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By

YAGOUB AMANI.

Theme :

Neuroprotective activity of Sweet chestnut «*Castanea sativa* Mill.» honey on Alzheimer disease –*In vivo* study-

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Board of Examiners:

Chairperson	Pr. Djibaoui Rachid	Professor	University of Mostaganem
Supervisor	Pr. Djebli Noureddine	Professor	University of Mostaganem
Examiner	Mr. Dahmouni Said	MAA	University of Mostaganem

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Dedication

*I heartily dedicate this accomplishment to my "**Mama**" the root of my successes ; this work and my accomplishment in life are the consequence of your hard work and advice, I value your sacrifices, and your prayers in my favor, may God bless you and give you the strength and courage. Thank you for being my mom.*

*To my brother "**Djamel Eddine**", I am extremely thankful to have you as my brother. Your Encouragement and immense support is the reason that I am here and what keeps me going through everything. Nothing makes me more proud than to call you my brother.*

*To my grandmother "**Mima**" for providing me guidance, wisdom, and insight. Your prayers and support always remains a source of comfort to me.*

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Abstract

Alzheimer's disease (AD) dementia refers to a particular onset and course of cognitive and functional decline associated with age. It causes the death of nerve cells and loss of tissues throughout the brain, which results in memory loss. Nowadays, only symptomatic treatments exist for this disease to block the progression of the disease but their side effects are frequently disappointing. For that, many heads for alternative therapies, such as Api-therapy. This study aimed to analyze whether a long-intake of Sweet Chestnut "*Castanea sativa* Mill*"* honey, can counteract the neurodegeneration occurring in Alzheimer model mice. The experimental protocol is divided into two phases; the first phase represents the treatment for 45 days consisting of daily administration by gastric gavage of two doses of sweet chestnut honey solution at 150mg/kg and 300mg/kg. Followed by the induction of Alzheimer's disease by oral administration of aluminium chloride ($AlCl_3$) at 100mg/kg combined with D-galactose at 120mg/kg intraperitoneally for the second 45 days. The results followed by the neurological test and the histological study showed that the Alzheimer model mice showed significant depression and anxiety-like behaviours, and cognitive decline. The histological analysis of the brain, liver, and renal tissue sections indicated significant histopathological alterations. The neurological tests and histological changes caused by "$AlCl_3$ combined with D-galactose" previously reported were improved by Sweet chestnut honey, in which, positive results were observed concerning anxiety and depression activity in Alzheimer's mice treated with the sweet chestnut honey solution at 150mg/kg (Alz-D1) and 300mg/kg (Alz-D2). While compared to Alzheimer model mice, the memory tests results indicate that Alzheimer's mice treated with Sweet chestnut honey solution at 300mg/kg (Alz-D2) had a remarkable recovery of memory and an improvement in learning ability. As for the histological study, Both doses showed positive results, however, the dose at 300mg/kg (Alz-D2) results were much better than 150mg/kg (Alz-D1). These results suggest that Sweet Chestnut "*Castanea sativa* Mill" Honey daily intake at respected doses can reduce the burden of neurological illnesses such as "Alzheimer's disease", which have been increasingly prevalent worldwide in recent decades.

Keywords: Alzheimer's disease, Neurological tests, Mice, "*Castanea sativa* Mill*",* Honey.

Résumé

La démence de la maladie d'Alzheimer (MA) se réfère à un début particulier et au cours du déclin cognitif et fonctionnel associé à l'âge. Il provoque la mort des cellules nerveuses et la perte de tissus dans tout le cerveau, ce qui entraîne une perte de mémoire. De nos jours, seuls les traitements symptomatiques existent pour bloquer la progression de la maladie mais leurs effets secondaires sont souvent décevants. Pour cela, beaucoup de têtes pour des thérapies alternatives, telles que l'Api-thérapie. Cette étude visait à analyser si une consommation prolongée de miel de châtaignier « *Castanea sativa* Mill » peut contrer la neurodégénérescence chez les souris modèles Alzheimer. Le protocole expérimental est divisé en deux phases, la première phase représente le traitement pendant 45 jours consistant en l'administration quotidienne par gavage gastrique de deux doses de solution de miel de châtaignier à 150mg/kg et 300mg/kg. Suivi de l'induction de la maladie d'Alzheimer par administration orale de chlorure d'aluminium ($AlCl_3$) à 100 mg/kg combiné avec du D-galactose à 120 mg/kg par voie intrapéritonéale pendant les 45 jours suivants. Les résultats suivis du test neurologique et de l'étude histologique ont montré que les souris Alzheimer modèles ont montré une dépression et des comportements anxieux significatifs, ainsi qu'un déclin cognitif. L'analyse histologique des coupes de tissus cérébraux, hépatiques et rénaux a révélé d'importantes altérations histopathologiques. Les tests neurologiques et les changements histologiques causés par "$AlCl_3$ combiné avec D-galactose" précédemment signalés ont été améliorés par le miel de châtaigne douce, dans lesquels des résultats positifs ont été observés concernant l'anxiété et l'activité dépressive chez des souris Alzheimer traitées avec la solution de miel de châtaignier à 150mg/kg (Alz-D1) et 300mg/kg (Alz-D2). Par rapport aux souris Alzheimer, les résultats des tests de mémoire indiquent que les souris Alzheimer traitées avec la solution de miel de châtaignier à 300mg/kg (Alz-D2) ont une remarquable récupération de la mémoire et une amélioration de la capacité d'apprentissage. En ce qui concerne l'étude histologique, les deux doses ont donné des résultats positifs, mais la dose à 300mg/kg (Alz-D2) était bien supérieure à 150mg/kg (Alz-D1). Ces résultats suggèrent que l'apport quotidien de miel « *Castanea sativa* Mill » à des doses respectées peut réduire le fardeau des maladies neurologiques comme la « maladie d'Alzheimer », qui sont de plus en plus répandues dans le monde au cours des dernières décennies.

Mot clés : Maladie d'Alzheimer, Tests neurologiques, Souris, "*Castanea sativa* Mill", Miel.

ملخص

يشير مرض الزهايمر إلى بداية ومسار معين للتدهور المعرفي والوظيفي المرتبط بالعمر. يتسبب في موت الخلايا العصبية وفقدان الأنسجة في جميع أنحاء الدماغ، مما يؤدي إلى فقدان الذاكرة. في الوقت الحاضر، توجد فقط علاجات للأعراض لهذا المرض لمنع تطور المرض ولكن آثاره الجانبية غالبًا ما تكون مخيبة للآمال. لذلك، يتجه العديد إلى العلاجات البديلة، مثل علاج بمنتجات خلية النحل, تهدف هذه الدراسة إلى تحليل ما إذا كان تناول كمية طويلة من عسل "الكستناء الحلو"، يمكنه مواجهة التنكس العصبي الذي يحدث في الفئران النموذجية لمرض الزهايمر. ينقسم البروتوكول التجريبي إلى مرحلتين، تمثل المرحلة الأولى العلاج لمدة 45 يومًا تتكون من إعطاء يومي عن طريق هضم المعدة لجرعتين ,من ,محلول عسل الكستناء الحلو عند 150 و 300 ملغ/كغ. يليه تحريض مرض الزهايمر عن طريق إعطاء كلوريد الألومنيوم عن طريق الفم عند 100 ملغ/كغ مع د-غلاكتوز عند 120 ملغ/كغ داخل الصبغة خلال الـ 45 يومًا الثانية. أظهرت النتائج التي أعقبها الاختبار العصبي والدراسة النسيجية أن فئران نموذج الزهايمر أظهرت اكتئابًا كبيرًا وسلوكيات شبيهة بالقلق وتدهورًا إدراكيًا, أشار التحليل النسيجي لأقسام الدماغ والكبد والأنسجة الكلوية إلى تغيرات كبيرة في الأنسجة المرضية. تم تحسين الاختبارات العصبية والتغيرات النسيجية الناجمة عن «ألومنيوم الكلوريد جنبًا إلى جنب مع د-غلاكتوز» التي تم الإبلاغ عنها سابقًا بواسطة عسل الكستناء الحلو، حيث لوحظت نتائج إيجابية بشأن القلق ونشاط الاكتئاب في فئران الزهايمر المعالجة بمحلول عسل الكستناء الحلو عند 150 مجم/كجم و 300 مجم/ كجم, بينما مقارنة بفئران نموذج الزهايمر تشير نتائج اختبارات الذاكرة إلى أن فئران الزهايمر التي عولجت بمحلول عسل الكستناء الحلو عند 300 مجم/كجم قد تعافت بشكل ملحوظ من الذاكرة وتحسن في القدرة على التعلم. أما بالنسبة للدراسة النسيجية، فقد أظهرت كلتا الجرعتين نتائج إيجابية، ومع ذلك، كانت نتائج الجرعة عند 300 ملجم/كجم أفضل بكثير من 150 ملجم/كجم. تشير هذه النتائج إلى أن تناول عسل "الكستناء الحلو" يوميًا من بجرعات محترمة يمكن أن يقلل من عبء الأمراض العصبية مثل «مرض الزهايمر»، الذي انتشر بشكل متزايد في جميع أنحاء العالم في العقود الأخيرة.

الكلمات المفتاحية: مرض الزهايمر، الاختبارات العصبية، الفئران، كستناء الحلو، العسل،

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Abbreviations list

Ach: acetylcholin.

AchE: acetylcholinesterase.

AD: Alzheimer diseases

$AlCl_3$: Aluminum chloride.

ALZ: Alzheimer's.

APOE: Encoding apolipoprotein E.

APP: the precursor protein beta-amyloid.

Aβ plaques: beta-amyloid plaques.

Aβ: Amyloid beta.

BVT: Bee venom therapy.

C°: Celsius degree.

CA : Cornu Ammonis.

D-gal: D-galactose

FDA: Food and drug administration.

FST : Forced swim test.

IP : intraperetenial.

MAPT: Microtubule-associated tau protein.

MWM : Morris water maze.

NFTs: Neurofibrillary tangles.

Ptau: Phosphorylated tau

STD: Standard.

SCH: Sweet chestnut honey

45d: 45 Days**.**

90d: 90 Days.

Introduction

General introduction

Alzheimer's disease (AD) is a complex progressive neurodegenerative disorder and the leading cause of dementia **(Cummings, J. L et *al*., 2019).** 40 million people are estimated to suffer from dementia throughout the world, and this number is supposed to become twice as much every 20 years, until approximately 2050 (**Yiannopoulou, K. G & Papageorgiou, S. G., 2020).**

Alzheimer disease (AD) is a heterogeneous disease with a complex pathobiology. The presence of extracellular β-amyloid deposition as neuritic plaques and intracellular accumulation of hyperphosphorylated tau as neurofibrillary tangles remains the primary neuropathologic criteria for AD diagnosis **(Long, J. M & Holtzman, D. M., 2019).**

Given the complexity of AD, the treatment remains challenging. Indeed, no new drug has been approved by FDA (Food and drug administration), for treatment of AD since 2003 **(Yiannopoulou, K. G & Papageorgiou, S. G., 2020)**. As of now, there is only two classes of drugs approved to treat AD, including inhibitors to cholinesterase enzyme (naturally derived, synthetic and hybrid analogues) and antagonists to N-methyl d-aspartate (NMDA). Despite the therapeutic effect of these two classes, they are effective only in treating the symptoms of AD, but do not cure or prevent the disease **(Breijyeh, Z & Karaman, R., 2020).**

Due to side effects of these drugs **(Marucci, G et *al.*, 2021).** In recent years, attention has grown towards the use of natural therapies given the numerous advantages offered by natural products compared to synthetic drugs. Currently, more preference is given to apitherapy, due to the high safety margin lower cost, and broad bioactivity compared to synthetic medicine **(Kumar, M et *al.*, 2022).**

The sweet chestnut tree (*Castanea sativa* Mill) is common in Europe where it was introduced from Sardis (in what is now Turkey) thousands of years ago. Sweet chestnut honey has a strong aromatic taste and a slightly bitter after taste, rich in pollen content, mineral salts and tannin **(Beşir, D. A. Ğ., 2017).** A sweet dark-colored honey with a distinct bitter aftertaste. It contains numerous phenolic compounds and alkaloids **(Kim, J et al., 2022).** Antifungal, anti-inflammatory, cytotoxic, wound healing, urease inhibitory, tyrosinase inhibitory, xanthine

oxidase inhibitory and monoamine oxidase inhibitory, antioxidant and antibacterial potentials of chestnut honeys have been reported **(Taş-Küçükaydın, M et al., 2023).**

This study aimed to investigate whether sweet chestnut honey has protective effects on the brain in the Aluminum chloride ($AlCl_3$) and D-galactose (D-gal), induced-Alzheimer's disease (AD) mice model. Before starting our experimental research, a bibliographical part was developed in two chapters: general information on Alzheimer's disease, and treatment (synthetic and natural "phytotherapy and apitherapy") and finally a description of the natural product used *(castanea sativa* Mill.). The second part is devoted to an experiment, which is based on *in vivo* evaluation of the neuroprotective activity of (*castanea sativa* Mill.). The results of this research ended with a general discussion and conclusion.

Bibliography part

Chapter I

«Alzheimer disease» and Treatment.

I.1. Brain anatomy and function

The brain is arguably the most important organ in the human body. While the brain only weighs about 3 kilograms, it is a highly complex organ made up of many parts. Years of scientific study of the brain have made it possible for scientists to identify the various areas of the brain and determine their specific functions **(Atlanta Brain and Spine Care., 2023).**

I.1.1. The Nervous System

The nervous system is commonly divided into the central nervous system and the peripheral nervous system. The central nervous system is made up of the brain, its cranial nerves and the spinal cord. The peripheral nervous system is composed of the spinal nerves that branch from the spinal cord and the autonomous nervous system (divided into the sympathetic and parasympathetic nervous system) **(American Association of Neurological Surgeon., 2023) (Fig.1).**

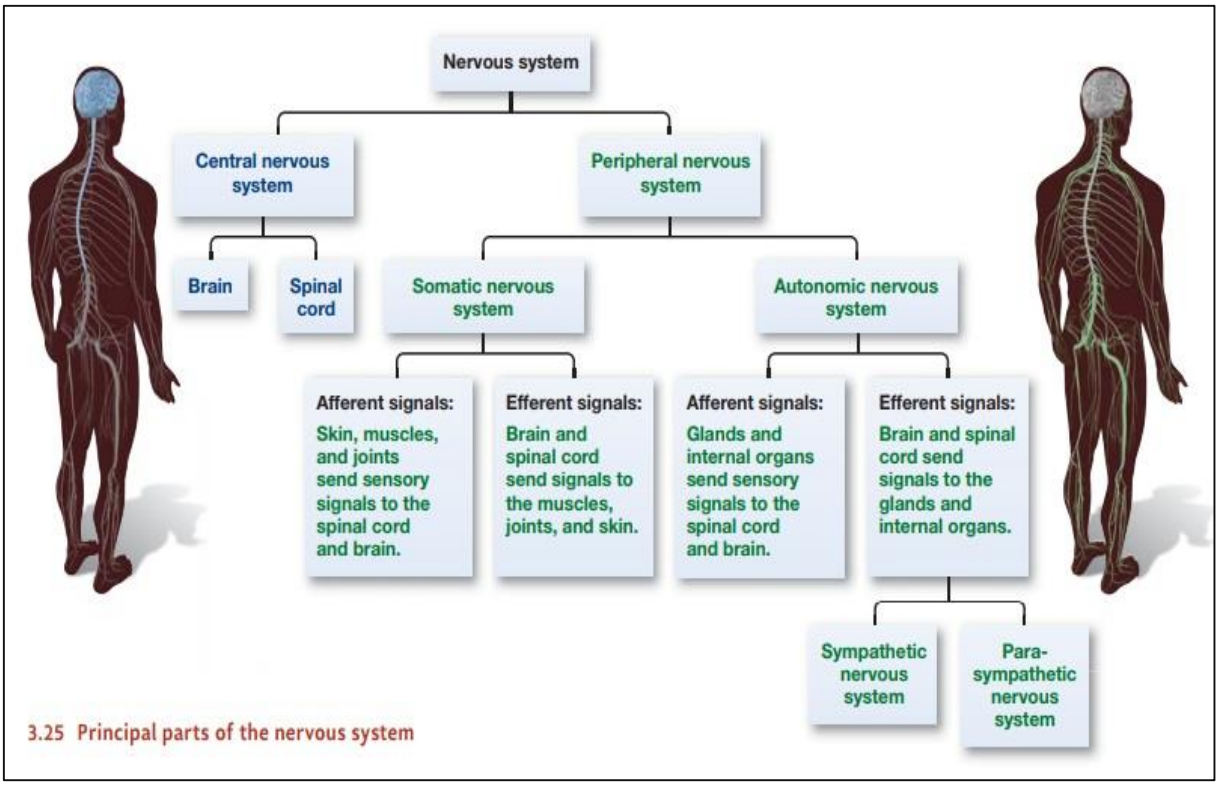


Figure 1: The Central and Peripheral Nervous Systems **(Anonymous[1]., 2018).**

I.1.2. Main Parts of the Brain and Their Functions

The human brain is perhaps the most complex of all biological systems. Protected within the skull, the three main parts of the human brain are the cerebrum, cerebellum, and brainstem **(Maldonado, K. A & Alsayouri, K., 2021) (Fig.2).**

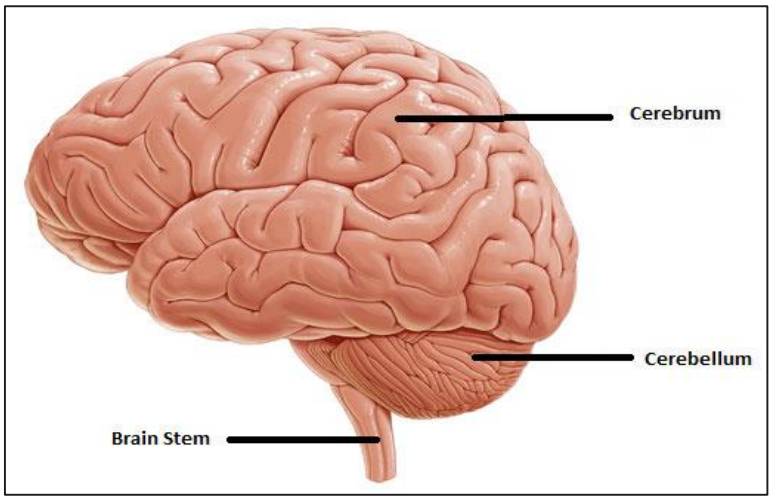


Figure 2: The main brain parts, the cerebrum, cerebellum, and brainstem **(Anonymous[2]., 2018).**

I.1.2.1. The cerebrum

Cerebrum, the largest and uppermost portion of the brain. The cerebrum consists of the cerebral hemispheres and accounts for two-thirds of the total weight of the brain. One hemisphere, usually the left, is functionally dominant, controlling language and speech. The other hemisphere interprets visual and spatial information. The cerebral hemispheres consist of an inner core of myelinated nerve fibres, the white matter, and an outer cortex of gray matter **(Britannica., 2023).**

The cerebral cortex has a left and a right hemisphere. Each hemisphere can be divided into four lobes: the frontal lobe, temporal lobe, occipital lobe, and parietal lobe. The lobes are functional segments. They specialize in various areas of thought and memory, of planning and decision making, and of speech and sense perception **(Anonymous[4]., 2023) (Fig.3).**

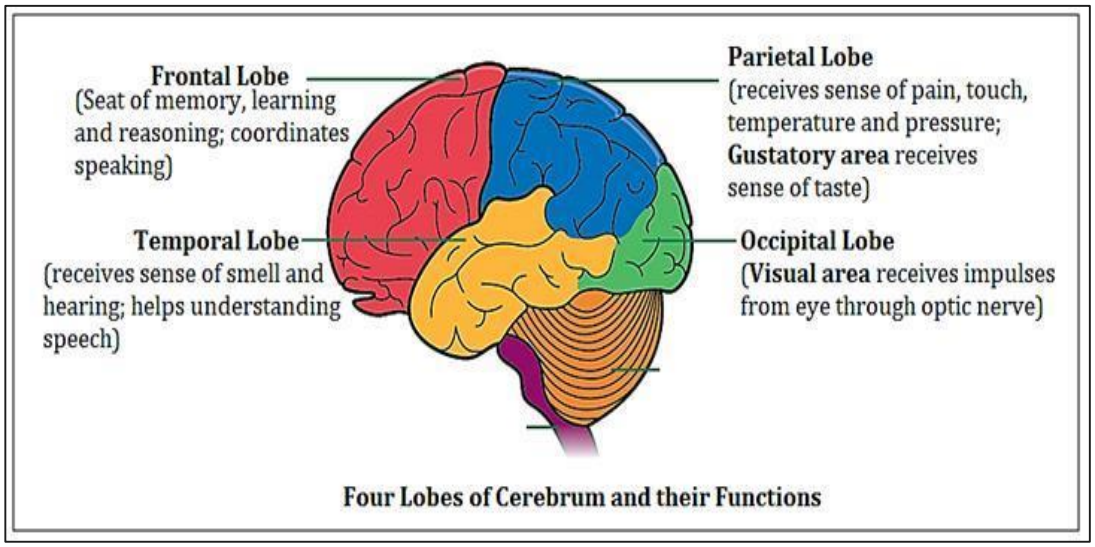


Figure 3: Represent four lobes of cerebrum and their functions **(Anonymous[3]., 2015).**

I.1.2.2. The cerebellum

The cerebellum is the second largest part of the brain. It sits below the posterior (occipital) lobes of the cerebrum and behind the brain stem, as part of the hindbrain. The cerebellum has left and right hemispheres. Within the interior tissue rises a central white stem, called the arbor vitae because it spreads branches and sub-branches through the hemispheres. The primary function of the cerebellum is to maintain posture and balance **(Anonymous[4], 2023) (Fig.4).**

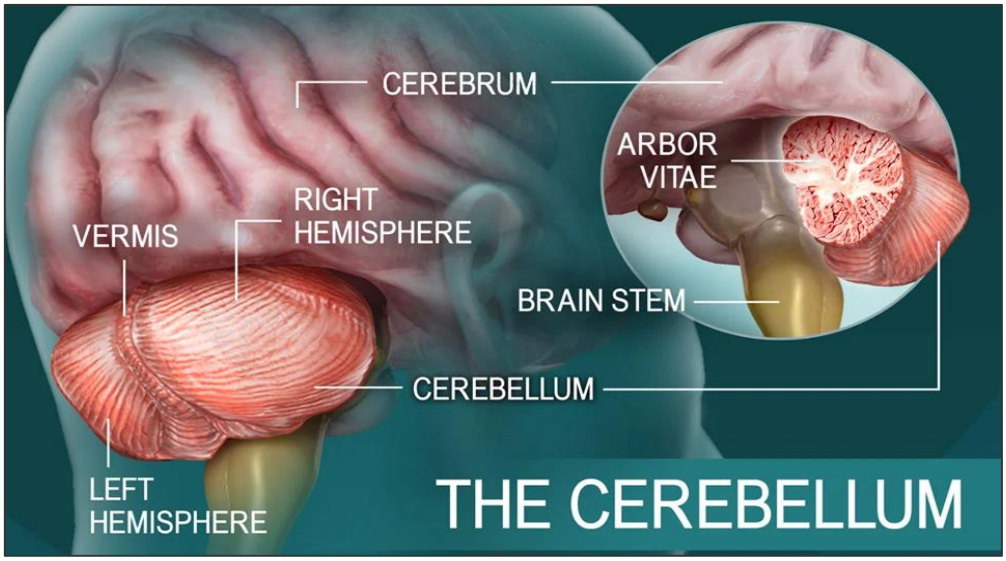


Figure 4: Represent the cerebellum anatomy **(Anonymous[4]., 2023).**

I.1.2.3. The Brain Stem

The brainstem acts as a relay center connecting the cerebrum and cerebellum to the spinal cord. The brainstem includes the midbrain, the pons and the medulla. It performs many automatic functions such as breathing, heart rate, body temperature, wake and sleep cycles, digestion, sneezing, coughing, vomiting, and swallowing **(Anonymous., 2023) (Fig.5).**

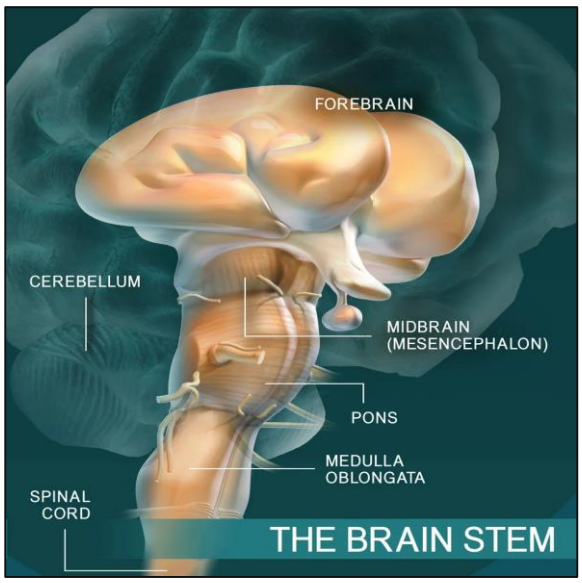


Figure 5: A representive image of the brain stem anatomy **(Anonymous[4], 2023).**

I.1.3. The Limbic system parts and functions

The limbic system is an aggregation of brain structures. The structures included in the limbic system are in the general region that borders the cerebral hemisphere and brainstem, lateral to the thalamus, underneath the cerebral cortex, but above the brainstem **(Torrico TJ, Abdijadid S., 2019)**. Limbic system, group of structures in the brain that governs emotions, motivation, olfaction (sense of smell), and behaviour, is also involved in the formation of long-term memory **(Raikar, Sanat Pai., 2023) (Fig.6).**

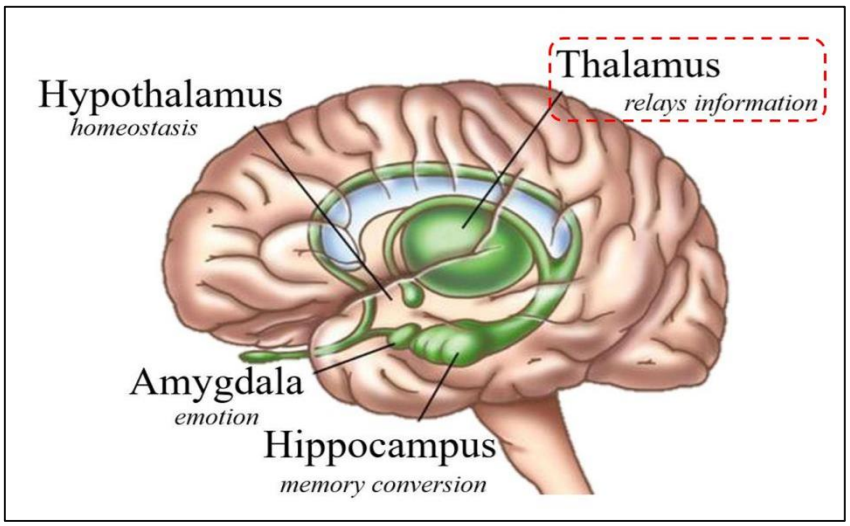


Figure 6: A representive image of the limbic system parts **(Anonymous[5], 2020).**

I.1.3.1. The thalamus

The thalamus is the largest part of the diencephalon and is located between the third ventricle medially and the internal capsule laterally. The thalamus is extremely heterogeneous in its anatomical structure, functional connectivity, and neuroimaging appearance, and different classifications have been proposed **(Capone, F et *al*., 2020).**

While the thalamus is classically known for its roles as a sensory relay in visual, auditory, somatosensory, and gustatory systems, it also has significant roles in motor activity, emotion, memory, arousal, and other sensorimotor association functions **(Blumenfeld, H and Gummadavelli, A., 2023) (Fig.7).**

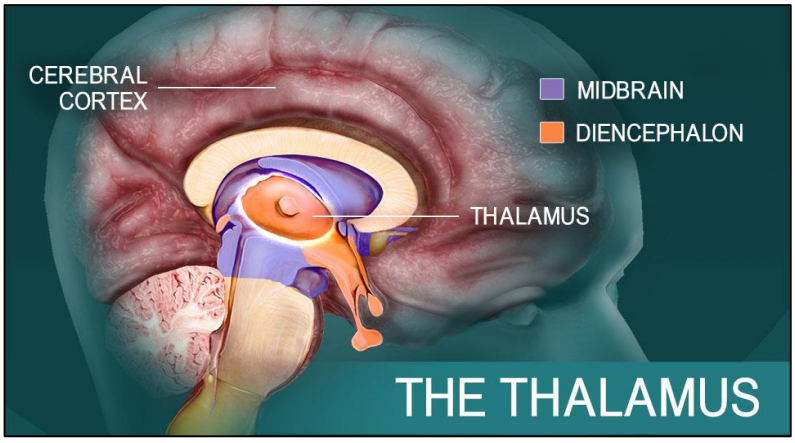


Figure 7: Represent the thalamus **(Aonymous[4], 2023).**

I.1.3.2. The Hypothalamus

The hypothalamus is a cerebral structure, that is part of the diencephalon, and located below the thalamus. The hypothalamus plays a central role in controlling many vital functions, including food intake and perception of satiety, circadian rhythms (i.e., sleep-wake pattern), immune and endocrine response, thermoregulation and cardiovascular activity **(Billot, B et *al*., 2020) (Fig.8).**

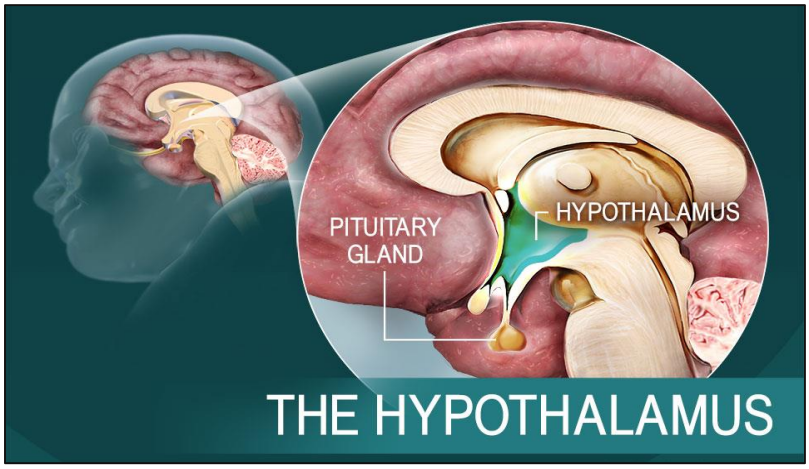


Figure 8: A representive image of the hypothalamus **(Anonymous[4], 2023).**

The lateral hypothalamus contains genetically-heterogeneous neurons many of which act as nutrient-sensors, project brain-wide, and also integrate direct neural inputs from much of the brain. More recent work suggested that the hypothalamus might act as an interface for various types of cognitive functions, such as learning and memory **(Burdakov, D & Peleg-Raibstein, D., 2020).**

I.1.3.3. The Amygdala

The amygdala is an almond-shaped structure that lies in the temporal lobe, lying just beneath the uncus. The amygdala is diverse and complex in structure and comprises approximately 13 nuclei. They further subdivide into extensive internuclear and intranuclear connections **(AbuHasan, Q et *al*., 2021) (Fig.9).**

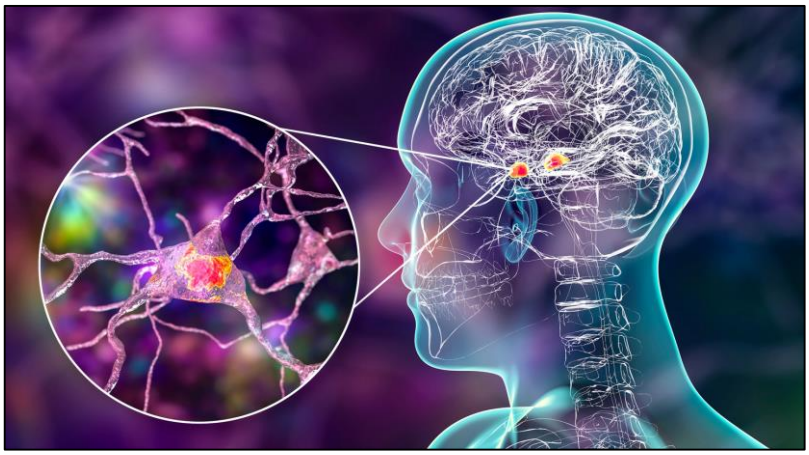


Figure 9: A representive image of amygdala **(Anonymous[6], 2022**).

A limbic brain region the amygdala plays an important role in emotional-affective aspects of behaviors, stress integration, and related disorders such as anxiety, depression, and addiction, as well as pain modulation **(Neugebauer, V et *al*., 2020).**

I.1.3.4. The hippocampus

The hippocampus is a paired structure present in each temporal lobe of the brain and part of a larger structure of the temporal lobe called the hippocampal formation. The hippocampal formation extends from the amygdala anteriorly, to the splenium of the corpus callosum posteriorly **(Roberto Grujičić MD., 2022).** The hippocampus is thought to be principally involved in storing long-term memories and in making those memories resistant to forgetting. It is also thought to play an important role in spatial processing and navigation **(Yassa, Michael A., 2023).** It receives information from the cerebral cortex and may play a role in Alzheimer's disease **(Anonymous, 2023) (Fig.10).**

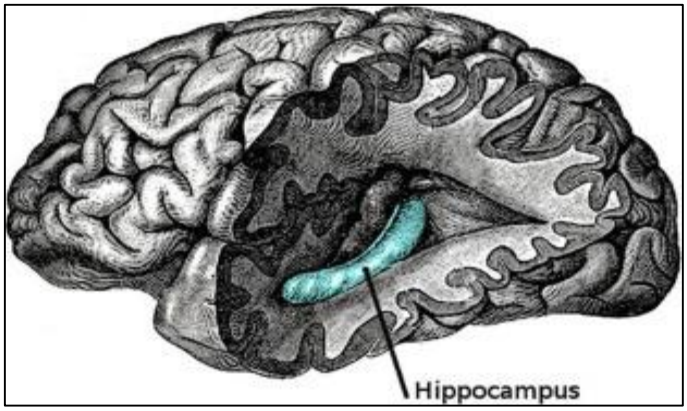


Figure 10: A representative image of hippocampus **(Hamilton, G. F & Rhodes, J. S., 2015).**

The Cornu Ammonis (CA) is a seahorse-like or ram's horn-like structure that describes the different layers of the hippocampus. There are four hippocampal subfields CA1, CA2, CA3, and CA4 **(Fogwe, L. A et *al.,* 2018)** but nowdays this field is considered a part of the dentate gyrus **(Roberto Grujičić MD., 2022).** Both hippocampal areas CA1 and CA3 contribute to the context dependence of extinguished fear. CA1 and CA3 are both required for contextual encoding of extinction, whereas area CA1 is essential for context-dependent retrieval **(Ji, J., & Maren, S., 2008) (Fig.11).**

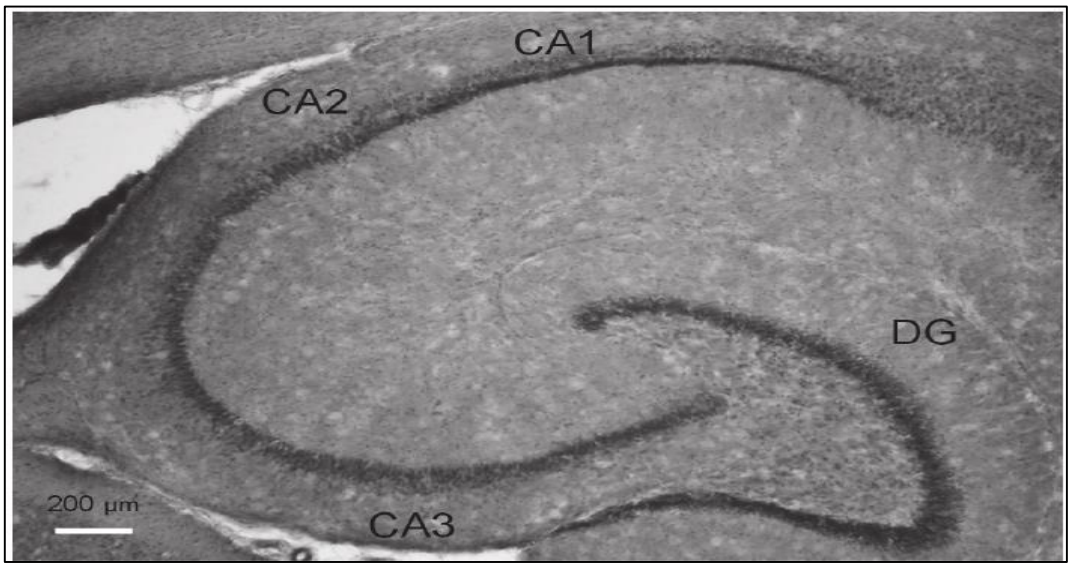


Figure 11: A representative image for rat hippocampus CA1, CA2 and CA3 subfields. CA1: hippocampus CA field, CA2: hippocampus CA2 field, CA3: hippocampus CA3 field, DG: dentate gyrus **(Akdogan, I & Yonguc, N. G., 2011).**

I.2. Alzheimer disease (AD)

The term ‘dementia’, otherwise known as ‘major neurocognitive disorder’ **(World Alzheimer report., 2021).** Nearly 9.9 million people are expected to develop dementia each year, which translates to one new case every three seconds **(Pot, A. M et *al.*, 2019).** Dementia is a group of symptoms that affect neurons and cause some mental disorders, such as losing memory. Alzheimer's disease (AD) which is known as the most common cause of dementia **(Ashrafian, H et *al*., 2021).** Alzheimer’s disease (AD) is a disease that’s caused due to the damage of memory cells permanently. It causes the death of nerve cells and loss of tissues throughout the brain, which results in memory loss **(Ghazal, T. M & Issa, G., 2022).**

Alzheimer's disease (AD) dementia refers to a particular onset and course of cognitive and functional decline associated with age which ultimately results in death **(Alzheimer's Association, 2019).** It was first described by *Alois Alzheimer in 1906* when he described the case of Auguste Deter, a 51-year-old woman with cognitive disturbance, disorientation, delusions, and other behavioral changes whom he first saw in 1901 **(Lopez, J. A. S et *al*., 2019).**

The whole aetiology of the illness is still not clear, but available data suggest AD is more than just a neurodegenerative brain disorder. It is a systemic illness with the signs in blood and the peripheral tissues provoked by inflammatory, metabolic, oxidative, and other biochemical mechanisms **(Zvěřová, M., 2019).**

This Primer conceives of AD biology as the brain disorder that results from a complex interplay of loss of synaptic homeostasis and dysfunction in the highly interrelated endosomal/lysosomal clearance pathways. **(McKhann, G. M et *al*., 2011)**. Although the exact sources of the disease are not understood, is it believed that aggregation of amyloid-beta (Aβ) outside of neuron cells and tau aggregation or neurofibrillary tangles (NFTs) formation inside the cell may play crucial roles **(Ashrafian, H et *al*., 2021).**

Given the continual increase in the number of patients and the lack of curative treatment, the development of new therapies to treat Alzheimer’s disease (AD) is becoming ever more urgent **(Séguy, L et *al*., 2022).**

I.2.1. Epidemiology and prevalence

The World Health Organization defines Alzheimer disease (AD) as a neurodegenerative disease of unknown etiology, characterised by progressive memory and cognitive impairment which accounts for 50% to 75% of all cases of dementia **(Niu, H et *al*., 2017).** About 50 million people are living with dementia around the world, due to the aging population, the number of patients is predicted to triple by 2050 projected to reach 152 million by mid-century, with the greatest increase expected in low-and middle-income countries **(Zhang, X. X et *al*., 2021).**

The estimated prevalence of all-cause dementia varies from 4.7% in Central Europe to 8.7% in North Africa/Middle East, with North America falling between at 6.4%. Currently, over 46 million individuals live with dementia worldwide and this number is projected to increase to 131.5 million by 2050 **(Podcasy, J. L & Epperson, C. N., 2022).**

Several papers have associated AD with aging, obviously because AD generally (in about 90% of cases) affects individuals from the age of 65 and its prevalence doubles each 5 years, generating a time-dependent exponential increase **(Trevisan, K et *al*., 2019).**

According to **A cross-sectional, door-to-door study in the Department of Sidi M'Hamed in the city of Algiers "Algeria"**, conducted in the general population, was carried out, between June 2012 and August 2014. The analysis and calculation of the prevalence of dementia in the department of Sidi M'Hamed were estimated from a sample of 3896 subjects. Age ranged from 60 to 105 years, on average (73.15 ± 8.35) years. The sample included 1406 men (36.1%) and 2490 women (63, 9%). There was a clear predominance of women with a sex ratio of 0.56.

The prevalence of dementia was estimated at *5.33% in men* and *4.70% in women*. It did not differ significantly by sex (p=0.674). On the other hand, the prevalence of dementia increases significantly (p<0.00001) with age in both men and women. The estimated prevalence rate of all types of dementia recorded was *4.93% in people aged 60 years and older*, *7.06% in people aged 70 years and older,* and *13.04% in people aged 80 years and older* **(Belarbi, S et al., 2022).**

I.2.2. Risk factors

AD is a multifactorial disease with a number of well established genetic and environmental risk factors **(Uchoa, M. et *al*., 2016).** But after all "Age" remains the greatest risk factor for Alzheimer's and is thus a fundamental driver for development of the disease **(Riedel, B. C et *al.,* 2016).** Moreover, there are also modifiable risk factors—in terms of treatable medical conditions and lifestyle choices—that play a role in developing AD **(Edwards III, G. A et *al*., 2019).**

I.2.2.1. Age

Systems driving brain aging are contributors to risk of AD and include multiple conditions that emerge during aging, such as metabolic dysregulation, cholesterol dyshomeostasis, insomnia, depression, subjective memory complaints and cognitive decline are associated with increased risk of neurodegenerative diseases later in life such as Alzheimer's **(Riedel, B. C et *al.,* 2016).** According to **Alzheimer's Association (2023)** The percentage of people with Alzheimer's dementia increases dramatically with age. 5% of people age 65 to 74, 13.1% of people age 75 to 84, and 33.3% of people age 85 or older have Alzheimer's dementia**.**

I.2.2.2. Sex

Advanced age is the strongest predictor; however, sex and gender differences have been noted in prevalence, clinical manifestation, disease course, and prognosis **(Podcasy, J. L & Epperson, C. N., 2022).** Sex differences also impact AD risk, with women accounting for approximately two-thirds of AD patients **(Uchoa, M. et *al*., 2016).** Typically, the greater risk of AD in females is attributed to their greater longevity of, on average, 4.5 years. Postmenopausal women constitute >60% of the affected Alzheimer population and are those who will bear the greatest burden of the disease **(Riedel, B. C et *al.,* 2016).**

I.2.2.3. Genetic factors

Genetic studies of AD have revealed several risk factors, (1) family history, (2) AD PGRS, and (3) APOE ε4 carrier status. With the APOE locus (encoding apolipoprotein E) being among the strongest **(McCartney, D. L et *al*., 2018).** There are three different forms of the APOE gene, called APOE ε2, APOE ε3, and APOE4. People who inherit one copy of the APOE ε4 gene have an increased risk of developing Alzheimer's disease, and those who inherit two copies of the APOE ε4 gene are at an even greater risk. Other genes that have been implicated in the

development of Alzheimer's disease include PSEN1, PSEN2, and APP. These genes are involved in the processing of a protein called amyloid-beta **(Karch, C. M & Goate, A. M., 2015).**

I.2.2.4. Associated pathologies

Various epidemiological studies revealed Cardiovascular disease as an important contributor to the development and progression of AD. Vascular risk factors like hypertension, diabetes, and hyperhomocysteinemia, significantly increase risk of developing Alzheimer disease, suggesting an additive or even synergistic effect. These vascular risk factors are often linked to a reduction in cerebral blood flow and the resulting chronic cerebral hypoperfusion is suggested to play a key role in the onset of AD **(Scheffer, S et *al*., 2021).**

I.2.2.5. Others risk factors

I.2.2.5.1. Metal

Environmental exposures and/or alterations in the homeostasis of essential transition metals (ETM), such as Fe, Cu, Zn or Mn, are known to contribute to neurodegenerative diseases (ND), such as Alzheimer's Disease (AD) and Parkinson's Disease (PD) **(de Andrade, V. L et al., 2021).** Virtually the only metal considered by earlier reviews as a risk factor for AD was aluminium, exposure to which was considered then, and remains today, controversial **(Armstrong, R. A., 2019).** Neurotoxic events caused by aluminium, such as oxidative stress, apoptosis, inflammatory events, calcium dyshomeostasis, Aβ deposition, and neurofibrillary tangle formation in the brain, are all potential risk factors for AD **(Dey, M & Singh, R. K., 2022).**

I.2.2.5.2. Sleep Disturbance

Sleep disturbance is a common symptom in patients with various neurodegenerative diseases, including AD, and it can manifest in the early stages of the disease. Studies have demonstrated that sleep deprivation or restriction in various AD models exacerbates AD-related biochemical or pathological changes in mice brains, such as an increase in Aβ or phosphorylated tau, and an increase in Aβ40, Aβ42 and β-site amyloid-precursor-protein-cleaving enzyme 1 (BACE1), which produce toxic Aβ species **(Minakawa, E. N et *al.*, 2019).**

I.2.3. Symptoms

Alzheimer's disease causes both cognitive and non-cognitive symptoms **(Gottesman, R. T & Stern, Y., 2019).**

I.2.3.1. Cognitive symptoms of AD mainly include deficits in short-term memory, praxis and visuospatial and executive dysfunction. Primary progressive aphasia, posterior cortical atrophy and frontal variant of AD are rarer and atypical variants of Alzheimer's disease with relative preservation of memory **(Zvěřová, M., 2019).**

I.2.3.2. Non-cognitive symptoms associated with Alzheimer's disease and related dementias include psychosis, mood disturbances, personality changes, agitation, aggression, pacing, wandering, changed sleep patterns, and appetite disturbances **(Forester, B. P & Oxman, T. E., 2003) (Fig.12).**

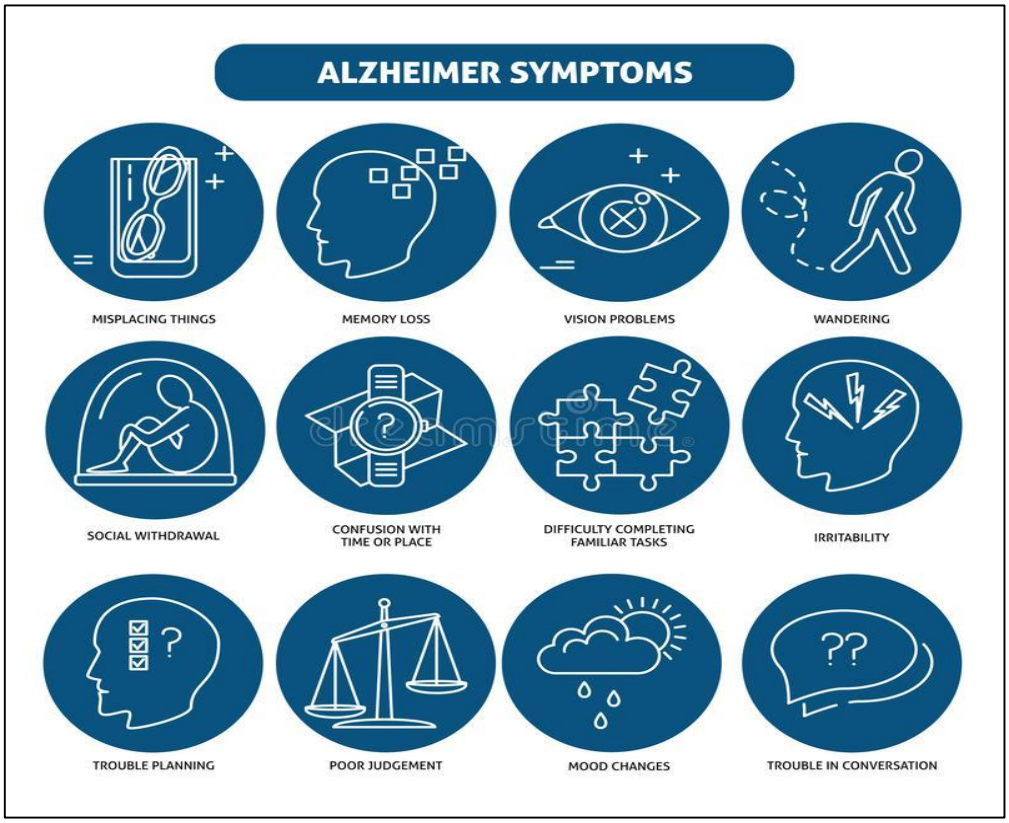


Figure 12: Set of Alzheimer's disease symptoms **(Anonymous[7], 2022).**

I.2.4. Pathophysiology of Alzheimer's disease

In AD patients, the size of the ventricles increases in the brain, and the size of the cerebral cortex and hippocampus shrinks. When the size of the hippocampus is reduced the episodic memory and spatial memory are damaged. This damage between neurons leads to communication defects in planning, judgment, and short-term memory. This reduction causes impairment of the synapses, neuron ends, and further cell loss **(Ghazal, T. M & Issa, G., 2022) (Fig.13).**

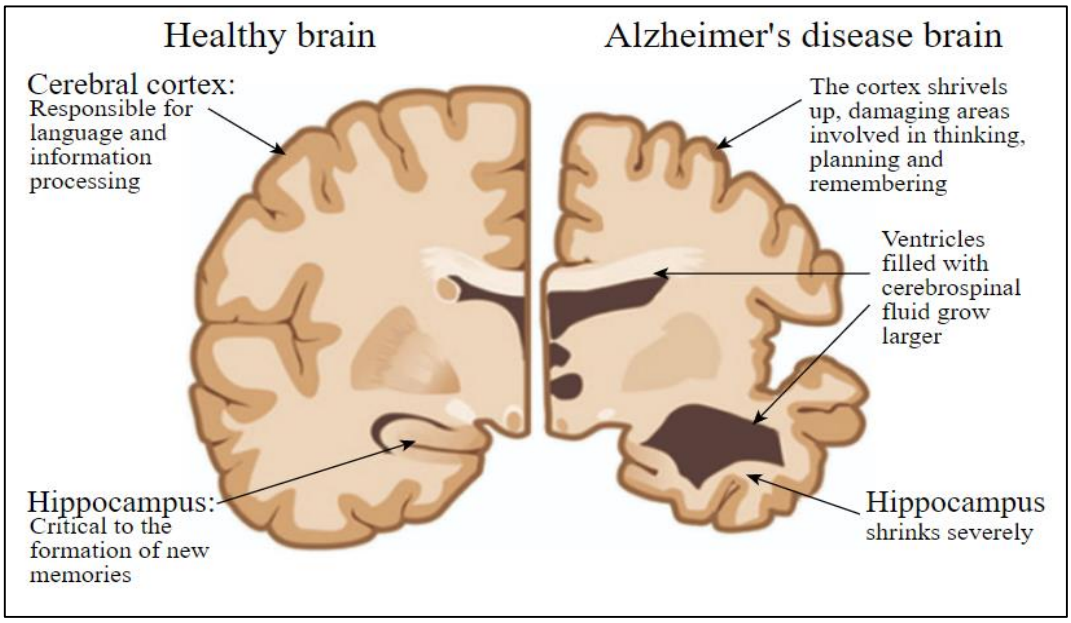


Figure 13: The difference between healthy brain and AD brain **(Anonymous[8], 2023).**

Synapse loss and damage are central features of AD and contribute to the onset and progression of its behavioural and physiological features. However, molecular biological and proteomic studies in the last two decades have revealed that synapses are far from simple connectors. Instead, they contain thousands of proteins with complex cell biological and signalling properties **(Griffiths, J & Grant, S. G., 2023).**

AD as a distinct entity is now defined biologically by the presence of a specific neuropathological profile: extracellular deposition of β-amyloid (Aβ) in the form of diffuse and neuritic plaques and the presence of intraneuronal neurofibrillary tangles (NFTs) and neuropil threads within dystrophic neurites consisting of aggregated hyperphosphorylated tau protein **(Long, J. M & Holtzman, D. M., 2019).**

I.2.4.1. β-amyloid (Aβ)

Abnormal accumulation of β-amyloid (Aβ) peptide aggregates in the brain is a major hallmark of AD **(Jung, H et *al.*, 2020).** According to this hypothesis, the Aβ over-production, aggregation, and accumulation in the human brain trigger a cascade of molecular and cellular events leading to a progressive synaptic and neuritic injury, disturbance of ionic homeostasis, oxidative damage of cells that result in neuronal death, and consequently, dementia **(Mrdenovic, D et *al.*, 2022) (Fig.14).**

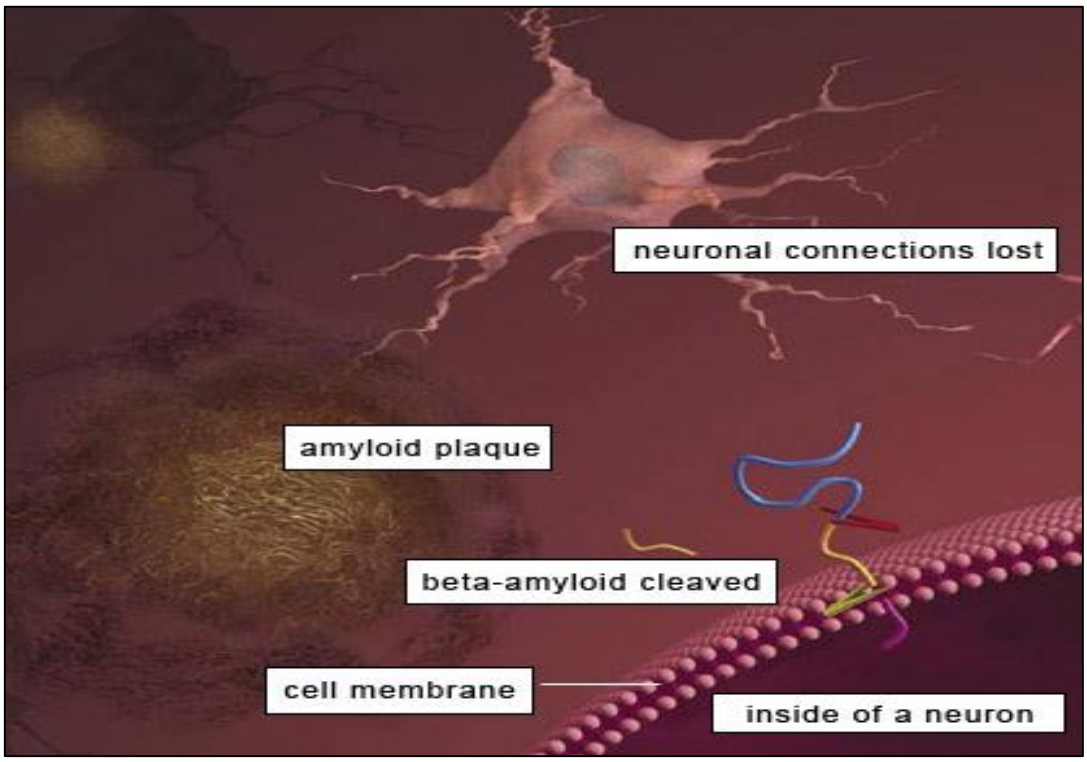


Figure 14: Amyloid-β Peptide Impact on Synaptic Function **(Liu, L et *al.*, 2012).**

One form, beta-amyloid 42, is thought to be especially toxic. In the AD brain, abnormal levels of this naturally occurring protein clump together to form plaques that collect between neurons and disrupt cell function **(National Institute on Aging (NIA)., 2017).** Aβ deposition does not always follow a stereotypic spatio-temporal pattern of progression. Nevertheless, amyloid plaques in general first appear in the neocortex from where they spread into the allocortex and the subcortical regions **(d 'Errico, P & Meyer-Luehmann, M., 2020) (Fig14).**

I.2.4.2. Neurofibrillary tangles (NFTs)

NFTs begin to appear in the first decade of life, increase with aging, and are present in 100% of people in their 40s. NFTs increase very slowly with age **(Sengoku, R., 2020)**. They are associated with neuronal death in AD **(Luna-Viramontes, N. I et *al*., 2020).** Evidence is accumulating that Tau protein is a critical predictor of AD progression, and it exhibits a closer correlation to the degree of cognitive impairment in patients than Aβ **(Ji, L et *al*., 2023).**

NFTs primarily consist of phosphorylated tau (pTau). In the healthy brain tau binds to microtubules, providing stability and facilitating axonal transport. In AD tau becomes hyperphosphorylated, causing it to disassociate from microtubules and aggregate into the paired helical filaments that are present in NFTs and dystrophic neurites **(Drummond, E et *al*., 2020).** The development of NFTs evolves in the brain with a predictable and hierarchical distribution pattern that starts from layer II of the entorhinal cortex, spreads through the limbic and associations areas to finally reach the hippocampus and neocortex **(d 'Errico, P & Meyer-Luehmann, M., 2020) (Fig.15).**

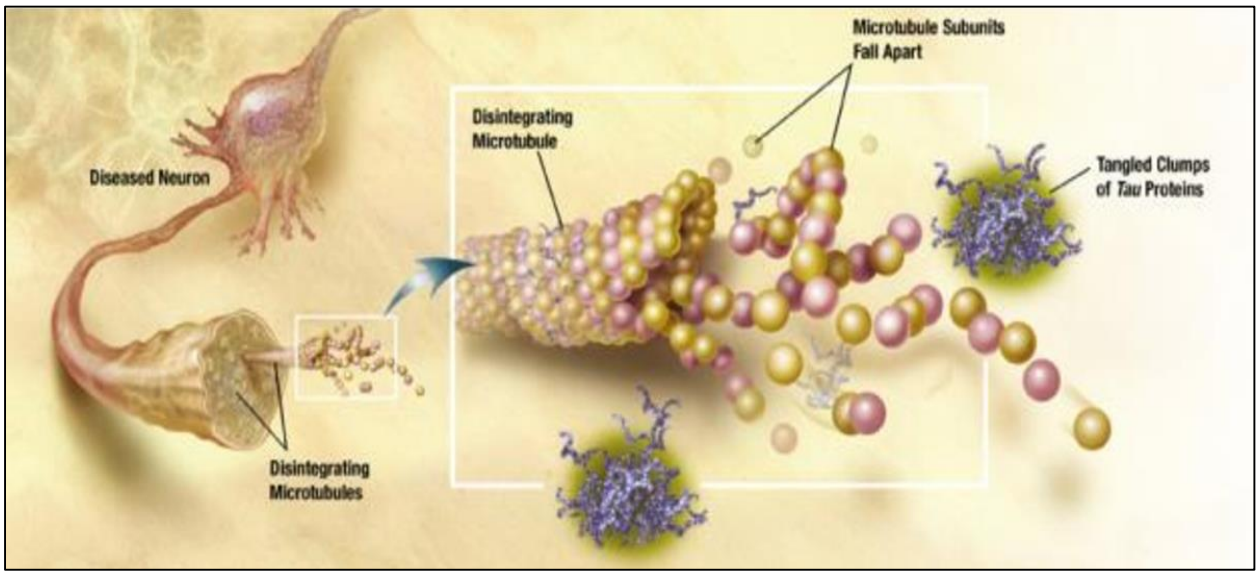


Figure 15: Tau protein accumulation **(Liu, L et *al.*, 2012).**

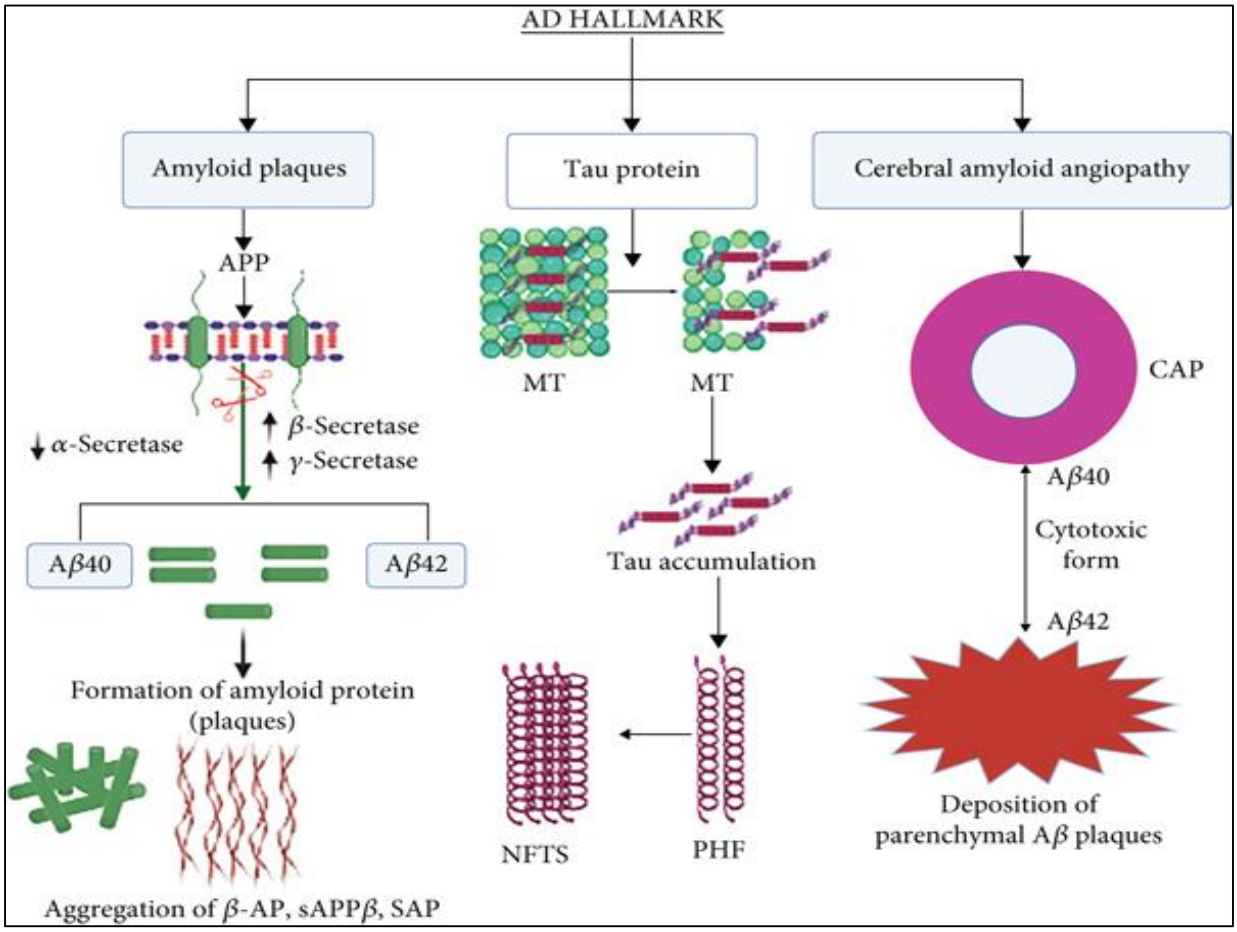


Figure 16: This figure depicts the overview of pathological hallmark of AD through amyloid plaque formation, NFT formation through Tau protein, and deposition of parenchymal Aβ plaques. **(Minocha, T et *al*., 2022).**

I.2.5. Alzheimer's disease stages

AD is classified into preclinical or presymptomatic, mild, and dementia-stage depending on the degree of cognitive impairment **(Singh, N. A et *al*., 2018).** AD typically progresses slowly in three stages: mild AD, moderate AD and severe AD. Since Alzheimer's affects people in different ways, each person may experience dementia symptoms — or progress through the stages — differently **(Alzheimer's Association., 2023) (Fig.17).**

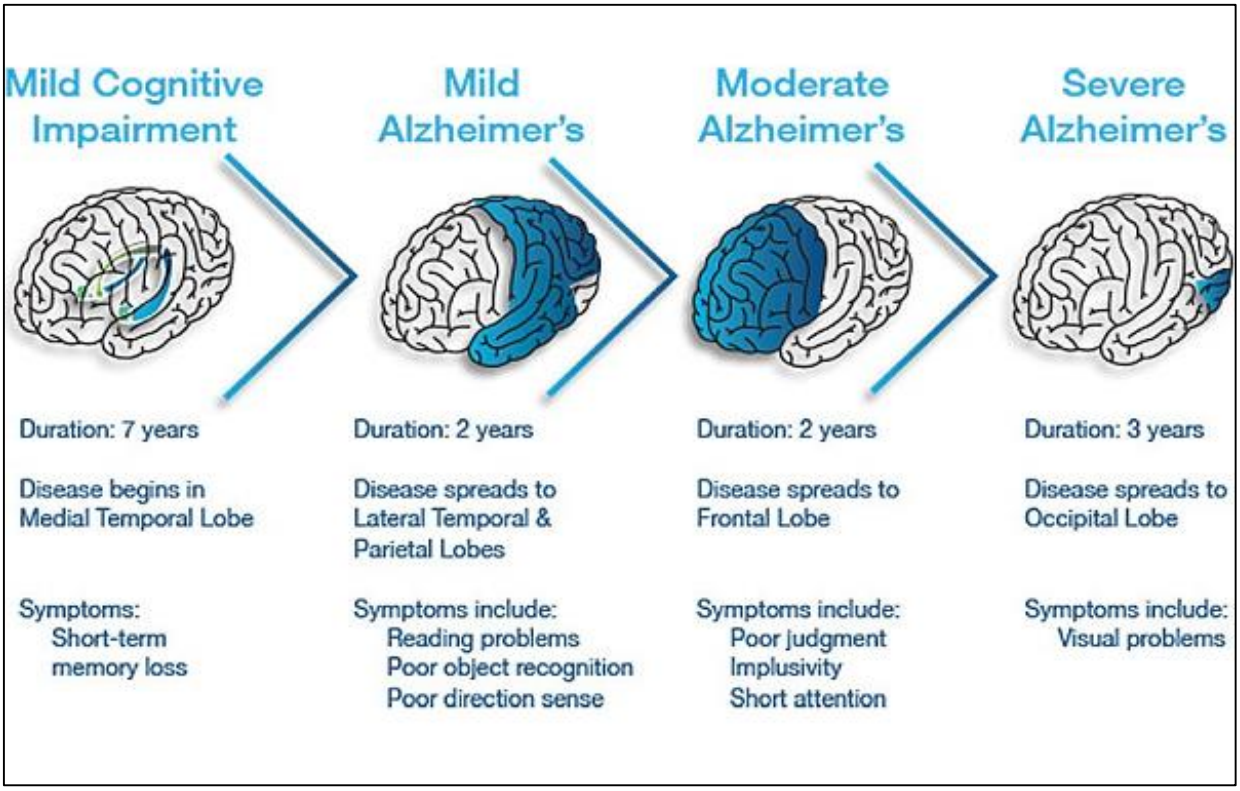


Figure 17: The Stages of Alzheimer's Disease **(Anonymous[9]., 2017).**

I.2.6. Diagnosis

To diagnose Alzheimer's, physicians may use medical history, mental status tests, physical and neurological exams, diagnostic tests and brain imaging **(Alzheimer's Association., 2023).**

Current AD diagnosis is primarily based on neuropsycho-logical testing. A clinical diagnosis of AD requires neu-roimaging and monitoring accepted biomarker. Magneticresonance imaging (MRI) at increasingfield strength andresolution is another helpful, noninvasive approach foridentification of functional abnormalities. MRI is utilizedfor detection and identification of amyloid plaques utiliz-ing iron oxide NPs as contrast agents or tagged withfluorescent probes to make detection efficient **(Tiwari, S et *al*., 2019) (Fig.18).**

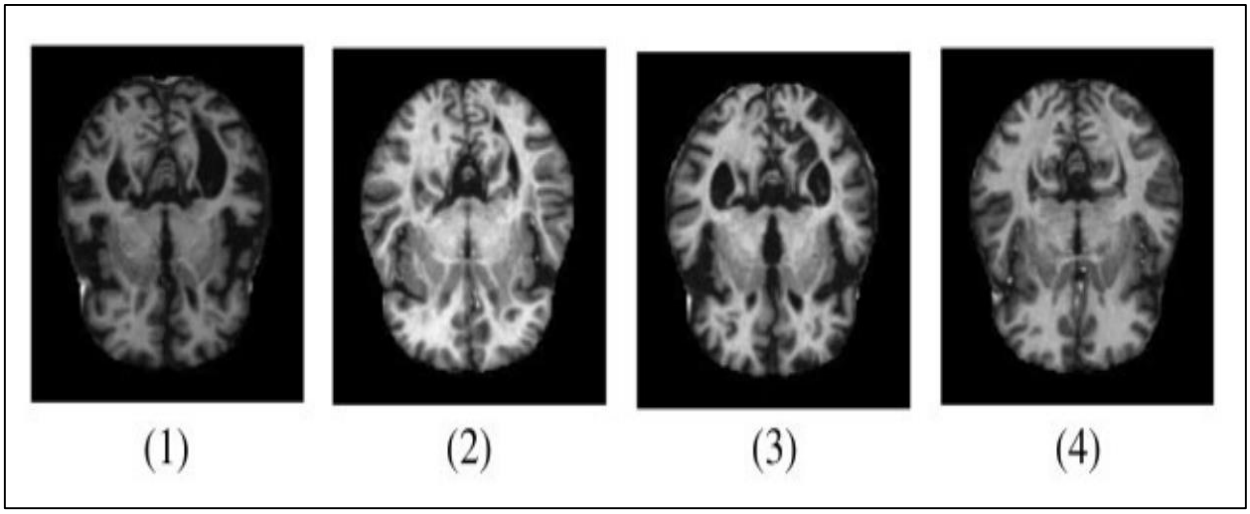


Figure 18: Samples of MRI images representing different AD stages. (1) MD; (2) MOD; (3) ND; (4) VMD **(Ghazal, T. M & Issa, G., 2022).**

I.3. Alzheimer disease Treatment

I.3.1. Synthetic treatment

The foundation of current Alzheimer's disease (AD) treatment involves pharmacological and nonpharmacological management and care planning predicated on patient-centered psychoeducation, shared goal-setting, and decision-making forged by a strong triadic relationship between clinician and the patient-caregiver dyad **(Atri, A., 2019).**

There are currently four FDA-approved medications for the management of cognitive impairment and dysfunction in global activities in symptomatic AD. These include three cholinesterase inhibitors (ChEIs; donepezil, rivastigmine, and galantamine) and memantine, an uncompetitive NMDA receptor modulator (**Long, J. M & Holtzman, D. M., 2019).**

I.3.1.1. Cholinesterase inhibitors (ChEIs):

For exemple "Rivastigmine" works as a reversible inhibitor of AChE and butyrylcholinesterase (BChE), obtained as a semi-synthetic derivative of physostigmine, a natural AChE inhibitor. Rivastigmine is used to treat memory loss associated with mild, moderate, or severe AD **(Plascencia-Villa, G & Perry, G., 2022).** Facilitate central cholinergic activity by reducing the physiological breakdown of (ACh) by the enzyme acetylcholinesterase (AChE) in the synaptic cleft **(Atri, A., 2019).** The primary route of elimination for rivastigmine is liver and intestine metabolism; the elimination half-life of rivastigmine is very short (1-2 hours for oral and 3-4 hours for transdermal administration), but the duration of action is longer as acetylcholinesterase and butyrylcholinesterase are blocked for around 8.5 and 3.5 hours, respectively **(Yiannopoulou, K. G & Papageorgiou, S. G., 2020).**

The most common adverse effects are triggered by the cholinomimetic action of the AChEIs on the gastrointestinal tract and often include diarrhea, nausea, and vomiting. Rapid eye movement sleep behavior disorder has been also remarked in some individuals **(Yiannopoulou, K. G & Papageorgiou, S. G., 2020)**. While certain FDA-approved medications are approved for the management of AD, the results are frequently disappointing, and there is a space for alternative therapies, particularly herbal therapy **(John, O. O et al., 2022).**

I.3.2. Phytotherapy: A promising approach for the treatment of AD

Herbal medicine (Phytotherapy) is one of the oldest forms of therapy on our planet. In all parts of the world, independent forms of healing with plants have developed over the centuries, such as Ayurveda in India, Kampo medicine in Japan, and traditional Chinese medicine (TCM) **(Zimmermann-Klemd, A. M et *al*., 2022).**

The specificity of phytoconstituents and extracted chemicals for Brain receptors suggests that herbal medicines may play an essential role in the management of neurological illnesses. Numerous herbs have been shown to improve brain function and may have a role to play in AD treatment **(John, O. O et *al*., 2022).** Herbs such as Thymus vulgaris, Hypericum perforatum, Matricaria chamomilla, Salvia officinalis, Allium sativum, Ziziphus jujube, Lavandula officinalis, Curcuma longa, are considered to treat AD **(Karimi, E., 2020).**

I.3.2.1. Some medicinal plants

I.3.2.1.1. *Thymus vulgaris* L.

Thymus vulgaris L. is a flowering plant of the family Lamiaceae commonly known as thyme, native to Southern Europe, and has a worldwide distribution **(Kuete, V., 2017).** Plant of Thyme grows in the western Mediterranean region **(Rizwan, B., 2021) (Fig.19).**

Figure 19: *Thymus vulgaris* L.
(Anonymous[10], 2023)

The phenols, thymol (40%) and carvacrol (15%) are main components of TV. It was contains less amounts of phenol during the winter. Also, thymol methyl ether (2%), cineol, cymen, pinene, borneol and esters are components in the essential oil. Researchers have suggested that neuroprotective effects of thymol can attribute to its potential effect on GABA-mediated inhibition of synaptic transmission **(Khazdair, M. R et *al*., 2019).**

I.3.2.1.2. *Moringa oleifera* L.

Moringa oleifera L. is a traditional Indian herb **(Mundkar, M et *al*., 2022).** (Moringaceae family, commonly known as Horseradish tree) is a perennial herb tree that extensively grows in topical and subtropical countries **(González-Burgos, E *et al*., 2021) (Fig.20).**

Figure 20: *Moringa oleifera* L. **(Anonymous[11], 2018).**

Showing neuroprotective effects in several neurodegenerative disorders including AD. Owing to its several phytoconstituents including β-carotene, quercetin, kaempferol, ascorbic acid, flavonoids, phenolic acid, glucomoringin, and isothiocyanates **(Mundkar, M et *al*., 2022).** These phytochemicals reduce the level of lipid peroxides by increasing the production of catalase and SOD thereby leading to improved cognition in the brain **(John, O. O et *al*., 2022).**

I.3.2.1.3. *Glycyrrhiza glabra* L.

Glycyrrhiza glabra L. commonly known as liquorice, perennial herb of the pea family (Fabaceae), and the flavouring, and folk medicine made from its roots. Native to southern Europe, mainly cultivated around the Mediterranean and in parts of the united-**States (Britannica., 2023) (Fig.21) .**

Figure 21: *Glycyrrhiza glabra* L. **(Anonymous[12]., 2020).**

Glycyrrhizin's effects in neurological disorders are mainly attributed to the attenuation of neuronal damage by inhibiting HMGB1 expression and translocation as well as by downregulating the expression of inflammatory cytokines **(Paudel, Y. N et *al*., 2020).** The neuroprotective activity is due to the major active *isoflavan* "Glabridin" **(PRAJWAL, S & KUMAR, M. R., 2022)**. it's bioactive component, glycyrrhizin, a triterpenoid saponin glycoside **(Hamad, G et *al*., 2020).**

I.3.2.1.4. *Nigella Sativa* L.

Nigella sativa L. is commonly known as Black cumin, belongs to the family Ranunculaceae. It is used from hundreds of years ago in various systems of medicine like Ayurveda, The seed It has numerous pharmacological activates like neuroprotective **(PRAJWAL, S & KUMAR, M. R., 2022) (Fig.22).**

Figure 22: *Nigella Sativa*L.
(Anonymous[13], 2023)

Several bioactive compounds from the seed of N. sativa have been reported among those the most important bioactive ones are thymoquinones: include sterols and saponins, phenolic compounds, alkaloids, fatty acids, and volatile oils of varying composition **(Yimer, E. M et *al*., 2019). Hosseini M et *al*.** investigated neuroprotective activity of hydro-alcoholic extract of seeds of N.sativa against scopolamine-induced spatial memory impairment in malewistar rats. The extract at a dose of 400 mg/kg proved marked neuroprotective activity **PRAJWAL, S & KUMAR, M. R., 2022).**

I.3.2.1.5. *Withania somnifera* L.

Withania somnifera L. (solanaceae family); commonly known as Indian Ginseng", found in the drier parts of tropical and subtropical zones, the Mediterranean, Africa, South Asian countries, and the Middle East. been widely used in the indigenous (Ayurvedic) system of medicine for over 3,000 years **(Speers, A. B et *al*., 2021) (Fig.23).**

Figure 23: *Withania somnifera* L.
(Anonymous[14], 2023)

"WS" contain Withanolides, a group of steroidal lactones, and Withanamide A and C which are mostly responsible in protective effect against free radicals damage, Withanamide binds to Aβ inhibit fibril formation in the neurons andreduce Aβ toxicity to the cells **(Abdullah, F et *al*., 2022).**

I.3.3. Apitherapy: as a Possible Complementary Treatment for AD

Honeybees, also known as the ‘’Golden insect‘’, belong to the genus Apis, which is the Latin word for ‘’bee’’. These marvelous social insects live in a well-organized community and belong to the order Hymenoptera and to the family Apidae. Honeybees can be found all around the world and are used for their vital role as pollinators in agriculture, but the main used species for crop pollination is Apis mellifera. Since ancient times, honeybee products have been used for medicinal purposes **(Nader, R. A et *al.*, 2021) (Fig.24).**

Figure 24: ***Apis mellifera*** L**. (Wehbe, R et *al.*, 2019).**

Bee (*Apis mellifera* L.) products have a wide space among complementary medicinal methods. The use of bee products in medicine, called apitherapy. Apitherapy is a complementary medical technique that has an old history and is applied in various diseases worldwide. Apitherapeutical applications are not treatment methods by themself, but they can be substantial parts of multidisciplinary approaches **(SİG, A. K et *al.*, 2019).**

Cumulative evidence shows that bee products may prevent neurodegeneration in Alzheimer’s disease by protecting the blood brain barrier against leakage, improvement of neuronal energy supply, downregulation of inflammatory and oxidative signaling, improvement of signal transduction (e.g., acetylcholine) increasing the production of neurotrophic factors, increasing beta amyloid clearance from the brain and discouraging its accumulation inside neurons **(Ali, A. M & Kunugi, H., 2020).**

I.3.3.1. Beehive products

Beehive products have been used for thousands of years in many cultures for the treatment of various diseases. Their healing properties have been documented in many religious texts like the Noble Quran and the Holy Bible. Honey, bee venom, propolis, pollen and royal jelly all demonstrated a richness in their bioactive compounds **(Nader, R. A et *al*., 2021).**

I.3.3.1.1. Honey

Honey is a natural sweet substance constituted by hundreds of compounds **(Combarros-Fuertes, P et *al*., 2020)** made by bees using nectar from flowers. The flower from which bees gather nectar, determines the colour, chemical composition, flavour and aroma of the honey **(Adamu, H. I et *al*., 2021) (Fig.25).**

Figure 25: Represent "Honey" **(Anonymous[15], 2021)**

Honey is an extensively consumed plant-based natural food not just because of its unique organoleptic properties and nutritional values but also have high functional properties with numerous health benefits **(Brar, D. S et *al*., 2023).**

I.3.3.1.2. Royal jelly

Royal jelly (RJ) is a white or yellowish gelatinous substance secreted from the mandibular and hypopharyngeal glands of young nurse worker bees (Apis mellifera). It has a pungent smell, a distinct sweet-sour taste, and an acidic pH (3.4–4.5) **(Ali, A. M & Kunugi, H., 2020) (Fig.26).**

Figure 26: Royal jelly **(Anonymous[16]., 2018).**

One hypothesis is that RJ regulates the formation of Aβ via reducing cholesterol levels. Hypercholesterolemia, a known risk factor for AD, has been shown to promote Aβ neurotoxicity, Aβ accumulation and local neuronal loss across epidemiological. Moreover, RJ has anti-oxidative effects and that it enhances neuronal metabolic activities and prevents neuronal loss **(Pan, Y et al., 2018).**

I.3.3.1.3. Propolis

Propolis (bee glue) is a mixture of substances used by bees to defend the hive. Bees collect resins from buds, exudates and other parts of plants, mix them with their own salivary enzymes and beeswax which creates propolis **(Przybyłek, I & Karpiński, T. M et al., 2019) (Fig.27)**.

Figure 27: Propolis **(Anonymous[17], 2021).**

Propolis contains pharmacologically active constituents such as polyphenols, terpenoids, steroids, and amino acids. In vivo studies of Indian propolis have shown that propolis is a potential treatment for Alzheimer's disease by inhibiting AChE and increasing brain monoamines. Brazilian propolis, which contains kaempferol, has been reported to have a neuroprotective effect on preventing oxytosis/ferroptosis in HT22 cells **(Syaifie, P. H et *al.*, 2022).**

I.3.3.1.4. Pollen

Bee pollen, also known as apicultural or bee-collected or corbicular pollen, is a microscopic structure like grains, found in the anther of stamen in the angiosperms **(Thakur, M & Nanda, V., 2020).** Pollen is a combination of plant pollen and honeybee secretions and **nectar (Khalifa, S. A et *al.*, 2021) (Fig.28).**

Figure 28: Pollen **(Anonymous[18], 2012).**

It is considered a gold mine of nutrition due to its active components that have significant health and medicinal properties. Bee pollen contains bioactive compounds including proteins, amino acids, lipids, carbohydrates, minerals, vitamins, and polyphenols, mainly flavonoids **(Khalifa, S. A et *al.*, 2021).**

I.3.3.1.5. Bee venom

Bee venom (commercially known as Apitox or Apitoxin) is secreted by a gland located in the abdominal cavity of the bees (Apis mellifera L.). It is an odorless and transparent acidic liquid with bitter taste that bees often use as a defense tool against predators **(Khalil, A et al., 2021) (Fig.29).**

Figure 29: Bee stinger **(Anonymous[19], 2023).**

BVT, bee sting therapy or acupuncture is a complementary and integrative medicinal technique by application BV via bee sting to the particular points of the patient's body **(SİG, A. K et *al.*, 2019).** BV also possesses a neuroprotective potential in neurodegenerative diseases such as AD by significantly blocking their progression and improving cognitive functioning in mice models **(Wehbe, R et *al*., 2019).**

Chapter II

Sweet chestnut «*Castanea sativa* Mill.» honey

II. Honey

II.1. Generality

Honeybees make honey "known as 'asl (Arabic), angabīn (Persian), shehed (Urdu), and madhu (Hindi)" **(Nikhat, S & Fazil, M., 2022)** after feeding on flower nectar, blossoms, or by sucking on the flower's secretions. The collected substances are mixed together with other specific compounds from the honeybees and are then deposited by the honeybees in the wax honeycomb and allowed to mature over time **(Almasaudi, S., 2021).** Honey contains macro and micronutrients which depends basically on various factors: 1) bee type, 2) floral source, and 3) environmental and processing factors **(Ranneh, Y et *al.*, 2021).** Historically, the medicinal use of honey has been recorded in the ancient age as early as 8000 years ago **(Nikhat, S & Fazil, M., 2022).**

The color can be white, yellow, brown, among others, and is determined, in part, by the presence of phenolic compounds, flavonoids, and minerals, The development of the color is also linked to monosaccharide content, which are the sugars (fructose and glucose, maltose) that are found in the highest percentage and are responsible for some other sensory and functional properties such as flavor, texture, moisture retention, shelf life, conservation **(Becerril-Sánchez, A. L et *al.*, 2021) (Fig.30).**

Figure 30 : Different honey color **(Anonymous[20]., 2022).**

The chemical composition of honey primarily depends on the botanical origin of species or cultivars. However, climatic conditions and/or geographical origin can also affect the chemical composition even within the same honey type **(Sedláčková, V. H et *al.*, 2021).** However, honey of all origins is composed mainly of the sugars glucose sucrose, and fructose, which constitute ~80% of its weight,

with water composing the remaining 20%. In addition, vitamins, flavonoids, amino acids, enzymes, minerals, and phenolic acids are also present in honey **(Almasaudi, S., 2021).**

Honey is characterized as a natural and raw foodstuff that can be consumed not only as a sweetener but also as medicine due to its therapeutic impact on human health **(Fakhlaei, R et al., 2020).** Recent researches have revealed the presence of many bioactive compounds in honey with promising health effects like antioxidant, anti-diabetic, anticancer **(Nikhat, S & Fazil, M., 2022),** anti-inflammatory, antimicrobial, and antihypertensive to hypoglycemic effects, also, for the treatment of coughs and sore throats, dry eye symptoms, leg ulcers, wounds, earache, gastric ulcers, and constipation (**Hossain, M. et *al*., 2021),** honey possess nutritious properties which alleviate AD **(Shahar, S., 2020).**

In the last years, several studies have shown that bioactive compounds from natural products possess neuroprotective properties and are capable of relieving AD symptoms, indicating that natural source-based drugs could be a valid alternative in this therapeutic area **(Cianciosi, D et *al.*, 2021).**

Investigations of honey's influence on neurodegenerative disease prevention mainly focuses on the presence of polyphenol or phenolic compounds. Various established cell models and polyphenols were used for neurodegenerative studies and enzyme analysis associated with the excretion of inflammatory cytokines and inhibiting enzymes that trigger cell inflammation responses. Polyphenols are also investigated for their potential to ameliorate the effects of dysfunctional proteins or pathological aggregates in the brain **(Fadzil, M. A. M et *al.*, 2023).**

II.2. Chestnut honey

II.2.1. Sweet chestnut plant

Castanea sativa Mill., commonly known as European sweet chestnut, belongs the genus *Castanea* of the (Fagaceae family). The genus *Castanea* Mill. Is found in southern Europe countries such as (Italy, Spain, France, Greece, Portugal and Turkey), eastern North America, northern Africa, Asia Minor and eastern **Asia (Massantini, R et *al*., 2021) (Fig.31).**

Figure 31 : Sweet chestnut « *Castanea sativa* Mill. » **(Anonymous[21], 2023).**

Sweet chestnut trees are valuable not only as food but also as a honey source plant, are already classified as major honey plants in many countries **(Sedláčková, V. H et *al*., 2021).**

II.2.2. Sweet chestnut honey

Sweet chestnut honey (SCH) (monofloral honey) is produced from flowers of the chestnut tree (*Castanea sativa mill*) by honeybees, and it is consumed mainly as a nutritional food **(Sánchez-Martín, V et *al*., 2022)** also in the pharmaceutical and cosmetic industries **(Machado, A. M et *al*., 2020) (Fig.32).**

Figure 32 : Flowering chestnut trees **(Anonymous[22]., 2022).**

Sweet chestnut honey has a strong aromatic taste and a slightly bitter after taste. Dark in color, ranging from yellowish brown to almost black, sometimes with amber hues, it has an aromatic, pungent herbal aroma and taste and slightly tannic (due to the tannin in the tree) **(Beşir, D. A. Ğ., 2017).**

II.2.3. Botanical source

Honey composition is strongly associated with its botanical source and the geographical area of collection. Chemical volatile composition has great importance in characterizing honey's botanical source, which directly influences its organoleptic characteristics **(Machado, A. M et *al*., 2020).**

The sweet chestnut (*Castanea sativa* Mill.) is the only native species of the genus in Europe. The distribution area ranges from Southern Europe (Iberian Peninsula, Italy, Balkans, Mediterranean Islands) and North Africa, to North-Western Europe (England, Belgium) and eastward to Western Asia (North East Turkey, Armenia, Georgia, Azerbaijan, Syria), with an altitudinal range between 200 and 1800m, depending on the latitude and site aspect **(Conedera, M et *al.*, 2016).**

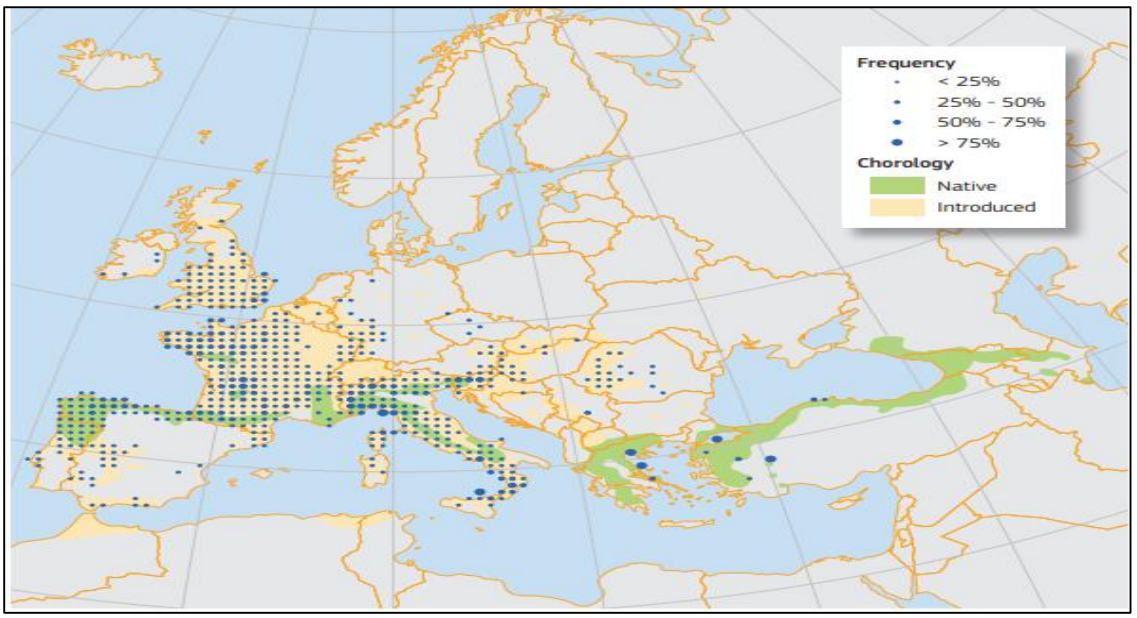


Figure 33 : Map of Plot distribution and simplified chorology map for *Castanea sativa* Mill. Frequency of *Castanea sativa* occurrences within the field observations as reported by the National Forest Inventories. **(Conedera, M et al., 2016).**

II.2.4. Chemical composition

The main compound of the chestnut is water, where the moisture content ranges from 40 and 64 g/100 g fresh weight. Studies conducted on chestnuts' chemical and nutritional composition confirm that this fruit is low in fat, cholesterol-free, and gluten-free. On the other hand, it is a rich source of starch (carbohydrates), protein, dietary fiber, vitamins, minerals (such as potassium, phosphorous, and magnesium), lipids, and nutrients **(Santos, M. J et *al*., 2022) (Tab.1, Tab.2).**

Table 1: The Chemical Composition of sweet chestnut Honey **(Rodríguez-Flores, S et *al.*, 2016; Temizer İ.K et *al*., 2018).**

Chemical	Mean
pH	4.20 - 6.59
DIASTASE NUMBER	24.3 DN
PROLINE mg/kg	585 mg/kg
FRUCTOSE %	37.2%
GLUCOSE %	25.9%
MALTOSE %	< 5%

Table 2 : The composition of mineral elements in chestnut honey (in mg/100g of honey) **(João C. M. Barreira et *al*., 2019).**

Mineral elements	Content in mg/100g of honey
Potassium (K)	473–974
Phosphorous (P)	104–148
Magnesium (Mg)	63–93
Calcium (Ca)	41–51
Iron (Fe)	5.3–10.9

II.2.4.1. Sweet chestnut honey (SCH) phytochemicals

SCH contain nutraceutical compounds (vitamin C, vitamin E) **(Wani, T. A et *al.*, 2020)**, and is a known source of phenolic bioactive compounds, such as L-ascorbic acid, carotenoids, and phenolic compounds such as gallic and ellagic acid and flavonoid particularly rich in kaempferol and quercetin **(João C. M. Barreira et *al.*, 2019)**, but, in particular of tannins. Tannins have been classified into two majors groups: hydrolysable and condensed tannins. Hydrolysable tannins can be subdivided into two subclasses: gallotannins and ellagitannins. **(Chiarini, A et *al.*, 2013) (Tab.3, Tab.4).**

Table 3 : Polyphenolics bioactive in SCH (Tanin – Flavonoid) **(PubChem., 2023).**

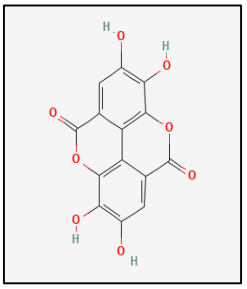
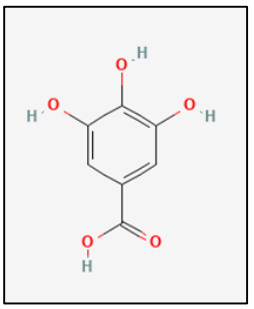
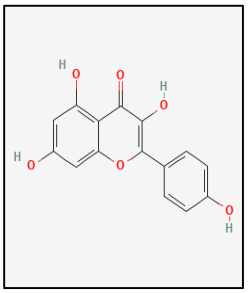
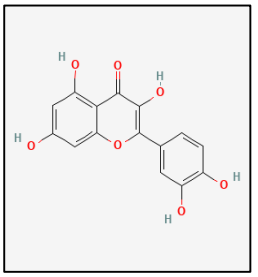
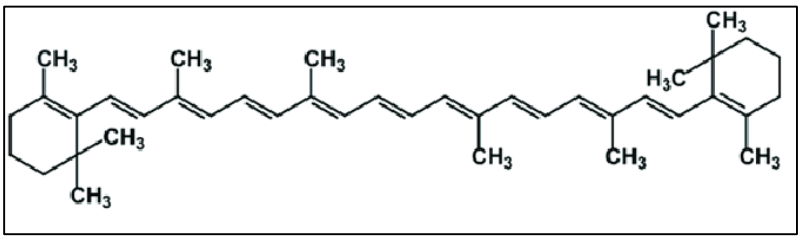
Tanins		**Flavonoid**	
 - Ellagic acid -	 - Gallic acid-	 - Kaempferol -	 - Quercetin -

Table 4: Terpene bioactive (Carotenoids) **(Jeyakodi, S et *al.*, 2018).**

Terpene : Tetraterpenoids.
 **- Carotenoids -**

II.2.5. Importance and Usage

"SCH" compounds have antioxidant activities, anti-carcinogenic activities, cardioprotective activities and antimicrobial activities. As such, sweet chestnut and its by-products could be a great prospect for exploitation as medical foods **(Wani, T. A et *al*., 2020).** Different studies indicate that ellagitannins have antiatherogenic, anti-thrombotic, anti-inflammatory, and antiangiogenic properties **(Chiarini, A et *al*., 2013).**

II.2.5.1. Antioxidant activity

In recent years, studies focused on the prevention of aging, degenerative heart and nervous system diseases and the protection of foods from oxidation, especially related to human health, have increased by utilizing the antioxidant properties of chestnut honey. The activity was due to its rich content of phenolics, flavonoids, vitamins, and sugar components **(Keskin, M et *a*l., 2023).**

II.2.5.2. Cardioprotective activity

Honey has cardioprotective and therapeutic effects against the heart diseases and vasomotor dysfunctions brought on by adrenaline. SCH contains flavonoids that enhance coronary vasodilation, reduce platelet clotting potential, limit low-density lipoprotein (LDL) oxidation, raise HDL levels, and enhance endothelial function. Consuming honey significantly decreased the risk factors for cardiovascular and metabolic disorders **(Karapetkovska-Hristova, V & Mustafa, S. K., 2022).**

II.2.5.3. Anti-carcinogenic activity

Natural products with bioactive components are widely studied on various cancer cell lines for their possible cytotoxic effects, recently. Among these products, honey stands out as a valuable bee product containing many active phenolic compounds and flavonoids. Chestnut found to have an outstanding cytotoxic effect on breast cancer cell lines, MCF-7, SKBR-3, and even on the most aggressive MDA-MB-231, representing triple negative breast cancer, which lacks of targeted anti-cancer therapy **(Seyhan, M. F et *al*., 2017).**

II.2.5.4. Antimicrobial activity

Sweet chestnut honey has already been reported to have high antimicrobial effect against E. coli. The tissue of chestnut plants contains compounds such as tannins and antioxidants, which have inhibitory effects on microorganisms, and 3-aminoacetophenone is the main volatile compound occurring specially in this floral source, known as having antibacterial properties (Bonaga and Giumanini) **(Oliveira, A et *al.*, 2018).**

II.2.5.5. Anti-inflammatory activity

Chestnut honeys protect against inflammation by regulating the main inflammatory biomarkers, including TNF-α, IL-10 and iNOS, through the decrease of NF-κB expression. Finally, they improved mitochondrial respiration and the main related parameters **(Cianciosi, D et *al.*, 2021).** Properties of honey flavonoids suggest that these compounds are important modulators of inflammatory processes **(Stavropoulou, E et *al.*, 2022).**

Experimental study

Materials and methods

I.1. Objective

This study aimed to investigate whether sweet chestnut honey has protective effects on the brain in the Aluminum chloride ($AlCl_3$) and D-galactose (D-gal), induced-Alzheimer's disease (AD) mice model. The experimental protocol is based, on the Alzheimer model described by **Feng et *al.* (2018); Xing et *al*. (2018).**

I.2. Hive product

The hive product chosen in our study is sweet chestnut honey (Balparmak) Harvested in 2019 in Western Black Sea, Turkey **(Fig.34).** The choice of this variety is selected because researchers suggests that honey supplementation can protect from neuroinflammation, reduce oxidative stress, and increase brain-derived neurotrophic factors.

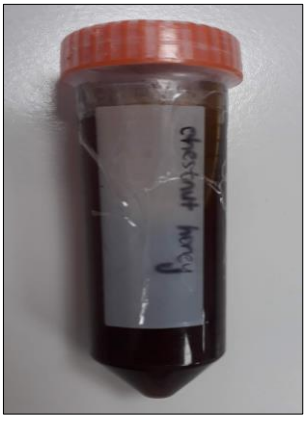


Figure 34: Represent Sweet chestnut honey **(Balparmak).**

I.3. Animal material

An effective of 37 female NMRI albino mice were purchased from the Institute pasteur, of body weight 35 ± 5 g were used for the present study. They were elevated in the Animal House (Part of the laboratory "LPAP") of the University Abdelhamid Ibn Badis -Mostaganem-. The animals were fed with ad libitum access to food and water in a room under a 12 h light/dark cycle. The room temperature was kept at 25°C.

An adaptation period of 15 days was necessary in order to acclimatize these animals to our working conditions.

I.4. Toxicity test

In order to avoid any possible toxicity during biological tests, it is necessary to carry out preliminary toxicity tests.

The dose toxicity study is established according to the method written by the Organization for Economic Cooperation and Development **(OECD, 2008) test No. 425.** The principle of this test is to observe the appearance of signs of toxicity, change in behavior (increased activity) and mortality. These signs are observed regularly from 30 min, 24 h, 48 h up to 14 days **(OECD, 2008).**

Four doses of sweet chestnut honey are chosen for each group, which correspond to 150mg/kg, 300mg/kg and limit doses of 1000mg/kg and 2000mg/kg. The doses are administered by gastric gavage. **(Fig.35)**

Groups: Aqueous solution of sweet chestnut honey.

Groupe 01 (n=3): Receives a single dose of 150mg/kg, by. gavage.

Groupe 02 (n=3): Receives a single dose of 300mg/kg by. gavage

Groupe 03 (n=3): Receives a limit dose of 1000mg / kg by. gavage.

Groupe 04 (n=3): Receives a limit dose of 2000mg/kg by. gavage.

Figure 35: Represent the toxicity test groups.

I.5. Evaluation of the neuroprotective activity of aqueous solution of sweet chestnut honey (SCH)

Our experiment is divided into two phases 45 days each. The first phase is where the treated Alzheimer mice (Alz-D1; Alz-D2; Alz-STD) received the preventive treatment during the first 45 days of our experimentation, followed by the induction of Alzheimer's disease to the Alzheimer model mice and (Alz-D1; Alz-D2; Alz-STD) during the second 45 days of our experimentation.

Experimental protocol of neuroprotective activity over a period of 90 days.

The first phase of the protocol (D1 - D45), preventive treatment by "gavage".

The second phase of the protocol (D46 - D90), induction of the « Alzheimer disease » orally and by IP. Injection.

Rivastigmine (1,5 mg/kg) for the Group (Alz-STD) and Two doses of sweet chestnut honey solution for the groups (Alz-D1) and (Alz-D2) at (150mg/kg, 300mg/kg).

Administration of aluminum chloride ($AlCl_3$) at 100 mg/kg, administered orally combined with a daily IP Injection of D-galactose at 120 mg *kg*.

I.5.1. Group distribution

After the toxicity test was done (after 14 days), an effective of 25 mice were divided according to their body weight, five groups each containing five mice.

Group control (n=5) – correspond to the group that received "food and water" during the whole experience (90days).

Group ALZ (n=5) – Correspond to Alzheimer's model mice.

Group Alz-STD (n=5) – Correspond to Alzheimer's mice treated with "Rivastigmine" at 1.5 mg/kg.m. by, *gavage* during the first 45 days of experimentation, followed by disease induction for the second 45 days of the experimentation;

Group Alz-D1 (n=5) – Correspond to Alzheimer's mice treated with SC honey at 150 mg/kg by., *gavage*, during the first 45 days of experimentation, followed by disease induction for the second 45 days of the experimentation;

Group Alz-D2 (n=5) – Correspond to Alzheimer's mice treated with SC honey at 300 mg/kg administered by., *gavage*, during the first 45 days of experimentation, followed by disease induction for the second 45 days of the experimentation **(Fig.36).**

Figure 36: Represent the experimental groups distribution.

I.5.2. Biological parameters studied

I.5.2.1. Body weight evolution

Performing weekly body weight measurements for each mouse of each groups during 14 weeks of the experimentation. The goal is to assess the growth and physiological development of the animals.

I.5.2.2. Solution consumed

The solution consumption was measured each week for each group; these measurements are essentially intended to evaluate the average volume of both "tap water" for the first 45 days of experimentation and "$AlCl_3$" solution for the second 45 days of experimentation.

At the end of the protocol (90 days), the mice are subjected to neurological tests, both "memory test" and "behavioural test",

I.5.2.3. Memory tests

I.5.2.3.1. Eight-arm radial maze (RAM)

It is one of the most used methods in behavioral laboratories, which is proposed by **Wan et *al*., (1997),** mainly due to the flexibility of its structure. The purpose of this device is to be able to test the spatial and non-spatial selection memory stimulus linked with the motivational elements.

The 8-arm radial maze is a widely validated device for learning tasks and the number of arm entries can be measured. Poor spatial working memory is correlated to increased feedback to arm choice and overall time to complete the task. **(Burette et *al.*, 2000).**

a- Spatial working memory

Spatial working memory is one of the most studied cognitive functions, serving as a model system to decipher computational principles of the brain **(Ayano Matsushima et *al*., 2014).**

In this test the food is placed at one end of a lane of eight. Then the mouse is placed on the central platform with free access to all the corridors. The mouse must look for food at the bottom of each corridor, *each error is recorded if the mouse visits the same corridor twice.* **(Fig.37).**

The number of visits for each mouse is counted during 4 practice trials over 4 days and the 5th day (essay) represents the test. Each test (essay) lasts 5 minutes. This test was developed by **Olton (1997).**

Figure 37: Spatial working memory (Radial arm maze).

b- Position distinction

During this test six arms of the maze are used. The mouse is placed on the central platform and the six arms are opened one after the other, three with food (baited arms) and the others without food (unbaited arms). The test will be carried out by opening the arms in a pair of baited and unbaited arms (3 pairs in total) **(Fig.38).** The score being recorded each time by connecting the number of baited arms chosen by each mouse measured during 4 learning essays for 4 days and the 5th day (essay) represents the test. Each session (essay) lasts 5 minutes. **(Lenck-Santini et al., 2001).**

Figure 38: Position distinction (Radial arm maze).

c- Spatial reference memory (MSR)

In this test, only two arms of the maze are used. One arm illuminated with food at its end, the other arm dark. The animal is dropped into the center of the maze and both arms are opened simultaneously, and this is when the test begins. Thus the residence time in the illuminated arm is measured during 4 practice essays for 4 days and the 5th day (essay) represents the test **(Fig.39).** Each session (essay) lasts 5 minutes **(Cole and Chappell-Stephenson, 2003).**

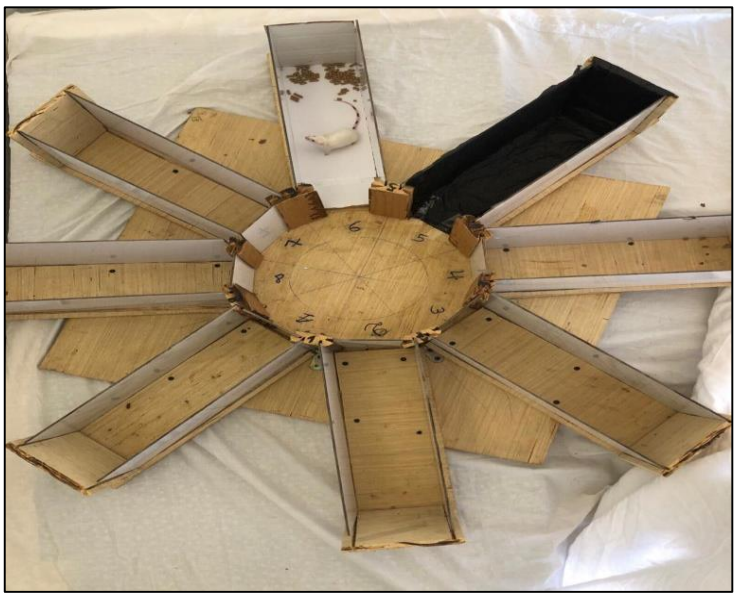


Figure 39: Spatial reference memory (Radial arm maze)

I.5.2.3.2. Morris water maze (MWM)

The Morris water maze is one of the most used tests, it was designed by Morris (1984) to assess the ability to memorize and manage spatial information in an unpleasant situation for the mouse.
The animal must escape an aversive situation by taking refuge on a platform. To do this, he must create and use a representation of his environment based on the available spatial cues. This representation is called allocentric. **(Seron and Van der Linden, 2014).**

a- Spatial working memory

The mouse is placed in a container 147 cm in diameter by 25 cm in height, containing lukewarm water maintained at 21°C. An exposed platform is placed in the pool which is surrounded by visual cues. The time taken by the mouse to climb on the platform is calculated during 4 practice essays for 4 days and the 5th day (essay) represents the test. (**Fig.40)** Each session (essay) lasts 5 minutes.

Figure 40: Morris/ Spatial working memory (Morris water maze).

b- Spatial reference memory (MSR)

The water in the container is colored with a non-toxic dye in order to hide the platform which is itself a little submerged. The time taken by the mouse to find the platform is calculated during 4 practice essays over 4 days and the 5th day (essay) represents the test. **(Fig.41)** Each (essay) session lasts 5 minutes.

Figure 41: Morris/ Spatial reference memory (Morris water maze).

I.5.2.4. Neurological behavior test

Behavioral assessment is a key component in examining nervous status (behavioral determination). These guidelines apply to animals in special essays **(Zerrouki, 2012).**

I.5.2.4.1. Locomotor activity

The open-field test is used to provide a qualitative and quantitative measurement of exploratory and locomotor activity in rodents. The apparatus consists of an arena surrounded by high walls (of 32 X 32 cm^2) and the floor of the open field is divided into squares (from 1 to 16). Crossings refer to the total number of square crossings during the test period, used to provide measurement of locomotor activity of the animals, each move counts as a score **(Valvassori, S. S et *al*, 2017) (Fig.42).** During this test, the behavior of the animal in a new environment is evaluated for 5 minutes in four consecutive phases.

Figure 42: Open field test (Locomotor activity).

I.5.2.4.2. Anxiety test

a- Black/white test box

The validity of a black and white test box to measure changes in mouse exploratory behaviour relevant to assessment of anxiety was investigated by variation of the illumination within the test box **(Costall, B et *al*., 1989). T**he test consists of putting the mouse in a closed cage (L=80cm; I=30cm; h=30cm) **(Rebai, O & Djebli, N. E., 2008) (Fig.43).** During this test, the behavior of the animal in the dark compartment is evaluated for 5 minutes in four consecutive phases.

Figure 43: Black/white test box.

b- The elevated plus maze

The elevated plus maze test is one of the most widely used tests for measuring anxiety-like behavior. The test is based on the natural aversion of mice for open and elevated areas, as well as on their natural spontaneous exploratory behavior in novel environments **(Komada, M et *al*., 2008).** The apparatus used for the elevated plus maze test is in the configuration of a + and comprises two open arms across from each other and perpendicular to two closed arms with a center platform. The entire apparatus is 50 cm above the floo **(Fig.44).** The mice are placed on the central platform Mice are allowed to move freely in the experimental device. This test is based on the calculation of the duration of time spent in the protected corridor for 5 minutes in four consecutive phases.

Figure 44: The elevated plus maze (EPM).

I.5.2.4.2. Persolt test (Forced swim test: FST)

The forced swim test is a rodent behavioral test used for evaluation of antidepressant drugs and experimental manipulations that are aimed at preventing depressive-like states **(Can, A et *al.*, 2012).** The FST is based on the assumption that when placing an animal in a container filled with water (25 C°) **(Fig.45).** It will first make efforts to escape but eventually will exhibit immobility that may be considered to reflect a measure of behavioral despair **(Yankelevitch-Yahav, R et *al*., 2015).** The goal of this test is to calculate the immobility time of each mouse for 5 min.

Figure 45: Persolt test (Forced swim test).

I.6. Histological study

The day after the end of the neurological tests, (Dissection day) the mice were euthanatized. The brain, liver and kidneys were collected and kept at formaline solution (10%) till the start of the historogical study **(Fig.46).**

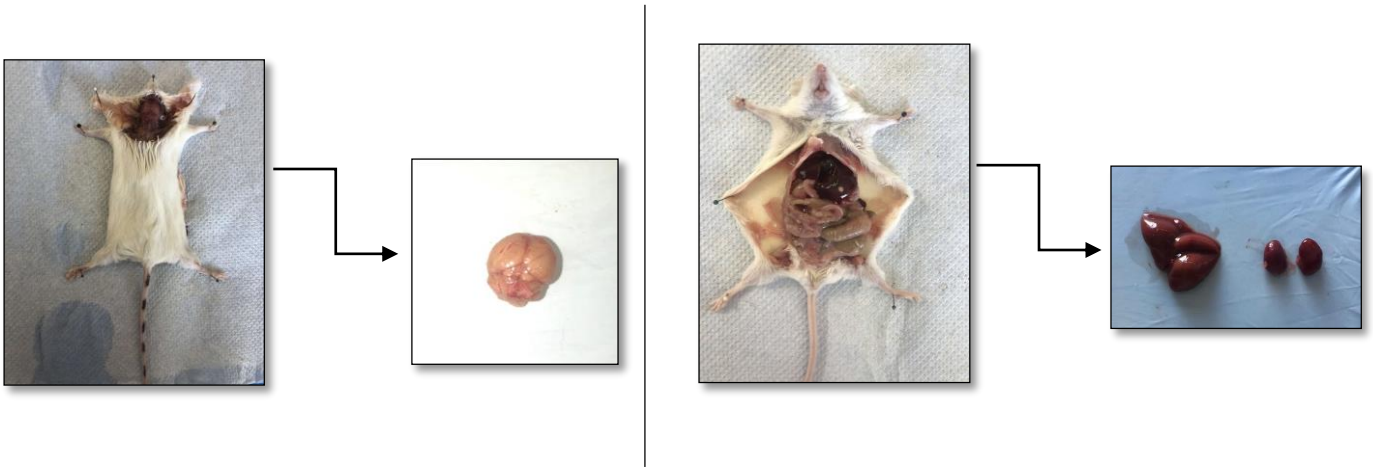


Figure 46: Represent mice dissection.

The anatomopathological study is carried out at the end of the experiment at the laboratory "Pharmacognosy & Api phytotherapy" of the University Abd El Hamid Ibn Badis -Mostaganem, according to the manual of anatomo cytopathology techniques **(Marck, 2010).**

I.6.1. Fixation

The brains are removed and then fixed in a 10% formalin solution.

I.6.2. Macroscopic

A detailed macroscopic examination is an essential part of the study of an operative piece, so our pieces are examined, measured, weighed, palpated and then dissected. Preparation of the fragments for each brain, and then we introduce these fragments into inclusion cassettes. The cassettes were marked on their edge.

I.6.3. Impregnation (circulation)

To obtain cuts of an appropriate thickness, the fabric must be hardened, this is achieved by impregnating it with a rigid material which gives it the desired mechanical resistance. This step is

based on the substitution of water in the tissues by a chemically inactive hydrophobic solution such as paraffin.

Several steps must be followed:

- Post fixation:

- 1 Glass staining dish of 10% formalin.

- Dehydration

- 1 Glass staining dish of 96% ethanol for 1 hour
- 1 Glass staining dish of 96% ethanol for 1 hour
- 1 Glass staining dish of acetone for 2 hours

- Substitution

- 1 Glass staining dish of toluene / xylene for 2 hours

- Impregnation

- 1 Glass staining dish of paraffin at 70°C for 1 hour.

I.6.4. Inclusion

Placing the sampled part in a steel mould and coating it with liquid paraffin. Once the block is prepared, it is stored in a freezer (-20°C).

I.6.5. Microtomy

This step allows the cuts to be made on the block using a microtome. All the slices obtained form a ribbon of very thin quality (2 to 4 µm). The sections are then spread on microscope slides using a hot plate to avoid the formation of wrinkles and streaks.

I.6.6. Coloring

Before the dewaxing step, the slides must be dried to facilitate the adhesion of the sections to the microscope slide. This firing is carried out in a laboratory oven at 58°C for 1 hour.

- **Dewaxing**

The first step in any staining of a histological section is to remove the paraffin from the tissue so that the dyes can penetrate it.

- 1 Glass staining dish of toluene / xylene for 10 min.

- **Rehydration**

Consists of gradually replacing the solvent of the tissue with ethanol baths to bring it to the water.

- 1 Glass staining dish of 70% ethanol for 5 minutes
- 1 Glass staining dish of 80% ethanol for 5 minutes
- 1 Glass staining dish of 96% ethanol for 5 minutes
- Rinse with water for 10 minutes

➢ **Coloring**

Staining is performed with Hematoxylin/Eosin

- 1 Glass staining dish of Harris hematoxylin for 5-10 minutes
- 1 Glass staining dish of acidified water
- 1 Glass staining dish water tank
- 1 Glass staining dish of 96% ethanol
- 1 Glass staining dish of eosin for 5 minutes
- 2 Glass staining dish of acetone, 5 min each
- 1 Glass staining dish of toluene or xylene until assembly.

I.6.7. Montage

This operation consists of using a synthetic resin (EUKITT solution) to fix a coverslip on the section (the slide) in order to protect it from the chemical degradation of the dyes which oxidize in the air and mechanical breakage.

I.6.8. Microscopic reading

The tissue to be studied is observed after assembly by an optical microscope (photo-microscopic) which uses visible light to evaluate certain forms or cellular and tissue anomalies. the microscopic reading is carried out after staining of the tissues which makes it possible to evaluate the cellular state highlighted by the structures, this step is carried out by Hematoxylin / Eosin which helps to colour the nuclei in mauve colour and the cytoplasm in light pink.

I.7. Statistical analysis

The statistical analysis of the experimental data obtained during the tests carried out was realise by using XLstat software. Results were expressed as average ± STDV. $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$.

Results and Discussion

Results

II.1. Toxicity test

The intra-gastric administration of aqueous solutions of *Castanea sativa* Mill. honey with doses 150mg/kg and 300mg/kg and two limit doses 1000mg/kg, 2000mg/kg did not induce any apparent signs of toxicity in the experimental animals during the 14 days of observation. The abnormal signs are Abnormality in mice behaviour, mortiality. **(Tab.5).**

Table 5: Results of the toxicity test of "*Castanea sativa* Mill" honey solution at 150 and 300, 1000 and 2000 mg/kg during 14 days of observation. (- : Nothing to report).

	After 24h	After 5 days	After 10 days	After 14 days
Groupe 01	-	-	-	-
Groupe 02	-	-	-	-
Groupe 03	-	-	-	-
Groupe 04	-	-	-	-

II.2. Parameters studied

II.2.1. Body weight evolution

As shown in **(Fig.47A),** at the end of the first phase of the experimental protocol (Preventive treatment), ALZ model mice were highly significantly heavier than the control mice. The Alzheimer mice treated with Rivastigmine at 1,5mg/kg (Alz-STD) and *Castanea sativa* Mill. honey at 150mg/kg (Alz-D1) presents an intermediate body weight gain between the control group and the ALZ model mice (very significant and highly significant compared with ALZ respectively). Although the daily caloric intake of *Castanea sativa* Mill. honey at 300mg/kg (Alz-D2) mice was higher than the ALZ group, group (Alz-D2) revealed virtually similar body weight averages.

On the contrary, during the second phases of the experimental protocol, all animals gained weight except ALZ model mice and (Alz-STD) who stayed almost the same body weight (34.86g and 31.57g, respectively), however, the groups (ALz-D1) and (Alz-D2) gained weight compared to the first phase which explain that the administration of $AlCl_3$ and D-galactose did not impact on their body weight **(Fig.47B).**

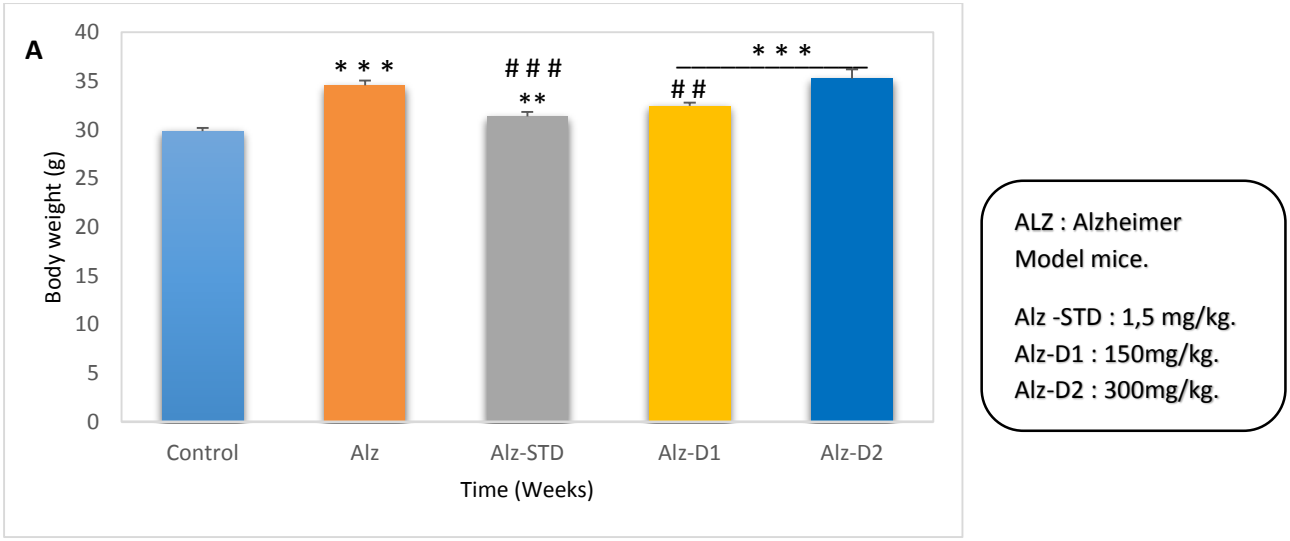


Figure 47.(A): Average body weight gain during the first phase.

Values were represented as means ± SD for each group (n=5). **P≤ 0.01, ***P≤ 0.001. ##P≤0.01, ###P≤ 0.001. (* Compared to the control group, # Compared to Alzheimer model mice).

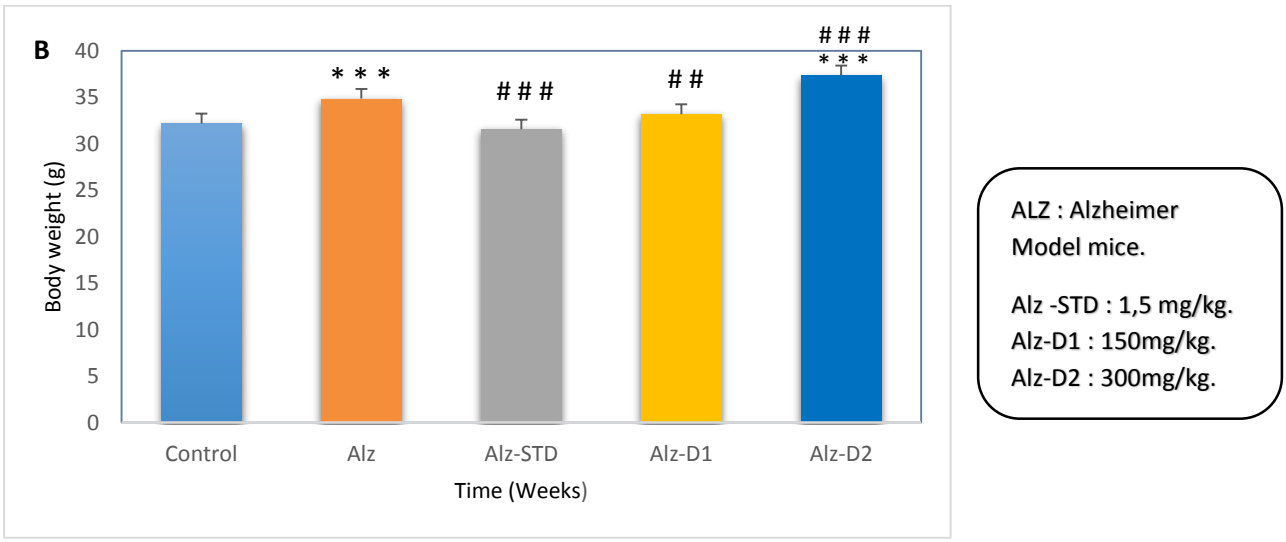


Figure 47.(B): Average body weight gain during the second phase.

Values were represented as means ± SD for each group (n=5). **P≤ 0.01, ***P≤ 0.001. ##P≤0.01, ###P≤ 0.001. (* Compared to the control group, # Compared to Alzheimer model mice).

II.2.2. Solution consumed

During the first phase (preventive treatment), the volume of the solution consumed is relatively unequal between the different experimental groups. Indeed, the results of the average volume of the solution consumed during the first 45 days range between 316ml to 355ml for the group's control, ALZ model mice, Alzheimer mice treated with "*Castanea sativa* Mill." at 150mg/kg and

Rivastigmine at 1.5mg/kg However, the Alzheimer mice treated with "*Cstanea sativa* Mill." honey at 300mg/kg presented a very significant increase (430ml) compared with the ALZ model mice and Control group **(Fig.48A).**

In the second phase, during the induction period, the group Control, ALZ model mice and (Alz-STD) showed a decrease in solution consumed, 308 ml to 316 ml (they revealed virtually similar solution consumed averages), as for the group (Alz-D2) went from an average volume of 430 ml to 413 ml, comparative with (Alz-D1) who shown an increase that went from an average volume of 355 ml to 377 ml. **(Fig.48B).**

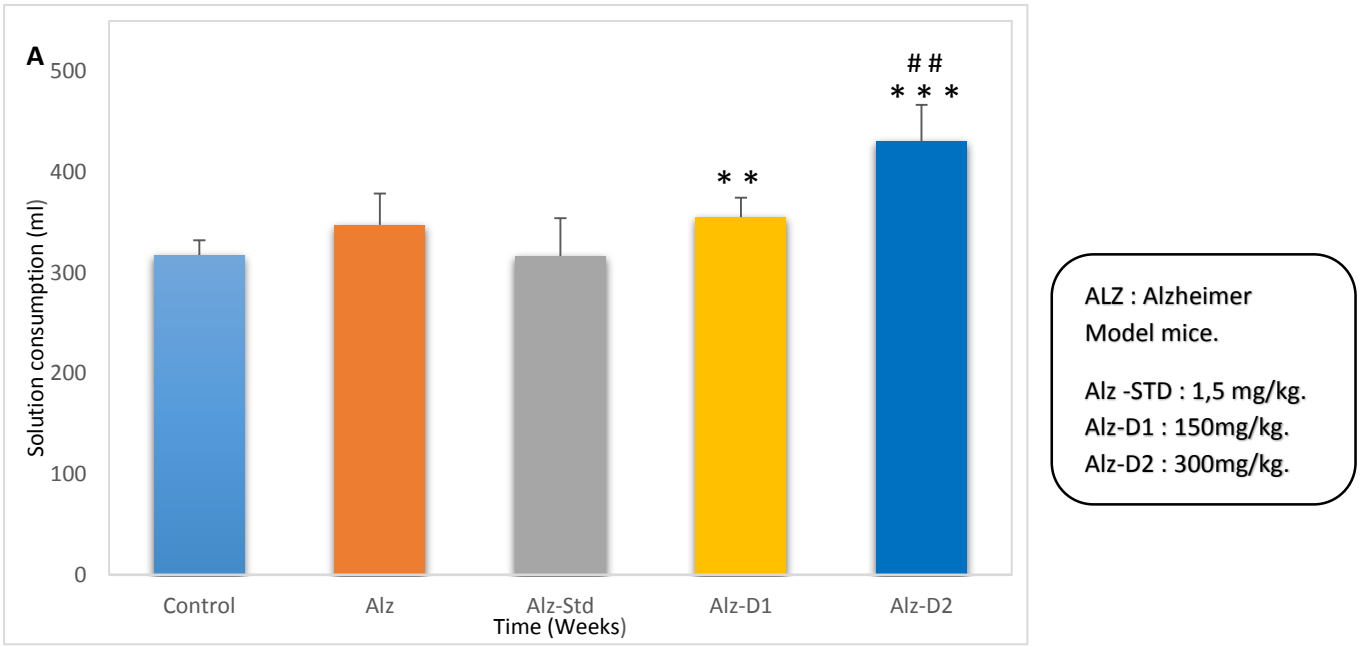


Figure 48.(A): Average volume of solution consumed during the first phase.
Values were represented as means ± SD for each group (n=5). **P≤ 0.01, ***P≤ 0.001. ##P≤0.01. (* Compared to the control group, # Compared to Alzheimer model mice).

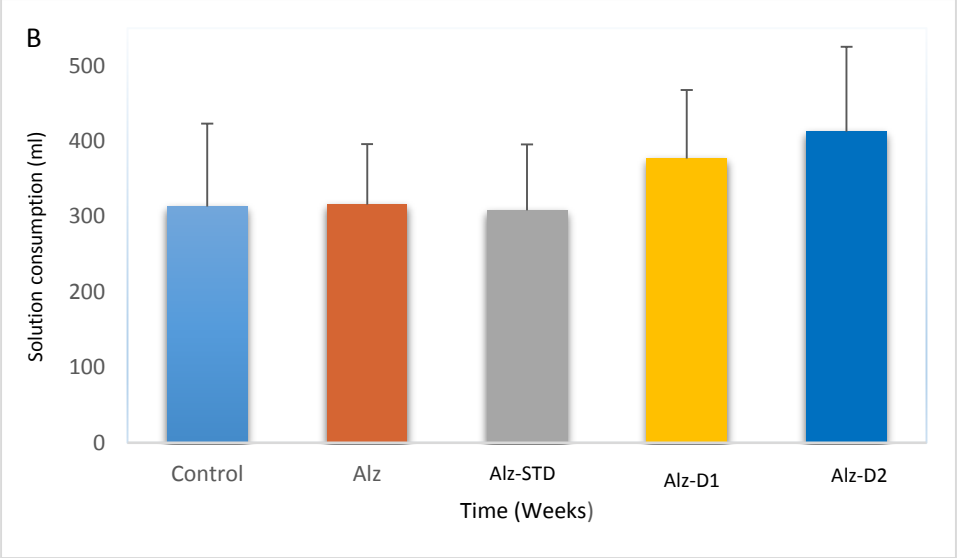


Figure 48.(B): Average volume of solution consumed during the second phase.

II.2.3. Memory tests

II.2.3.1. Eight-arm radial maze (RAM)

a- Spatial working memory

On this test, the number of repeat visits (errors number) recorded in the Alzheimer model mice (ALZ) was significantly higher ($p<0,010$) during the 3 days of practice compared to the control group. We also note that the number of errors in Alzheimer's mice treated with Rivastigmine at 1.5mg/kg (Alz-STD) is lower than that recorded in Alz's model mice during the 4 days of learning. The Azheimer mice treated with "*Castanea sativa* Mill." honey at 300mg/kg (ALZ-D2) showed a lower errors number compared with the ALZ model mice on the second day of practice, the same results were observed with the group treated with "*Castanea sativa* Mill." honey at 150mg/kg (ALZ-D1) on the third day of learning **(Fig.49A).**

As shown in figure **(Fig.49B)** on the fifth day of the test (Test day) the results obtained shown a high error number in the Alzheimer's model mice (ALZ) compared to the control group.

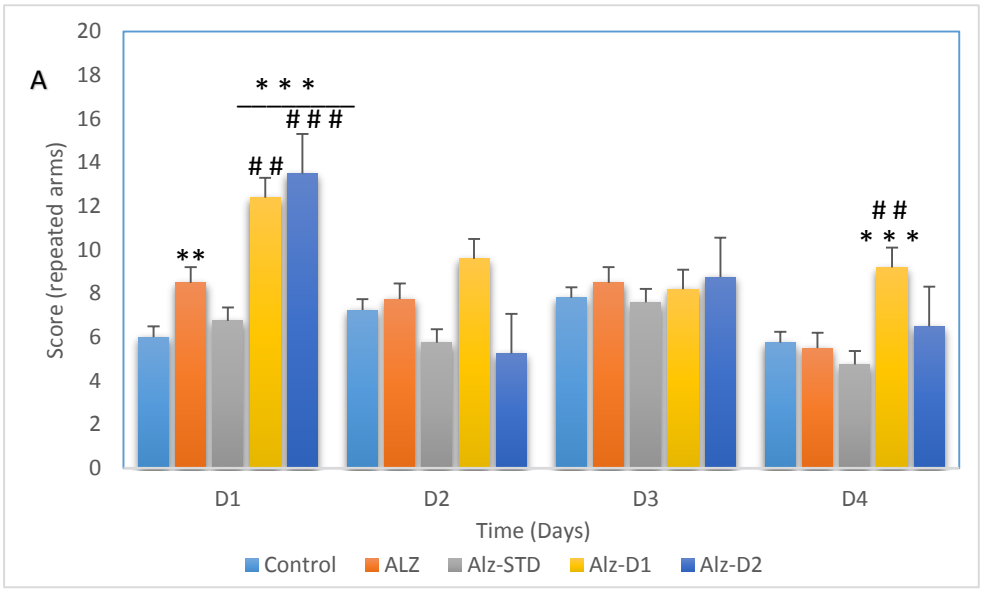


Figure 49.(A): Spatial working memory (4 days- of practice).

Values were represented as means ± SD for each group (n=5). *P≤ 0.05, **P≤ 0.01, ***P≤ 0.001. #P≤ 0.05, ##P≤0.01, ###P≤ 0.001. (* Compared to the control group, # Compared to Alzheimer model mice).

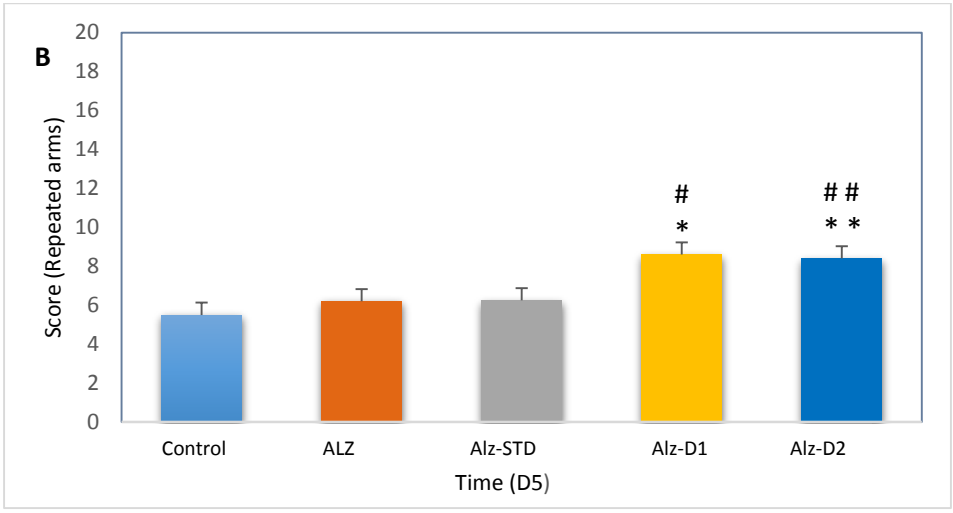


Figure 49. (B): Spatial working memory (Test day).

Values were represented as means ± SD for each group (n=5). *P≤ 0.05, **P≤ 0.01. #P≤ 0.05, ##P≤0.01. (* Compared to the control group, # Compared to Alzheimer model mice).

b- Position distinction (RAM)

Besides the first day of the learning days, the results of this test demonstrate that the number of visits to the baited arms in control mice is superior to that in the Alzheimer's model mice (ALZ). However, the Alzheimer mice treated with *Castanea sativa* Mill. honey solution at 150mg/kg (Alz-D1) display a score of visits in the baited arms similar to the control mice during the four practice days. Moreover, the alzheimer's mice treated with *Castanea sativa* Mill. honey at 300mg/kg (Alz-D2) and Rivastigmine at 1.5mg/kg (ALz-STD) results were between the Alzheimer model mice and the control group **(Fig.50A).**

On the fifth day (Test day), The control group recorded fewer visits to the baited arms compared to the Alzheimer model mice (ALZ), However the treated group (Alz-STD) showed similar results as the (ALZ), as for the Alzheimer treated groups (Alz-D1 and Alz-D2) the visits to the baited arms were superior to both (ALZ), and control group with a very significant difference (P<0.01) **(Fig.50B)**.

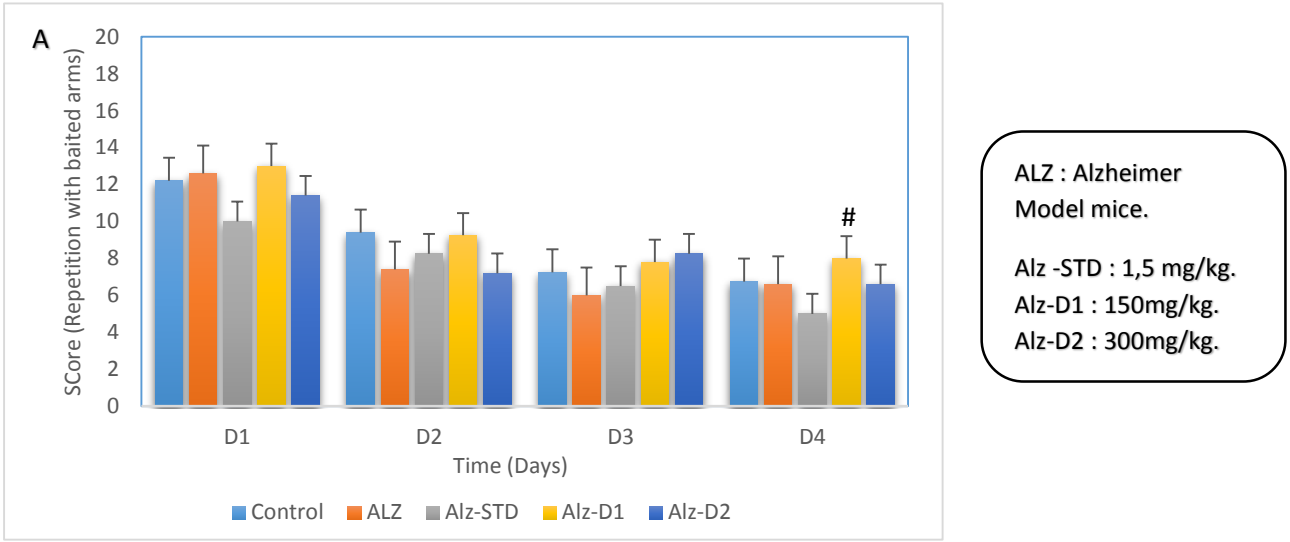


Figure 50.(A): Position distinction (4 days- of practice).
Values were represented as means ± SD for each group (n=5). #P≤ 0.05 compared to Alzheimer model mice.

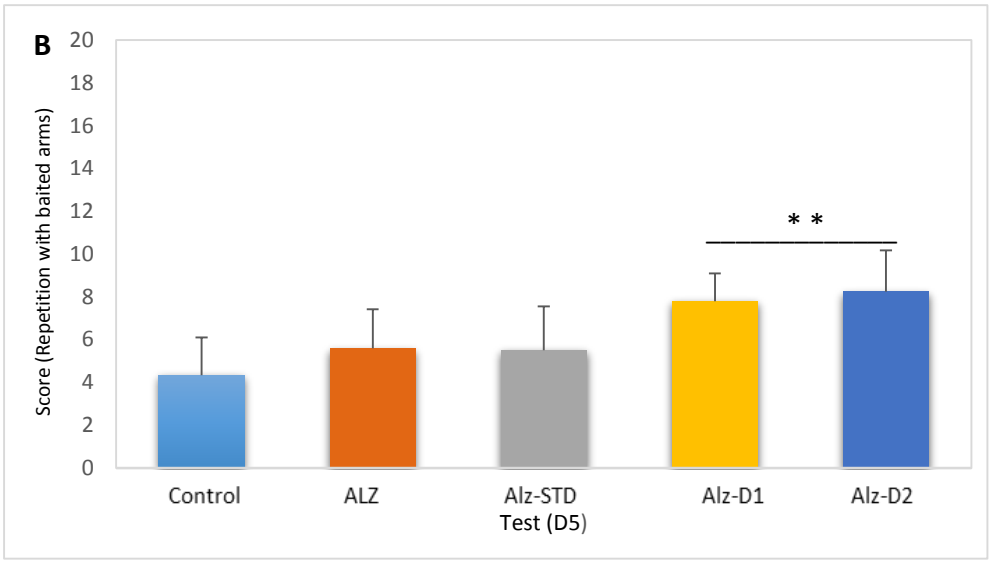


Figure 50.(B): Position distinction (Test day).

Values were represented as means ± SD for each group (n=5). **P≤ 0.01. #P≤ 0.05. (* Compared to the control group, # Compared to Alzheimer model mice).

c- Spatial reference memory (RAM)

As shown in **(Fig.51A)**, the first and last two days of the practice days, the results show that the residence time in the lighted arm in Alzheimer model mice is greater compared to the controls group, except the second day where the control group shown more residence time in the lighted arm than the Alz's model mice. The results obtained for the Alzheimer mice treated with both Rivastigmine at 1.5mg/kg (Alz-STD) and those treated with the *Castanea sativa* Mill. honey at 150mg/kg (Alz-D1) and at 300mg/kg (Alz- D2) show a highly significant ($p<0.001$), very significant ($p<0.01$) and significant ($p<0.05$) stay preference in the lighted arm compared to the ALZ's model mice on the first two days and last day of the practice days.

On the fifth day (Test day), there's a significant difference between the control group and the ALZ's model mice, in which the control group showed more residence time in the lighted arm than the ALZ group. The treated Alzheimer's mice (Alz-STD and Alz-D2) indicated a residence time in the lighted arm practically close to that recorded in the control group **(Fig.51B).**

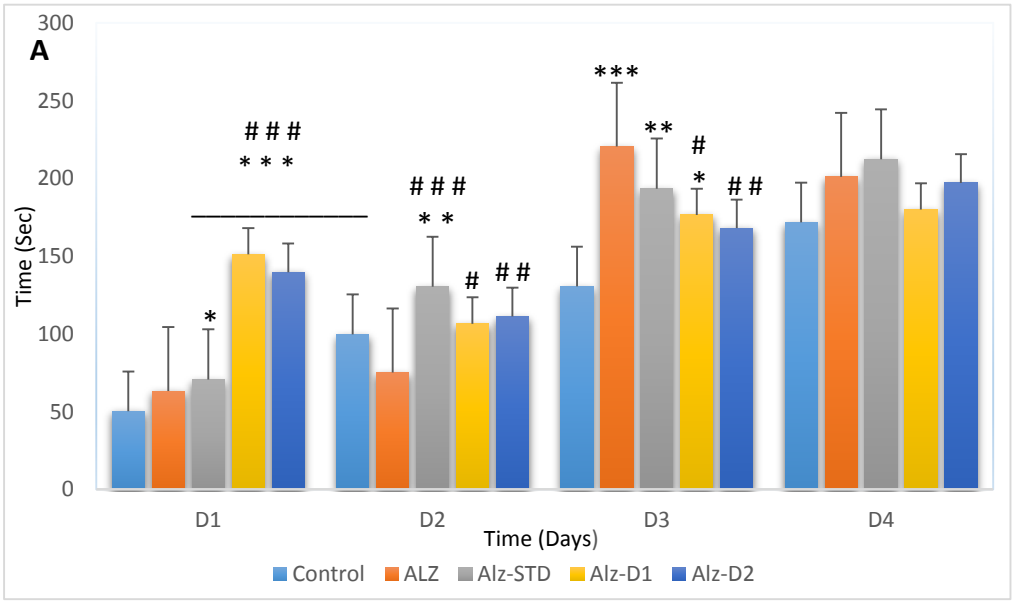


Figure 51.(A): Spatial reference memory (4 days- of practice).

Values were represented as means ± SD for each group (n=5). *P≤ 0.05, **P≤ 0.01, ***P≤ 0.001,. #P≤ 0.05, ##P≤0.01, ###P≤ 0.001. (* Compared to the control group, # Compared to Alzheimer model mice).

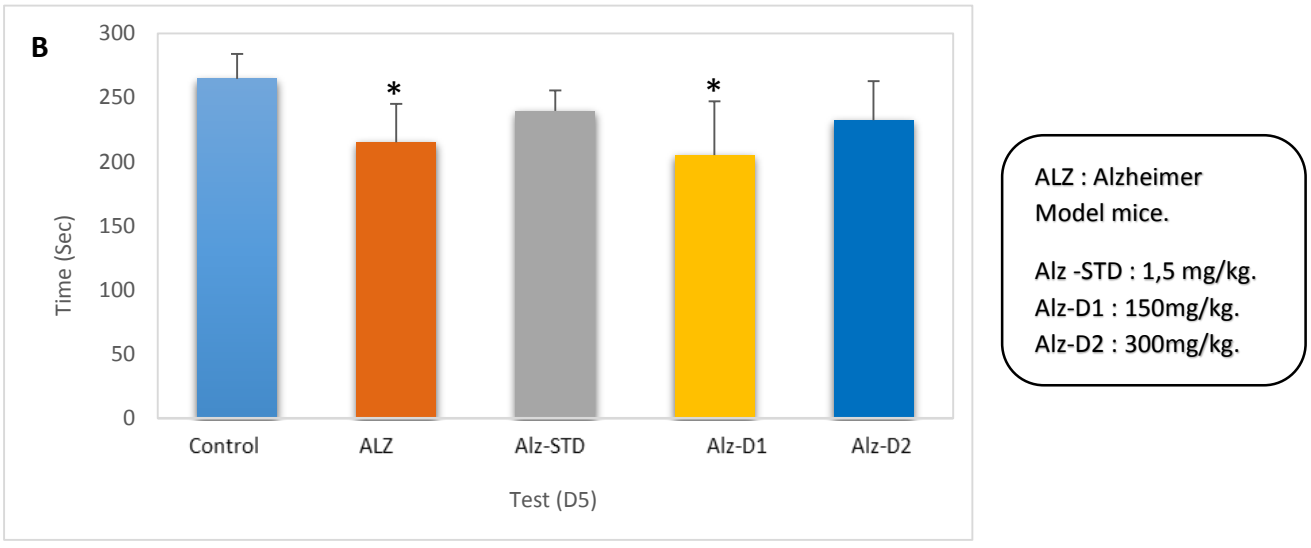


Figure 51.(B): Spatial reference memory **(Test day).**

Values were represented as means ± SD for each group (n=5). *P≤ 0.05, compared to control.

II.2.3.2. Morris water maze (MWM)

a- Spatial working memory

After the four days of practice, the results show a very significant ($p<0.010$) and a highly significant ($p<0.001$) long time to find the visible platform in the Alzheimer model mice compared with the control group who showed a short time into finding the platform during the practice days. As for the Alzheimer mice treated with *Castanea sativa* Mill. honey at 150mg/kg (Alz-D1) and 300mg/kg (Alz-D2) record a very significant short time to detect the visible platform compared to Alzheimer model mice **(Fig.52A).**

On the fifth day (test day), Alzheimer model mice (ALZ) showed highly significant ($P<0.001$) times to find the visible platform compared to the control group. In contrast, the Alzheimer mice treated with *Castanea sativa* Mill. honey at 300mg/kg (Alz-D2) had significantly shorter escape latency compared to the Alz group, with a very significant difference ($P<0.01$) **(Fig.52B).**

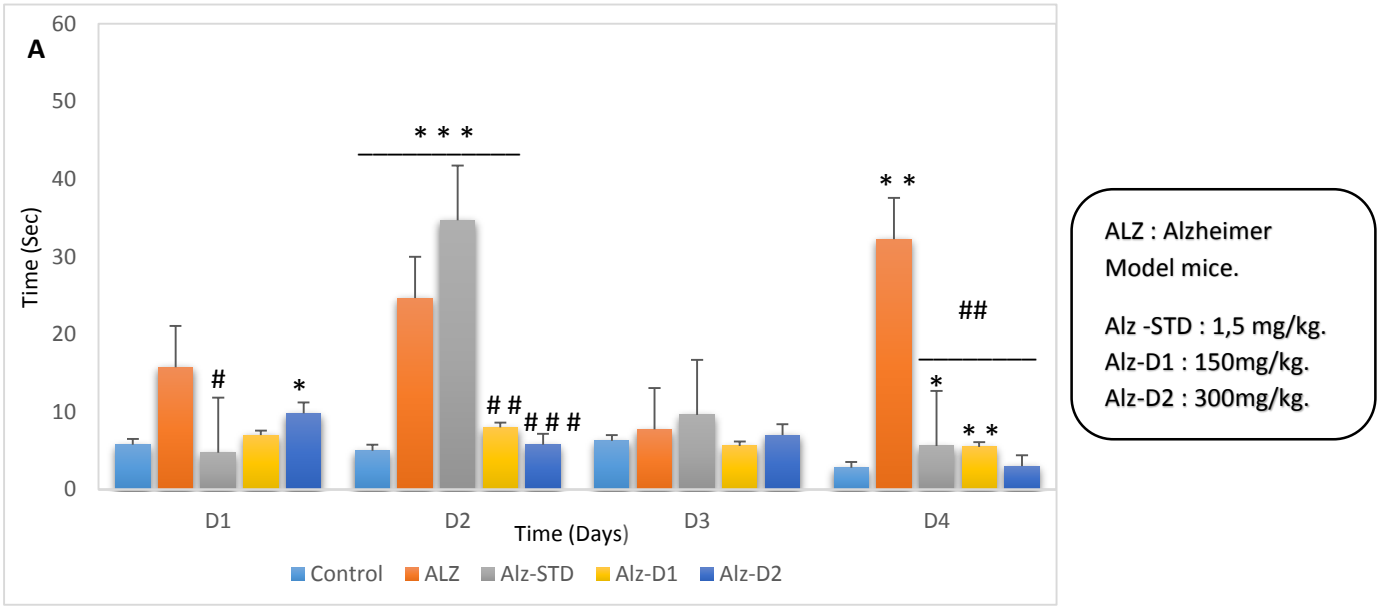


Figure 52.(A): Morris water maze- Spatial working memory (4 days- of practice).
Values were represented as means ± SD for each group (n=5). *P≤ 0.05, **P≤ 0.01, ***P≤ 0.001, #P≤ 0.05, ##P≤0.01, ###P≤ 0.001. (* Compared to the control group, # Compared to Alzheimer model mice).

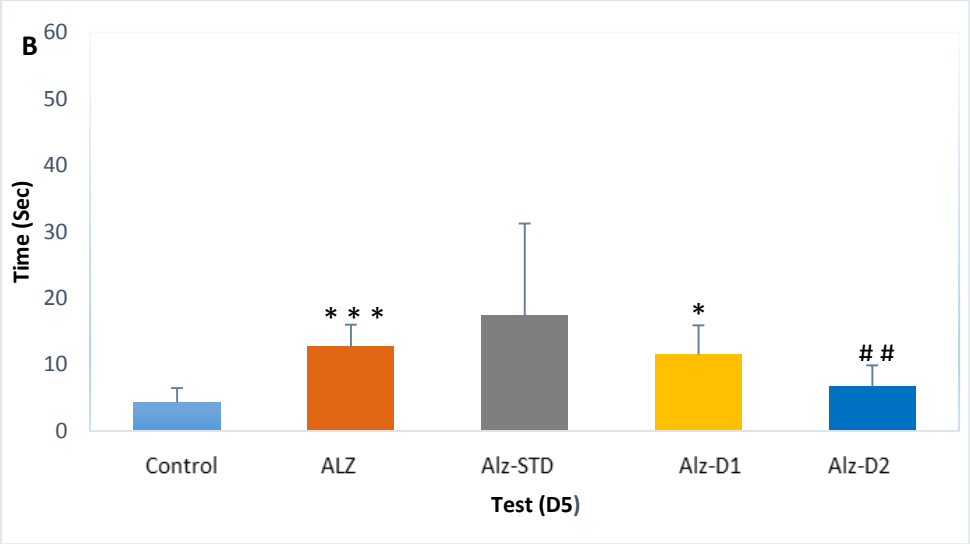


Figure 52.(B): Morris water maze- Spatial working memory (Test day).
Values were represented as means ± SD for each group (n=5). *P≤ 0.05, ***P≤ 0.001. ##P≤0.01. (* Compared to the control group, # Compared to Alzheimer model mice).

b- Spatial reference memory (MSR)

The results of the practice days show that the Alzheimer model mice take a significantly long time to reach the invisible platform compared with the control group, who showed a short time finding the platform with a highly significant difference ($p<0.001$). Besides the third day, the Alzheimer mice treated with *Castanea sativa* Mill. honey at 150mg/kg (Alz-D1), 300mg/kg (Alz-D2) and Rivastigmine at 1.5mg/kg recorded a highly significant ($P<0.001$) short time to detect the invisible platform compared to Alzheimer model mice during the practice days **(Fig.53A).**

As shown in **(Fig.53B),** the Control group, the ALZ model mice, and ALZ treated with *Castanea sativa* Mill. honey at 300mg/kg showed similar time records finding the invisible platform.

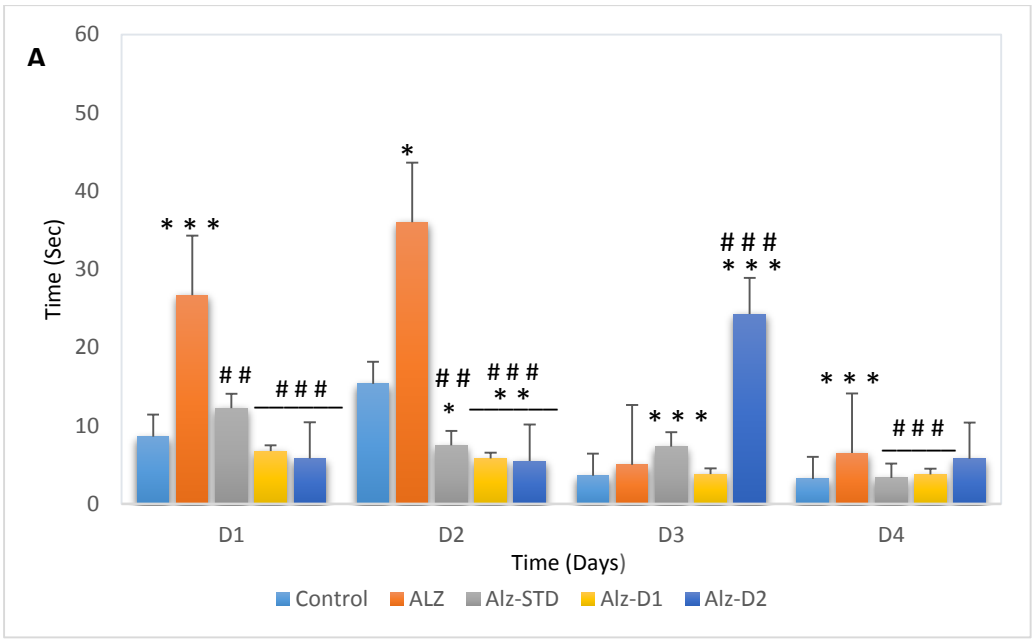


Figure 53.(A): Morris water maze- Spatial reference memory (4 days- of practice);
Values were represented as means ± SD for each group (n=5). *P≤ 0.05, **P≤ 0.01, ***P≤ 0.001, compared to control. #P≤ 0.05, ##P≤0.01, ###P≤ 0.001, compared to the ALZ's group.

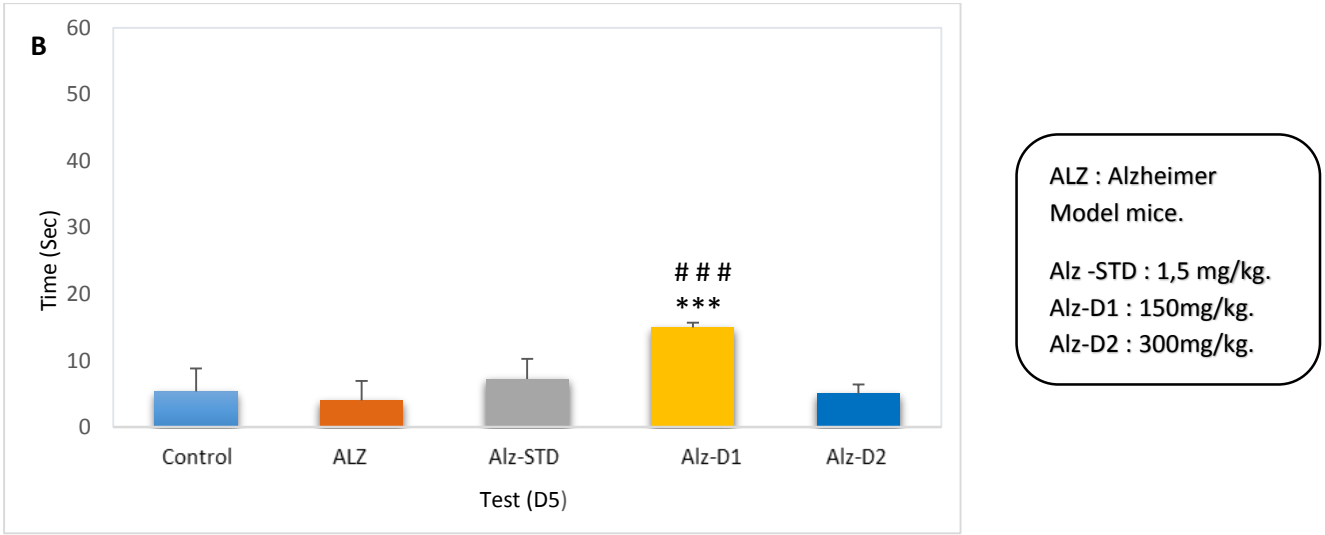


Figure 53.(B): Morris water maze- Spatial reference memory (Test day).
Values were represented as means ± SD for each group (n=5). *P≤ 0.05, **P≤ 0.01, ***P≤ 0.001, #P≤ 0.05, ##P≤0.01, ###P≤ 0.001. (* Compared to the control group, # Compared to Alzheimer model mice).

II.2.4. Behavioral test

II.2.4.1. Locomotor activity

The results of the average of the four phases of locomotor activity show more activity in the control group compared with the Alzheimer model mice. However, both Alzheimer mice treated with *Castanea sativa* Mill. honey at 150mg/kg (Alz-D1) and 300mg/kg (Alz-D2) showed hyperactivity compared with the other groups. Moreover, the Alzheimer's mice treated with Rivastigmine at 1.5mg/kg showed hypoactivity compared to the other groups **(Fig.54).**

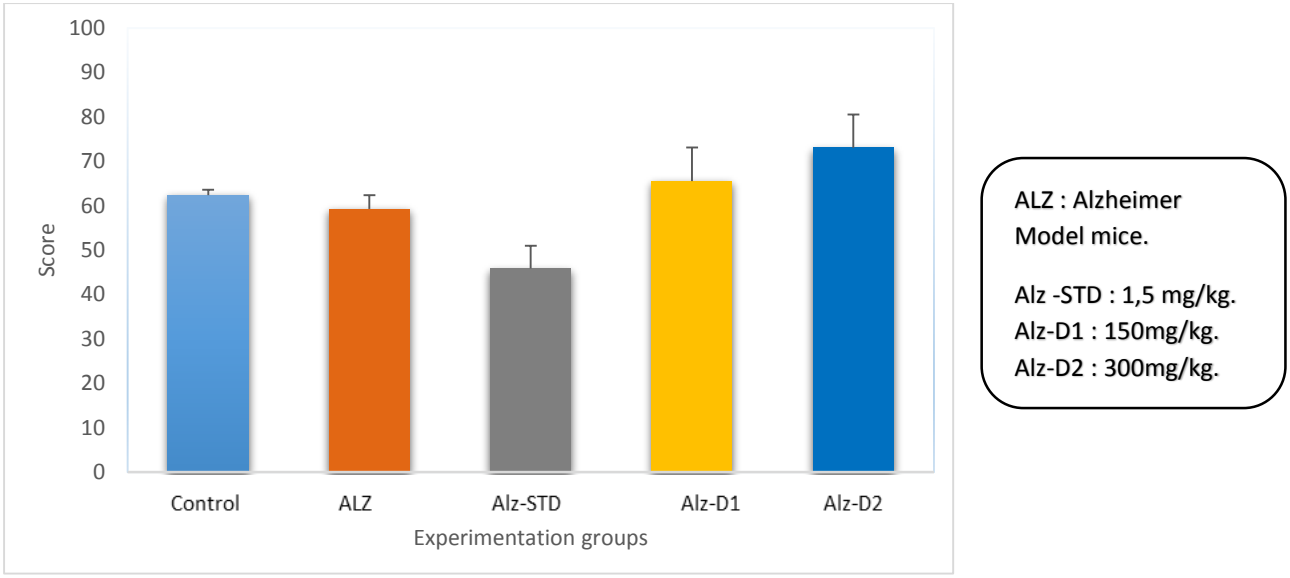


Figure 54: Average of the four phases of locomotor activity.

II.2.4.2. Anxiety test

II.2.4.2.1. Black/White Test Box

The average of the 4 phases recorded for this test reveals a significant ($P<0.05$) preference for the illuminated compartment for the Alzheimer model mice compared with the control mice, whom showed a preference for the dark compartment. The results were similar for both the Alzheimer's mice treated with *Castanea sativa* Mill. honey at 300mg/kg (Alz-D2) and Rivastigmine at 1.5mg/kg (Alz-STD), who had a preference for the dark compartment **(Fig.55).**

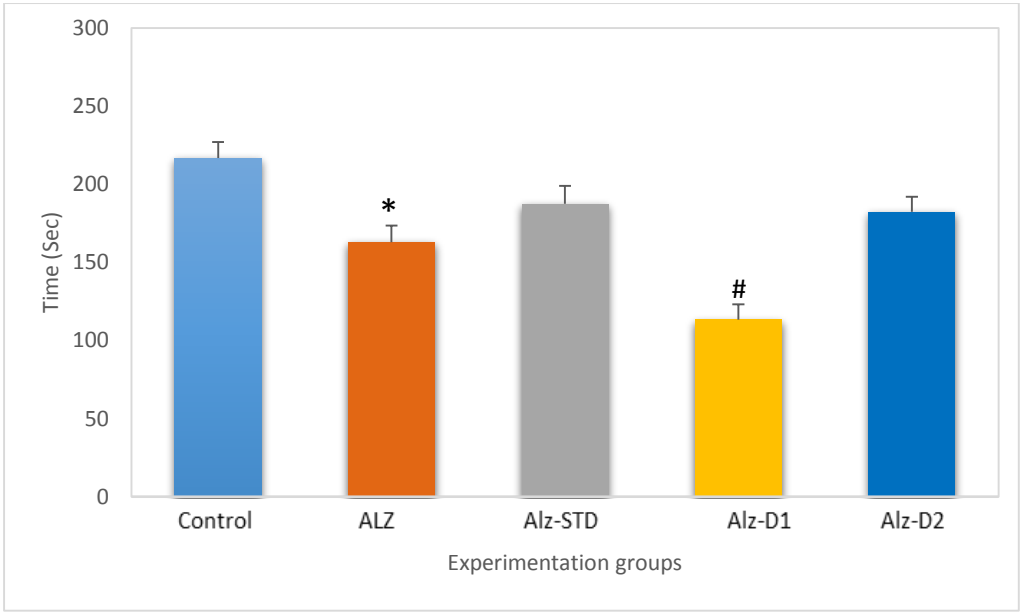


Figure 55: Average of the four phases of B/W Test Box.

Values were represented as means ± SD for each group (n=5). *P≤ 0.05. #P≤ 0.05. (* Compared to the control group, # Compared to Alzheimer model mice).

II.2.4.2.2. Elevated plus maze (EPM)

As shown in **(Fig.56)** the results of the average of the four phases of this test showed a preference stays in the protected arms in the Control mice compared with the Alzheimer model mice with a significant difference (P<0.05). Moreover, the Alzheimer mice treated with *Castanea sativa* Mill. honey at 150mg/kg (Alz-D1) and 300mg/kg (Alz-D2) showed preference for the protected arms like the Control group compared to the ALZ model mice with a very significant difference (P<0.01), same results, were observed for the ALZ mice treated with Rivastigmine at 1.5mg/kg with a significant difference (P<0.05).

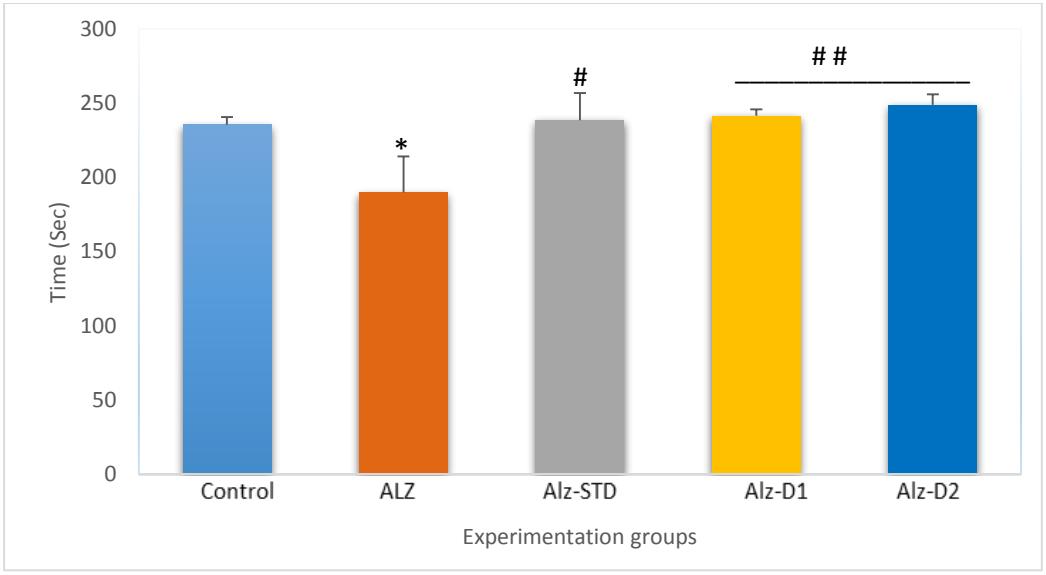


Figure 56: Average of the four phases of the elevated plus maze;

Values were represented as means ± SD for each group (n=5). *P≤ 0.05. #P≤ 0.05, ##P≤0.01. (* Compared to the control group, # Compared to Alzheimer model mice).

II.2.4.2. Persolt test (Forced swim)

On this test (Persolt test), the record immobility times were superior for the Control group compared with the Alzheimer model mice with a highly significant (P<0.001) difference, while the Alzheimer mice treated with *Castanea sativa* Mill. honey at 150mg/kg (Alz-D1) showed a very significant (P<0.01) difference compared with the ALZ model mice. The same results were recorded, for both the Alz mice treated with Rivastigmine at 1.5mg/kg (Alz-STD) and *Castanea sativa* Mill. honey at 300mg/kg (Alz-D2), in which the latter showed a higher immobility time than both the control group and ALZ model mice with a very significant (P<0.01) and highly significants (P<0.001) difference respectively **(Fig.57).**

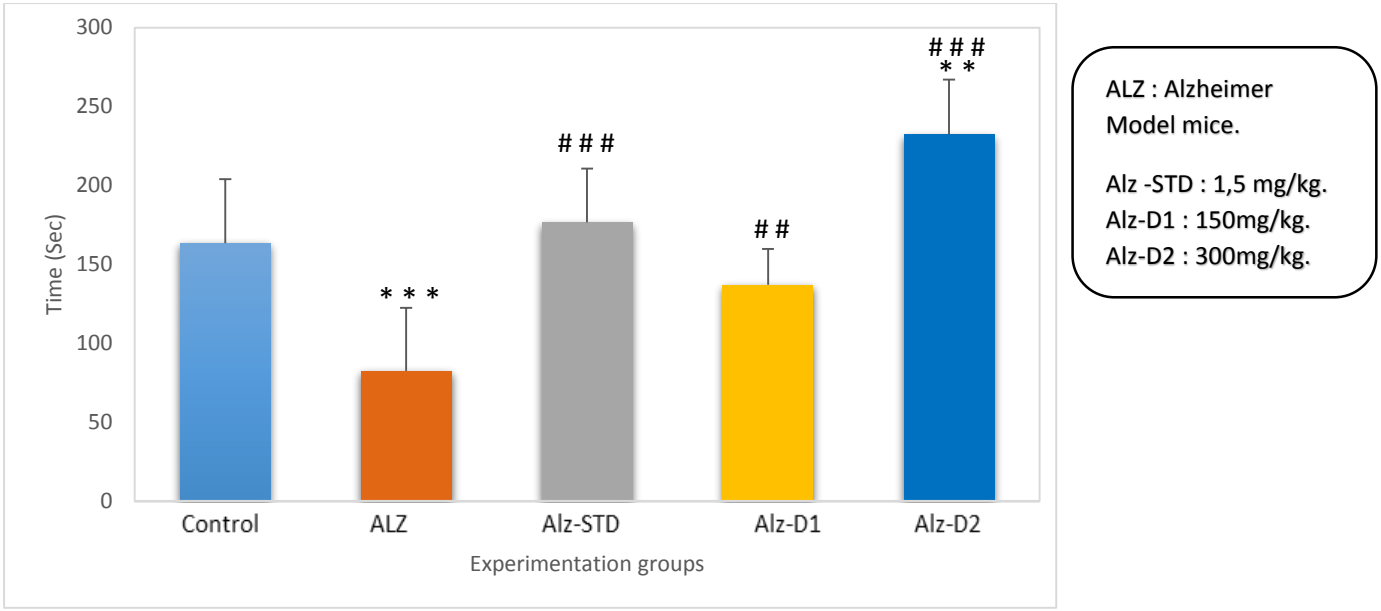


Figure 57: Persolt test (Forced swim);

Values were represented as means ± SD for each group (n=5). **P≤ 0.01,*** P≤ 0.001, ##P≤0.01, ###P≤0.001 (* Compared to the control group, # Compared to Alzheimer model mice).

II.3. Histology

II.3.1. Brain tissus

The brain histological examination was carried out at different levels of the brain tissues.

II.3.1.1. Hippocampus

Histological examination of the hippocampus showed a decreased cell density, represented in, the deposition of both amyloid plaques (AP), and neurofibrillary tangles (NFTs) at the CA area of the hippocampus in the ALZ group after exposure to Alcl3 and D-galactose for 7 weeks; compared with the control group who shown a normal hippocampal structure. Chronic *Castanea sativa* Mill. honey treatment markedly reduced hippocampal atrophy and neuronal loss in the CA region of the hippocampus for both groups D1 (150mg/kg) and D2 (300mg/kg). The Alzheimer mice treated with Rivastigmine at 1,5mg/kg (Alz-STD) showed an apparently normal structure of the hippocampus **(Fig.58).**

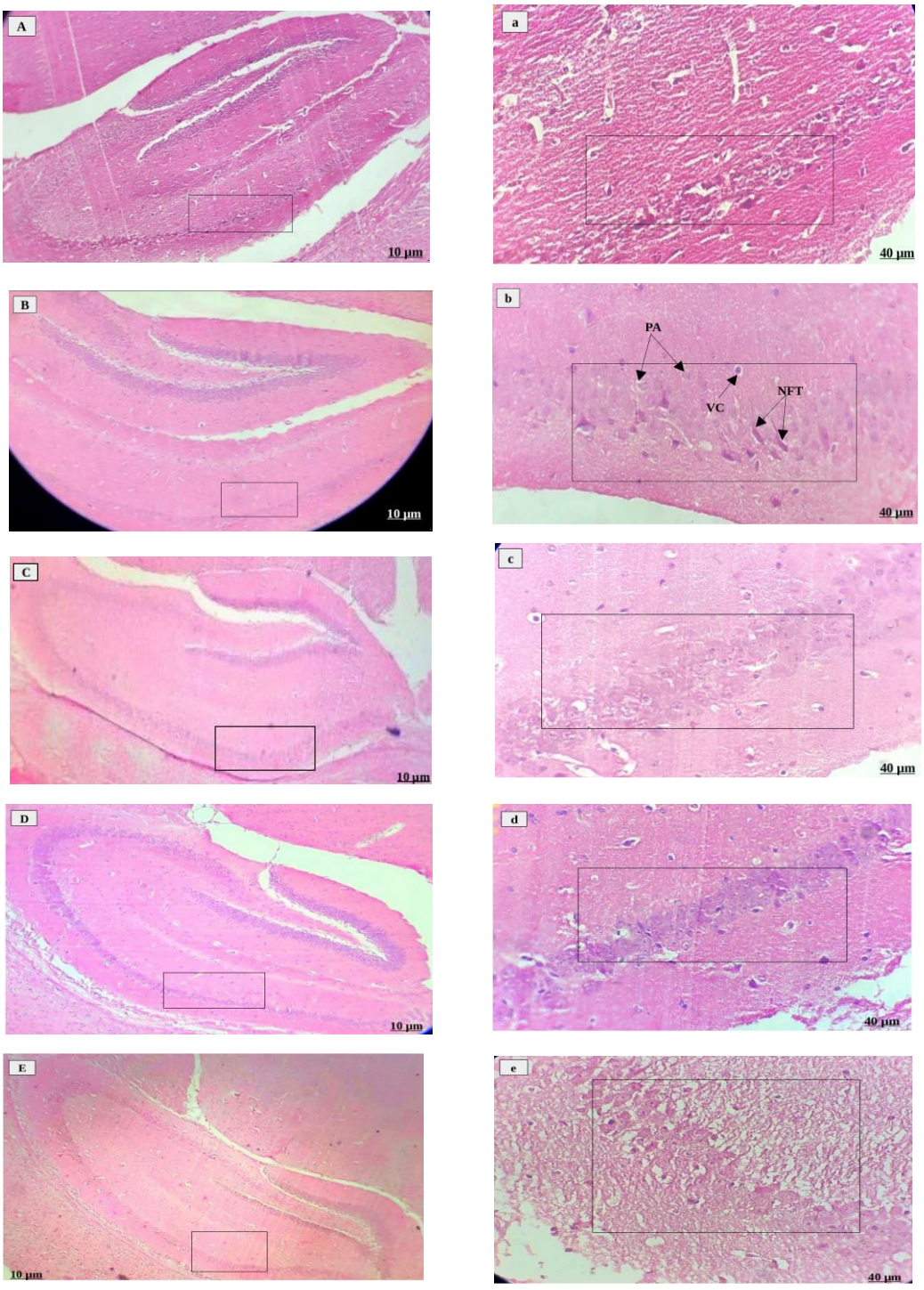


Figure 58: Photomicrograph of mice brain, stained (H&E X10 - X40) sections of the hippocampus of each group; **(A;a)** Control group **(B;b)** Alzheimer model mice; **(C;c)** Alzheimer mice treated with SCH solution 150mg/kg; **(D;d)** Alzheimer mice treated with SCH solution 300mg/kg; **(E;e)** Alzheimer mice treated with Rivastigmine 1.5mg/kg. **PA:** Plaque amyloïdes. **NFT:** Neurofibrillary tangles. **VC:** Vacuole cells. (a,b,c,d,e, are **CA** part of A,B,C,D,E respectively).

II.3.1.2. Cortex

Compared with the control group, the cortex histological examination for the ALZ group shows perivascular haemorrhage on certain parts of the cortex, neuronal degeneration and neuronophagia, also the presence of amyloid plaques and neurofibrillary tangles within neurons. However, the Alzheimer treated mice with Rivastigmine 1,5mg/kg and *Castanea sativa* Mill. honey at 150mg/kg, 300mg/kg showed an apparently normal structure of the cortex. **(Fig.59).**

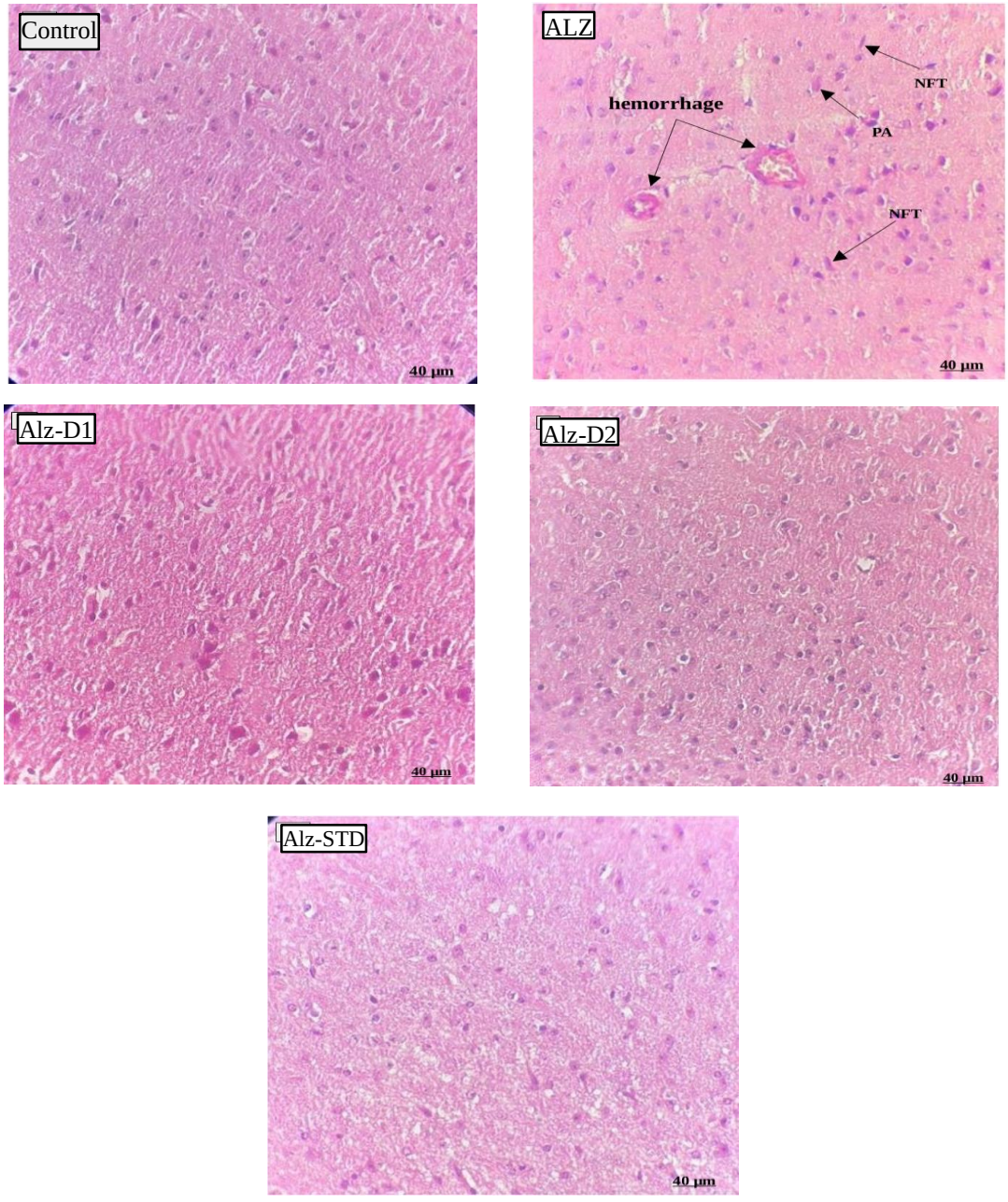


Figure 59: Photomicrograph of mice brain, stained (H&E X40) sections of cortex of each group; **PA:** Plaque amyloïdes. **NFT:** Neurofibrillary tangles. **VC:** Vacuole cells.

II.3.1.3. Cerebellum

After the cerebellum histological examination, the results shown that all the groups except the ALZ has normals cells structure, when comparing with the control group, in the ALZ group cells damaged and purkinje cells necrosis was detected after $AlCl_3$ and D-galactose exposure. In the control group the purkinje cells are aligned between the granular and molecular layers. As for the Alzheimer treated groups with *Castanea sativa* Mill. honey at 150mg/kg, 300mg/kg (Alz-D1; ALz-D2), the cerebellum cells apparently have normals structure revealed normally appearing molecular layer, Purkinje cell layer, and the same results was for the group STD (Rivastigmine 1.5mg/kg) **(Fig.60).**

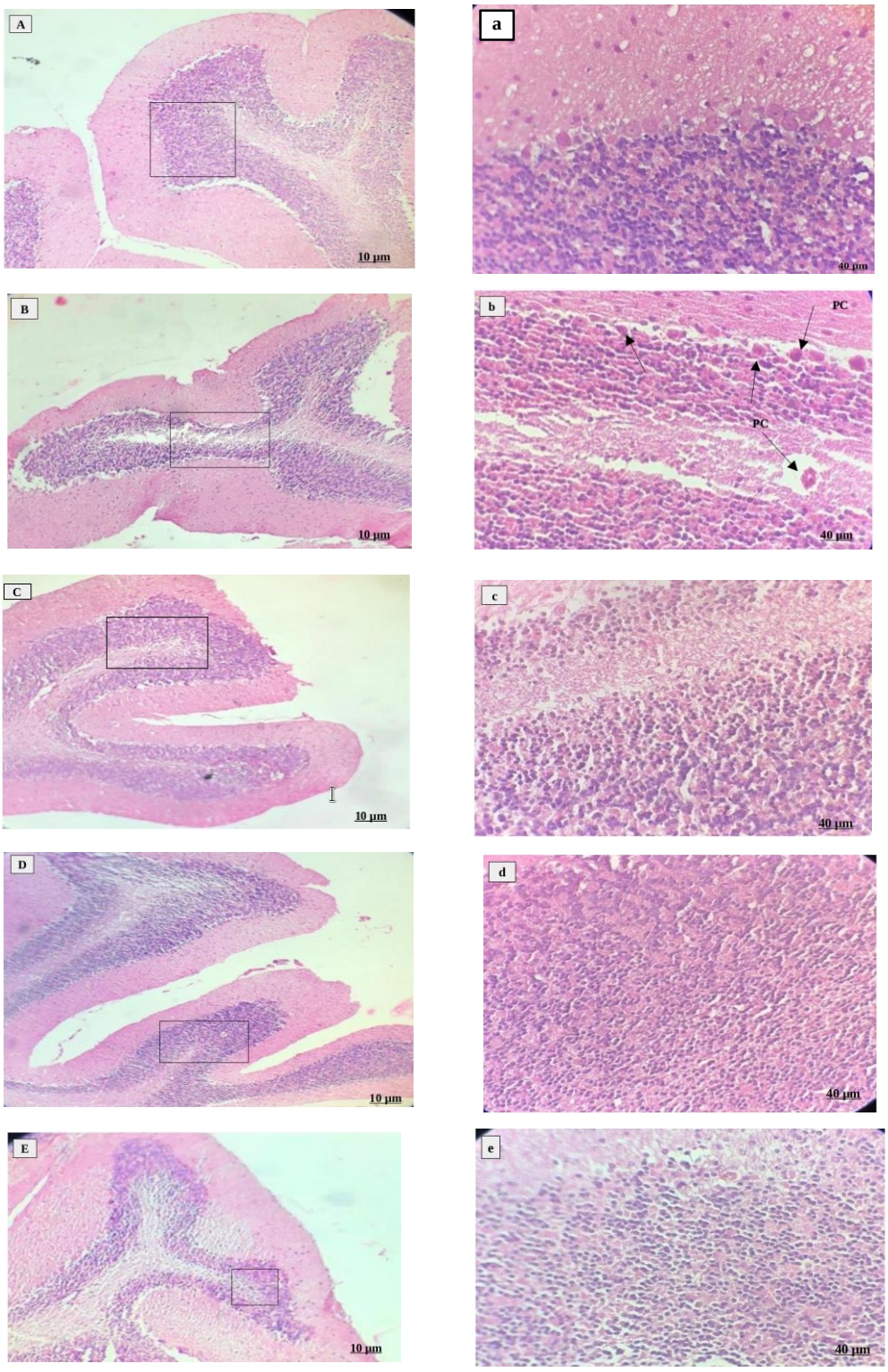


Figure 60: Photomicrograph of mice brain, stained (H&E X10 - X40) sections of the cerebellum of each group; **(A;a)** Control group, **(B;b)** Alzheimer model mice; **(C;c)** Alzheimer mice treated with SCH solution 150mg/kg; **(D;d)** Alzheimer mice treated with SCH solution 300mg/kg; **(E;e)** Alzheimer mice treated with Rivastigmine 1.5mg/kg. **PC**: Purkinje cells.

II.3.2. Liver tissues

On the present histological examination of $AlCl_3$ effect on the liver tissue, the control group compared with the ALZ group shown normal liver cell structure relative to central veins and sinusoids with a characteristic pattern of hexagonal lobules, however, after $AlCl_3$ exposure, hepatic alteration were observed in the ALZ group, the damage was characterized by the appearance of numerous apoptotic cells and haemorrhage. Chronic *Castanea sativa* Mill. honey administration for (Alz-D1; Alz-D2) prevented some liver tissue damage caused by $AlCl_3$ with the presence of normal cell architecture. As for the group (Alz-STD) certain liver cell damage was detected with the appearance of apoptotic cells. **(Fig.61).**

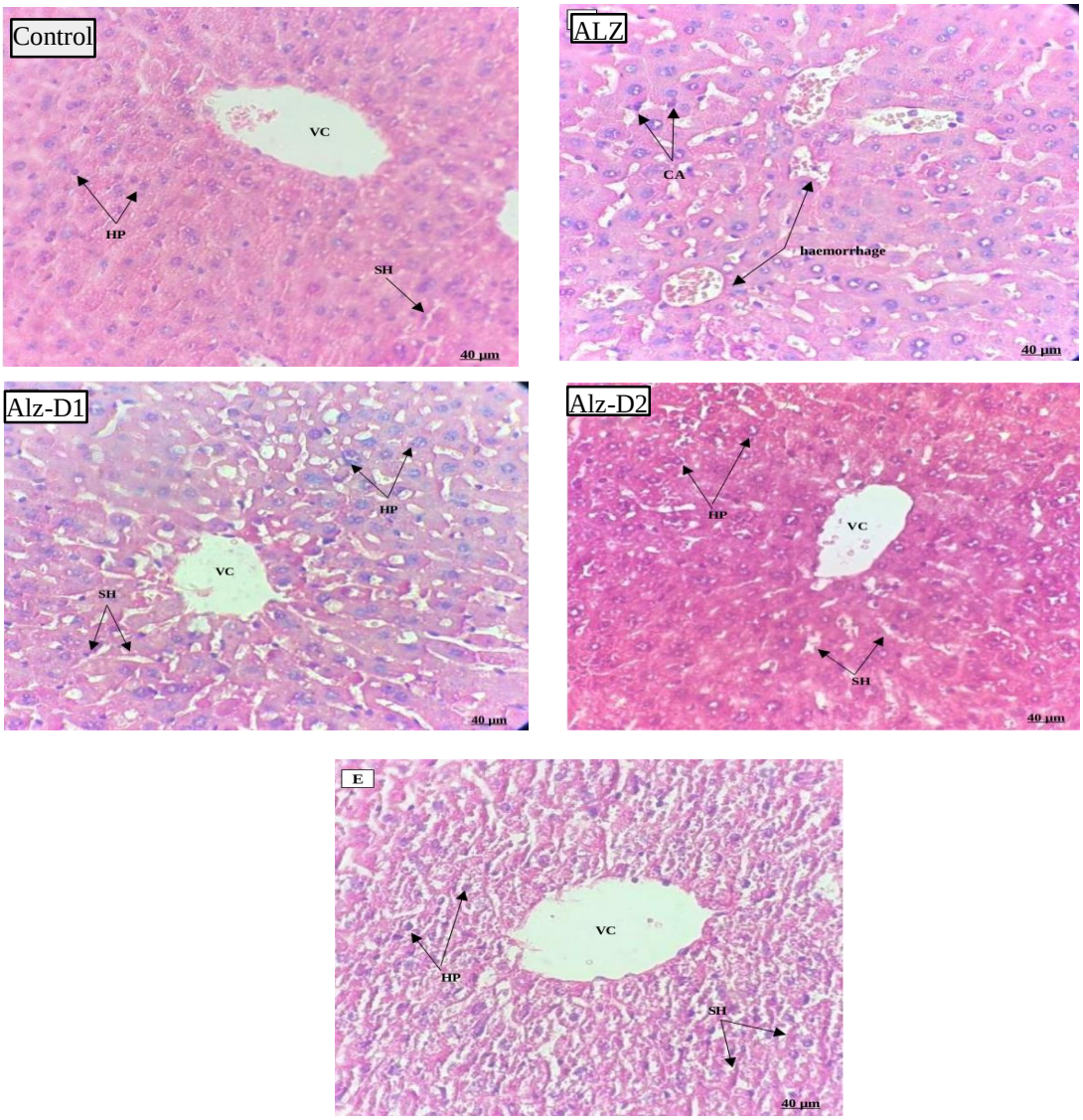


Figure 61: Photomicrograph of liver tissue, stained (H&E X40) sections of the liver of each group; **CV:** central vein; **SH:** hepatic sinusoid; **CA:** Apoptotic cell.

II.3.3. Kidney tissues

Kidney histological examination showed $AlCl_3$ effects on the tissues for the ALZ group, in which we see a narrowing of the renal corpuscles with heterogeneous architecture surrounded by haemorrhage in the tissues, shrinkage of Bowman space was observed, as for Proximal convoluted tubule appeared to be burst and surrounded by. The control group showed a normal architectural cell structure consisting of glomeruli surrounded by a regular Bowman's capsule, as the proximal and distal convoluted tubules appeared with normal thickness. The histological examination of renal tissues in the treated groups with the *Castanea sativa* Mill. honey at 150mg/kg and 300mg/kg as well as with Rivastigmine at 1.5mg/kg, showed apparent similarities to the control group **(Fig. 62).**

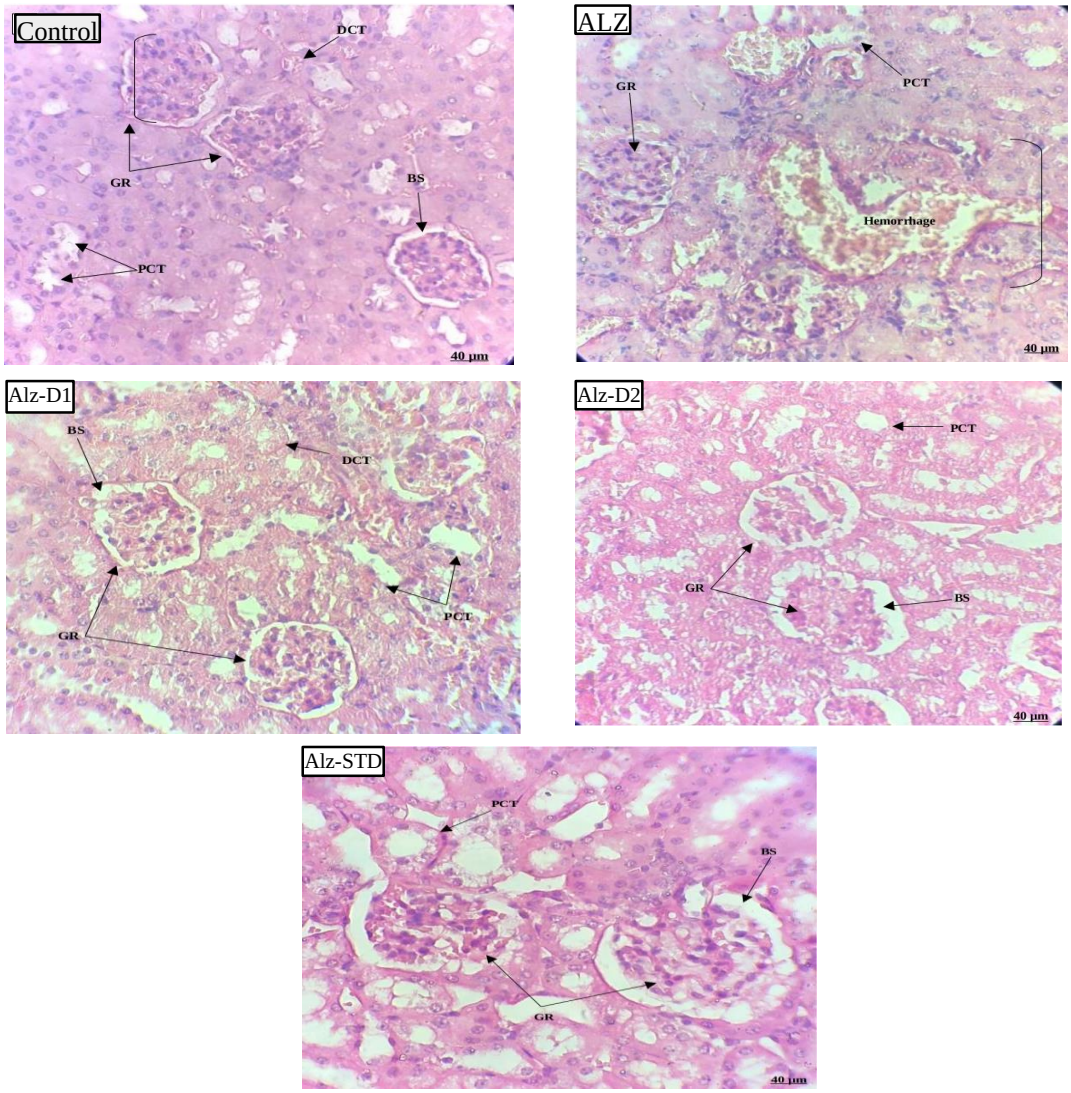


Figure 62: Photomicrograph of renal tissue, stained (H&E X40) sections of the kidney of each group; **BS:** Bowman space. **DCT:** Distal convoluted tubule. **PCT:** Proximal convoluted tubule.

Discussion

This study aims to determine whether *Castanea sativa* Mill. honey at 150 and 300 mg/kg has neuroprotective effects on Alzheimer's mice. We were able to arrive at several resolutions.

A toxicity test was completed before the evaluation of the sweet chestnut honey solution's potential as a neuroprotective agent. The latter showed that *Castanea sativa* Mill. solution at 150, 300, 1000, and 2000 mg/kg had no harmful effects. These results show that our solution is non-toxic at the experimental levels. These results agree with those reported by **Mustafa, M. Z et *al.* (2019)**, in which the use of a high dose of honey supplementation of up to 2000 mg/kg did not result in any drawback.

To evaluate the physiognomy and growth of mice under our working conditions, Bodyweight and solution consumption intake were mesured weekly throughout the study (90Dyas). The results obtained during the first phase of the protocol (Treatment) show that Alzheimer's mice with a daily intake of *Castanea sativa* Mill. honey were heavier than the control mice. These results agree with those reported by **Yaacob, W.M. et *al.* (2020)** where Neuroinflammation and cognitive decline were induced by injecting lipopolysaccharide (LPS), in which the LPS rats treated with Tualang honey showed weight gain compared to controls at the end of the protocol. And by **Liyanage, D., & Mawatha, B. (2017),** whom claim that Honey increases body weight.

In the second phase, the mice were exposed to oral administration of $AlCl_3$ at 100mg/kg and D-galactose at 120 mg/kg intraperitoneally (i.p) for (7 Weeks) to induce Alzheimer's disease. According to **(Hamdan, A. M et *al.* 2022),** Aluminum's (Al) is a neurotoxic agent that causes oxidative stress, which is linked to AD progression. Whereas d-galactose (d-gal) has been established as a senescence agent **(Mahdi, O et *al*., 2021).** This phase shows that after the induction, ALZ model mice weight stayed almost the same as it was in the first phase. However, the Alzheimer's mice treated with *Castanea sativa* Mill. honey at 150mg/kg and 300mg/kg gained weight compared to the first phase. These results explain that chronic administration of $AlCl_3$ (100mg/kg) and D-gal (120mg/kg) did not impact on their body weight. These results agree with those reported by **Feng, L et *al.* (2018)**, who reported that D-gal and $AlCl_3$ did not affect the body weight and appetite of mice.

Regarding solution consumption volume. During the first phase, the results show an unequal average of solution consumption between the experimental groups in which all groups showed a significant water intake during (7 weeks). According to **Ribes. D et *al,* (2008)** transgenicmice drunk more water than wild type mice. However, after $AlCl_3$ (100mg/kg) administration for (7 weeks), all groups exepts the control group, showed a decrease in solution consumption. The results consistent with

Kowalczyk. E et *al.* (2004) in which, after administration of aluminium chloride solution in drinking water (80 mg/l) to rats for three months resulted decreases in water intake.

At the end of the protocol of our study, memory and behavioural tests, were performed to measure the mice's memory and behaviour change caused by Aluminium chloride (100mg/kg) and D-galactose (120mg/kg). Acording to **Al-Amin, M. M et *al.* (2016); Singh, N. A et al. (2018),** aluminium exposure significantly reduces memory and learning curve in rats in the spatial memory performance.

Following 14 weeks of the protocol, spatial learning of experimental mice was assessed by using both Rdial arm-maze (RAM) and Morris water maze (MWM). An animal's re-entry into a radial arm that has already been visited counts as working memory error, whereas an entry into an un-baited arm counts as a reference memory error **(Mei, J. et *al*., 2020).** Learning in the MWM is quantified using specific parameters such as latency to reach the platform, average distance from the platform, path efficacy, or swimming distance **(Curdt, N. et *al*., 2022).** In our study the spatial learning ability of the mice was assessed by the escape latency.

The results obtained from the eight-arm radial maze (RAM): The Spatial working memory and Position distinction show that the number of errors recorded in the Alzheimer model mice is significantly higher compared to the control mice. These results agree with those reported by **Shunan, D et *al.* (2021),** in which the rats received $AlCl_3$ (100 mg/kg b.wt) prepared in drinking water for 28 days. The results showed that $AlCl_3$-induced rats significantly elevated ($p > 0.05$) the reference memory errors (RMEs), and working memory errors (WMEs) when compared with the rats in the control group. According to **Al-Amin, M.M et *al.* (2019)** Aluminum exposure leads to impairment of spatial working memory in mice, this memory deficit could be associated with the oxidative stress in the cortical and subcortical brain tissues.

However, Alzheimer's mice treated with *Castanea sativa* Mill. honey at 150mg/kg (Alz-D1) and 300mg/kg, (Alz-D2) error scores were significantly higher compared to the control group and Alzheimer model mice (Alz). These results don't match those found by **Kamarulzaidi, M.A et *al.* (2016)** in which they investigated the effect of *Tualang honey* on spatial memory performance (SMP) of rats using the radial arm maze (RAM), the results were consumption of *Tualang honey* could improve both working memory and reference memory in RAM evaluation in male adult rats after the Honey group rats committed significantly less ($p<0.05$) number of errors than the Control group rats.

In the Radial Arm Maze (RAM) Test: Spatial Reference Memory (two-arm maze), the control group generally spent more time in the illuminated arm compared to the Alzheimer model mice. The treated group with *Castanea sativa* Mill. honey at 300mg/kg (Alz-D2) and Rivastigmine at 1,5mg/kg (Alz-STD) showed similar results to the control group in which they shown more residence time in the lighted arm.

Concerning the Morris water maze (MWM) test: Spatial working memory, reference memory, the Alzheimer model mice (ALZ) escape latency time during four consecutive training days were much longer compared with the Control group. The results consistent with **Hassanzadeh, A. et *al.* (2023)**; **He, X. et *al.* (2022),** in which the escape latencies of the Alzheimer's group showed statistically longer than that in the Control group.

On the fifth day of the Spatial Working Memory (MWM) test, the results showed that learning and memory abilities were impaired in the Alzheimer model mice. However, the Alzheimer mice treated with Castanea sativa Mill. honey at 150mg/kg (Alz-D1), 300mg/kg (Alz-D2) revealed a significant improvement and restoration of learning and Memory. These results agreed with those found by **Yaacob, W.M. et** *al.* **(2020)** in which the treated rats with Tualang honey rapidly learned the location of the platform and reached it within 23 and 22s on the fifth day of the trials. And **Şener, G. et *al.* (2022**) whom used *Petroselinum crispum* (Rich with Flavonoids and carotenoids, in which they are the main components of *Castanea sativa* honey) extract to ameliorates scopolamine-induced cognitive dysfunction, where the *Petroselinum crispum* treated rats found the platform in a short time. As for spatial reference memory, the treated groups with Castanea sativa honey and Rivastigmine showed similar results to the control group during the learning days. The results are consistent with **Mustafa, M. Z et *al.* (2019)**, which used stingless bee honey (SBH) to evaluate spatial working memory in mice with the Morris water maze test (Spatial Working Memory).

On the fifth day, the results observed were the Control group, the ALZ model mice, and ALZ treated with SCH solution at 300mg/kg showed similar time records finding the invisible platform. which does not agree with the work of **Yaacob, W.M. et *al*. (2020).**

The results revealed that spatial memory was affected in D-galactose/$AlCl_3$ alzheimer model mice. According to **Song, X. et *al.* (2022),** long-term D-gal/$AlCl_3$-exposure contributes to AD-like symptoms. **Haider, S. et *al.* (2020)** results show that cognitive deficits, decreased cholinergic activity, and AD-like lesions in brain were observed after administration of $AlCl_3$ and D-gal.

Aluminum trichloride ($AlCl_3$) exposure was proven to encourage some behavioral deficits and eventually induces anxiety and depression in rodents animals **(Abu-Taweel, G. M., & Al-Mutary, M. G., 2021**). Therefore, the purpose of this study was to investigate the effects of *Castanea sativa* honey on the anxiety- and depression-like behaviors in mice.

The results of the locomotor activity test showed locomotor hypoactivity in the Alzheimer model mice compared with the control group. These results agree with those of **Houari ADLI, D. E. et *al.* (2021)**, where The $AlCl_3$-intoxicated rats show locomotor hypoactivity compared to control rats. According to **Liaquat et *al.* (2017)** this hypoactivity is due to altered signaling pathways between neurons as well as loss of synapses and neurons and decreased synthesis of neurotransmitters such as dopamine, glutamate, and serotonin. However, Alzheimer's mice treated with SCH solution at 300mg/kg (Alz-D2) showed hyperactivity compared with the other groups. According to **Gohar, A. et *al.* (2020)**, natural honey increases energy expenditure and increases locomotor activity and energy expenditure.

Regarding the black and white Test Box, the test was to assess anxiety-like behaviour in mice in the present work. The Alzheimer model mice showed a significant ($P<0.05$) preference for the illuminated compartment compared with the control mice. The results were consistent with **Houari ADLI, D. E. et *al.* (2021),** in which the mice induced by AlCl3 spend more time in the illuminated compartment compared to the control. According to **Liaquat et *al.* (2017)** this gives a clear picture of ithe psychological state that the $AlCl_3$ intoxicated rats live in a state of permanent stress due to a neurophysiological impairment. The results were similar for the Alzheimer's mice treated with SCH solution at 300mg/kg (Alz-D2), who had a preference for the dark compartment.

The results obtained from Elevated plus maze (EPM) test show a shorter residence time in the protected arm in the Alzheimer model mice compared with the control group. The results consistent with **Kumar, A. et *al.* (2011)**, in which the D-galactose induced mice showed more latency time to enter into closed arm compared with the control. **Várkonyi, D. et *al.* (2022)** claim that female mice spent even more time in the open arm in their experiment. According to **Anand, T et *al.* (2017)** elevated plus maze results, the memory deficits were due to $AlCl_3$ administration.

In contrast, the Alzheimer mice treated with SCH solution at 150mg/kg (Alz-D1) and 300mg/kg (Alz-D2) showed preference for the protected arms similar to the control group. Similar results were found by **Anand, T et *al.* (2017)** in which they used "Brahmi Herbal Drink" (prepared with a combination of date syrup (4%), honey (10 %), Ginger extract (1.5%) with lemon juice (2%) and sodium benzoate (0.015%)). same results, were observed for the ALZ mice treated with Rivastigmine at 1.5mg/kg with a significant difference ($P<0.05$). These results agreed with those found by **Thippeswamy, A.H. et *al.* (2013)** in which animals treated with rivastigmine (5 mg/kg) showed preference for the protected arms.

As for the Porsolt test (Forced swim) it demonstrates a highly significant increase ($p<0.001$) in the time of immobility in the control group comparde with Alzheimer model mice. **Lin et *al.* (2015)** found that reduced motility time is an indicator of increased depressive state (behavioral hopelessness), indicating disruption of serotonergic neurotransmission. The Alzheimer treated mice with chestnut at 150mg/kg and Rivastigmine at 1.5mg/kg showed similar results as the control group.

Excessive exposure to Al can lead to the overexpression of (amyloid-beta precursor protein) AβPP consequently resulting in the deposition of (amyloid-beta) Aβ plaque on the brain cells, thus making Al a potential alzheimerogenic chemical **(Mahdi, O. et *al.*, 2021)**. Honey play a role as a nutraceutical agent in improving memory and cognitionm, the effectiveness of honey in minimizing neurodegeneration is attributed to its neuroprotective effects on the brain, including the prefrontal cortex and hippocampus **(Shaikh, A. et *al.*, 2023).**

$AlCl_3$ and D-gal effects on the histopathological alterations in the brain, liver, and kidney tissues were examined under a microscope using paraffin-embedded tissue sections stained with haematoxylin and eosin. To evaluate morphological alterations in mice brain hippocampus, cortex, and cerebellum histopathological studies were done.

Alzheimer model mice showed histopathological abnormalities, these alterations were characterized by a decrease in cell density, represented by the deposition of amyloid plaques (AP) and neurofibrillary tangles (NFTs) and granulovacuolar degenerations in the CA area of the hippocampus and the cortex compared with the control group. The results are consistent with **Almuhayawi, M.S et *al*. (2020); Song, X. et *al*. (2022); Eraky, S.M et *al*. (2023).** In addition, **Mahdi O et *al*. (2021),** found that rats exposed to AlCl3 and d-gal alone showed cognitive impairments and marked neuronal loss in their hippocampal conus ammonis 1 (CA1). Same results as the one in our study.

Chronic honey treatment at 150mg/kg (Alz-D1) and 300mg/kg (Alz-D2) markedly reduced hippocampal and cortex atrophy and neuronal loss. These results agreed with those found by **Terzo, S et *al.* (2022); Terzo, S. et *al*. (2023).** The Alzheimer's mice treated with Rivastigmine at 1.5mg/kg (Alz-STD) showed similar results. The results are consistent with **Abdel-Aal, R.A. et *al.* (2021)** in which they combined Rivastigmine with *celecoxib*. And **Xing, Z et *al*. (2018),** they used donepezil (1 mg/kg) as a standard drug.

While compared with the control group, Purkinje cell necrosis were observed in the Alzheimer model mice after the cerebellum histological examination. No alteration was observed in the treated groups. According to **Khalil, H.M. et *al.* (2020)** administration of Rivastigmine had an ameliorative effect against neurotoxicity exerted by $AlCl_3$, and prevented Purkinj cell necrosis.

The histological examination, results of liver and kidney tissues showed hepatic and renal alteration in the Alzheimer model mice. According to **Keshava, R. et *al*. (2019)**, $AlCl_3$ causes hepatocellular necrosis, increased inflammatory cell infiltration, dilated sinusoid, vacuolation in the cytoplasm and pyknotic nuclei in rat liver. As for the kidney alteration **Obafemi, T.O. et *al.* (2022)** claim that aluminum chloride ($AlCl_3$)-induced nephrotoxicity in rats also lowered the activities of antioxidant enzymes in the kidney of rats. The Alzheimer treated with chestnut at 150mg/kg and 300mg/kg showed apparent similarities to the control group in the kidney tissues. The results are consistent with **Obafemi, T.O. et *al*. (2022)**, in which they used gallic acid. In our study, gallic acid is one of the main components of chestnut honey.

The results of the present study suggest that long-term intake of chestnut *Castanea sativa* L. honey showed that it significantly enhanced the spatial reference memory in Alzheimer mice in the Radial arm maze test and the spatial learning and memory in the Morris water maze. According to **Terzo, S. et *al*. (2023)**, *Quercetin* as well as *kaempferol* can cross the blood–brain barrier. Quercetin has been reported to protect neurons from oxidative stress and inflammation and to have beneficial properties against mechanisms involved in AD in different in vitro and in vivo models. In addition, according to **Fadzil, M. A. M et *al*. (2023)**, gallic acid was proven to reverse the plaque-caused Abeta deposition, thus preventing synaptic and neuronal damage. Moreover, gallic acid is also a potent cholinesterase inhibitor, thus preventing neurotransmitter deficiency.

Conclusion and Perspective

Complementary and integrative medicine (CIM), techniques are used by people from many cultural backgrounds. Apitherapy, one of these methods, is the supplementary and supportive use of bees and bee products in the treatment of certain chronic diseases. This study was acomplished with the aim is to evaluate the neuroprotective activity of *Castanea sativa* Mill. honey.

The experimental protocol is diveded into two phases the first phase, is the therapeutic period, the mice were treated with *Castanea sativa* Mill. Honey at 150mg/kg and 300mg/kg and a standard drug "Rivastigmine" at 1.5mg/kg by gastric gavage, for the first 45 days. Followed, by the deases induction, with the oral administration of aluminum chloride at 100 mg/kg combined with D-gal at 120 mg/kg by daily intraperitoneal injection for the second 45 days. At the end of the protocol of our study, neurological tests were performed to measure the mice's memory and behaviour. These tests consist of behavioural tests, namely locomotor activity, anxiety test, and memory test, namely radial arm-maze and Morris water maze.

When compared to control mice, the behavioural and memory abnormalities associated with the neurological disorders seen in Alzheimer's model mice are more pronounced. These memory deficits are likely caused by the accumulation of aluminium chloride ($AlCl_3$) in the brain. As for the treated groups with *Castanea sativa* honey at 150mg/kg and 300mg/kg, we note a remarkable improvement in spatial learning and behavioural improvement compared with the Alzheimer model mice. The same results were observed in histopathological studies, in which, Alzheimer model mice showed histopathological abnormalities in brain, liver and kidney tissues compared with the treated groups where *Castanea sativa* honey markedly reduced any tissues atrophy and neuronal loss.

According to the results obtained during our in vivo experimentation, the positive effect of Chestnut "*Castanea sativa* Mill." might be due to the presence of active compounds, such as quercetin and gallic acid, which according to researchers, these compounds show the most promising potential as anti-neurodegenerative agents in terms of their potential negating neurodegenerative pathways. In conclusion, this study investigated the protective effect of *Castanea sativa* Mill. honey on neurodegenerative induced by d-gal and $AlCl_3$ in mice. Because of its high antioxidant activity, *Castanea sativa* honey from Turkey's Black Sea area, may be useful in protecting brain neurons from neurogeneration, according to the results we found.

Even though, Alzheimer's disease is the most common type of dementia with over 40 million worldwide, there is still a lack of information when it comes to natural treatment, especially Apitherapy. According to the results we found, Having being proven to positively affect cognition and memory, honey can be used in complementary and alternative medicine for the prevention of neurological disorders like Alzheimer's disease, due to its powerful compounds like, Flavonoid (Quercetin as well as kaempferol) and Tannin (gallic acid).

This present study represents one of the preliminary steps in the neuroprotective activity *of Castanea sativa* Mill. honey. Further research is needed to understand and determine the bioactive molecules contained in *Castanea sativa* Mill. honey, to be able to isolate them and understand their mechanism action and their neurological activity. Morever in vitro experiments such as the determination of anti-cholinesterase activity can help to understand the neurological mechanism better. Thus, we can conclude that more research, on the advantages of honey in the treatment and management of neurodegenerative diseases, such as Alzheimer's, is needed.

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Annexes

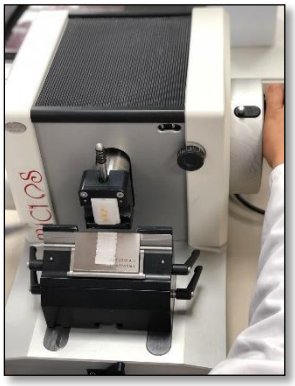
Annex 01

Histological study steps.

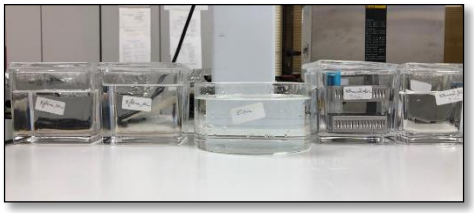
1- Circulation

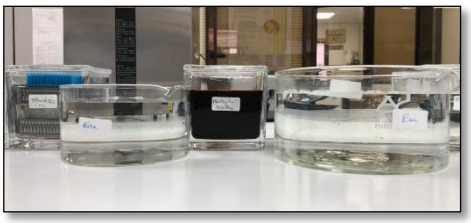
2- Inclusion.

3- Microtomy.



4- Coloring+Montage





5- Microscopic reading.

Annex 02

Body weight evolution dusring the first phase

	Control	*Alz*	*Alz-Std*	*Alz-D1*	*Alz-D2*
19/12/22	**29,6 ± 3,13**	**32,8 ± 2,17**	**32,5 ± 1,29**	**33,2 ± 1,48**	**34,6 ± 4,72**
26/12/22	**29,8 ± 2,17**	**33,2 ± 1,64**	**32 ± 0,82**	**32,6 ± 1,34**	**35,2 ± 2,28**
02/01/23	**28,2 ± 2,49**	**34,2 ± 1,64**	**31,75 ± 0,96**	**31 ± 0,71**	**34,2 ± 2,59**
09/01/23	**30,2 ± 2,59**	**35,4 ± 2,41**	**32,25 ± 0,96**	**32,8 ± 0,84**	**35,6 ± 2,07**
16/01/23	**30,2 ± 2,59**	**35,4 ± 2,41**	**32,25 ± 0,96**	**32,8 ± 0,84**	**35,6 ± 2,07**
23/01/23	**30,6 ± 2,30**	**35,8 ± 3,11**	**30,25 ± 1,89**	**32,4 ± 1,14**	**35,4 ± 2,07**
30/01/23	**30,4 ± 2,30**	**35 ± 2,12**	**30,25 ± 1,89**	**32,4 ± 1,52**	**36 ± 2,92**

Body weight evolution dusring the second phase

	Control	*Alz*	*AlzStd*	*Alz-D1*	*Alz-D2*
02/02/23	**32 ± 2,74**	**34,8 ± 2,59**	**29,75 ± 1,50**	**31,8 ± 0,84**	**35,8 ± 2,39**
09/02/23	**31,8 ± 3,11**	**34,8 ± 2,05**	**30,5 ± 2,89**	**32,8 ± 1,79**	**37 ± 3,16**
16/02/23	**32,6 ± 3,13**	**34,4 ± 3,05**	**31 ± 1,41**	**31,6 ± 2,19**	**35,8 ± 2,49**
23/03/23	**30,6 ± 2,07**	**34,6 ± 1,67**	**31 ± 1,41**	**33,8 ± 1,30**	**39 ± 2,92**
02/03/23	**32,4 ± 3,21**	**34,6 ± 2,61**	**33 ± 1,41**	**33,4 ± 1,82**	**38,2 ± 3,27**
09/03/23	**33 ± 2,65**	**35 ± 2,24**	**32,25 ± 2,06**	**34,6 ± 2,07**	**38,2 ± 3,11**
16/03/23	**33 ± 2,92**	**35,8 ± 2,17**	**33,5 ± 1,29**	**34,4 ± 1,82**	**37,6 ± 2,79**

Annex 03

Solution consumption during the first phase

	Control	*Alz*	*Std*	*D1*	*D2*
26/12/22	**295**	**315**	**320**	**375**	**405**
02/01/23	**331**	**367**	**385**	**379**	**488**
09/01/23	**308**	**325**	**315**	**351**	**460**
16/01/23	**328**	**340**	**315**	**355**	**425**
23/01/23	**330**	**400**	**280**	**342**	**410**
30/01/23	**313**	**338**	**285**	**328**	**397**
$\bar{x}$	317,5	347,5	316,67	355	430,83
σ	14,60	31,13	37,51	19,44	35,77

Solution consumption during the second phase

	Control	*Alz*	*Std*	*D1*	*D2*
09/02/23	**310**	**357**	**335**	**375**	**400**
16/02/23	**342**	**330**	**340**	**400**	**412**
23/02/23	**392**	**335**	**365**	**385**	**440**
02/03/20	**370**	**370**	**380**	**410**	**494**
09/03/23	**350**	**365**	**320**	**450**	**540**
16/03/23	**360**	**315**	**300**	**440**	**420**
19/03/23	70	140	120	180	185
$\bar{x}$	313,43	316	308,57	377,14	413,00
σ	110,28	80,14	87,31	91,10	112,27

Annex 04

Memory test : Radial arm maze

a- Spatial working memory

	Control	***ALZ***	***STD***	***D1***	***D2***
D1	6 ± 0,71	8,5±1,5	6,75±0,83	12,4±1,67	13,5±1,5
D2	7,25±1,3	7,75±1,48	5,75±1,48	9,6±2,41	5,25±2,17
D3	7,8±1,3	8,5±2,29	7,6±0,41	8,2±1,64	8,75±2,28
D4	5,75±1,09	5,5±2,06	4,75±1,09	9,2±0,84	6,5±1,8
D5	5,5±1,5	6,2±0,84	6,25±0,83	8,6±1,52	8,4±1,14

b- Position distinctio

	Control	*ALZ*	*Alz- STD*	*Alz-D1*	*D2*
D1	12,2 ± 2,95	12,6 ± 1,52	10 ± 2,12	13 ± 3,61	11,4 ± 1,95
D2	9,4 ± 2,07	7,4 ± 3,29	8,25 ± 2,05	9,25 ± 2,86	7,2 ± 3,9
D3	7,25 ± 1,09	6 ± 1,87	6,5 ± 2,29	7,8 ± 1,92	8,25 ± 1,48
D4	6,75 ± 1,64	6,6 ± 0,89	5 ± 0,71	8 ± 2,65	6,6 ± 2,97
D5	4,33 ± 1,78	5,6 ± 1,82	5,5 ± 2,06	7,8 ± 1,3	8,25 ± 1,92

c- Spatial reference memory

	Control	***ALZ***	***Alz-STD***	***Alz-D1***	***Alz-D2***
D1	50,25 ± 6,87	63,25 ± 13,5	70,75 ± 17,75	151,25 ± 32,33	139,75 ± 29,97
D2	99,75 ± 17,46	75,25 ± 19,72	130,33 ± 4,26	106,6 ± 15,18	111,25 ± 9,63
D3	130,5 ± 29,38	220,5 ± 21,27	193,5 ± 26,46	176,5 ± 28,22	168 ± 26,62
D4	171,75 ± 22	201 ± 45,71	212,25 ± 40,27	180 ± 23,18	197,2 ± 17,89
D5	264,4 ± 19,59	215,2 ± 29,73	239,5 ± 16,04	205 ± 41,93	232,4 ± 30,35

Morris water maze

a- Spatial working memory

	Control	*ALZ*	*Alz-STD*	*Alz-D1*	*Alz-D2*
D1	5,75 ± 2,49	15,75 ± 10,06	4,75 ± 2,49	7 ± 3,67	9,8 ± 2,17
D2	5 ± 2	24,66 ± 6,34	34,66 ± 9,93	8 ± 6,63	5,75 ± 2,83
D3	6,25 ± 1,92	7,75 ± 4,92	9,6 ± 2,94	5,6 ± 0,41	7 ± 3,94
D4	2,8 ± 0,84	32,25 ± 3,34	5,6 ± 2,16	5,5 ± 1,5	3
D5	4,25 ± 2,17	12,66 ± 3,34	17,33 ± 13,9	11,5 ± 4,39	6,66 ± 3,19

b- Spatial Reference memory

	Control	*ALZ*	*Alz-STD*	*Alz-D1*	*Alz-D2*
D1	8,6 ± 5,77	26,66 ± 1,08	12,25 ± 6,87	6,75 ± 3,03	5,8 ± 2,17
D2	15,33 ± 6,01	36 ± 13,84	7,5 ± 4,39	5,8 ± 1,79	5,5 ± 1,66
D3	3,6 ± 0,89	5 ± 3,46	7,33 ± 1,63	3,8 ± 1,3	24,25 ± 7,29
D4	3,2 ± 0,84	6,5 ± 1,5	3,33 ± 1,08	3,75 ± 0,83	5,75 ± 3,56
D5	5,33 ±3,49	4 ± 2,92	7,25 ± 3,03	15 ± 0,71	5 ± 1,41

Annex 05

Behavioural test

a- Locomotor activity

Control	***Alz***	***Alz-STD***	***Alz-D1***	***Alz-D2***
62,37 ± 1,26	59,32 ± 3,06	45,85 ± 5,15	65,56 ± 7,53	73,06 ± 7,49

b- Anxiety test

Elevated plus maze

Control	*ALZ*	*Alz-STD*	*Alz-D1*	*Alz-D2*
235,53 ± 5,16	189,63 ± 24,46	238,52 ± 18,26	241,56 ± 4,32	248,85 ± 7,18

Black/WHITE test box

Control	*Alz*	*Alz-STD*	*Alz-D1*	*Alz-D2*
216,91 ± 10,32	162,88 ± 10,76	187,63 ± 11,34	113,46 ± 9,8	182,46 ± 9,65

C- Persolt test (FST)

Control	*ALZ*	*Alz-STD*	*Alz-D1*	*Alz-D2*
163,4 ± 40,54	82,5 ± 39,9	176,5 ± 34,25	137 ± 22,77	232,4 ± 34,65