition (encoding), consolidation (formation), storage, and retrieval. Extinction and reconsolidation are two significant outcomes of retrieval [35].

Short-term memory (STM): Short-term storage, also known as primary or active memory, refers to a number of memory systems that are involved in the recall of factual information (memory chunks) for a brief amount of time (usually up to 30 seconds) [36].

Long-term memory (LTM): Long-term memory relates to what can be remembered from the past when the information to be learnt no longer fills the present stream of thought, either because immediate memory capacity has been exhausted or attention has been redirected away from the memoranda [37].

LTM can hold an infinite amount of data. However, the two memories differ not only in terms of the "time" variable but also in terms of functionality. Despite this, the two systems are linked [36].

These memories were previously categorized according to their duration and storing capacity. The STM's capacity is limited in terms of the amount and time of information it can store. LTM, on the other hand, appears to have an infinite capacity and can continue for years. The functional disparities between memory storage systems, as well as the precise methods by which memories migrate from STM to LTM, remain contentious issues. Although the STM is most likely a substructure of the LTM, that is a type of long-term active storage, it appears more relevant to test the processes of transition from transitory memory to permanent memory rather than seeking a physical division [36].

The data gathered in the LTM storage, on the other hand, contains of memories for performing skills or actions as well as memories of factual information, rules, ideas, and events. Semantic and episodic memories comprise declarative memory [36].

According to neurofunctional definitions, the LTM differs from the STM in that a number of events are required to completely fix the engram(s) before it can be said that it has been fixed. As a result of the development of neural networks, this effect manifests as the phenomena of neurofunctional such as long-term potentiation (LTP), whichever is an increase in the strength of neural transmission brought on by the fortification of synaptic connections. In the case of declarative memories, this process necessitates the expression of genes and the new protein synthesis, and it is associated with long-term structural changes in the synapses (synaptic consolidation) of the brain areas involved, such as the hippocampus [36].

Long-term potentiation: LTP is the most well-known and widely accepted theory of memory formation in the nervous system. This popularity is most likely due to the lack of other convincing models, as well as the fact that such use-dependent changes in synaptic response fit theoretical predictions so well**.**

LTP was discovered for the first time in the hippocampus, a structure known to be involved in learning processes It was created in a rabbit's perforant track and lobed by inserting electrodes. As a result, a new technique for studying the cellular mechanisms of synaptic plasticity has been developed. such as LTP, that allows direct and controlled access to selective neurons, was developed [38].

Working memory: WM refers to the system or systems thought to be necessary for maintaining information in memory while carrying out difficult tasks like reasoning, comprehension, and learning. In the past 30 years, WM has gained popularity, moving from its roots in cognitive psychology to numerous branches of cognitive science and neuroscience. It has been used in a variety of fields, including education, psychiatry, and paleoanthropology. [39].

WM and short-term memory are not different. WM is a term used to describe memory, which is used to plan and carry out behavior. Working memory is commonly regarded as a collection of interconnected components. Some people even combine

working memory with long-term memory, which makes a significant contribution to lowering the burden of working memory by classifying and grouping information in working memory into fewer units [34].

Spatial memory: Spatial learning and memory were evaluated by using the MWM test, which included hidden platform acquisition and probe trial testing [40].

The Hippocampal Network's Role: LTP maintenance is monitored by hippocampal neurogenesis. The hippocampus appears to have limited capacity, as it acquires information automatically and quickly without retaining it for long periods. Independent of hippocampus activity, initially accessible information is permanently stored in other brain regions over time (in the cortex). The reactivation (replay) of neural activity configurations is a critical mechanism of this transfer [36].

Memory processes involve other brain areas; for example, motor skill learning is linked to cerebellar and brainstem nuclei activation. Furthermore, while learning perceptive activities (improvements in the processing of perceptual stimuli required in daily activities such as understanding spoken and written language) involves the basal ganglia and sensory and associative cortices at first, learning cognitive skills (related to problem-solving) involves the medial temporal lobes at first [36].

Neurotrophins

The neurotrophins are a group of secreted proteins that potently regulate different neural responses. Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin 4/5 (NT4/5) are all members of the group. Neurotrophins, secreted by target neurons, govern the type and the number of afferent synapses by supporting the survival of distinct neuronal subpopulations. Neurotrophins also affect differentiation, cell fate decisions, and neurite shape. Neurotrophins influence the developing brain synaptic connections and perhaps synaptic strength in mature animals. Neurotrophins elicit impressively diverse but specialized responses and are critical regulators of nervous system structure and function throughout development and maturity [41].

1. **BDNF:** Brain-derived neurotrophic factor (BDNF) is a neurotrophic factor that promotes neuron differentiation, maturation, and survival in the nervous systemIt also has a neuroprotective effect in cases of glutamatergic stimulation, cerebral ischemia, hypoglycemia, and neurotoxicity. BDNF protein and mRNA have been found in most brain locations, including the olfactory bulb, cortex, hippocampus, basal forebrain, mesencephalon, hypothalamus, brainstem, and spinal cord, and they promote and regulate the development of new neurons from neural stem cells (neurogenesis). In several neurodegenerative diseases, including Parkinson's disease (PD), multiple sclerosis (MS), and Huntington's disease, BDNF levels are reduced. BDNF has a significant impact on energy homeostasis in addition to having neuroprotective properties. Body weight is decreased, and energy intake is suppressed by peripheral or intracerebroventricular (ICV) injection of BDNF [42].

   BDNF, contribute significantly to the maintenance of a functional neural system in both healthy and sick conditions. The conversion of pro-BDNF to mBDNF is required for synaptic plasticity, neuronal survival, and neural growth under physiological conditions. In pathological conditions such as AD, BDNF is linked to apoptosis, tau phosphorylation, Aβ accumulation, and a neuroinflammatory response. Reduced BDNF levels at synapses are linked to memory deficits caused by AD. Aβ has been shown to impair BDNF processing both activity-dependent and activity-independently. While Aβ inhibits activity-dependent BDNF transcription by impairing CREB phosphorylation, Aβ induced decreases in basal BDNF levels are linked to decreased CREB transcription [18].

2. **CREB:** is a transcription factor found inside the nucleus that belongs to the leucine zipper transcription factor family, which includes CREB, CRE modulator (CREM), and activating transcription factor-1 (ATF-1). Because of its ability to bind to DNA and thus control gene expression, CREB was expected to play an important role in the LTM that requires structural modifications. CREB is essential for neurogenesis during the several stages of neural cell development (such as proliferation, differentiation, and survival), as well as for adult brain neuronal plasticity, learning, and memory. It was discovered that CREB is crucial for the formation of synapses and the expression of various neurotrophins, such as BDNF [41].
3. **NLRP3:** Neuroinflammation is thought to be involved in AD pathogenesis. The innate immune system's large multiprotein inflammasome is capable of inducing both

inflammatory responses and pyroptosis, which causes neurodegeneration. Among the various types of inflammasomes, the NLRP3 inflammasome has received the most attention in neurodegenerative diseases, particularly Alzheimer's disease. Recent research indicates that activation of the NOD-like receptor pyrin domain containing 3 (NLRP3) inflammasome can regulate the formation of IL-1 and IL-18 and is linked to the etiology of neurological diseases. When the NLRP3 inflammasome is activated, caspase-1-mediated IL-1 and IL-18 are produced in microglia cells, where neuroinflammation plays a role in the development and progression of AD. NLRP3 can also detect a wide range of inflammatory crystals and aggregated proteins, including Aβ, and is highly expressed in microglia cells, which have been linked to a number of chronic inflammatory disorders. In the postmortem brains of Alzheimer's disease patients, researchers discovered NLRP3 inflammasome overexpression. Additionally, it appears that inflammasome complexes are essential for the spread of Aβ pathology within and among different brain regions. Because of this, the NLRP3 inflammasome signalling pathway is important in the pathogenesis of Alzheimer's disease, indicating that it could be a useful therapeutic target for AD [43].

3. MATERIALS and METHODS

3.1 Animals

Thirty Male BalbC Mice (8-10 weeks old) were obtained and used from Yeditepe University Experimental Animal Research Center. The mice were grown under standard environmental conditions; 12:12 light-dark cycle. Animals had access to standard laboratory chow and tap water. All mice exhibited both normal health conditions and behavior for being accepted in the study, else were excluded. In all the stages of this work, mice have been used and handled in a proper way that met all the requirements and instructions of the Laboratory Animals Ethics Committee of Yeditepe University.

3.2 Drug Preparation

Scopolamine was prepared and dissolved in a 0.9% saline solution for an appropriate final concentration of 1 mg/ml. Memantine was prepared and dissolved in 0.9% saline solution for an appropriate final concentration of 5 mg/ml, and Donepezil was prepared and dissolved in PBS for an appropriate final concentration of 1 mg/ml. In the study, scopolamine and memantine were administered intraperitoneal (i. p.), and donepezil was administered subcutaneously (s.c.).

3.3.Creating Experimental Groups

This study followed the Turkish guidelines for the use and care of laboratory animals plus, the animal research studies following the Animal Research Ethics Committee of Yeditepe University were applied in the experimental protocol. Animal euthanasia and experiments on Bulb C were performed at the Yeditepe University Faculty of Medicine Experimental Research Center.

The experimental groups were created to determine the effect of various AD treatment strategies on working spatial memory (WSM) in the scopolamine-induced AD model in mice.

1. Control group: 6 mice were used; the animals in this group were fed Ad libitum till the end of the experiment.

2. Scopolamine group: 8 mice were used, and the animals in this group were injected with scopolamine (1 mg/kg/day, i.p.) for 15 days. Scopolamine was dissolved in saline.

3. AD + Memantine group: 8 mice were used, first, Alzheimer-like memory loss was stimulated by scopolamine injection for 15 days, then memantine (NMDA receptor antagonist) was injected (5 mg/kg/day, i.p) for 15 consecutive days.

4. AD + Donepezil group: 8 mice were used, first, Alzheimer-like memory loss was stimulated by scopolamine injection for 15 days, then, Donepezil was dissolved in PBS and injected into animals (1mg/kg/day) sc. for 15 days [44].

On the 16th day the Morris Water Maze test was performed, blood was taken out via cardiac puncture and plasma was separated, animals were decapitated, the brain was taken out, the hippocampus was dissected. Brain acetylcholinesterase enzyme (AChE) activity, NLRP3 inflammasome level, and brain oxidative stress levels were determined.

3.4. Behavioral test

3.4.1. Morris's water maze (MWM) test

Mice were subjected to behavioral tests. The MWM was used to assess the mice's spatial learning and memory. The maze is made up of a circular tank 90 cm in diameter and 45 cm in depth. The tank will be filled with white dye-dyed water to a height of 30 cm. (22 ‡ 1 °C). The platform wae white 6 cm diameter 29 cm height then placed in the center of one quadrant of the tank. The test lasted six days, with five training sessions and one probe session. Two training trials per day with the platform in the tank during the five subsequent training sessions, the mice were subjected to two training trials per day in the tank with the platform. Within 60 seconds, also they were trained to remember where the platform was hidden in the tank. When the mouse reached the platform within 60 s, it will be allowed to stay on the platform for 10 s. (If the mouse does not find the platform within

60 seconds, it is instructed to stay on the platform for 15 seconds). During training sessions, 3 minutes was the interval time between the trails and trails.

On day 6, the probe day the mouse was subjected to the trial without the platform in the pool also 60 s were givin to the mice. A video camera-based Ethovision system was used to record the time in the target quadrant, the swimming speed, and the distance.

3.5. Collection of Tissue Samples and Blood

The experiment was terminated by exsanguination. Blood samples were taken from the heart from the left ventricle using 19 gauge needles. About 5 ml was collected from each mouse, and then it was transferred immediately into a centrifuge tube containing EDTA and placed on ice. For all blood samples, centrifugation was performed at 2000 RPM for 20 minutes at +4 °. All samples were put on dry ice and then stored at -80^{o}C. The whole brain was removed, the hippocampus was isolated carefully and stored in a formalin solution.

3.6. Enzyme-Linked Immunosorbent Assay (ELISA)

NLRP3 in vitro SimpleStep ELISA® (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of NLRP3 protein in mouse cell and tissue extract.

The SimpleStep ELISA® employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB Development Solution is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm (Figure 5).

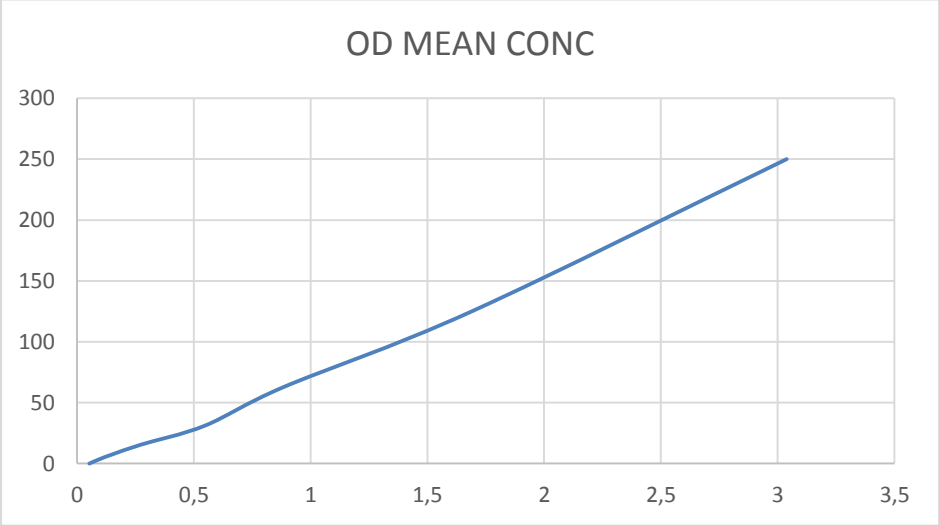

Figure 5: Mice NLRP3 (ng/ml), (O.D. 450 nm)

3.7. Statistical analysis

The conformity of the data to the normal distribution was evaluated with Kolmogorov-Smirnov and Shapiro-Wilk tests. Comparison of the data, non-parametrically, multiple comparisons were made with the Kruskal-Wallis test, and pairwise comparisons were made with the Mann-Whitney U test. Values were expressed as the mean ± standard error of the mean (SEM). A value of $p<0.05$ was considered statistically significant. Also for NLRP3 analysis Data were assembled in Microsoft Excel and statistical analyses were conducted in IBM SPSS version 22. The level of statistical significance was set at p-values < 0.050. One-way analysis of variance (ANOVA) followed by the Tukey's post-hoc test was used to compare the difference of the mean between the groups.

4. RESULTS

4.1. Memory results

4.1.1. Morris's water maze (MWM) analysis for spatial memory

Figures and tables (6-15) demonstrated the MWM test analysis for spatial memory for scopolamine, memantine, and donepezil. Results showed that there is no change in memory performance in the memantine and donepezil group that compered with control and scopolamine group.

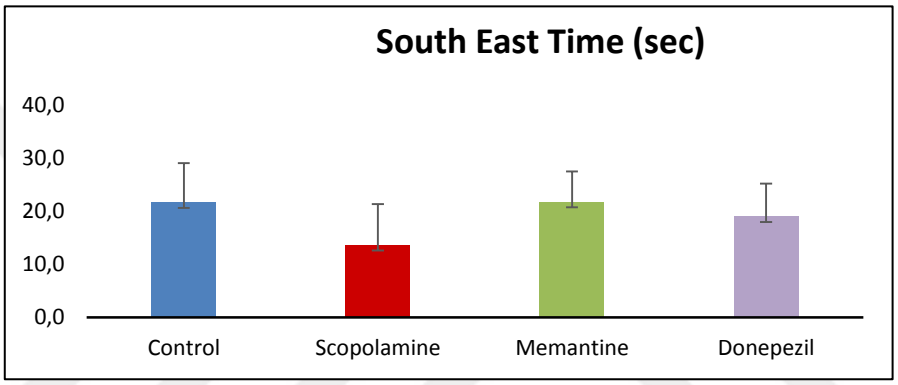

Figure 6: Morris's water maze (MWM) test, South East Time

SE (%)	Average	STD DEV
Control	37.8	12.9
Scopolamine	23.9	13.6
Memantine	39.7	10.7
Donepezil	34.3	11.3

Table 1: Morris's water maze (MWM) test, South East Time

Figure (6) evaluate the average time in second and table (1) evaluate the average time in percent in the probe day (day 6^{th}), that each mouse need to find the hidden platform place below the surface which was in the south east direction, which prove that scopolamine group found it with the lowest degree which mean that the mouse was forgetting.

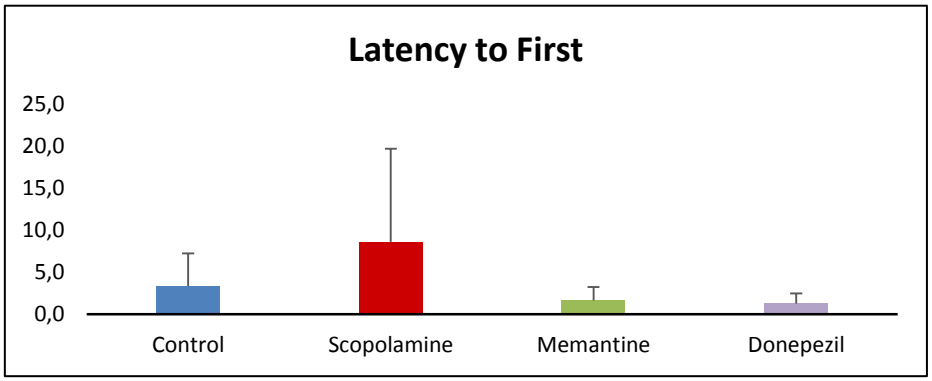

Figure 7: Morris's water maze (MWM) test, Latency to First Time.

Latency to first	Average	STD DEV
Control	3.4	3.9
Scopolamine	8.6	11.1
Memantine	1.7	1.6
Donepezil	1.3	1.2

Table 2: Morris's water maze (MWM) test, Latency to First Time.

Figure (7) and table (2), evaluate the average time in second for latency to first which is the time that required to find a hidden platform to escape from swimming in the water pool, for each mouse in the probe day, which shows that scopolamine group took the most time, while the memantine and donepezile groups took less time compered with control group.

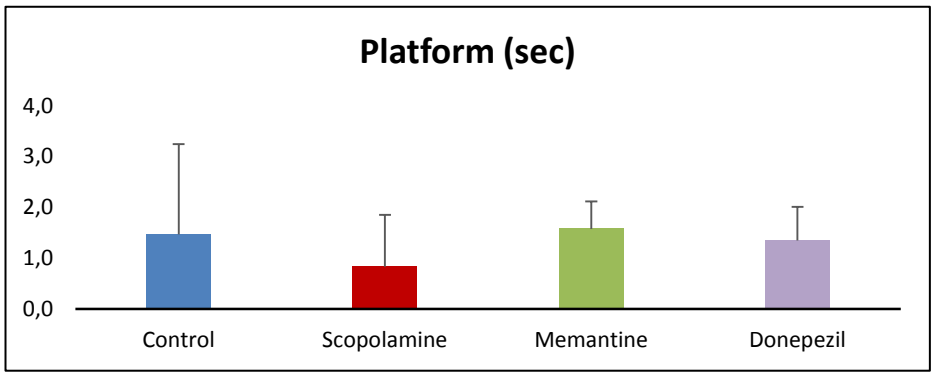

Figure 8: Morris's water maze (MWM) test, Platform Time

Platform (sec)	Average	STD DEV
Control	1.5	1.8
Scopolamine	0.8	1.0
Memantine	1.6	0.5
Donepezil	1.4	0.7

Table 3: Morris's water maze (MWM) test, Platform Time

Figure (8) and table (3) evaluate the average time in second for platform. Which evaluate the time that each mouse spent in the platform place, which shows that the scopolamine group took the less time in the platform comperd with the control, memantine and donepezil group.

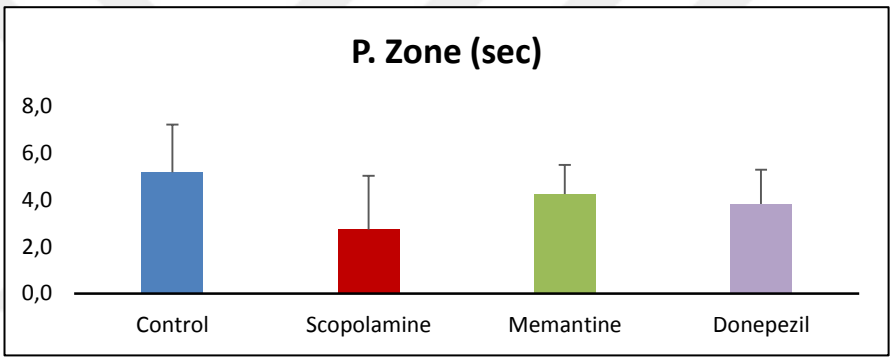

Figure 9: Morris's water maze (MWM) test, Platform Zone Time

P.Zone (sec)	Average	STD DEV
Control	5.2	2.0
Scopolamine	2.7	2.3
Memantine	4.3	1.2
Donepezil	3.8	1.5

Table 4: Morris's water maze (MWM) test, Platform Zone Time

Figure (9) and table (4) shows the platform zone time in seconed, which evaluate the time that each mouse spent in the zone around the platform, which shows that the scopolamine group took the less time around the platform comperd with the control, memantine and donepezil group.

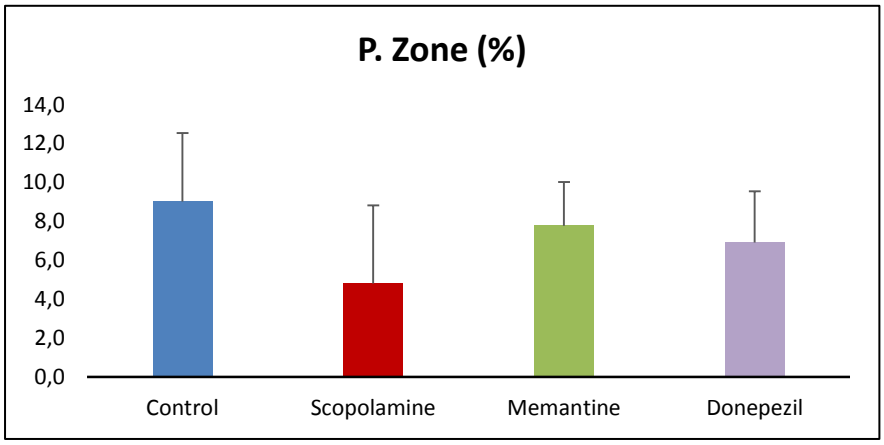

Figure 10: Morris's water maze (MWM) test, Platform Zone

P.Zone (%)	Average	STD DEV
Control	9.0	3.5
Scopolamine	4.8	4.0
Memantine	7.8	2.3
Donepezil	6.9	2.6

Table 5: Morris's water maze (MWM) test, Platform Zone

Figure (10) and table (5) shows the platform zone time in percent, which is the time that each mouse spent it in the area around the platform.

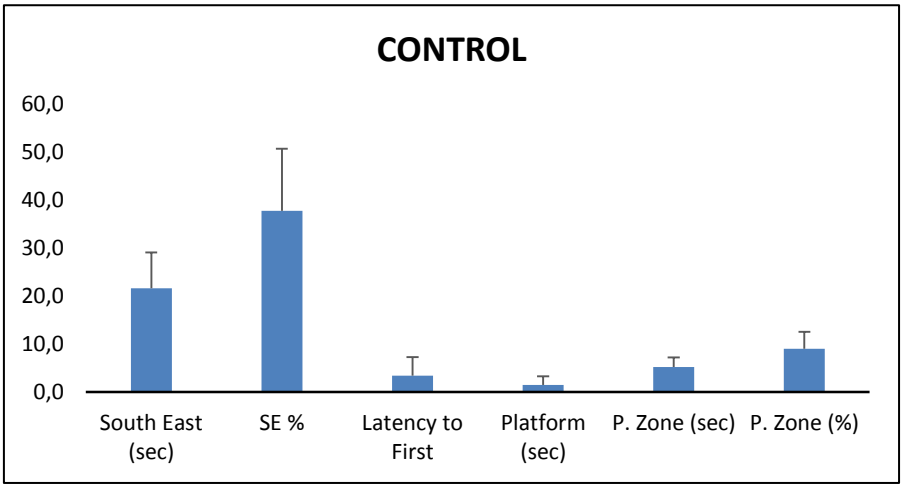

Figure 11: Morris's water maze (MWM) test, Control Group Data

Control	average	std.dev
South East (sec)	21.6	7.5
SE %	37.8	12.9
Latency to First	3.4	3.9
Platform (sec)	1.5	1.8
P. Zone (sec)	5.2	2.0
P. Zone (%)	9.0	3.5

Table 6: Morris's water maze (MWM) test, Control Group Data

Figure (11) and table (6) shows all values for the control group, all required data to study the spatial memory.

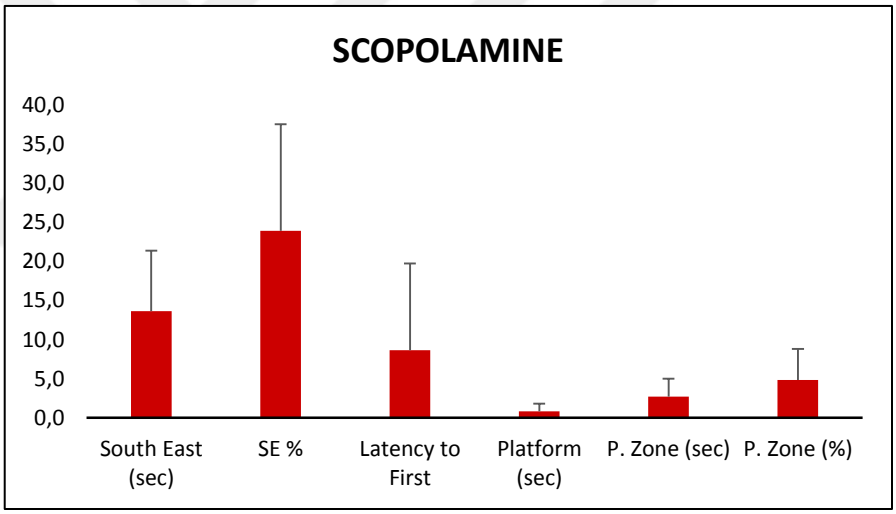

Figure 12: Morris's water maze (MWM) test, Scopolamine Group Data

Scopolamine	average	std.dev
South East (sec)	13.6	7.8
SE %	23.9	13.6
Latency to First	8.6	11.1
Platform (sec)	0.8	1.0
P. Zone (sec)	2.7	2.3
P. Zone (%)	4.8	4.0

Table 7: Morris's water maze (MWM) test, Scopolamine Group Data

Figure (12) and table (7) shows all values for the scopolamine group, all required data to study the spatial memory.

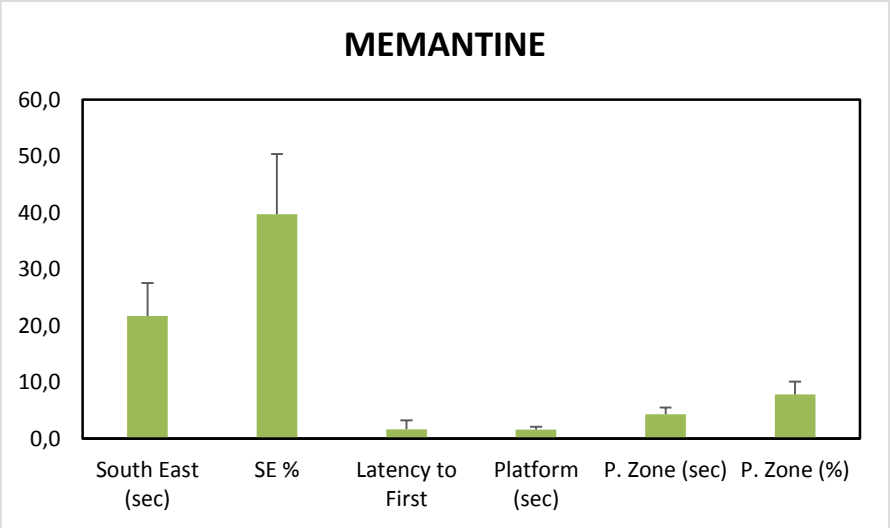

Figure 13: Morris's water maze (MWM) test, Memantine Group Data.

Memantine	average	std.dev
South East (sec)	21.7	5.8
SE %	39.7	10.7
Latency to First	1.7	1.6
Platform (sec)	1.6	0.5
P. Zone (sec)	4.3	1.2
P. Zone (%)	7.8	2.3

Table 8: Morris's water maze (MWM) test, Memantine Group Data.

Figure (13) and table (8) shows all values for the memantine group, all required data to study the spatial memory.

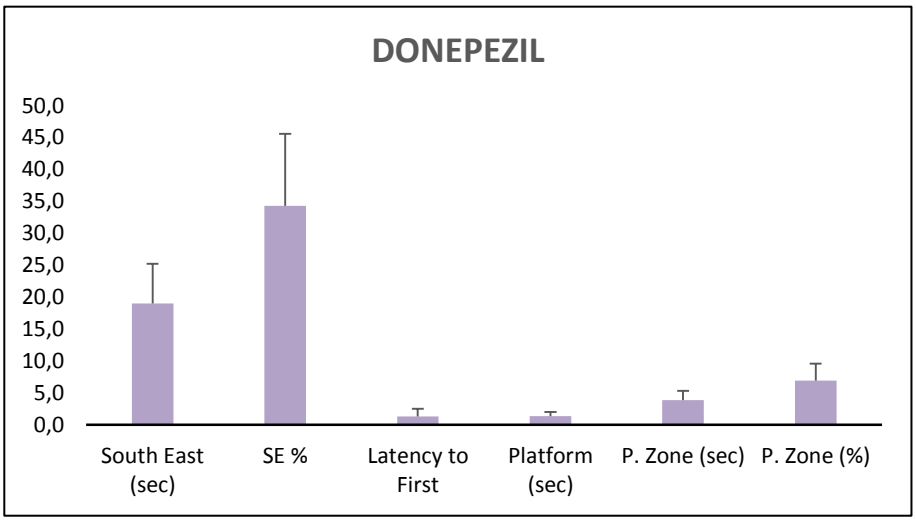

Figure 14: Morris's water maze (MWM) test, Donepezil Group Data.

Donepezil	average	std.dev
South East (sec)	19.0	6.2
SE %	34.3	11.3
Latency to First	1.3	1.2
Platform (sec)	1.4	0.7
P. Zone (sec)	3.8	1.5
P. Zone (%)	6.9	2.6

Table 9: Morris's water maze (MWM) test, Donepezil Group Data.

Figure (14) and table (9) shows all values for the donepezil group, all required data to study the spatial memory.

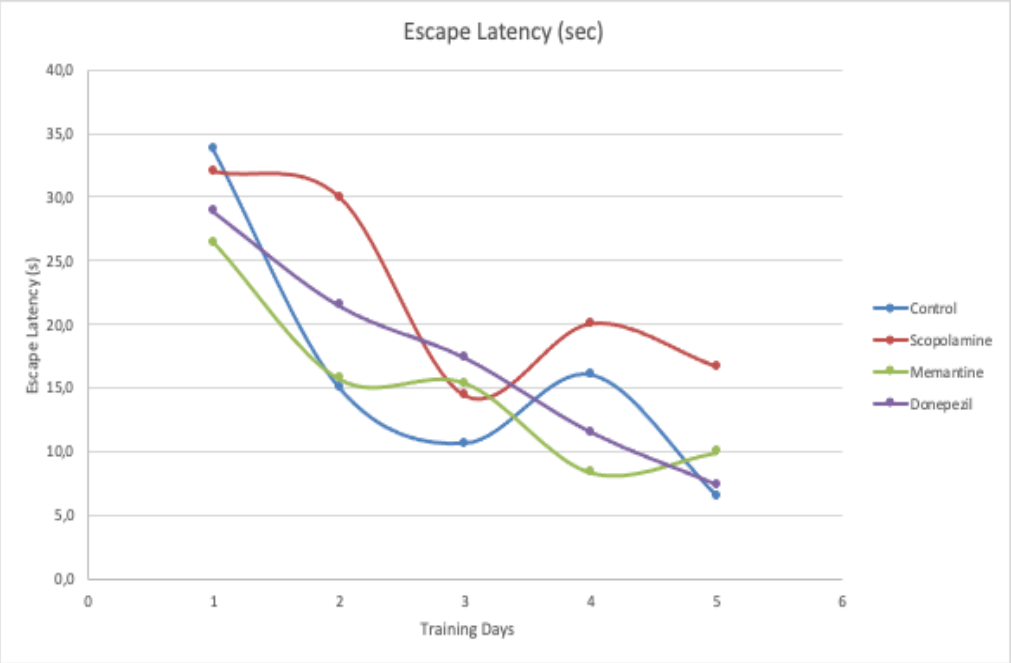

Figure 15: Morris's water maze (MWM) test, Escape Latency Time for Contol, Scopolamine, Memantine, and Donepezil groups In The Furst Five Days.

Fig(15): The escape latency time to find the hidden platform place (which was removed) in the probe day. The length of time required for each mouse to find the hidden platform in the maze was recorded. Comparing to the control group, scopolamine group take more time which was impose from them which was more than 15s , memantine group and donepizle group show less time which almost 10s or less than 10s respictevly.

4.2. NOD-like receptor pyrin domain containing 3 (NLRP3) Levels

The mean of NLRP3 concentration was different between four studied group. The higher elevation of NLRP3 was reported among the Donepezil group (0.099634± 0.000153) in comparison to Memantine group (0.0996030± 0.000098), Scopolamine group (0.099502± 0.000136), and Control group (0.099485± 0.000094) (Figure 16). No significant difference of NLRP3 concentration was reported among those groups (*p*-Value= 0.091).

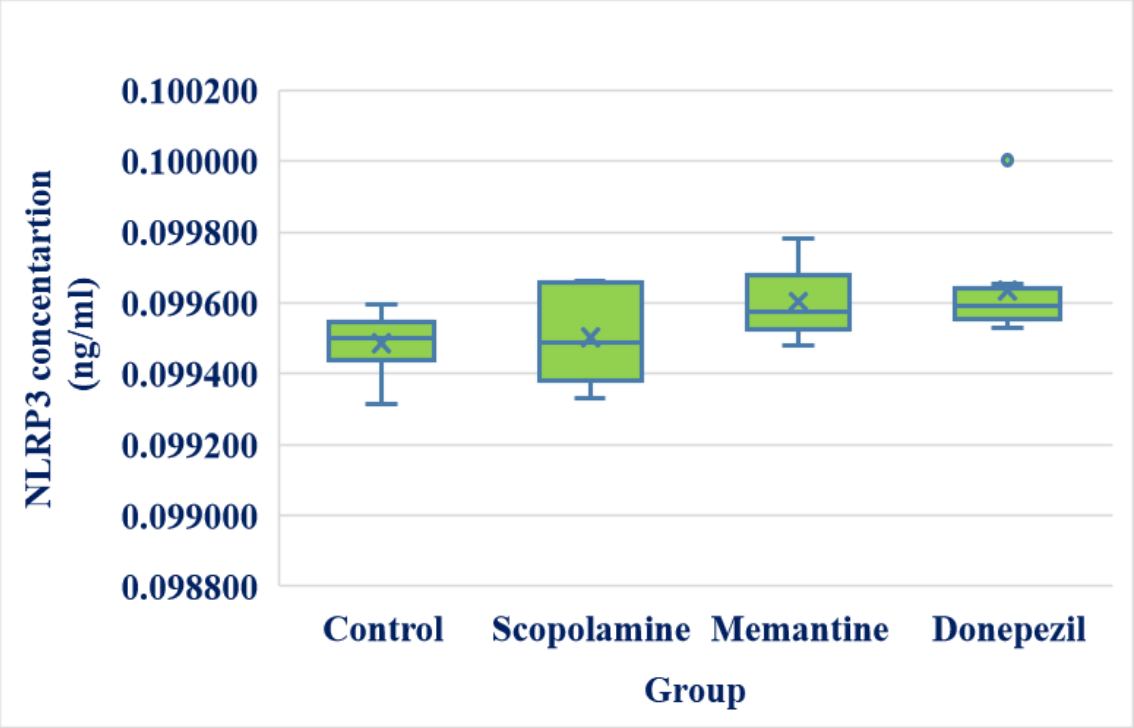

Figure 16: Mean of NLRP3 concentration among the control group, scopolamine group, memantine group, and donepezil group.

		Mean	Std. Deviation	*p*-Value
NLRP3 concentration	**Control**	.099485	.000094	0.091
	Scopolamine	.099502	.000136	
	Memantine	.099603	.000098	
	Donepezil	.099634	.000153	

Table 10: Mean of NLRP3 concentration among the control group, scopolamine group, memantine group, and donepezil group.

4.3. Total Antioxidant Status (TAS) Level

Treating the AD- induced mice with Memantine lead to one-fold decrease of TAS levels (0.627733± 0.201447) where as Donepezil led to 1.2 folds increase of TAS levels (0.802186± 0.127409) in comparison to control group (0.663684± 0.361121). No significant decrease of TAS levels while using those treatment in comparison to positive and negative control group (p = 0.306) (Figure 17).

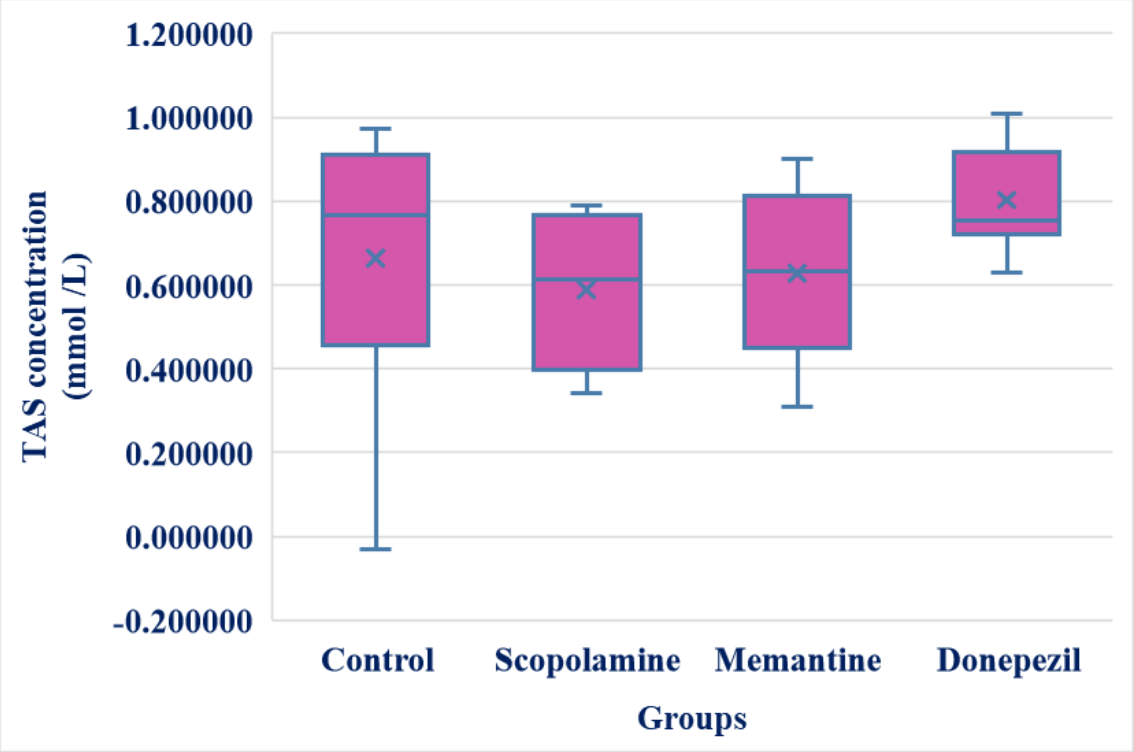

Figure 17: Mean of TAS concentration among the control group, scopolamine group, memantine group, and donepezil group.

		Mean	Std. Deviation	*p*-Value
TAS concentration	**Control**	0.663684	0.361121	0.306
	Scopolamine	0.588469	0.179887	
	Memantine	0.627733	0.201447	
	Donepezil	0.802186	0.127409	

Table 11: : Mean of TAS concentration among the control group, scopolamine group, memantine group, and donepezil group.

4.4. Total Oxidant Status (TOS) Level

Although there is no significant difference of TOS concentration in comparison to Scopolamine-induced Alzheimer disease model (0.892256± 0.547384, *p*= 0.628). Inject the rats with Memantine lead to neutralize the reactive-oxygen species (ROS) and subsequently decrease the TOS-concentration (0.719697± 0.499063). Donepezil led to elevated TOS concentration (0.921717± 1.020777) (Figure 18).

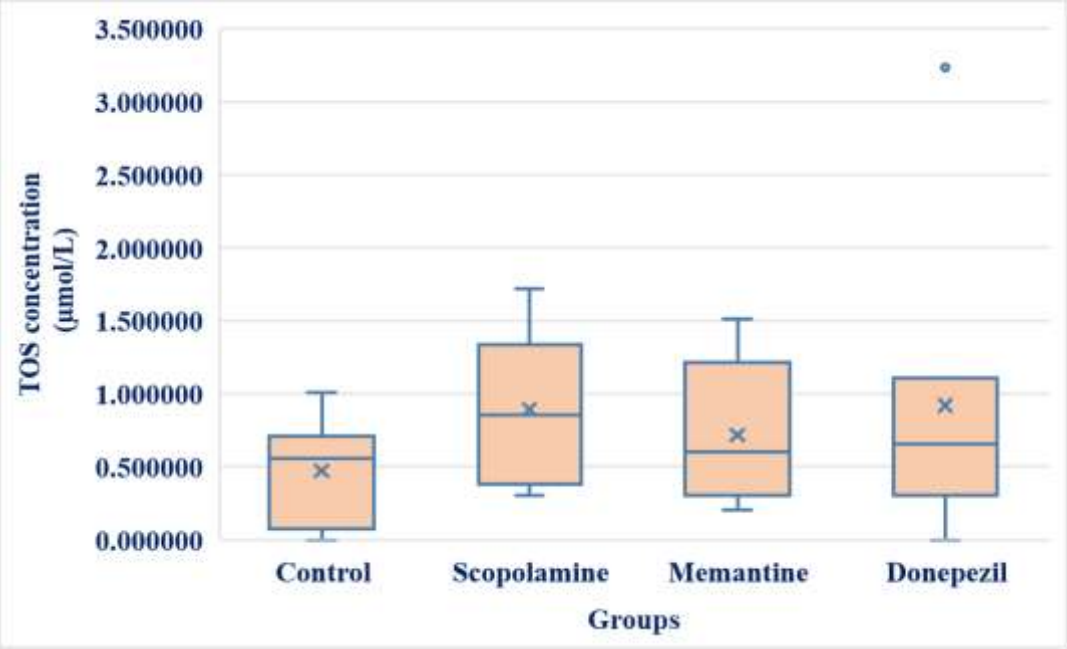

Figure 18: Mean of TOS concentration among the control group, scopolamine group, memantine group, and donepezil group.

		Mean	Std. Deviation	p-Value
TOS concentration	**Control**	0.471381	0.370676	0.628
	Scopolamine	0.892256	0.547384	
	Memantine	0.719697	0.499063	
	Donepezil	0.921717	1.020777	

Table *12*: Mean of TOS concentration among the control group, scopolamine group, memantine group, and donepezil group.

4.5. Acetylecholinesterase (AchE) Activity.

The Donepezil group (0.265585± 0.0194624) was significantly associated with elevated AchE activity in comparison to control group (0.230648± 0.006002) and scopolamine model group (0.253976± 0.020865) (p= 0.020) (Figure 19).

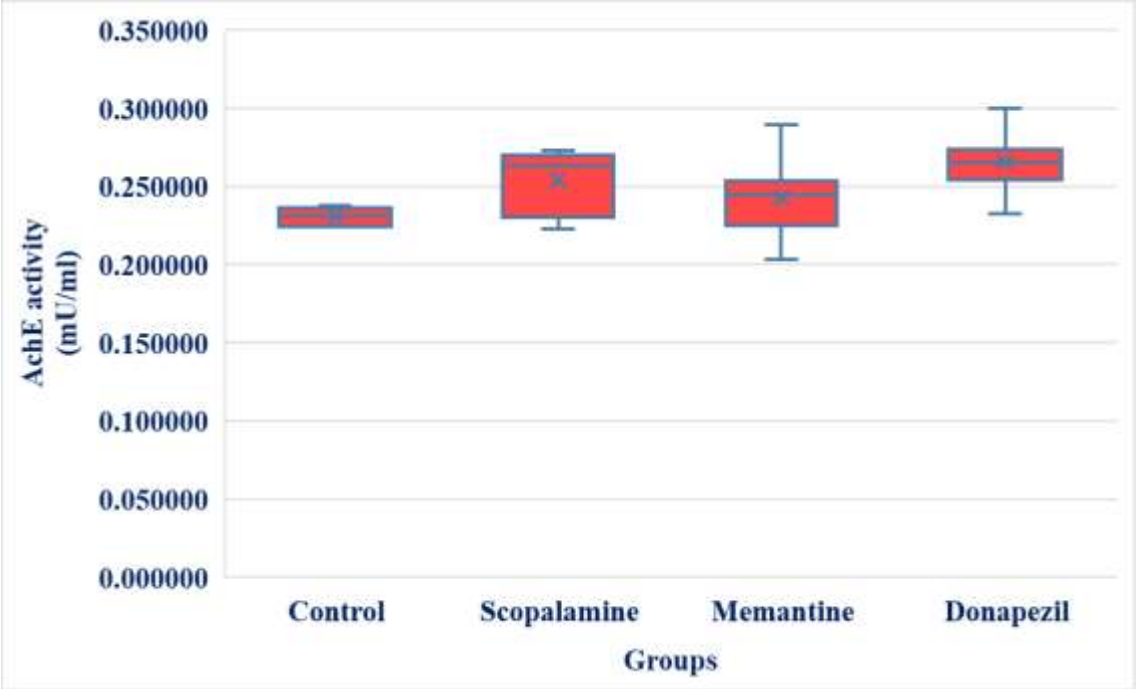

Figure 19: Mean of AchE activity concentration among the control group, scopolamine group, memantine group, and donepezil group.

		Mean	Std. Deviation	p-Value
AchE activity	**Control**	0.230648	0.006002	**0.020***
	Scopolamine	0.253976	0.020865	
	Memantine	0.242753	0.025070	
	Donepezil	0.265585	.0194624	

Table 13: Mean of AchE activity concentration among the control group, scopolamine group, memantine group, and donepezil group.

5. DISCUSSION and CONCLUSION:

The current study aims to evaluate if donepezil and memantine have any potential anti-amnesia activity in a mice model with amnesia-like symptoms in AD. Scopolamine was employed in the current work to induce amnesia-like symptoms in a mice model that simulates AD. Numerous studies have demonstrated the effectiveness of injecting mice with the selective muscarinic acetylcholine receptor antagonist Scopolamine (1 mg/kg) intraperitoneally (i.p.) to cause memory impairment and assess the behavioral and biochemical effects on the mice [24].

The research work was divided into two parts: Disease part and treatment part.

Disease part: 15 days of scopolamine injection for each group, to assess anti-amnesic potential activity which stimulates AD.

Treatment parts: two drugs have been chosen to see the potential anti-amnesia activity in a mice model with amnesia-like symptoms in AD, they have been used for 15 days for each drug.

Treatment groups were divided into two different groups: Memantine group and Donepezil group. There is no change in memory performance in the memantine and donepezil group that compered with control and scopolamine group.

MWM was applied as a behavioral test to evaluate the spatial learning and memory of animal cognitive function of mice (i. e. learning and memory). This task is important for evaluating spatial WM, STM, and LTM.

Brain samples was studied by measurement of NLRP3 protein in mouse, and there is no significant difference of NLRP3 concentration in memantine, donepezil groups that compared with control group.

But, the studied of AchE showed that the Donepezil group was significantly associated with elevated AchE activity in comparison to control group and scopolamine group.

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APPENDIX 1. Curriculum Vitae

Personal Informations

Name	Shaima	**Surname**	Almahameed

Education

Degree	**Department**	**The name of the Institution Graduated From**	**Graduation year**
Master	Department of Physiology	**Yeditepe University**	2023
University	Medical laboratory scince	**Mutah University**	2019
High school	Mutah University model school	**Mutah University model school**	2016

Languages	**Grades**
English	75

Work Experience

Position	**Institute**	**Duration (Year - Year)**

APPENDIX 2. Ethical Committee Approval