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Effects of Telmisartan and Rosemary on the Experimental Alzheimer's Model Induced in Rats

A thesis Submitted to Council of College of Medicine, University of Babylon, as a Partial Fulfillment of Requirements for Degree of Master in Pharmacology /Pharmacology and Toxicology

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالَ رَبِّ اشْرَحْ لِي صَدْرِي (٢٥) وَيَسِّرْ لِي أَمْرِي (٢٦) وَاحْلُلْ عُقْدَةً مِنْ لِسَانِي (٢٧) يَفْقَهُوا قَوْلِي (٢٨)

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was prepared by (**Saba Abdulrahim Mukheef Hasan)** under our supervision at the department of Pharmacology. College of Medicine, University of Babylon (Iraq) in partial fulfillment of the requirements for the master degree of sciences in Pharmacology and Toxicology.

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Summary:

Alzheimer's disease is a neurodegenerative condition characterized by a gradual onset and progressive decline in cognitive and behavioral abilities, including logic, language, attention, vision, and memory.Till now,the main physiological mechanisims of the disease still unknown.This disease responsible for two thirds of all cases of dementia in patients aged 65 years and above.There are several theories about its emerging but the main characteristics are the presence of neurofibrillary tangles and neurotic plaques.There is no known curative treatment that cure it of stop its progression,but there are several treatments that delay its progression or attenuate its symptoms so the patient can practice his daily activities. Studies.All the drugs that available are not perfectly effective and cause a lot of side effects.Currently the focus is on herbal medicine , natural products have little side effects.Previous studies have indicated that the angiotensin receptor blocker (telmisartan) has antioxidant and anti-inflammatory effects that has good impact on the patients.Rosemary also known to have these effects.The aim of this study is to verify if combining these two compounds will have a greater effect if given together. The results were compared to FDA approved drug (donepezil). Thirty rats were devided to six groups and each group contained five rats.The control group (groupI) rats have received normal saline by i.p injection for about 60 days,the Alzheimer's disease induced group (GroupII), rats received aluminum chloride(AlCl3) (10 mg/kg I.P for 60 days),telmisartan group(group III) rats received both (10 mg/kg telmisartan orally via gastric gavage needle tube) and AlCl3 for 60 days by intraperitoneal injection, rosemary group (group IV) received both (300 mg/kg rosemary liquid extract orally by gastric gavage needle tube) and AlCl3 intraperitoneal injection for 60 days , combination group(group V),rats received both previous doses of telmisartan(10 mg/kg orally) with rosemary extract (300

mg/kg) given orally via gastric gavage needle tube and AlCl3 intraperitoneal injection for 60 days and donepezil group (grouVI) rats received both donepezil (5 mg/kg orally by gastric gavage needle tube) and AlCl3 intraperitoneal injection for 60 days.The memory enhancing activity of telmisartan, rosemary and the combination groups was testified in comparing to FDA approved drug (donepezil) by using behavioural test method including Y-maze spontaneous alteration test to measure the spatial memory. The behavioural test results have showed that in group II,spontaneous alteration percentage(SAP) were highly significantly decreased ($p<0.001$), anyway, in groups III, IV, V, and VI, SAP was highly significantly increased ($p<0.001$).

Twenty-four hours after the last dose,rats were sacrificed by decapitation and rat's brains were removed from each group then histopathological and biochemical analysis have been performed.

The histopathological examination illustrated there are a clear morphological changes and moderate inflammation in rats group that received AlCl3,while rats that received telmisartan,rosemary and donepezil have found to have less inflammation in their brain tissues.

The oxidative stress tissue biomarkers (reduced glutathione(GSH), malondialdehyde (MDA) , total antioxidant Capacity(TAOC) and the inflammatory parameters (interleukin -1 , interleukin - 6, and tumor necrosis factor alpha (TNF – α) were detected by using enzyme linked immunosorbent assays kits (ELISA).

In group II (AlCl3 group), TAOC and GSH were highly significantly decreased ($p<0.001$) as compared to group I (the control group) which related to the oxidative damage. Also, we found that IL-1, IL-6, TNF-α and MDA were highly significantly increased ($p<0.001$). In group III ,GSH significantly increased ($p<0.05$) ,while in group IV to group VI , GSH was highly significantly increased ($p<0.001$).In group IV

to group VI,TAOA was highly significantly increased($p<0.001$) while in group III there was significant increasing ($p<0.05$), but in group III to group VI ,MDA, IL-1, IL-6, and TNF-α were highly significantly decreased($p<0.001$) in comparison to group II (the induction group).

In conclusion, telmisartan and rosemary have been shown to enhance spatial memory in rats having neurodegeneration also they have antioxidant and anti-inflammatory capacities by decreasing proinflammatory parameters and increasing antioxidant biomarkers.

List of Contents

List of tables

Lists of Figures

List of abbreviations

Abbreviation	Meaning
AAB-003	Humanized Monoclonal Antibody to Beta Amyloid
AAD-vac1	Active Immunotherapy for Alzheimer's Disease
ABTS	2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
AChEI	Acetyl-Cholinesterase-Inhibitor
AD	Alzheimer's Disease
AlCl3	Alumnium Chloride
Ang	Angiopoietin
AngII	Angiotensin II Receptor
ApoE4	Apolipoprotein E
APP	Amyloid Precursor Protein
ARBs	Angiotensin Receptor Blockers
ARE	Antioxidant Response Element
AT1	AngiotensinII Type 1 Receptor
AT1Rs	Angiotensin II Type1 Receptors
AT2Rs	Angiotensin II Type2 Receptors
ATP	Adenosine Triphosphate
Aβ	Beta-Amyloid
BACE1	Beta-Secretase,Beta-Site Amyloid Precursor Protein Cleaving Enzyme
BBB	Blood Brain Barrier
BCL2	B-Cell Lymphoma-2
BFL-1	B-Cell Lymphoma-2-Related Protein A1
BMS932481	Beta-Amyloid Secretase Modulator
Bu-ChE	Butyrylcholinestearase
CA	Carnosic Acid
CAT	Catalase
c-FLIP	Cellular FLICE-Like Inhibitory Protein
COX-2	Cyclooxygenase-2
CSF	Cerebrospinal Fluid
CT	Computerized Tomography
ct	Chemotype
CTF99/CTF89	C-Terminal Fragments
CXCL-1	Chemokine(C-X-C motif) Ligand 1
DMTs	Disease-Modifying Therapies
ECAM-1	Epithelial Cell Adhesion Molecule-1
ECG	Electrocardiogram
Fas	Fatty Acid Synthase

FDA	Food and Drug Adminstration
fMRI	Functional Magnetic Resonance Imagining
GC	Glutamylcysteine
GCL	Glutamate-Cystine Ligase
GCLC	Glutamate-Cystine Ligase Catalytic Subunit
GCLM	Glutamate-Cystine Ligase Modifier Subunit
GSH	Reduced Glutathione
GST	Glutathione Transferase
H2O2	Hydrogen Peroxide
HCL	Hydrochloric Acid
ICAM	Intercellular Adhesion Molecule-1
IL-1	Interleukin-1
IL-6	Interleukin-6
IRAK	Interleukin-1 Receptor-Associated Kinase
KEAP1	Kelch Like ECH Associated Protein1
LAPs	Leucine Aminopeptidase
LPS	Lipopolysaccarride
MCI	Mild Cognitive Impairment
MCP-1	Macrophage Inflammatory Protein-1
MDA	Malondialdehyde
MIP-2	Macrophage Inflammatory Protein-2
MMPs	Matrix Metalloproteinases
MRI	Magnetic Resonance Imagining
MyD88	Myloid Differentiation Primary Response88
nAChR	Nicotinic Acetylcholine Receptor
NADPH	Reduced Nicotinamide Adenine Dinucleotide Phosphate
NF-B	Nuclear Factor-B
NF-kB	Nuclear Factor Kappa-Light -Chain-Enhancer of Activated B Cells
NGF	Nerve Growth Factor
NLRP3	Nucleotide-Binding Domain,Leucine-Rich-Containig Family,Pyrin Domain-Containing-3
NMDA	N-Methyl-D-Asparate
NO	Nitric Oxide
NOX	Nicotinamide Adenine Dinucleotide Phosohate Oxidase
NRF2	Nuclear Factor Erythroid2-Related Factor2
PAI2	Plasminogen Activator Inhibitor-2
PET	Positron Emission Tomography
PI3K	Phosphoinositol-3 Kinase

PPAR	Peroxisome Proliferator-Activated Receptor
PSEN	Presenilin
RAS	Renin-Angiotensin-System
RCT	Randomized Controlled Trial
RNS	Reactive Nitrogen Species
RORyt	Retinoic Acid Receptor Gamma-Related Orphan Nuclear Receptor Transcription Factor
ROS	Reactive Oxygen Species
RXR	Retinoid X Receptor
SAP	Spontaneous Alteration Percentage
sAPPβ	Soluble Amyloid Precursor Protein Beta
SOD	Superoxide Dismutase
SPPAR-M	Selective Peroxisome Proliferator-Activated Receptor
-Syn	Synuclein
TAOC	Total Antioxidant Capacity
TCA	Trichloroacetic Acid
TDP-43	Transactive Response DNA Binding Protein-43
TLM	Telmisartan
TMB	3,3,5,5-Tetra Methyl Benzidine
TNF-α	Tumor Necrosis Factor-Alpha
TRAF	Tumor Necrosis Factor Associated Factor
TRx0237	Tau Protein Aggregation Inhibitor
TSH	Thyroid Stimulating Hormone
TXNIP	Thioredoxin-Interacting Protein
VCAM-1	Vascular Cell Adhesion Molecule-1

Chapter one
Introduction
and
Literature Review

1.Introduction

Dementia, in broad terms, is the decline in cognitive functions to a point where it hampers one's daily functioning. The predominant form of dementia is Alzheimer's disease, accounting for at least two-thirds of cases among individuals aged 65 and above (Zilberzwige-Tal *et al*., 2018).

Alzheimer's disease is a neurodegenerative condition characterized by a gradual onset and progressive decline in cognitive and behavioral abilities, including logic, language, attention, vision, and memory. The fact that Alzheimer's disease ranks as the sixth most common cause of mortality in the US should not be overlooked. About 10% of people with Alzheimer's disease have early onset Alzheimer's disease, which commonly manifests before the age of 65. Alzheimer's disease does not currently have a known treatment. There are, however, a number of treatments that can help reduce some of its symptoms. Depending on what stage of the disease a person is experiencing, the symptoms of Alzheimer's disease might change (Tang *et al*., 2019).

Alzheimer's disease is divided into several stages, early (mild),middle-stage (moderate) and the late stage (severe) depending on the severity of cognitive impairment.In the early stage, the patient will be asymptomatic at this time, but the laboratory findings are certain. Currently, the biomarkers can be used to diagnose Alzheimer's disease. Although they are not specific for the condition, amyloid and tau proteins in cerebrospinal fluid (CSF) function as biomarkers. Another analysis has also revealed the interaction of a number of factors, including the gene ApoE4 and whether it is present in one or two copies, as well as the recorded results on the paired associates instant recall test and the digits symbol substitution test,increased tau protein in CSF, right entorhinal cortex thickness, and right hippocampus volume on magnetic resonance imagining (MRI), can all predict the development of mild cognitive impairment in the preclinical period (Tahami *et al*.,2022).

In the second stage, the patient will have impairment in both memory and non-memory functions, such as language or daily activities. At this time, the patient can still live independently and go out with friends, work, etc. There is a 10% per year progression from mild cognitive impairment (MCI) to dementia. It relies on the presence of risk factors for Alzheimer's disease and the level of decline at the time of diagnosis.In the last stage of dementia, the patient will obviously experience a deterioration in memory. Anomia, paraphasic mistakes, rambling, a loss in visuospatial ability, and apraxia.Delusions may be present in 20% up to 40% of patients. In addition to visual hallucinations, patients may also have auditory and olfactory hallucinations.

The majority of the patients—50%—show aberrant behaviours. Patients' interrupted sleep will result in problems since their biological circadian sleep-wake cycle has changed. The most frequent early sign is short-term memory impairment, which is followed by impairment in other functions. Such as the ability to think critically, make decisions, lack of assistance, and disorganisation, which affect daily chores and logical reasoning. In the early stages, the impairment in practical functioning might range from slight to significant.In the mild to late stages there will be a neuropsychiatric disorders like social withdrawal , psychosis, agitation, dyspraxia, dystonia, akathesia and parkinsonian symptoms occur in late stages of the disease and that is followed by primitive reflexes, enuresis and finally total dependence on others for caring. (Maccioni *et al*., 2018)

Aims of the Study

1.Investigate the effects of telmisartan and rosemary on Alzheimer's disease consequences, like memory by measuring SAP in Y maze.

2.Investigate the effects of telmisartan and rosemary on oxidative stress parameters like malondialdehyde (MDA), reduced glutathione (GSH) , and total antioxidant capacity (TAOC) in male rat's model of Alzheimer's disease.

3.Explore the impacts of telmisartan and rosemary liquid extract on some of proinflammatory biomarkers (IL1,IL6, TNF-α) on Alzheimer's induced rat's model.

4.Compare oxidative stress and proinflammatory parameters results of telmesartan and rosemary when administered separately to their combined impacts when given together.

1.1. Historical Overview

Alzheimer's disease' as a term has been used in the past 100 years and the first time it used was in 1910 , the number of patients with dementia is increasing over time.The drugs that smoothen the symptoms without curing it also the diagnosis techniques have improved since then. There were a lot of agents that used as attempts to reduce the disease either in its symptoms or its primary physiological abnormalities.Thus, disease-modifying therapeutics (DMTs) and symptomatic treatments are the two categories under which the pharmaceutical treatments for Alzheimer's disease are classified (Cummings *et al*., 2020).

1.2. Epidemiology

Alzheimer's disease primarily affects elderly people.Additionally, there are roughly 24 million people with dementia worldwide, it is predicted that number would more than double by the year 2050. Financial losses in the United States related to the treatment of these diseases alone are estimated to be around 172 billion dollars annually.The number of AD patients (≥ 65 years) might increase greatly from 5.8 million to 13.8 million by 2050 (Zhang et al .,2021) .The incidence of Alzheimer's disease is doubled,usually after the age of 65.The incidence of the disease is increased considerably form about only 1% per year at the age before 65 years to about 6% per year above this age.The frequency rates of the disease is increased from 10% in the age above 65 years to about 40% at the age above 85.The incidence for the disease is a little bit higher for females especially at the age of 85 (Nicolas *et al*.,2018).

1.3. Etiology

Alzheimer's disease is progressive and gradual onset disease, basically it’s a neurodegenerative disease that is caused by neuronal cell death. Usually it starts in the entrohinal cortex of the hippocampus and then spread to other regions.

There are several risk factors for this disease but the most common one is the increasing age,other risk factors include cardiovascular, cerebrovascular disease,traumatic head injuries, depression,family history of dementia,increased level of homocystine,smoking,the disease also has genetic aspect,in the early and late stages of it,a risk factor for early onset dementia is trisomy 21,also the presence of APOEe4 allele increase the chance of getting the disease.Having a first degree relative with Alzheimer's diases can increase the risk by 10% to 30%, and individual with more than one sibling with Alzheimer' disease increase their possibility of getting the disease by 3 folds than the other individuals (Liljegren *et al*.,2018).

Higher education can help reduce the risk of disease, but so can regular aerobic exercise, the use of anti-inflammatory drugs, oestrogen use in women, a good diet, and some intellectual hobbies (Tong *et al*.,2018)

1.4. Pathophysiology and Disease Development Mechanisms

An accumulation of aberrant neurofibrillary tangles and neuritic plaques is what distinguishes Alzheimer's disease.

1.4.1 Plaques Formation

The extracellular amyloid beta-peptide core of plaques, which are globular, microscopic lesions surrounded by larger axonal terminals, is produced from a transmembrane protein called the amyloid precursor protein (APP) (Vik-Mo *et al*.,2019).Proteases called (alpha, beta, and gamma)-secretase work on APP to break the beta-amyloid peptide off it,typically, either alpha-secretase or beta-secretase will cleave APP, and the tiny fragments they produce are not neurotoxic. But later cleavage by the beta and gamma-secretase enzymes produces the 42 amino acid peptide known as beta-amyloid 42, a higher level of beta-amyloid 42 causes amyloid to accumulate, which in turn promotes neuronal damage. In contrast to the typical breakdown of amyloid precursor proteins, beta-amyloid 42 favours the development of clustered fibrillary amyloid protein.

1.4.2. Neurofibrillary Tangles Formation

A protein called tau forms the fibrillary intracytoplasmic aggregates known as neurofibrillary tangles in neurons. The stabilisation of axonal microtubules is the tau protein's primary function. Microtubules are important for intracellular transport and are present along neuronal axons. Tau protein holds together the microtubule cluster. Because of the buildup of extracellular amyloid peptide, which results in the aggregation of tau aggregates,there will be hyperphosphorylation of tau protein in Alzheimer's disease. Additionally, tau aggregates produce neurofibrillary tangles, which are twisted pairs of helical filaments. They begin to appear in the hippocampus before spreading to other parts of the cerebral cortex.In the neurons, tau-aggregates are deposited.Compared to plaques, tangles have a stronger correlation with Alzheimer's disease (Wallace *et al*., 2018).

1.4.3. Granulovacuolar Degeneration

Amyloid plaques cause the hippocampus pyramidal cells to deteriorate. According to previous study,cognitive decline is more closely related to a reduction in the density of presynaptic boutons from pyramidal neurons in laminae III and IV than it is to an increase in the number of plaques.

Low level of Acetylcholine also has been noted as a result of neuronal loss in the nucleus basalis of myenert.

Uncertainty surrounds the precise mechanism of the neurodegenerative process that takes place in Alzheimer's. According to studies, subcortical infarcts four times enhance the risk of dementia. In general, cerebrovascular illness magnifies the severity and rate of progression of dementia (Verma *et al*.,2018).

1.4.4. Genetic Basis of Alzheimer Disease

Alzheimer's disease can be inherited as an autosomal dominant disorder.The autosomal dominant pattern of the disease is caused by mutations in three genes: the amyloid precursor protein gene (APP) gene on chromosome

21, the presenilin 1 (PSEN1) gene on chromosome 14, and the presenilin 2 (PSEN2) gene on chromosome 1. Beta-amyloid peptide production and accumulation may be accelerated by APP mutations.Beta-amyloid aggregates and accumulates as a result of PSEN1 and PSEN2 mutations interfering with gamma-secretase processing. About 5 to 10% of all cases of Alzheimer's are due to mutations in one of these genes, and this gene is also the primary cause of the disease's early onset. Apolipoprotein E, a protein that controls lipid metabolism and has a strong affinity for beta-amyloid, is another hereditary factor that raises the risk of developing Alzheimer's disease (Nicolas *et al*.,2018).

After the age of 65, a family type of Alzheimer's disease is connected with the gene isoform e4 of this protein, which is located at chromosome 19.The presence of one APOE e4 allele does not guarantee the development of the disease, but it does increase the incidence to around 50%, and the presence of two alleles increases the incidence to about 90%,the age of disease onset will be lowered by the alleles' existence, both familial and sporadic types of Alzheimer's disease have been linked to mutations in the sortilin receptor SORT1 gene, which plays a key role in the transport of APP from the cell surface to the Golgi-endoplasmic reticulum complex(Jack *et al*.,2018;).

1.4.5. Oxidative Stress in Alzheimer's Disease

Although reactive oxygen species (ROS) are essential for metabolism, cell communication, and the proper development of learning and memory processes in a healthy state (Chandel, 2014), too much of them can cause oxidative stress. (Li *et al*., 2013). Nicotinamide adenine dinucleaotide phosphate oxidase (NADPH-oxidase (NOX)) produces ROS as a primary byproduct, and numerous other enzymatic activities, such as those involving xanthine oxidase, cyclooxygenases, uncoupled nitric oxide synthase (NOS).The mitochondrial electron transport chain, also produce ROS as secondary byproducts (De Silva and Faraci, 2013).

In addition, accumulating ROS can damage mitochondrial structure, reduce adenosine triphosophate(ATP) synthesis, and result in the creation of even more ROS from the mitochondria. It is well established that mitochondrial-derived ROS cause cellular malfunction by making their way to the cytoplasm, exhibiting further negative effects (Valenzuela *et al*., 2016).

Phosphoinositol-3 kinase (PI3K) and protein kinase C activation are coupled with nuclear and mitochondrial angiotensinII type1 receptors (AT1R) activation to create NOX4-derived ROS in neurons (Villar-Cheda *et al*., 2017). It's significant to note that activation of nuclear (AT1Rs) controls gene expression and activates a number of defense mechanisms that shield cells from oxidative stress (Villar-Cheda *et al*., 2017). To specifically counterbalance AT1R effects in neurons, nuclear renin angiotensin system (RAS) activates Ang II/AT2R and Ang (1-7)/MasR axes, increases AT2R mRNA expression (Villar-Cheda *et al*., 2010, 2017; Costa-Besada *et al*., 2018).

Angiotensin II receptors type I overexpression increased basal ganglia cell death and dysfunction in dopaminergic neurons. (Zawada *et al*., 2015; Ou *et al*., 2016). Ang (1-7)/MasR and Ang II/AT2R axes, in contrast, inhibited the generation of ROS and mitochondrial respiration in the same type of cells (Costa-Besada *et al*., 2018).

The release of a pro-inflammatory response is caused by the RAS-mediated elevation of oxidative stress, which exacerbates cell death (Ou *et al*., 2016).

1.4.6. Neuroinflammation in Alzheimer's Disease

The majority of Nicotinamide adenine dinucleaotide phosphate oxidase (NADPH-oxidase (NOX))-derived ROS in the brain is contributed by microglial cells. NOX2 is the main producer of extracellular ROS in microglia among the NOX isoforms. The activation, proliferation, and release of pro-inflammatory signals by microglia are likewise impacted by NOX-derived ROS intracellular signaling pathways.

AngiotensinII/AT1R axis plays a role in the transition of microglia between the activated substates of proinflammatory M1 and antiinflammatory M2. The transition between macrophages (M1) and (M2) is specifically regulated by AT1R-induced NOX activation, with overexpression of the AT1R/NOX axis favoring pro-inflammatory and decreasing anti-inflammatory substates. Microglia further increase ROS production with this signal cascade. Another study that demonstrated how AT1R antagonists reduced M1 microglia activation and promoted M2 microglia polarization corroborated this theory (Saavedra, 2017).

Through the nuclear factor kappa B (NF-kB) pathway, Rho-kinase activation promotes AT1R expression in microglial cells. Microglial RhoA/Rho kinase pathway activation, a crucial regulator of the actin cytoskeleton, mediates microglial polarization and neurodegeneration. (Labandeira-Garcia *et al*., 2015, 2017).

The Ang II/AT1R axis can also enhance the toll-like receptor 4, which is known to promote neuroinflammation and microglial activation. (Biancardi *et al*., 2016; Rietdijk *et al*., 2016). It has been demonstrated that Ang II and prorenin decrease levels of the anti-inflammatory cytokine IL-10 while increasing levels of ROS and pro-inflammatory cytokines, such as interleukin-1 beta (IL-1 beta) and IL-6 (Winklewski *et al*., 2015). For instance, a recent study revealed that Ang II treatment causes increasing the number of CD68-positive cells in hippocampus which cause the inflammatory response (Takane *et al*., 2017).

The Ang II/AT1R axis mediates IL-6 and ROS generation in astrocytes via the NF-k/ROS pathway (Gowrisankar and Clark, 2016). Additionally, Ang III can activate the transcription factor signal transducer and activator of transcription 3, which is essential for mediating cell proliferation,

differentiation, apoptosis, and inflammation in astrocytes (Kandalam *et al*., 2015).

Renin angiotensin system receptors also activate mechanisms that regulate inflammation. Neuronal AT2R signalling can reduce the phosphorylation of signal transducer and activator of transcription 1 and 3, which can stop microglia from getting activated (Jackson *et al*., 2018). In a mouse model of AD, AT4R was found to mediate anti-inflammatory effects (Royea *et al*., 2017). A perfect balance between the pro-oxidative and pro-inflammatory Ang II/AT1R/NOX and the antioxidant and anti-inflammatory Ang II/AT2R - Ang (1-7)/MasR axes must be preserved in order to modulate renin-angiotensin system (RAS) effects inside the brain.

As a summary of the pharmacological therapies available for AD from (2015–2020), figure.1.1 shows symptomatic therapies and DMTs along with figure.1.2 showing their mechanisms of action:

A-Disease Modifying Therapies

1-Targeting Aβ or its associated proteins at different stages of AD and in people with varying risk profiles is currently the most effective disease-modifying technique (Murphy and LeVine. , 2010).

2- Therapies that target tau protein are also being tried.The DMTs has no significant effect on reducing or delaying the progression of the disease (Novak *et al*.,2017; Gauthier *et al*.,2016; Malpas *et al*.,2016).

3-Nerve growth factor gene therapy (Tuszynski *et al*.,2015 ;Eyjolfsdottir *et al*.,2016 Ferreira *et a*l.,2015).

B-The Symptomatic Treatments

1-FDA approved acetylcholinestrase inhibiting agents (Rivastigmine, Galantamine, Donepezil) (Park *et al*., 2017; Isaacson *et al*.,2016)

2-N-methyl-D-aspartate receptors antagonists (memantine) (Hager *et al.*,2014).

3-5-hydroxytryptamine-6-receptor antagonist (idalopiridine) that is given in concomitant way with AChEI (Atri *et al*., 2018)

4-Cyclic neucleotide phosphodiestrase3 inhibitors (cilostazol) (Tai *et al*., 2017).

5- Other agents that used to reduce AD symptoms like Protein kinase C epsilon agonist (bryostatin) (Nelson *et al*., 2017); BI 409306 a cGMP specific selective phosphodiestrase 9A antagonist (Moschetti *et al*., 2016) ; Monoamine oxidase B inhibitors (sembragiline) (Sturm *et al*., 2017).

Symptomatic treatments, however, have shown to be more effective in delivering short-term improvements or longer-term stabilisation now that medicines that target the cholinergic system are in use (Chen *et al*., 2017).
Future studies would be more productive if they concentrate on subsequent processes, such as tau hyperphosphorylation and neuroinflammation, as well as mitochondrial damage in presynaptic neurons. For treating the disease, the simple suppression of Aβ or its associated proteins or enzymes appears inadequate (Wischik *et al*., 2015).

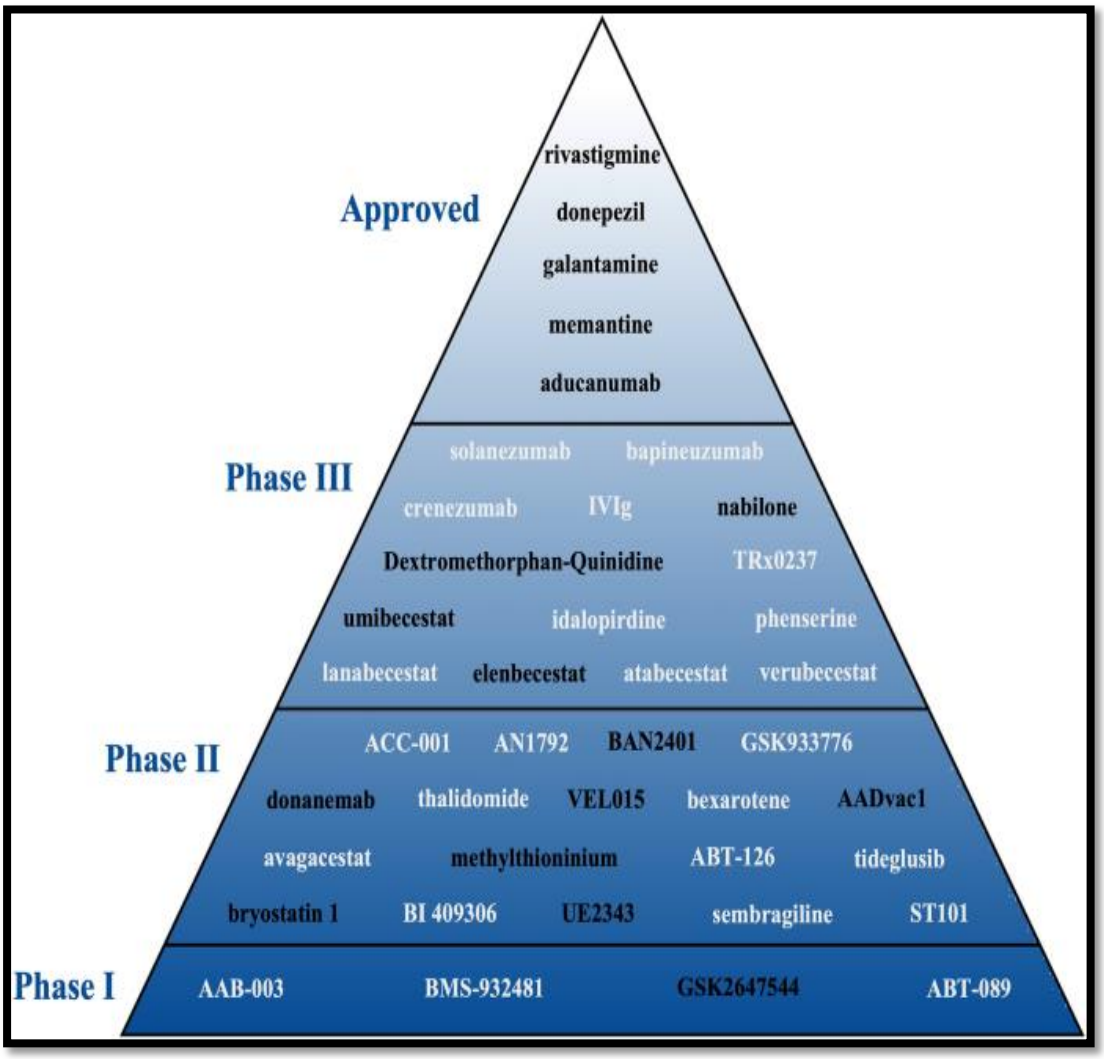


Figure 1.1. Symptomatic therapies and DMTs (Lin *et al*.,2021)

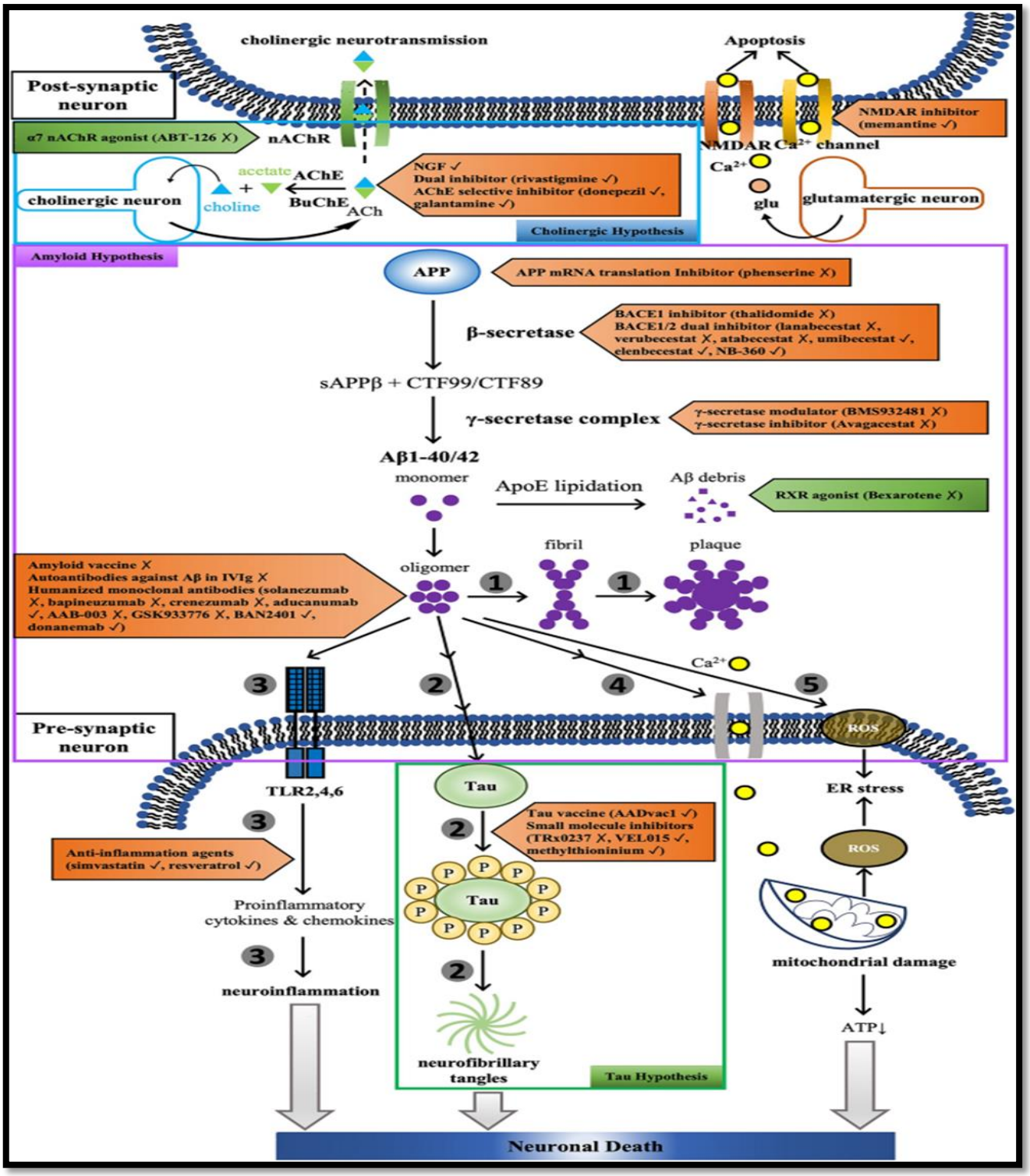


Figure.1.2. Symptomatic and DMTs treatments mechanisms of action (Lin *et al*.,2021). nAChR:Nicotinic acetylcholine receptor,TLR:Toll like receptor,NGF:Nerve growth factor,NMDA:N-methyl D-aspartate,APP:Amyloid precausor protein,CTF:C-terminal fragment,AB:Beta amyloid,ApoE:Apolipoprotein E,RXR:Retinoid X receptor, ER: Endoplasmic reticulum,ATP:Adenosine triphosphate,glu :glutamate, BuChE:Butyryl cholinesterase

1.5.Diagnosis

The key elements for the diagnosis are physical examinations and history of the symptoms, the evaluation is based on asking the patient's family members and carers about his cognitive abilities and daily activities since the patient may not be aware of his condition. It is crucial to recognise the disease's onset and early symptoms to distinguish the condition from other types of dementia (Kim.H *et al*.,2018).

The assessment in advanced stage of Alzheimer's disease must include the mental status condition, like the language, concentration, attention, visuospatial function,the practical activities , etc. Other forms of mental assessments like the mini mental status examination is not specific and less sensitive but it can be used for screening, the patient's follow up visits must include full mental status examination to evaluate the progression of the disease and the development of the neuropsychiatric symptoms (Petersen RC *et al*., 2018). Routine laboratory tests for Alzheimer's disease do not reveal any distinct abnormalities, tests are used to rule out other diseases may be presnt like thyroid stimulating hormone TSH,the complet metabolic panel,complete blood count,B12. The computed tomography of the brain will show extension in the third ventricle with cerebral atrophy, these factors could be informative but not conclusive because they could also be present in other illnesses and disorders linked to advancing age, the cerebrospinal fluid test can also be used to determine whether there is low beta amyloid due to its brain accumulation and elevated tau protein due to neurodegeneration in the disease's preclinical stage,a neuropsychological evaluation is the most effective way to diagnose mild cognitive impairment in the early stages of a disease.Some functional brain imaging techniques, such as positron emission tomography (PET),fuctional magnetic resonace imagining (fMRI), and the medial temporal and parietal lobes of the brain, as well as other smaller brain regions, can be evaluated using spectral imaging,the beta amyloid and neurofibrillary tangles,

two histological indicators of Alzheimer's disease that are currently under investigation, can be recognized using various contemporary techniques, It should be known that, despite a thorough physical examination, a complete clinical history, and other pertinent testing, determining the precise kind of dementia is difficult and may not always be achievable, although some people may complain of cognitive impairment, it may only be moderate cognitive impairment and not meet the criteria for dementia if it does not interfere with everyday activities,nevertheless a sizable portion of those people will eventually get some form of dementia, usually between 5 and 7 years later (Haapasalo and Hiltunen .,2018).

1.6.Prognosis

Alzheimer's disease is perpetually escalated.average life span for patient that's diagnosed with Alzheimer's disease with a 65-year-old or older is about 4 or 8 years, but some patient can live for more than 20 years .The main causative agent of death is pneumonia (Barnes *et al*.,2018)

1.7. Renin-Angiotensin System in Alzheimer's Disease

Through the above mentioned pathways,renin angiotensin system(RAS) is linked to many of the disorders relevant to Alzheimer's disease (AD), making it potentially crucial to the pathogenesis of AD. As a result, it was suggested that more investigation be done into the angiotensin theory. (Kehoe, 2018).

It has been demonstrated that the mitochondria and nuclei of neurons contain intracrine RAS, including AT1Rs, AT2Rs, and MasRs (Valenzuela *et al*., 2016; Villar-Cheda *et al*., 2017; Costa-Besada *et al*., 2018).)

Like mitochondrial RAS, nuclear RAS is essential for the control of oxidative stress. It controls the interaction between the detrimental and beneficial RAS pathways through transcription and the migration of several additional receptor types. (Zawada *et al*., 2015; Villar-Cheda *et al*., 2017; Costa-Besada *et al*., 2018).It illustrated in figure 1.3:

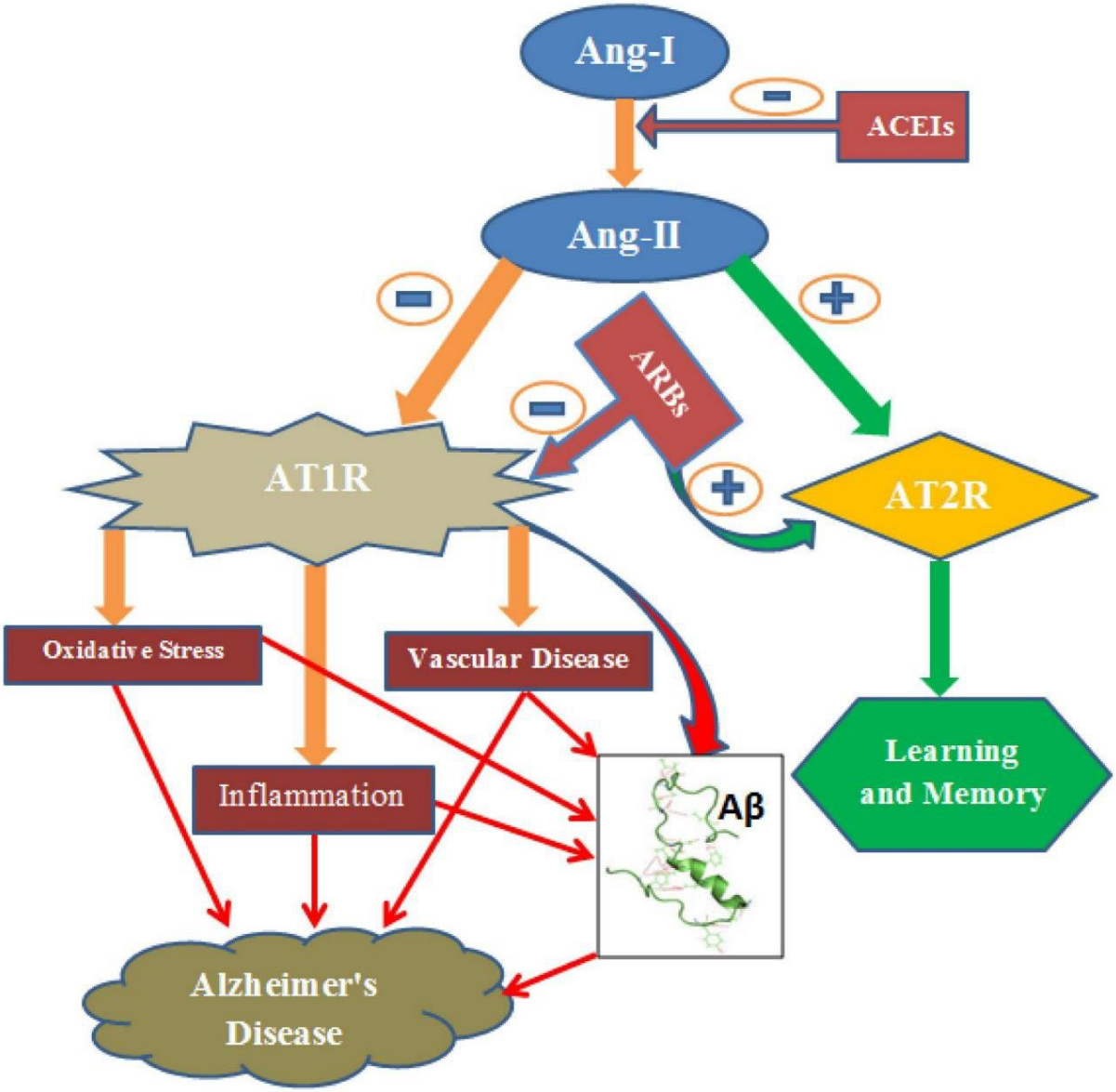


Figure1.3. (Ruginsk *et al* .,2015)

FIGURE 1.3. Ang-II induces oxidative stress, inflammation and vascular disease via AT1R. Consequently, it causes accumulation of amyloid-β resulting Alzheimer's disease. However, AT2 R signaling produces beneficial effect including learning and memory. ARBs inhibit AT1R signaling and this shifts the action of Ang-II toward the beneficial pathway (AT2R signaling). ACEIs, Angiotensin converting enzyme inhibitors; ARBs, Angiotensin Receptor blockers; AT1R, Angiotensin 1 Receptor; AT2R, Angiotensin 2 Receptor; Aβ, Amyloid-β; -, negative outcome or blockage; +, positive outcome.

1.7.1. Angiotensin II and Alzheimer's Disease

On their cell membrane, neurons have angiotensin receptors of every type, and they also have an independent intracellular angiotensin system.

Angiotensinogen can be produced by neurons as well as astrocytes in the brain, while astrocytes produce the majority of the substance. Angiotensinogen can be converted to active angiotensin peptides intracellularly in neurons due to the presence of RAS enzymes (such as intracellular renin, prorenin, etc). (Ladonya *et al*., 2018).

In neurons as well as other organs, the Ang II/AT1R axis generates reactive oxygen species (ROS) and causes oxidative stress via NADPH oxidase.On the other hand, the Ang II / AT2R axis and the Ang (1-7) / MasR axis both create NO. While the (Pro)renin/PRR axis and the Ang II/AT1R axis promote oxidative stress and inflammation in neurons, respectively, the Ang II/AT2R axis and the Ang (1-7)/MasR axis function as the RAS's protecting arm (Costa-Besada *et al*., 2018).

Both oxidative and antioxidative pathways start when nuclear AT1Rs are activated. More Ang II is generated inside of cells as a result of an increase in PRR, angiotensinogen, and renin mRNA levels (1–7). At the same time, protective AT2R levels are increased. With aging and cognitive problems, these compensatory systems may become faulty (Labandeira-Garcia *et al*., 2017).

There is evidence that the neurosecretory impact of AT1R is countered by the activation of AT2R and MasR. (De Kloet *et al*., 2016).

Ang II activates AT2R as opposed to AT1R signalling, promoting neuroprotective processes, NO production, neurite outgrowth, and brain development, which improves cognitive function. This is due to the fact that oxidative stress and neuroinflammation, which can impair cognition, are caused by altered AT2R signalling and are mediated by the AT1R. Ang (1-7)/MasR, which restricts the pressor, angiogenic, and proliferative effects of Ang II, is another neuroprotective axis.Anti-inflammatory and antioxidant actions are

produced by this axis.It is essential for learning, memory, neuroprotection, and cell survival. (Ho and Nation, 2018).

1.7.2. Astrocytes and Angiotensin

Oligodendrocytes, microglial cells, and astrocytes are the primary glial cells in the brain. Astrocytes are the most common type of glial cells. Maintaining brain tissue is one of astrocytes' essential roles. Managing the extracellular environment's chemical make-up, surrounding synaptic connections to stop neurotransmitter molecules from spreading there, and cleaning the synaptic cleft of many neurotransmitters. (Bear *et al*., 2016; Almad and Maragakis, 2018). These characteristics make them crucial for memory as well as long term potentiation(LTP) and support.

The majority of the brain's angiotensinogen comes from astrocytes. They have AT1R, AT2R, and MasR in particular on their mitochondria, nuclei, and cell membranes (Fogarty and Matute, 2001; Garrido-Gil *et al*., 2013; Costa-Besada *et al*., 2018).

Angiotensin II and angiotensin (1-7) function as protective factors, similar to neurons excessive AT1R activation in astrocytes causes oxidative stress, inflammation, cognitive impairment, and cell death. Despite the fact that it is generally accepted that BBB permeability is increased by an overactive Ang II/AT1R axis, astrocyte AT1Rs differ from those on neurons in that astrocyte activity of the AT1R can change BBB permeability. (Biancardi and Stern, 2016; Guo *et al*., 2019).

1.7.3. Oligodendrocytes and Angiotensin

Oligodendrocytes are axon-myelinating cells. As far as we know, they have AT1Rs and AT2Rs on their cell membrane, which are known to have opposite effects.To enhance synaptic transmission and neuronal communication, in particular, AT1Rs stimulate demyelination in oligodendrocytes while AT2Rs promote re-myelination. (Jackson *et al*., 2018).

1.7.4 Angiotensin Receptor Blockers in Alzheimer's Disease

Angiotensin receptor blockers can stop blood brain barrier (BBB) deterioration and lessen the influx of inflammatory mediators seen in many neurodegenerative diseases like AD. However, recent studies have shown that peroxisome proliferative activated receptor(PPAR)-modulation have neuroprotective characteristics that are independent of AT1 blockage (Gebre *et al*., 2018).

1.7.4.1.Telmisartan and Alzheimer's Disease

It is chemically described as [1,1_-biphenyl]-2- carboxylic acid, 4_-[(1,4_-dimethyl-2_-propyl[2,6_-bi-1*H*- benzimidazol]-1_-yl) methyl]-(CAS) (Fig.1.4). It is a white crystalline powder with a molecular weight of 514.6 and a melting point of 261 to 263°C (47). The solubility of telmisartan in aqueous solutions is strongly pH-dependent, with maximum solubility observed at high and low pH. In the range of pH 3–9 it is only poorly soluble. The telmisartan molecule is unusually stable. No Phase I-type metabolism has been observed. Among the AII antagonists, telmisartan is the most lipophilic compound with a partition coefficient log *P* = 3.2 (*n*-octanol_buffer at pH 7.4). Due to its physicochemical properties, telmisartan shows excellent oral absorption and tissue penetration.

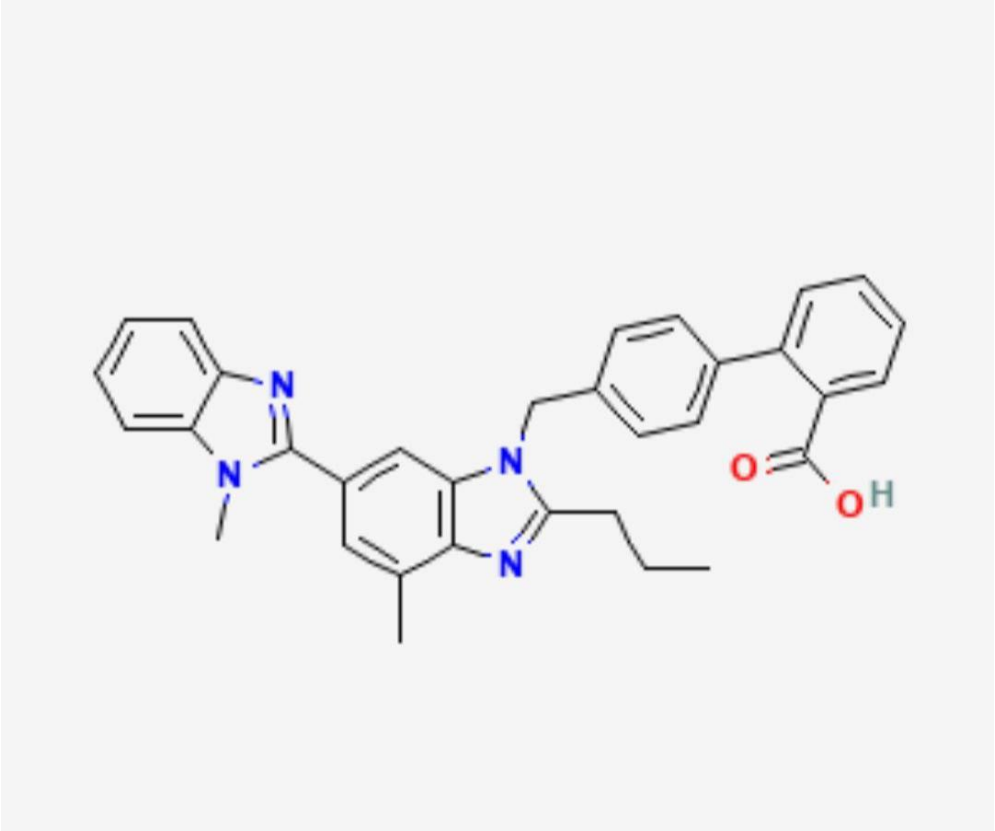


Figure1.4.Chemical Structure of Telmisartan

It was identified as a direct-acting AII receptor antagonist. On this basis, telmisartan was expected to possess therapeutic potential in the. The results presented here established that telmisartan is a potent and selective antagonist for the AT1 receptor subtype.It only comes as oral tablet.

Common side effects of telmisartan include sinus pain and congestion,back pain,diarrhea,sore throat,flu like symptoms such as fever and body aches,upset stomach,muscle pain,headache,dizziness,fatigue,nausea.

In addition to its cardiovascular effects,it also found to protects against the neurotoxicity induced by high glutamate concentrations and prevents apoptosis in neuronal cells exposed to starvation. telmisartan also lessens inflammatory processes in astrocytes, microglia, and cerebrovascular endothelial cells, which increases neuroprotection in various conditions.(Villapol *et al*.,2018), it is a promising treatment for amyloidogenesis and cognitive decline (Abo-Youssef *et al* .,2020) and has a

protective effect against cuprizone-induced demyelination and behavioral dysfunction in mice, additionally, it functions as a potential neuroprotective drug shielding individuals with vascular dementia from cognitive decline (Abd El Aziz *et al*.,2021).

1.8.*Rosemarinus officinalis L*

Figure.1.5.*Rosemarinus offisinalis* L **(González *et al*.,2007)**

The Lamiaceae family includes the plant species rosemary (*Rosmarinus officinalis* L). Rosemary is an evergreen perennial herb that blooms in lavender-blue or pink-lavender and has very aromatic leaves (Figure1.1). It can bloom early in the garden even though it typically blooms in the spring (as early as December in warmer climes), and it also has the potential to bloom at other times of the year.

Although rosemary is native to the Mediterranean region, it may thrive elsewhere with a similar climate. The leaves of rosemary are spiky, green, and

needle-like, resembling the shape of fir needles. Both the flowering tops and the leaves are gathered and distilled to create essential oils (Pérez *et al*.,2010).

Three main chemotypes of rosemary have historically been produced: Chemotype (ct).verbenone, ct.cineole, and ct.camphor.However, other chemotypes including ct. borneol, ct. bornyl acetate, ct. myrcene and ct. pinene also exist in the modern world (Brewer MS.,2011). When essential oils are collected from various plants to be used as therapeutic agents, they create essential oils with various chemical compositions, these changes may have occurred naturally in the wild or as a result of cross-pollination. Additional aspects that affect a plant's variation in chemotypes include the elevation at which it was grown, the conditions under which it was cultivated, the climate, and a variety of other environmental factors (Rašković *et al*., 2014). The aroma of rosemary essential oil varies depending on the chemotype and is often mild, camphoraceous, and slightly minty.Chemical composition of rosemary include: (Elhassan and Osman ., 2014):

1-Borneol, terpineol, linalool

2- Humulene and caryophyllene

3- Fenchyl acetate and bornyl acetate

4- Camphor, verbenone and thujone

5- 1, 8-cineole

6-Camphene, limonene, myrcene, and pinnene

7- Cuminic aldehyde

Rosemarinus officinalis L.'s essential oils and extracts can be used to identify a number of phytocompounds with pharmacological properties, with the concentration of these molecules varied in each plant specimen (**Fig.1.6**). The most often reported phytocompounds include alpha-pinene, camphor,

carnosol, eucalyptol, rosmadial, rosmanol, rosmaquinones A and B, secohinokio, and derivatives of eugenol and luteolin(Borges *et al*.,2019) They also include oleanolic acid, rosmarinic acid, ursolic acid, caffeic acid, carnosic acid.

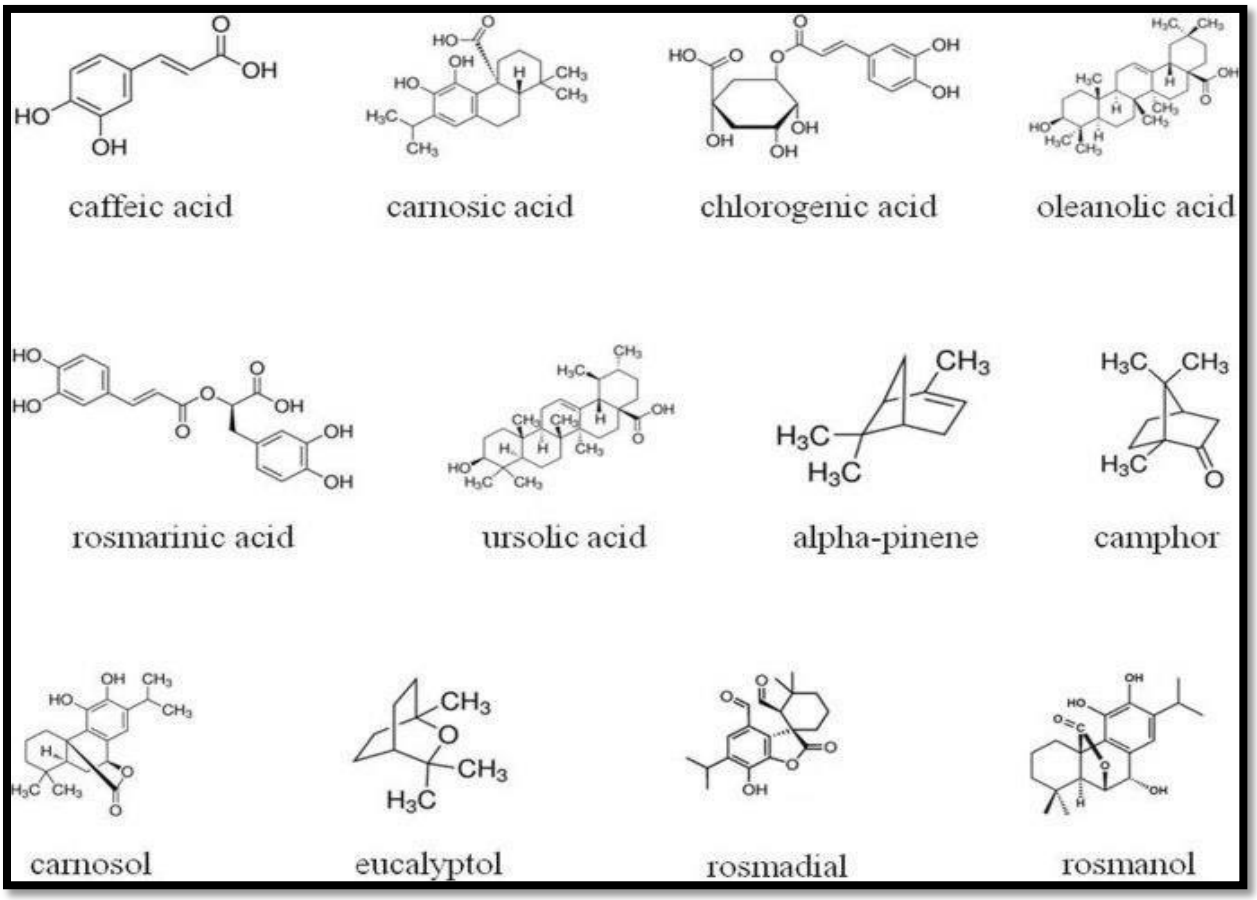


Figure.1.6.Phytocompounds in rosemary (Borges *et al*.,2019)

1.8.1. Clinical Studies with Rosemary Essential Oil, Memory in General

Positive outcomes have been seen in numerous clinical investigations including the use of rosemary essential oil for memory loss and, in particular, Alzheimer's disease. Sadly, a large number of these investigations omit to specify which specific chemotype of rosemary essential oil was utilized (Diego *et al.*,1998).

1.8.2. Neuroprotective Mechanisims of Rosemary

1.8.2.1. Anti-Inflammatory Mechanisms

In AD, carnosic acid (CA)-mediated reduction of nucleotide-binding domain, leucine-rich-containig family,pyrin domain-containing-3(NLRP3) that may have a considerable therapeutic advantage in regulating macrophage and microglial inflammatory responses (Figure 1.4). In keeping with this, research has shown that CA or rosemary extract can reduce the production of the inflammatory cytokine tumor necrosis factor (TNFα) in macrophages and microglia (Chae *et al.*,2012) Figure 1.4. that shows how inflammation in neurodegenerative diseases is mediated by microglia. Misfolded proteins from neurons or other cell types cause microglial inflammation in neurodegenerative illnesses such AD (Wang *et al.*,2020).

Carnosic acid reduces the amount of reactive nitrogen species (RNS) and nitric oxide (NO) that lipopolysaccharide (LPS) causes in these macrophage cells.Additionally, CA can block the nuclear factor-B (NF-B) pathway and increase heme oxygenase-1(HO-1) expression to block the TNFα signaling pathway (Foresti *et al.*,2013).

lipopolysaccharide cause increasing in proinflammatory cytokine levels, such as TNFα and interleukin (IL)-6, in vivo it can be counteracted by CA, this action is brought on by nuclear factor erythroid2-related factor2 (Nrf2) activation (Hadad and Levy *et al.*,2017).

In these *in vivo* trials, CA was gavaged (100 mg/kg once daily for three days) in a 0.5% solution suspension. Increased expression of antioxidant genes that are known to be transcriptionally controlled by the Nrf2 pathway and Nrf2 nuclear translocation were used to establish that Nrf2 was activated (Guo *et al*.,2016).

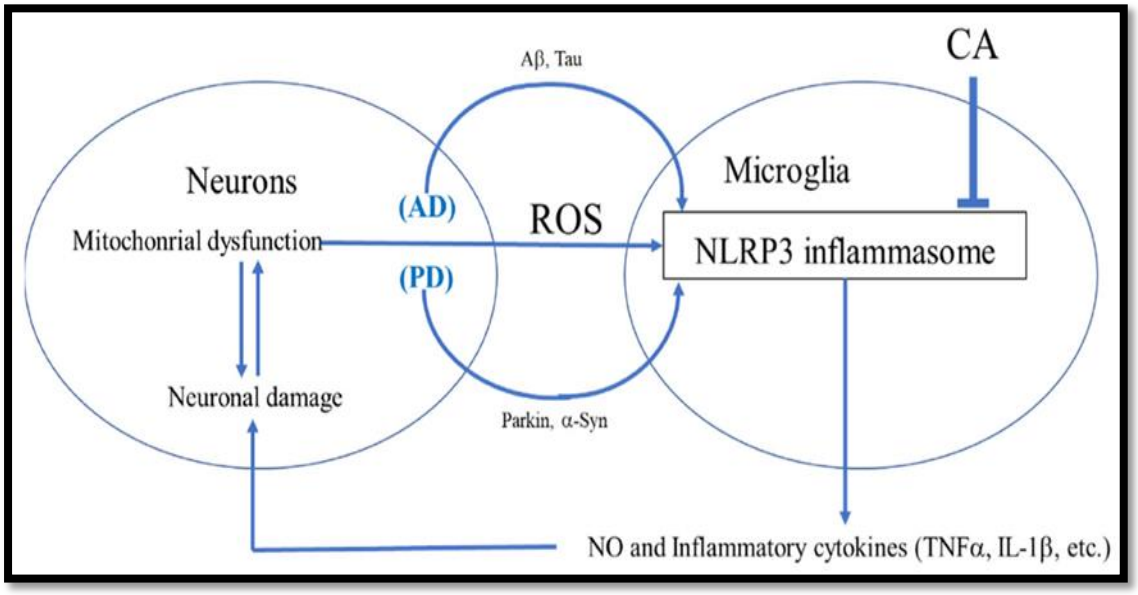


Figure 1.7.NLRP3 and neuroinflammation (Wang *et al*.,2020)

ROS:reactive oxygen species,NLRP3:nucleotide-binding domain, leucine-rich-containig family,pyrin domain-containing-3,AD:Alzheimer's disease,PD:Parkinson disease,CA:carnosic acid,NO:nitric oxide,TNF:tumor necrosis factor,IL:interleukin 1 beta.

Inflammatory cytokines and NO are released by activated microglia in response to these stimuli. Through suppression of the NLRP3 inflammasome, CA may prevent the inflammatory loop between neurons and microglia (Trudler *et al*.,2021).

The majority of the brain's resident innate immune inflammatory cells are called microglial cells, and when activated, they can release proinflammatory cytokines like IL-1, IL-6, and TNFα.CA was discovered to block mouse microglia's LPS-induced activation, hence reducing the release of

inflammatory cytokines including IL-1β and IL-6 in an in vitro model of brain infection. Inducible NO synthase-related NO generation has been observed to be reduced by CA(Li *et al*.,2018) .Misfolded proteins like -synuclein (-Syn), especially when combined with amyloid-β(Aβ) or potentially Tau, and the production of inflammatory cytokines from microglia are crucial for microglial-astrocyte-neuron communication in neurodegenerative illnesses, according to a number of studies .Additionally, it has been claimed that human AD brains exhibit an upregulation of microglial-mediated inflammation.The therapeutic potential of CA for a number of neurodegenerative illnesses, including AD, has been proposed based on several inflammatory models studies. (Ho MS., 2019).

1.8.2.2.Antioxidant Actions of Rosemary and CA

1.8.2.2.1. Direct Antioxidant Action

Oxidation processes and the defense of cells against oxidative cell death have been used to demonstrate the antioxidant effects of CA . For instance, it is believed that oxidative damage via ROS causes the catechol form of CA to change into an ortho-quinone ,Through this method, CA can defend neuronal cells from oxidative stress, which can be brought on by lipid hydroperoxides and hydrogen peroxide,As a result, catechol conversion to an ortho-quinone is responsible for part of the antioxidant benefits of polyphenolic natural compounds (Yu *et al*.,2016).

1.8.2.2.2. Indirect Antioxidant Action

In addition to these direct antioxidant mechanisms, CA also has considerable indirect antioxidant actions, which are primarily transcriptional in nature. The activation of phase 2 antioxidant enzymes is a crucial aspect of the cell's defence against the oxidative stress brought on by these chemicals. (Yu *et al*.,2016).The NRF2 transcription factor attaches to the antioxidant response element (ARE) on the enhancer/promoter region of DNA to activate the genes encoding phase 2 antioxidant and anti-inflammatory enzymes.This includes

glutathione (GSH)-regulating enzymes like GSH-S-transferase (GST) and -γ-glutamylcysteine synthetase (-GC synthase) .CA protects neurons by activating Keap1/Nrf2 pathway,its activation initiated by S-alkylation of the cysteine thiol of the Keap1 protein by the electrophilic quinone-type of carnosic acid (Yu *et al*., 2016).

Due to these direct and indirect antioxidant actions, the potential of rosemary extract or CA for the treatment of AD has been taken into consideration based on encouraging results in a number of model systems. (Maynard *et al*., 2019) . Numerous studies have shown that proteins including Aβ, Tau,TDP-43, Parkin, and -Syn, which are linked to several neurodegenerative illnesses, enhance ROS/RNS generation through mitochondrial malfunction and lead to synaptic damage and neuronal cell death (Esteraz and Abramov ., 2020). The production of ROS and RNS, as well as the ensuing synaptic damage and neuronal death, are also influenced by excessive activation of N-methyl-D-aspartate (NMDA)-type glutamate receptors on the surface of pyramidal neurons in the cortex and hippocampus (Guo *et al*.,2016).

There is substantial evidence to suggest that the generation of oxidative and nitrosative stress contributes to the aetiology of AD by harming synapses and neurons.(Lipton *et al*.,2016). When the KEAP1/NRF2 pathway is active, transcription levels of phase 2 antioxidant and anti-inflammatory genes are increased. This presents a promising therapeutic approach for the management of several neurodegenerative illnesses. After exposure to Aβ oligomers, both in vitro and in vivo models have been used to examine the protective effects of CA on primary neurons. Improvement in histology and neurobehavioral aspects was seen. According to the histological findings, CA (10 mg/kg, given twice weekly for three months through transnasal route to the brain) boosted synaptic and dendritic markers and decreased the concentration of amyloid plaques, astrogliosis, and phospho-tau staining in the hippocampus (Lipton *et al*., 2016).

1.9.Donepezil in Alzheimer's Disease

In this study,donepezil has been used to be compared with telmisartan and rosemary regarding to its cognitive effects, it is an FDA-approved medication used for the treatment of dementia in mild, moderate, and severe Alzheimer disease (Hussein *et al* .,2018). Although there is currently no evidence to suggest that donepezil can alter the progression of the disease, it can improve cognition and behavior, thereby alleviating certain symptoms. In 2014, the FDA approved a fixed-dose combination of donepezil and memantine for moderate-to-severe dementia associated with Alzheimer disease (Morant *et al* .,2020).

1.9.1.Mechanism of Action

Donepezil hydrochloride, a piperidine derivative, is a centrally acting, rapid, reversible acetylcholinesterase inhibitor. Acetylcholinesterase is an enzyme that breaks down acetylcholine after its release from the presynapse. Donepezil binds reversibly to acetylcholinesterase, preventing acetylcholine hydrolysis, thereby increasing the availability of acetylcholine at the synapses and enhancing cholinergic transmission. In vitro data suggest that the anticholinesterase activity of donepezil is relatively specific for acetylcholinesterase in the brain. Donepezil is structurally unrelated to other anticholinesterase agents such as tacrine and physostigmine .In addition to its cholinergic effects, some noncholinergic mechanisms have been proposed for donepezil. Notably, the drug upregulates nicotinic receptors in cortical neurons, which may contribute to its neuroprotective properties. The drug also exhibits reversible inhibition of voltage-activated sodium currents and delays in rectifier potassium currents and fast transient potassium currents. However, these actions are less likely to contribute significantly to its clinical effects. Recent findings suggest that neuroinflammation plays a vital role in the pathophysiology of neurodegenerative diseases. Notably, donepezil has been

found to have a significant impact on downregulating neuroinflammatory responses, including microglial and astrocytic activation, which are associated with Alzheimer disease (Kim *et al* .,2021).

1.10.Alumnium Chloride and Neurodegeneration

Aluminum chloride (AlCl3) was extensively utilized for the stimulation of dementia in numerous animal models. Aluminum is a well-recognized neurotoxin and concerned with the pathological progression of numerous neurologic. Aluminum can act as a cross-linkers of amyloid β-protein and results in oligomerization, thus stimulating neurotoxicity. Furthermore, it was already reported that continuous exposure to AlCl3 could cause dementia in Wistar rats (Khan *et al* .,2013).

Chapter Two

Materials

And

Methods

2.Materials and Methods

2.1.Animals

Thirty wistar Albino male rats,weighing (210-290) grams were employed in this study (purchased from Alkhadhraa farm,Alhilla,Iraq).All the possible efforts were taken to prevent any harm to the animals.The rats were kept at suitable conditions including the temperature25℃,humidity about 60-65 % and the invironment was kept on 12 hour light/dark cycle,the animals were fed on water and rat's food.The rats were allowed to be adapted to those new conditions for about two weeks before we started the study. This study started from 14/11/2022 to 13/1/2023 and was examined at the animal house in the College of Medicine/University of Babylon.

2. 2.Instruments and Equipments

Table 2.1 Instruments and Equipments

No.	Instrument / Equipment	Company/ country
1.	Deep freeze	GFL/Germany
2.	Disposable 1cc syringe	Shanachuan/China
3.	Disposable test tube (10 mL)	Meheco, China
4.	Distilled water	Pioneer/Iraq
5.	Electronic scale	SDT/ China
6.	ELISA(reader, washer, printer)	Biotek/USA
7.	Eppendorf plastic tubes	Gondong/ China
8.	Gloves	Top glove/ Malaysia
9.	Homogenizer drill	Jiao Jie/ China
10.	Incubator	Memmert/ Germany
11.	Micropipettes (different volumes)	Eppendorf/ Germany
12.	Normal saline	Pionner/Iraq
13.	Refrigerator	Concord/ lebanon
14.	Sensitive balance	Sartorius/ Germany
15.	Spectrophotometer	BioTek/USA
16.	Surgical set	China
17.	Tube rack	China
18.	Vortex	CYAN/ Belgium

2.3.Kits

Table 2.2. Kits used in this research, along with their countries of origin manufacturers

No.	Kit	Company	Country
1	Rat IL-1(Interleukin 1) ELISA Kit	Elabscience biotechnology	United states
2	Rat IL-6(Interleukin 6) ELISA Kit	Elabscience biotechnology	United states
3	Rat TNF-α (Tumor Necrosis Factor Alpha) ELISA Kit	Elabscience biotechnology	United states
4	GSH(Glutathione) ELISA Kit	Elabscience biotechnology	United states
5	Total Antioxidant Capacity (T-AOC) Colorimetric Assay Kit (ABTS, Enzyme Method)	Elabscience biotechnology	United states
6	Lipid Peroxidation kit	Bilişim Destek Hizmetleri	Turkey

2.4.Chemicals and Drugs

Table 2.3. Chemical materials with their remarks

No.	Chemicals	Company	Country
1	Alumnium chloride	THOMAS BAKER L.T.D	India
4	Donepezil(5 mg tablet)	Pfizer	USA
5	Formaldehyde	AL-Jubail	KSA
4	Phosphate buffer	HiMedia	India
2	Rosemary liquid extract (333mg/ml)	HAWAII PHARM LLC	USA
3	Telmisartan(40 mg tablet)	Boehringer Ingelheim Pharmaceuticals	USA

2.5.Ethical Approval

The research conducted in the College of Medicine, University of Babylon, Iraq, received approval from the publication ethics committee according to the document number 4-3, at 06/07/2022 to get this approval

2.6.Procedures

2.6.1. Induction of Alzheimer's Disease

Alzheimer's was induced by using alumnium chloride intraperitoneal injection of dissolved AlCl3 powder in normal saline,in a dose of 10 mg/kg intraperitoneal injection daily for 60 days (Khalifa *et al*.,2020).300mg of AlCl3 power was taken and has been dissolved in 15 ml of D.W,then 1 ml of this stock solution was taken and added to 3 ml D.W,then 0.5 ml of this solution was taken to be given intraperitoneally for each rat.

2.6.2. Experimental Design and Treatment Protocol

Rats were divided in six groups (five rats in each group).

***Group I*:** (normal control group), rats were administered normal saline intraperitoneally (i.p.) once daily for 60 days.

***Group II*:** (Alzheimer's induced group), rats were received AlCl3 (10 mg/kg, i.p.) once daily for 60 days (Khalifa *et al*., 2020).

Group III: (telmisartan group), rats were were undergoing same procedure as group II plus they were received telmisartan (10 mg/kg) orally for 60 days via gastric gavage at the same time (Khalifa *et al*., 2020).

***Group IV*: (**rosemary group), rats were were undergoing same procedure as group II plus they were received rosemary extract (300 mg/kg) ,3 ml of rosemary liquid extract was added to 12 ml D.W and 1 ml of this solution was given orally via gastric gavage for 60 days at the same time (Ozarowski *et al*.,2013).

***Group V* :** (Combination group), rats were undergoing same procedure as group II plus they were received telmisartan (10 mg/kg) orally along with

rosemary extract (300 mg/kg) (Khalifa *et al*., 2020; Ozarowski *et al*.,2013) orally via gastric gavage for 60 days at the same time.

***Group VI*:** (donepezil group), rats were undergoing same procedure as group II plus they were received donepezil (5 mg/kg) orally by gastric gavage for 60 days at the same time (Moosavirad *et al*., 2019).

2.6.3. Dilution Method of Drugs

1 -AlCl3+telmisartan group: We took 40 mg tablet of telmisartan and ground it then we added 16 ml of D.W .1 ml of the solution has been taken and given to the rats orally once daily for 60 days at the same time with AlCl3 injection (Khalifa *et al*.,2020)

2- AlCl3+rosemary group :3 ml of rosemary liquid extract was taken, diluted with 12 ml D.W and 1 ml has been given to the rat orally by using oral gavage syringe once daily for 60 days at the same time with AlCl3 injection (Ozarowski *et al*.,2013)

3-The combination group (telmisartan+rosemary extract):By using the same previous steps of the two agents dilution methods,both were taken orally by oral gastric gavage at the same time with AlCl3 injection for 60 days (Khalifa *et al*.,2020; Ozarowski *et al*.,2013).

4- AlCl3+Donepezil: group we took 5 mg tablet of donepezil,ground it completely and add 4 ml of D.W then 1 ml of this solution has been taken and administered to the rat orally once daily for 60 days,with the previously explained dose of AlCl3 at the same time(Moosavirad *et al*.,2019)

2.6.4. Behavioural Tests

2.6.4.1. Y maze Spontaneous Alteration Test

The Y maze was formed by using the wood by the researcher, three similar arms at the shape of letter Y, separated from each other by angle of 120 degree,(length 45 cm ,height 35 cm ,width 12 cm) and names A,B and C. After 24 hours from the last dose, behavioural test was performed, rats were put in the center of the maze and were allowed to discover the arms freely for about 8 minutes. each trial was recorded . The arms were cleansed with alcohol after each rat's entry to eliminate any lingering smells. A full arm entry was recorded when the hind paws of the rat completely entered the arm of the maze. The sequence of consecutive entries in the three arms followed a pattern such as ABC, CAB, BAC, and so on, which we referred to as alterations. It explained in figures (2.1,2.2, and 2.3).

To calculate the spontaneous alteration percentage (SAP), the following equation was employed (Office of Research.,2021):

Spontaneous alteration percentage (SAP) %=[(number of alterations) / (total arm entries -2)] x100.

Number of alterations indicated for the entry into different arms in three different sequences, and the total entries mean the total trials of the rats into every arm in the maze.

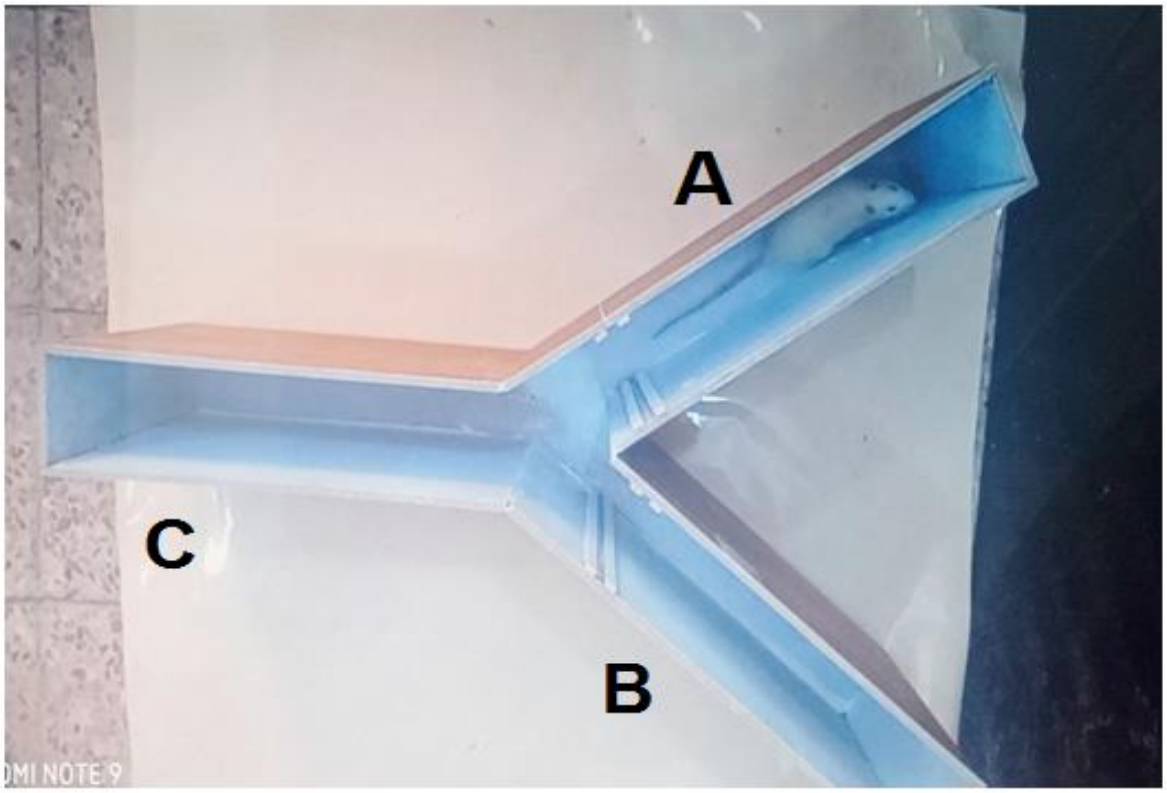


Figure.2.1.Y maze alteration A

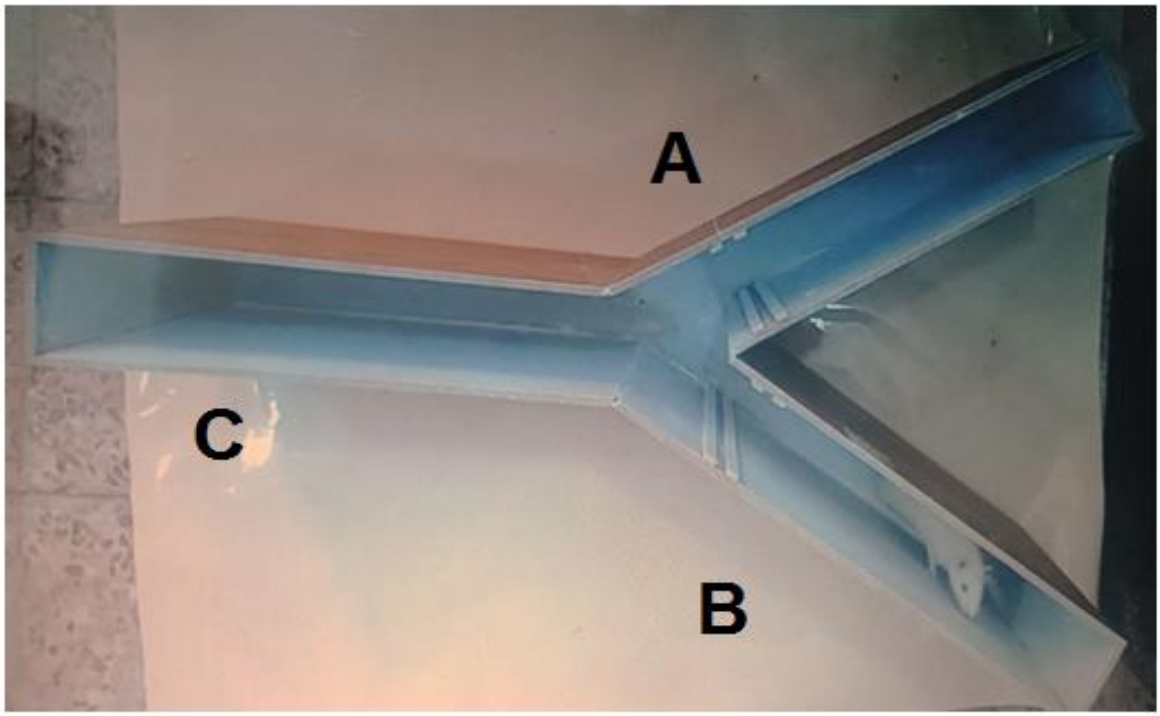


Figure.2.2.Y maze alteration B

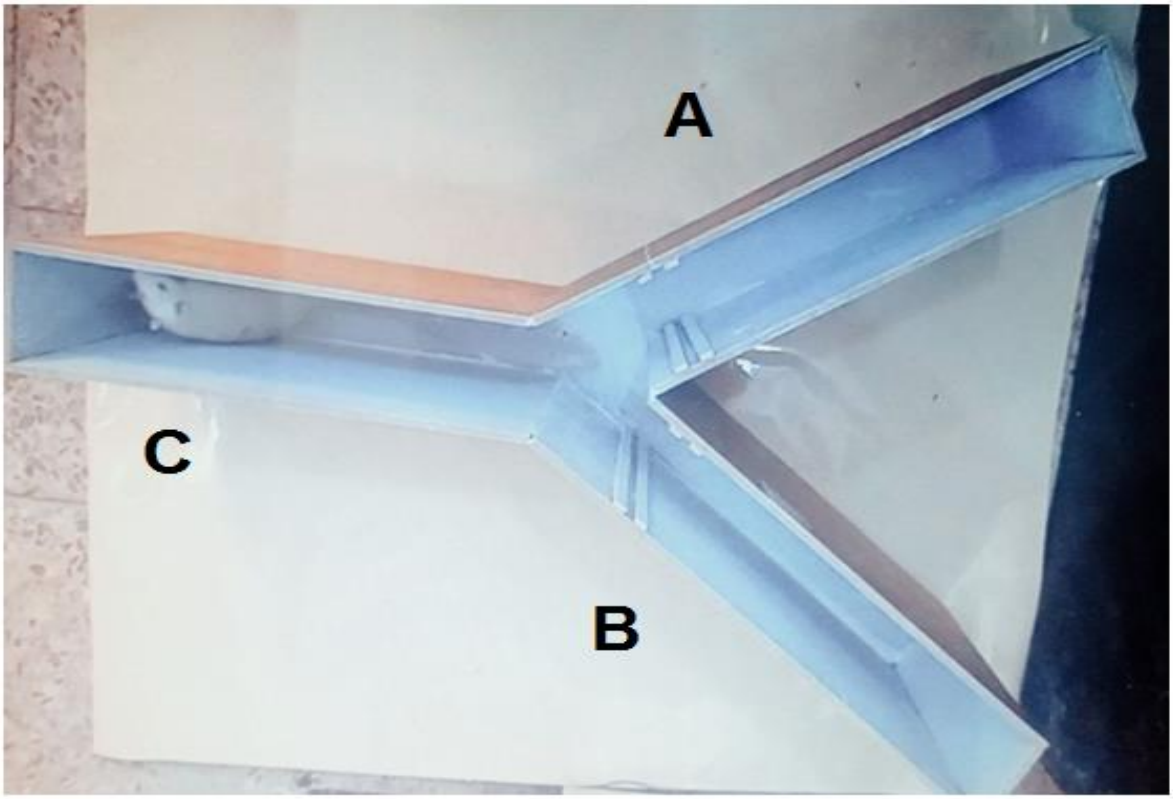


Figure.2.3.Y maze alteration C

2.6.5. Tissue Sampling

After 24 hours from the last dose and completion of the behavioral test, the animals were decapitated. Following the dissection of their skulls, the brains were carefully removed, and the cerebellum and olfactory bulbs were excised. Subsequently, the brain was gently extracted from the rat skull, we divided the brain into two halves (Figure 2.5 and 2.6).Hippocampi from each half of rat's brain from each group were dissected. The hippocampi were then rinsed with phosphate buffer solution (pH 7.4), weighed, and placed in sterilized Eppendorf tubes. These samples were subsequently frozen at -20°C. Moreover, the hippocampi were homogenized in phosphate buffer with the same pH as previously mentioned for further biochemical analysis. Additionally, the remaining half of rat's brains from each group were fixed in 10% formalin before being sent for histopathological evaluation (Zaky *et al*., 2013).

2.6.5.1. Preparation of Phosphate Buffer Saline (PBS) Solution

According to the instructions provided by the manufacturer, which involve dissolving 10.79 grams in 1000 milliliters of purified or distilled water. The solution is then sterilized by autoclaving at 115℃ for a duration of 10 minutes. It is important to store the solution in a cool environment, maintaining a temperature range of 15-30℃ and keeping it away from direct sunlight.

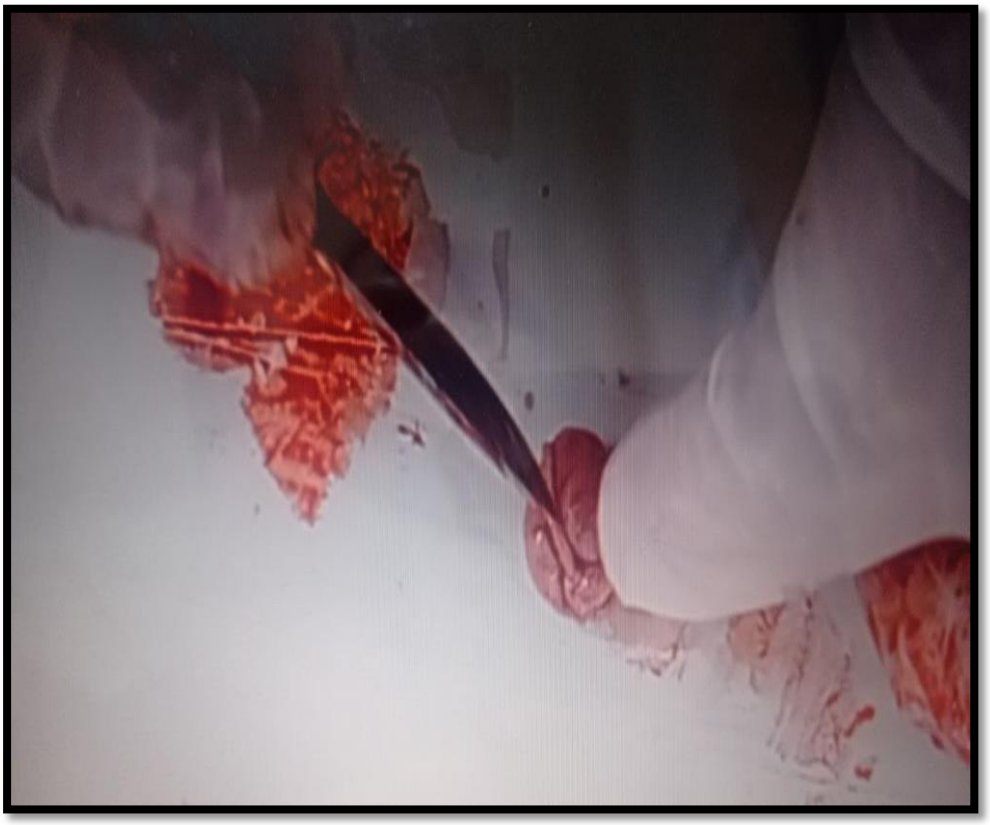


Figure.2.4.Taking out the rat's brain

2.6.5.2. Histopathological Examination

One half of each rat's brain had been fixed in formalin (10%) for about 24 hours, followed by tissue processing by using automated tissue processor for 20 hrs.Then dehydration process with ethanol alcohol was done in different concentrations after that clearing in xylene, then impregnation in wax, embedding, and formation of paraffin blocks.After that, they have been cut down to 5 μm with the help of a microtome and stained with Hematoxylin and Eosin (H&E) stains (khalifa *et al*.,2020)

2.6.6. Tissue Homogenization

To prepare the samples for biochemical analysis after washing out any remaining blood on the samples by using phosphate buffer solution, there are several steps in this procedure including the following: (Kulakowska *et al* .,2007)

1.A razor blade was used, and a single cut was made on a piece of tissue.

2. The weight of the tissue piece (0.2 gm) was measured and then returned it to ice to prevent warming.

3. While working on ice, scissors or a single-edge razor blade were used to chop the tissue sample into small pieces. It is crucial to keep the tissue cold.

4. 3ml of phosphate buffer (pH 7.4) was added to the sample.

5. On ice, the sample was homogenized by grinding it with a pestle for several minutes until no visible chunks remain.

6. The samples were transfered into Eppendorf tubes in small portions and centrifuged them in the cold room at 14,000 x g for 10 minutes.We carefully collected the supernatant.

2.7. Biochemical Assessments by Using ELISA kits

2.7.1. Interleukin 1 (IL-1)

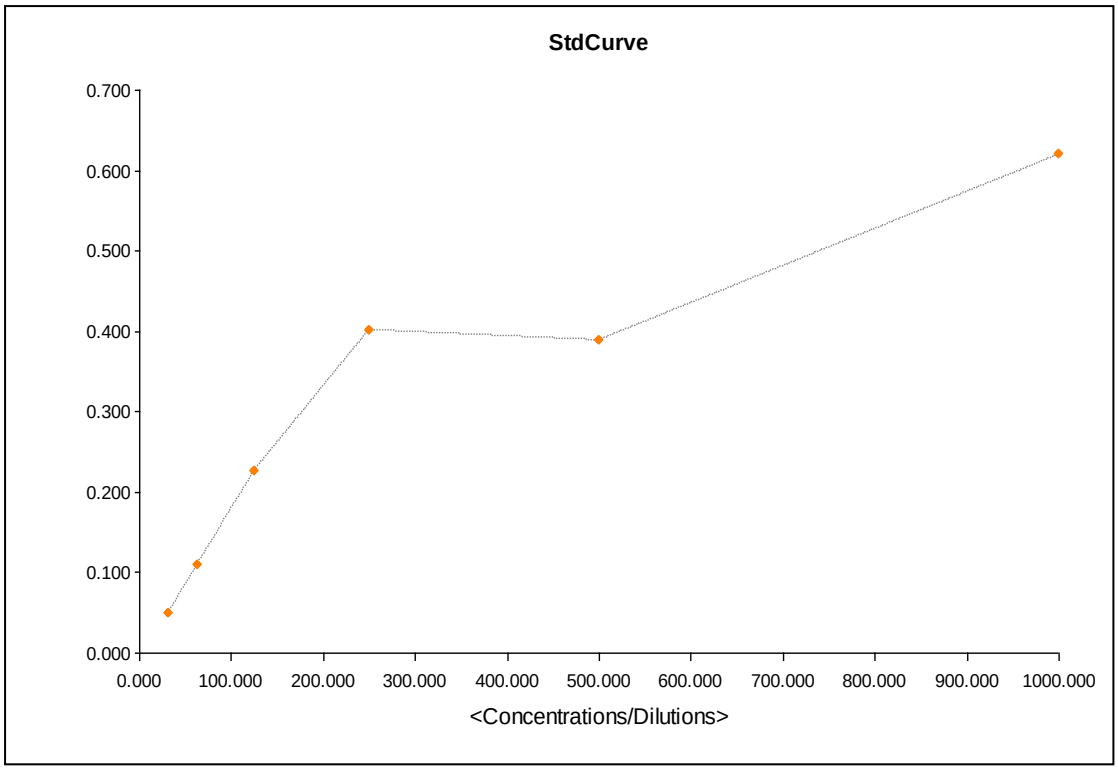
2.7.2.Interleukin 6(IL6)

2.7.3.Tumor Necrosis Factor Alpha (TNF-α)

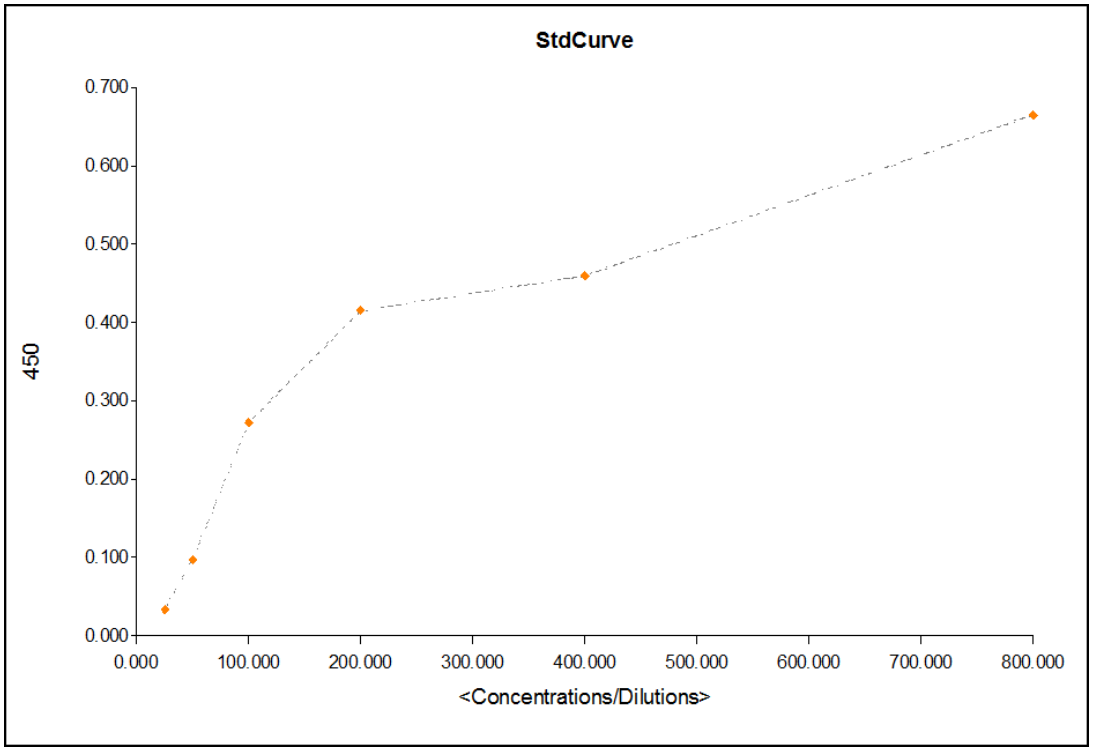
Tests principles

Theses ELISA kits use the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to (Rat IL-1a),(IL6), (TNF-α). Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Rat IL-la,IL6, TNF-α and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Rat IL-1a, IL6, TNF-α ,biotinylated detection antibody and Avidin - HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow.

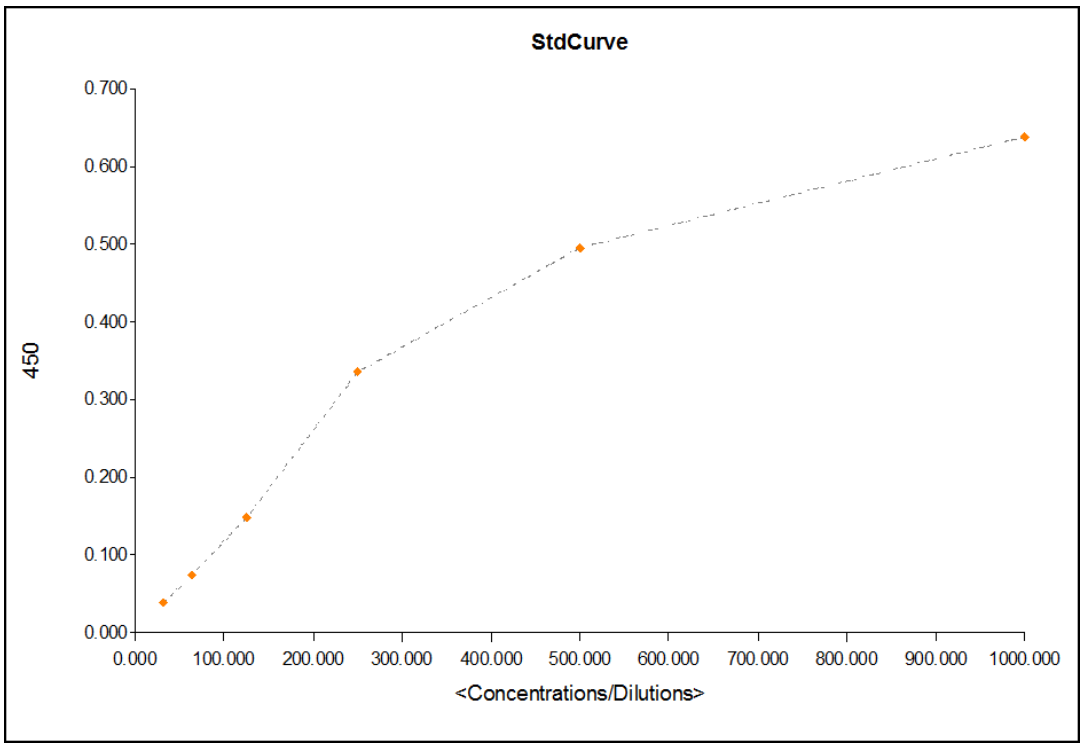
The optical density (OD) is measured spectrophotometrically at a wavelength of 450 ± 2 nm. The OD value is proportional to the concentration of Rat IL-1a, IL6, TNF-α. We can calculate the concentration of Rat IL-1a, IL6, TNF-α in the samples by comparing the OD of the samples to the standard curve.



Standard Curve for IL1



Standard Curve for IL6



Standard Curve for (TNF-α)

Reagent preparation

1- All reagents brought to room temperature (18-25°C) before use.

2- Wash Buffer: 30 mL of Concentrated Wash Buffer was diluted with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer.

3-Standard working solution: The standard was centrifuged at 10,000xg for 1 min. We added I mL of Reference Standard & Sample Diluent, let it stand for 10 min and invert it gently several times. After it dissolves fully, we mixed it thoroughly with a pipette. This reconstitution produces a working solution of 1000 pg/mL.Then we made serial dilutions as needed. The recommended dilution gradient is as follows: 1000, 500, 250, 125, 62.5. 31.25 15.63, 0 pg/mL.

Dilution method: 7 EP tubes have been taken, we added 500 µL of Reference Standard & Sample Diluent to each tube. Pipetted 500 µL of the 1000 pg/mL working solution to the first tube and mix up to produce a 500 pg/mL working solution. Pipetted 500 µL of the solution from the former

tube into the latter one according to this step. Note: the last tube is regarded as a blank. We did not pipette solution into it from the former tube.

4-Biotinylated Detection Ab working solution:We calculated the required amount before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. Centrifuged the Concentrated Biotinylated Detection Ab at 800xg for 1 min. Then we diluted the 100x Concentrated Biotinylated Detection Ab to 1x working solution with Biotinylated Detection Ab Diluent(Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent= 1:99).

5-HRP Conjugate working solution: The required amount was calculated before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. We Centrifuged the Concentrated HRP Conjugate at 800xg for 1 min, then diluted the 100x Concentrated HRP Conjugate to 1 working solution with HRP Conjugate Diluent (Concentrated HRP Conjugate: HRP Conjugate Diluent= 1:99).

Assay procedure:

1. wells for diluted standard, blank and sample were determined. 100 µL was added from each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate). The plate was covered with the sealer provided in the kit. Incubated for 90 min at 37°C. Note: We added solutions to the bottom of the micro ELISA plate well, and avoided touching the inside wall and causing foaming as much as possible.
2. The liquid from each well was decanted.Immediately added 100 µL of Biotinylated Detection Ab working solution to each well. The plate was covered with a new sealer and incubated for 1 hour at 37°C.

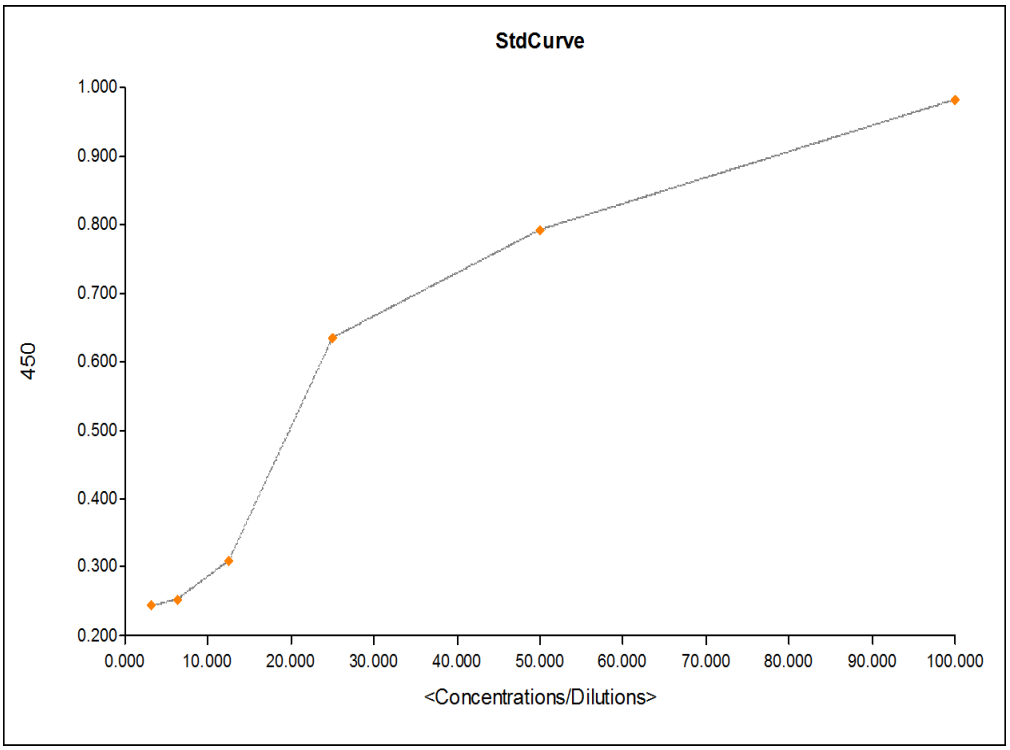
3. The solution from each well was decanted, 350 µL of wash buffer was added to each well. Soaked for 1 min and decanted the solution from each well and pat it dry against clean absorbent paper. This wash step was repeated 3 times.
4. 100 µL of HRP Conjugate working solution was added to each well. Covered the plate with a new sealer. Incubated for 30 min at 37°C.
5. The solution from each well was decanted, repeated the wash process for 5 times as conducted in step 3.
6. 90 µL of Substrate Reagent was added to each well. Covered the plate with a new sealer. Incubated for about 15 min at 37°C.The plate was protected from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.We Preheated the Microplate Reader for about 15 min before OD measurement.
7. 50 µL of Stop Solution was added to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
8. The optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 mm

2.7.4. Glutathione (GSH)

Test principle

This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with GSH. During the reaction, GSH in samples or Standard competes with a fixed amount of GSH on the solid phase supporter for sites on the Biotinylated Detection Ab specific to GSH. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) are added to each microplate well and incubated. Then a 3,3,5,5-Tetrsmethyl benzidine(TMB) substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color change is

measured spectrophotometrically at a wavelength of 450 2 nm. The concentration of GSH in the samples is then determined by comparing the OD of the samples to the standard curve.



Reagent preparation

1. All reagents brought to room temperature (18-25°C) before use.
2. Wash Buffer: 30 ml of Concentrated Wash Buffer was diluted with 720 ml of deionized or distilled water to prepare 750 ml of Wash Buffer.
3. Standard working solution: The standard was centrifuged at 10,000 x g for 1 min. ImL. of Reference Standard &Sample Diluent were added, we let it stand for 10 min and inverted it gently several times. After it dissolves fully,we mixed it thoroughly with a pipette. This reconstitution produces a working solution of 100 µg/mL. (or add 1 mL of Reference Standard & Sample Diluent), let it stand for 1-2 min and then mixed it thoroughly with a vortex meter of low speed. Then we made serial dilutions as needed. The recommended dilution gradient is as follows: 100, 50, 25. 12.5. 6.25. 3.13. 1.56. 0 µg/mL.

Dilution method: 7 EP tubes have been taken,we added 500 µL of Reference Standard & Sample Diluent to each tube. Pipetted 500 µL of the 100 µg/mL working solution to the first tube and mixed up to produce a 50 ug/mL working solution. We Pipetted 500 µL of the solution from the former tube into the latter one according to this step. Note: the last tube is regarded as a blank. We didn not pipette solution into it from the former tube.

4. Biotinylated Detection Ab working solution: The required amount was calculated before the experiment (50 µL/well). In preparation, slightly more than calculated should be prepared. The Concentrated Biotinylated Detection Ab was centrifuged at 800 x g for 1 min. Then we diluted the 100 Concentrated Biotinylated Detection Ab to 1 working solution with Biotinylated Detection Ab Diluent (Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent= 1: 99).
5. HRP Conjugate working solution: The required amount was calculated before the experiment (100 µL/well). In preparation, slightly more than calculated should be prepared. The Concentrated HRP Conjugate was centrifuged at 800xg for 1 min, then we diluted the 100x Concentrated HRP Conjugate to 1x working solution with HRP Conjugate Diluent(Concentrated HRP Conjugate: HRP Conjugate Diluent= 1:99).

Assay procedure

1. Wells for diluted standard, blank and sample were determined.We added 50 µL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate). Immediately 50 µL of Biotinylated Detection Ab working solution was added to each well.We covered the plate with the sealer provided in the kit. Incubated for 45 min at 37°C. Note: solutions should be added to the bottom of the micro ELISA plate well, we avoided touching the inside wall and causing foaming as much as possible.

2. The solution was decanted from each well, 350 µL of wash buffer was added to each well. We soaked for 1 min and decanted the solution from each well and pat it dry against clean absorbent paper. We repeated this wash step 3 times.We Made the tested strips in use immediately after the wash step. Wells were not allowed to be dried .
3. 100 µL of HRP Conjugate working solution was added to each well. We covered the plate with a new sealer. Incubated for 30 min at 37°C.
4. The solution was decanted from each well, the wash process was repeated for 5 times as conducted in step 2.
5. 90 µL of Substrate Reagent was added to each well.We covered the plate with a new sealer. Incubated for about 15 min at 37°C. The plate was protected from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min. We Preheated the Microplate Reader for about 15 min before OD measurement.
6. 50 µL of Stop Solution was added to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
7. The optical density (OD value) of each well was determined at once with a micro-plate reader set to 450 nm.

2.7.5. Malondialdehyde (MDA)

Malondialdehyhe was estimated by thiobarbituric acid (TBA) assay method of Buege & Aust, 1978 on spectrophotometer.

Principle

This method quantifies lipid peroxides by measuring aldehyde breakdown products of lipid peroxidation. Basic principle of the method is the reaction of one molecule of malondialdehyde and two molecules of thiobarbituric acid to form a red MDA-TBA complex which can be measure at 535 nm.

Stock TCA – TBA – HCl Reagent

It was prepared by dissolving 15gm trichloroacetic acid and 0.375gm thiobarbituric acid and 0.25N HCl (2.1 ml of concentrated HCl in 100 ml). This solution was mildly heated to assist in the dissolution of TBA and volume was made up to 100 ml with 0.25 N HCl.

Procedure

To 0.4 ml of sample, 0.6 ml TCA-TBA-HCl reagents were added. It was mixed well and kept in boiling water bath for 10 minutes. After cooling 1.0 ml freshly prepared 1N NaOH solution was added. The absorbance of pink colour was measured in spectrophotometer at 535 nm against blank which contained distilled water in place of sample. In blank 0.4 ml distilled water and 0.6 ml TCA-TBA-HCl reagent was mixed and boiled.

MDA μ mol /L =Absorbance (532)/ L*E0 ×D

Where:

L: Lightpath (1 cm)

E0: Extinction coefficient = 1.56*105 M−1.Cm−1

D: Dilution factor (1 ml volume). Used in ref./0.15=6.7.

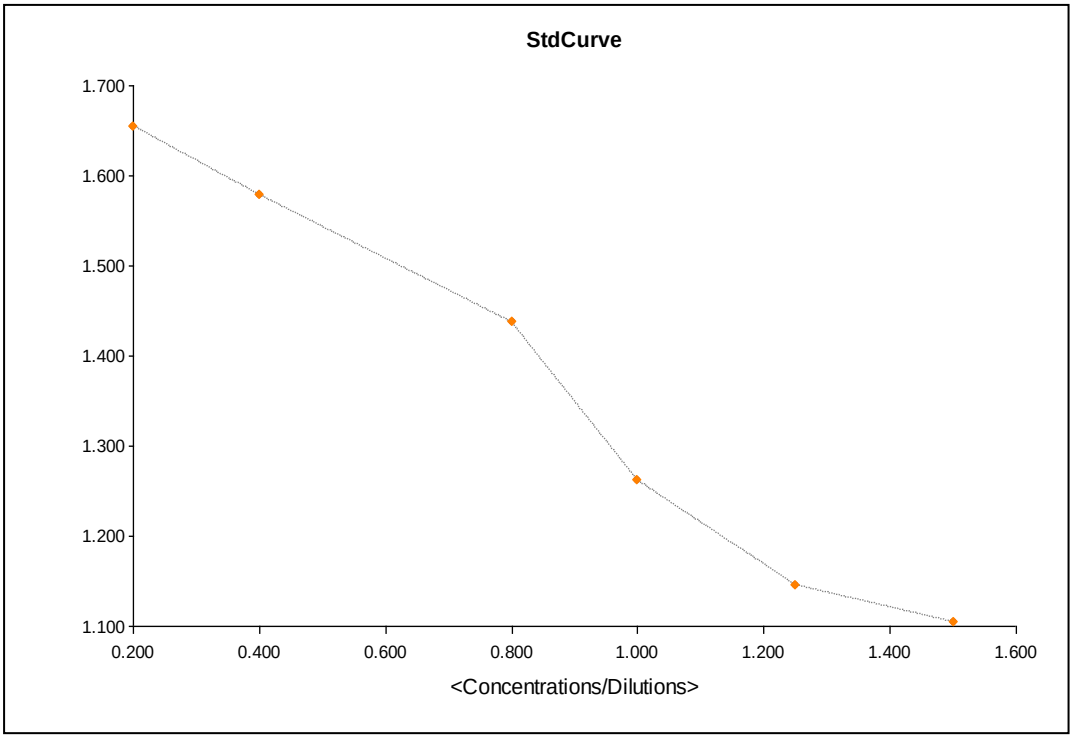
(Buege and Aust.,1978).

2.7.6. Total Antioxidant Capacity (TAOC) Colorimetric Assay

Principle

The principle of the 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method for determining the T-AOC is as follows. ABTS is oxidized to green ABTS by appropriate oxidant, which can be inhibited if there exist antioxidants. The T-AOC of the sample can be determined and calculated by measuring the absorbance of ABTS at 414 nm or 734 nm. Trolox is an analog of vitamin E and has a similar antioxidant capacity to that of vitamin E. Trolox is used as a reference for other antioxidant antioxidants. For example, the T-AOC of Trolox is 1, then the antioxidant capacity of the other substance with

the same concentration is showed by the ratio of its antioxidant capacity to Trolox antioxidant capacity.



Reagent preparation

1. Preparation of reagent 3 application solution: reagent 3 was diluted with double distilled water at a ratio of 1:39. The fresh solution was prepared before use.
2. Preparation of ABTS working solution: The needed amount of ABTS working solution was prepared according to the ratio (reagent 1: reagent 2: reagent 3 application solution = 152:10:8).The prepared solution was stored at room temperature with shading light and run out in 30 min.
3. Preparation of reagent 4 application solution: reagent 4 was diluted with reagent 1 at ratio of 1:9 before use. The fresh solution was prepared before use.

The measurement of samples

1. Standard well: 10 µL of standard with different concentration were added to the wells. Sample well : 10 µL of sample was added to the wells.
2. 20 µL of reagent 4 application solution was added to each well of step 1.

3. 170 µL of ABTS working solution was added to each well of step 2.
4. They were Mixed fully and stood for 6 min at room temperature. The OD values of each well was measured at 414 nm with Microplate reader.

2.8.Statistical analysis

The experiments involved three technical replicates, with five samples per group (i.e., n=5 per group). The results were presented as the mean value plus the standard deviation (SD). Statistical analysis was performed using SPSS version 1.0.0.1406, employing one-way ANOVA. For multiple comparisons, Dennett's correction was applied. Differences were considered statistically significant at $p \leq 0.05$. The means of each group were compared to those of the other groups. The data were visually represented using bar charts, where rectangular bars depicted categorical data, and the height of the bars corresponded to the represented data or values.

Chapter Three

Results

3.1. Behavioural Tests Results

3.1.1 Y Maze Spontaneous Alteration Percentage Test Result

The normal group was compared with Alzheimer's induced group, there is a significant difference. Normal group showed perfect SAP while Alzheimer's induced group showed highly significant ($p<0.001$) decrease in SAP. There is a symmetry in percentage of SAP between donepezil and telmisartan.On another hand, insignificant difference was found between rosemary group and the telmisartan group, combination group showed a symmetry to donepezil group .It showed in Table.3.1.and Figure 3.1

Table.3.1.Numerical results of Y-maze spontaneous alteration percentage

	Control group	Induced group	Telmisartan group	Rosemary group	Combination group	Donepezil group
Rat1	70%	50%	73%	75%	71%	62%
Rat2	77%	47%	70%	78%	63%	81%
Rat3	87%	66%	85%	68%	100%	58%
Rat4	92%	54%	70%	66%	71%	100%
Rat5	77%	63%	75%	85%	75%	83%
average	80.60%	56%	74.60	74%	76%	76.8%

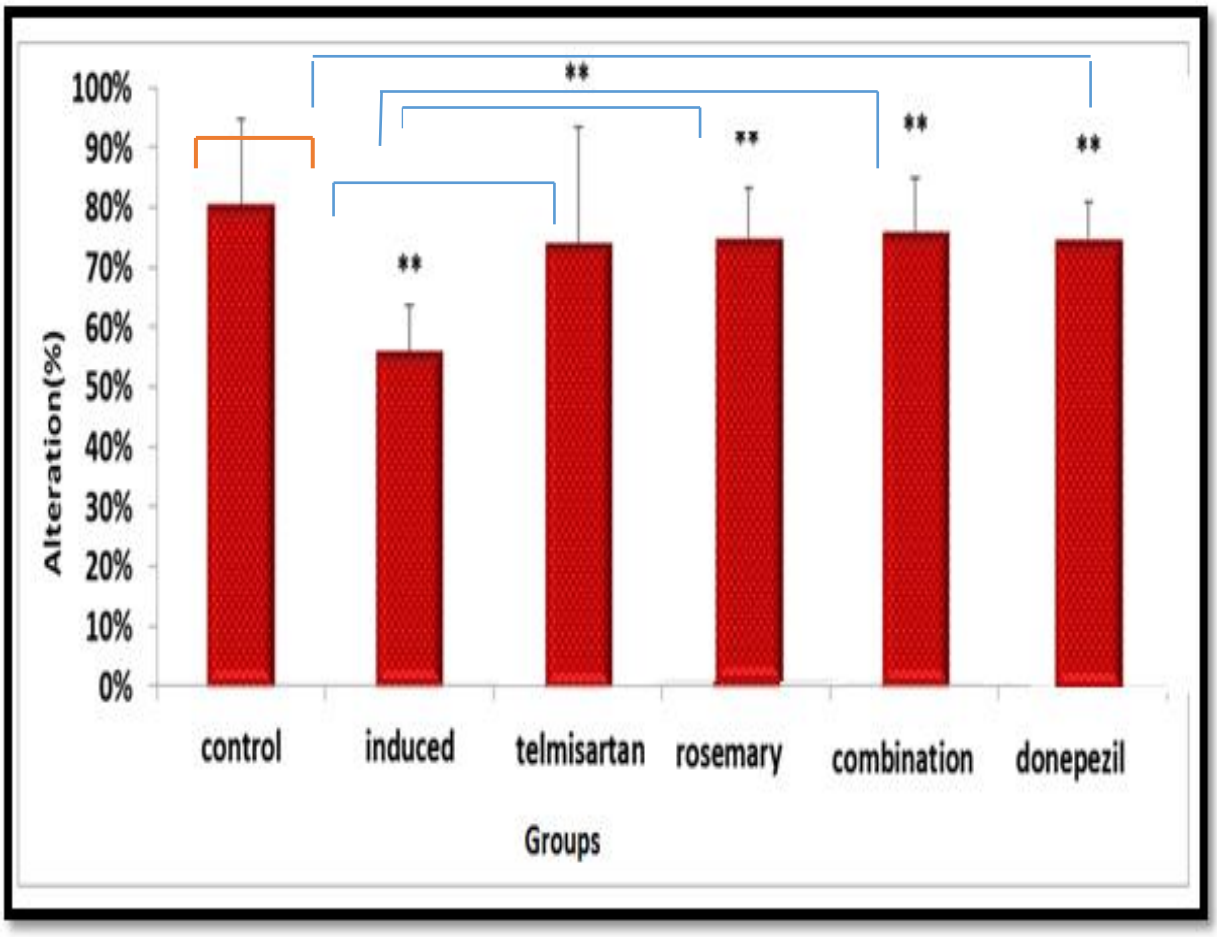


Figure3.1.SAP for control ,induced ,telmisartan+AlCl3 , rosemary+AlCl3 , telmisartan+rosemary+AlCl3 ,donepezil+AlCl3 .Results (n=5) are expressed as the mean ± SD *(p<0.001)*.This figure shows a significant decline in SAP of induced group in comparison to control group. However, in group 3 to 6,SAP surged significantly as compared to induced group.**

3.2.Tissue Biochemical Analysis Result

3.2.1 Inflammation Biomarkers tissue Results

The concentration of some inflammation biomarkers like interleukin 1,interlukin 6 ,tumor necrosis factor alpha were measured.

3.2.1.1.Interleukin-1 Level

For the normal group, the concentration of IL-1 was very low .There is a highly significant increase ($p<0.001$) in IL-1 level in induced group as compared to the normal group .While there was a highly significant ($p<0.001$) decrease in IL1 concentration in the telmisartan ,rosemary,combination,

donepezil groups,as compared to induced group.On another hand there is insignificant difference between telmisartan,rosemary,combination and the donepezil groups.It illustrated in figure 3.2.

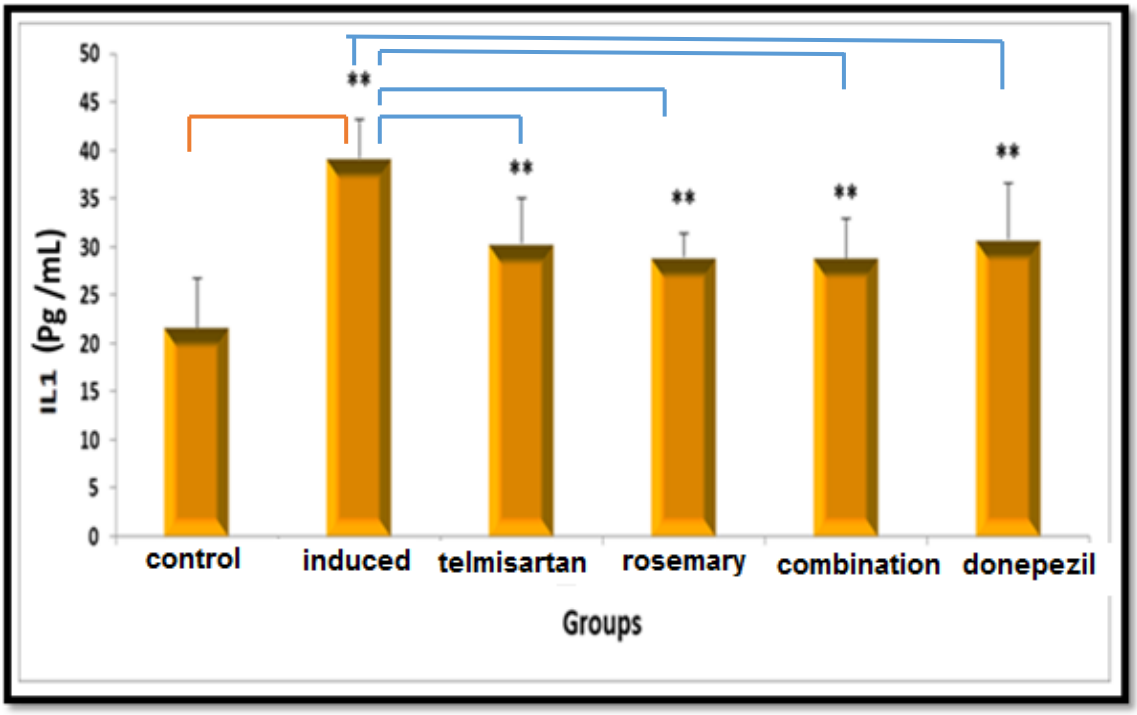


Figure3.2.IL1 concentration for control , induced, telmisartan+AlCl3 , rosemary+AlCl3 , telmisartan+rosemary+AlCl3,donepezil+AlCl3 groups.Results (n=5) are expressed as the mean (± SD). *(p<0.001)*.This figure shows a significant increase in IL1 level in induced group in comparison to control group.However,in group 3 to 6,IL1 level decreased significantly in comparison to induced group.**

3.2.1.2. Interleukin-6 Level :

normal group was compared to the induced group,there was highly significant difference.There is highly significant increase ($p<0.001$) in IL-6 level in the induced group as compared to the normal group,and highly significant decrease in IL-6 level in telmisartan,rosemary,donepezil and combination groups,as they compared to the induced group. It showed in figure 3.3.

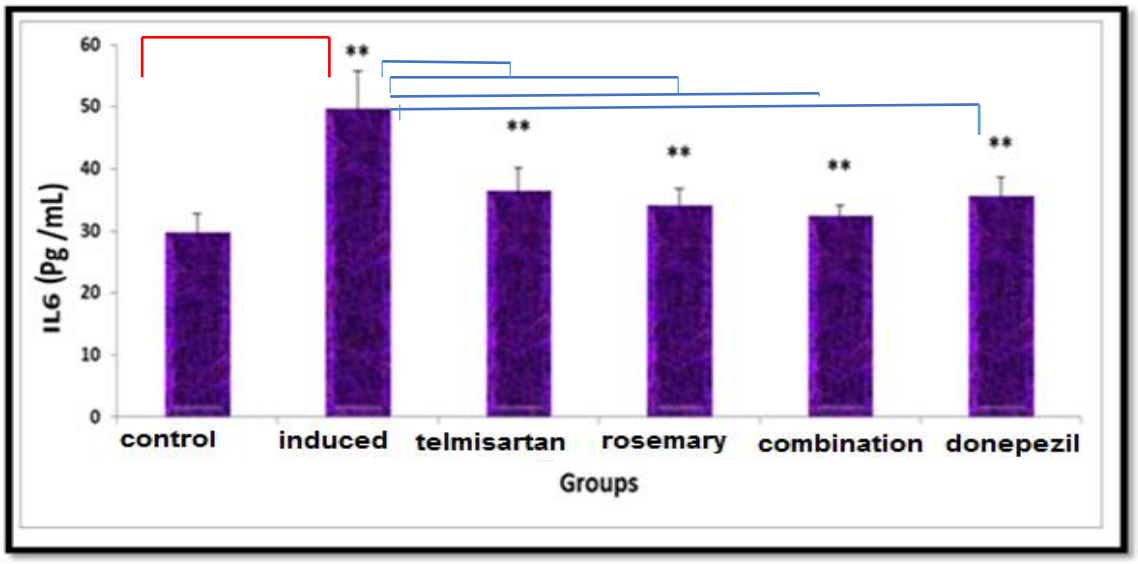


Figure3.3.IL6 level for control, induced, telmisartan+AlCl3 , rosemary+AlCl3, telmisartan+rosemary+AlCl3,donepezil+AlCl3 groups. Results (n=5) are expressed as the mean (± SD). (p<0.001).This figure shows a significant surge in IL6 level in induced group in comparison to control group.However,in group 3 to 6 there is a significant decrease in IL6 level in comparison to induced group.**

3.2.1.3 .Tumor Necrosis Factor Alpha Level

There was highly significant increase (p<0.001) in TNFα in induced group as compared to normal group. While there was highly significant decrease (p<0.001) in TNFα level in telmisartan,rosemary,donepezil and combination groups as compared to the induced group.Showed in figure 3.4.

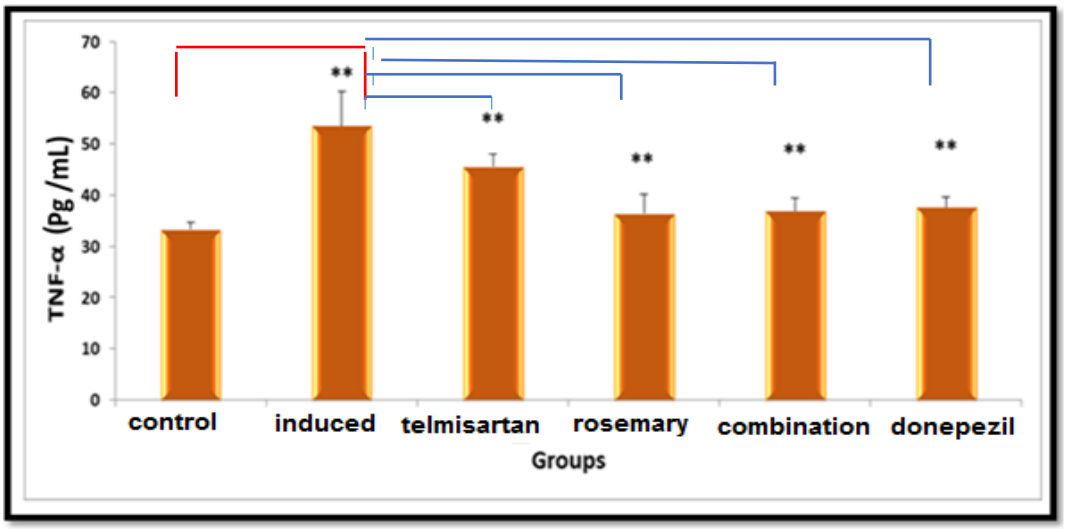


Figure3.4.TNFα level for control, induced, telmisartan+AlCl3, rosemary+AlCl3 , telmisartan+rosemary+AlCl3,donepezil+AlCl3 groups. Results (n=5) are expressed as the mean (± SD). *(p<0.001)*.This figure shows a significant increase in TNFα in induced group in comparison to control group.However,in group 3 to 6 there is a significant decrease in TNFα level in comparison to induced group**

3.2.2 Oxidative Stress Biomarkers:

3.2.2.1. Total Antioxidant Capacity

There was highly significant decrease ($p<0.001$) in total antioxidant capacity in Alzheimer's induced group as compared to normal group , while there was significant increase($p<0.05$) in total antioxidant in telmisartan and highly significant increase ($p<0.001$) in TAOC level in rosemary, donepezil and the combination group as compared to induced group. It showed in figure 3.5.

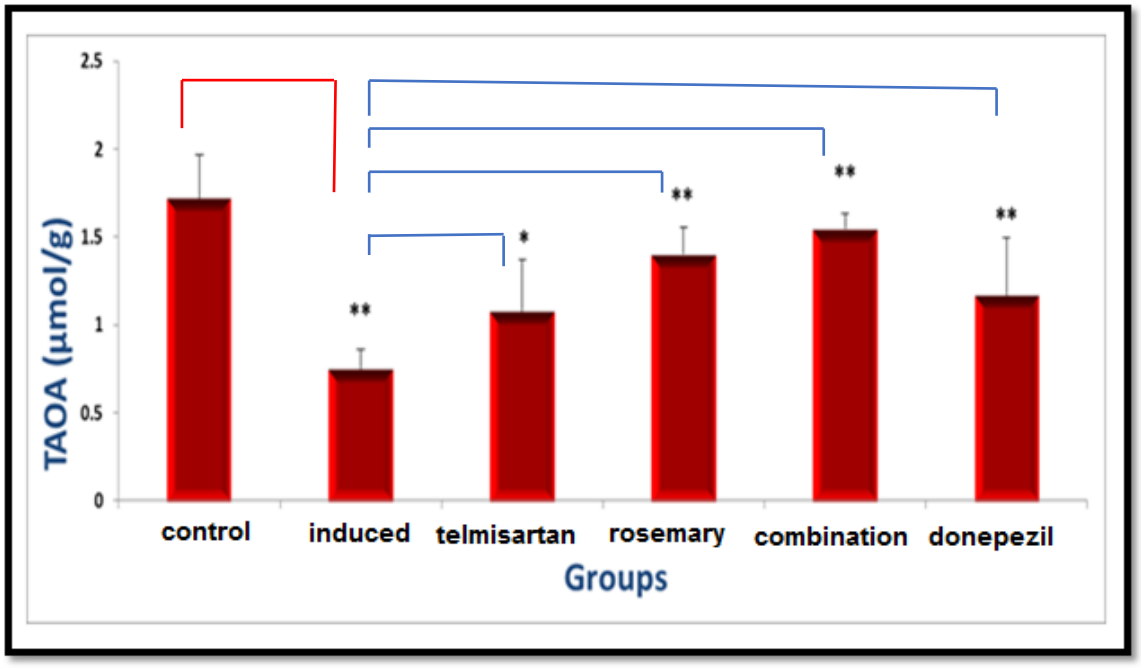


Figure3.5.TAOA for control, induced, telmisartan+AlCl3 , rosemary+AlCl3, telmisartan+rosemary+AlCl3,donepezil+AlCl3 groups. Results(n=5) are expressed as the mean (± SD). *(*p<0.05)*, *(p<0.001*).This figure shows a significant decrease in TAOC in induced group in comparison to control group.However,group 3 shows significant increase in TAOC and group 4 to 6 shows highly significant increase in TAOC in comparison to induced group.**

3.2.2.2. Glutathione(GSH) Level

There was a highly significant decrease ($p<0.001$) in glutathione level in induced group as compared to normal group and significant increase ($p<0.05$) in GSH level in telmisartan and highly significant increased ($p<0.001$) GSH level in rosemary,donepezil,and combination group as compared to induced group.Combination group showed insignificant difference from the normal group. It showed in figure 3.6.

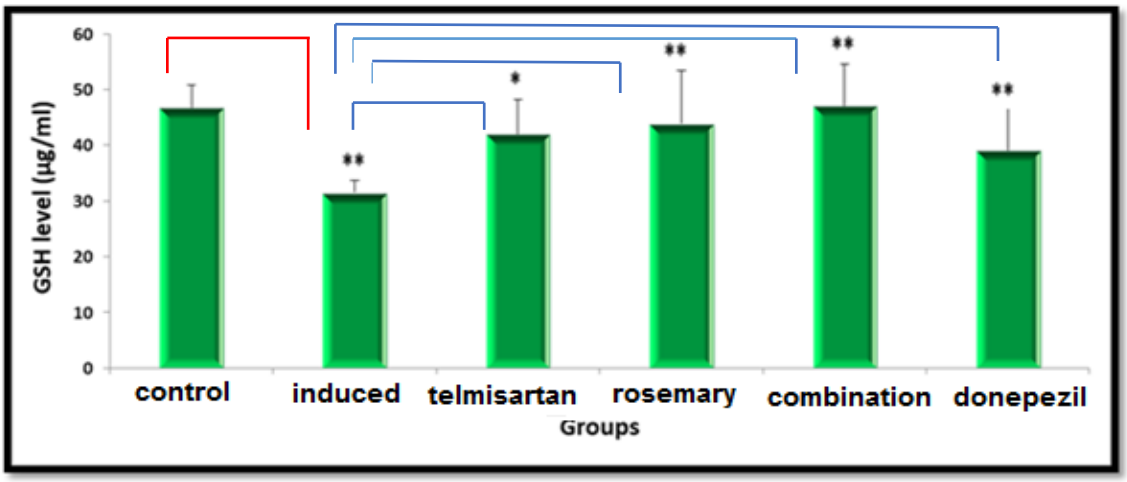


Figure3.6.glutathione level in control ,induced ,telmisartan+AlCl3 ,rosemary+AlCl3, telmisartan+rosemary+AlCl3,donepezil+AlCl3 groups.Results (n=5) are expressed as the mean (± SD). *(*p<0.05), (p<0.001*).This figure shows a significant decrease in GSH level in induced group in comparison to control group.However,group 3 shows significant increase in GSH and groups 4 to 6 show highly significant increase in GSH level in comparison to induced group.**

3.2.2.3. Malondialdehyde(MDA) Level

There was a highly significant increase ($p<0.001$) in malondialdehyde in induced group as compared to normal group and highly significant decrease ($p<0.001$) in its MDA level in telmisartan, rosemary, donepezil, and combination group as compared to the induced group.On another hand,combination group shows a better result than donepezil group.It illustrated in figure 3.7.

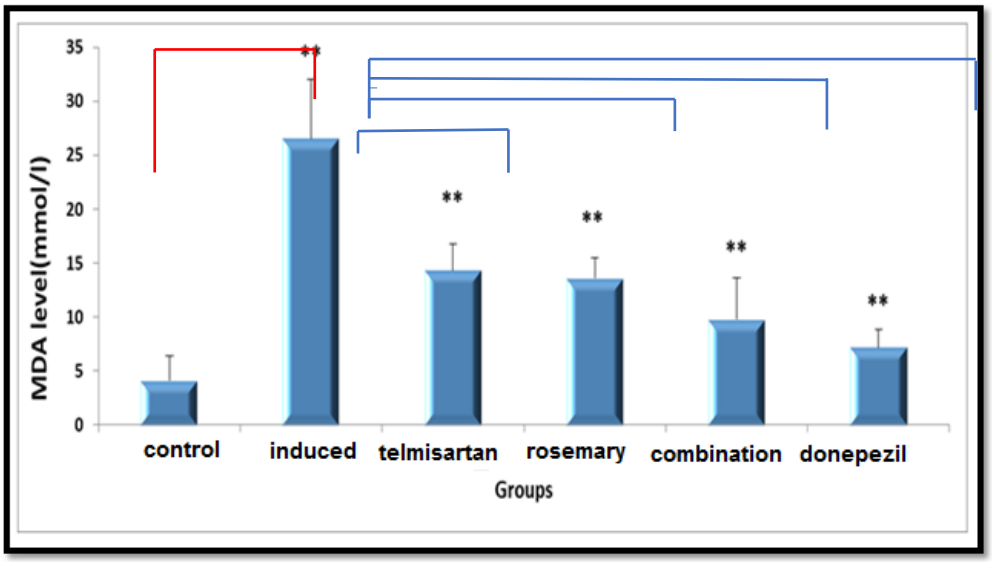


Figure3.7.Malondialdehyde level in control , induced ,telmisartan+AlCl3 ,rosemary+AlCl3, telmisartan+rosemary+AlCl3, donepezil+AlCl3 groups.Results (n=5) are expressed as the mean (± SD). *(p<0.001)*.This figure shows significant increase in MDA level in induced group in comparison to control group.However,in group 3 to 6 there is a significant decrease in MDA level in comparison to induced group.**

3.3.Histopathological Results

The brain histopathological analysis results confirmed the results of biochemical analysis.The microscopic brain examination of the normal control group showed no inflammation within the sections.Normal organization and appearance of the neuronal cells with normal architecture of the hippocampus.

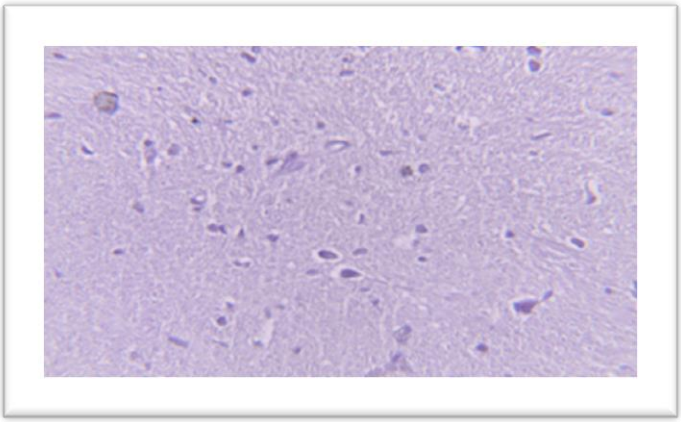


Figure 3.8. Rat brain, normal histology shows astrocytes with capillary blood vessels (H&E× 400) (control group 1)

While the AlCl3 group, showed moderate inflammation, with degeneration and necrosis of the neuronal cells.

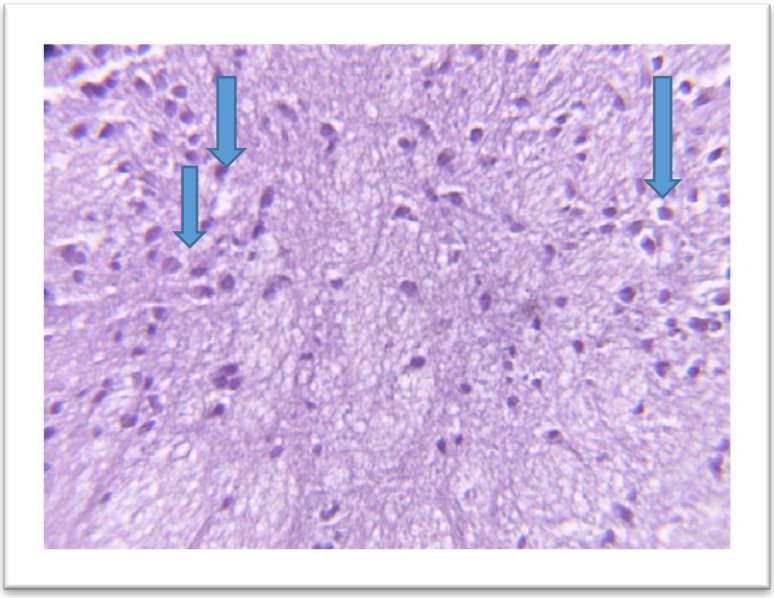


Figure 3.9: Rat brain, show moderate inflammation with frequent acute inflammatory cells infiltrate arrow (H&E× 400) (AlCl3 group 2)

The telmisartan, rosemary, combination and donepezil groups showed mild inflammation and the drugs has been showed to alleviate the degenerative effect of AlCl3.

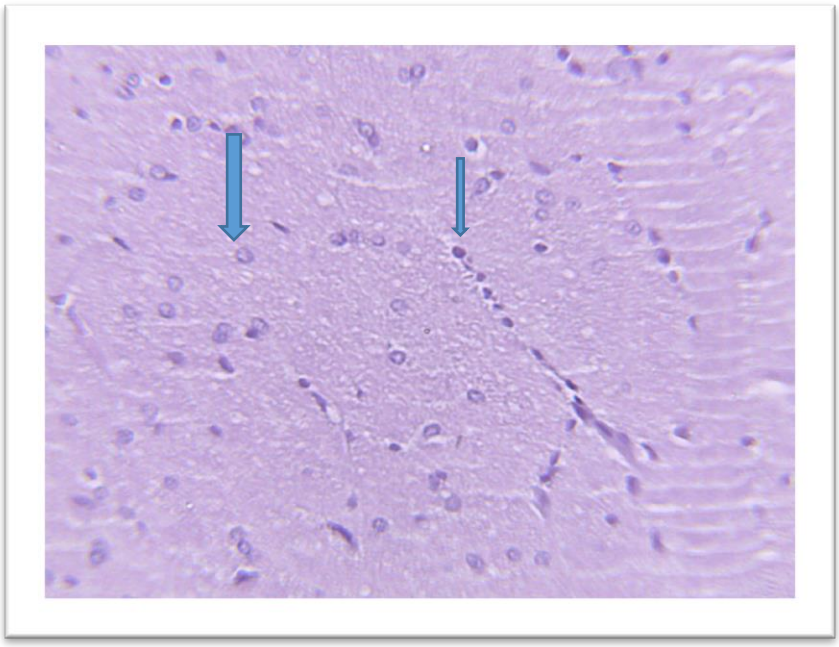


Figure 3.10. Rat brain, mild inflammation shows astrocytes with acute inflammatory cells infiltrate arrow (H&E× 400) (telmisartan+AlCl3 group)

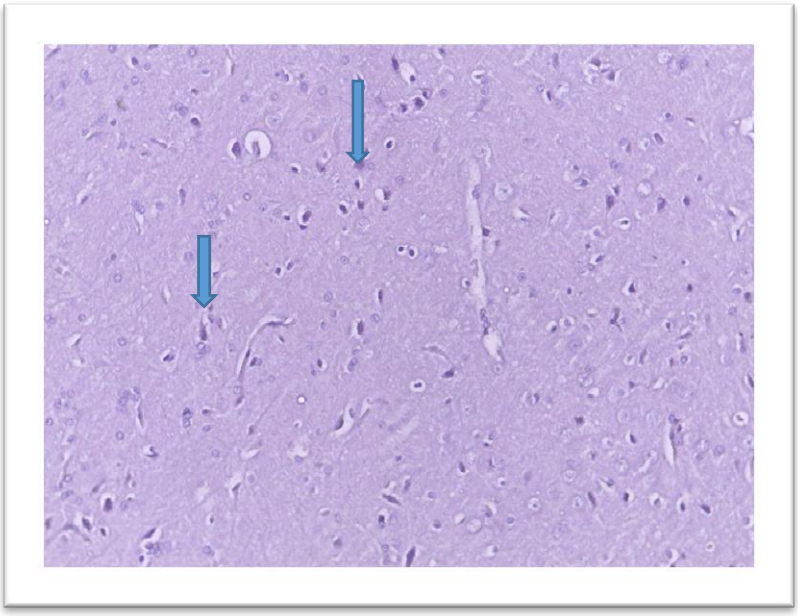


Figure 3.11. Rat brain, mild inflammation shows astrocytes with acute inflammatory cells infiltrate arrow (H&E× 400) (Rosemarly liquid extract +AlCl3 group)

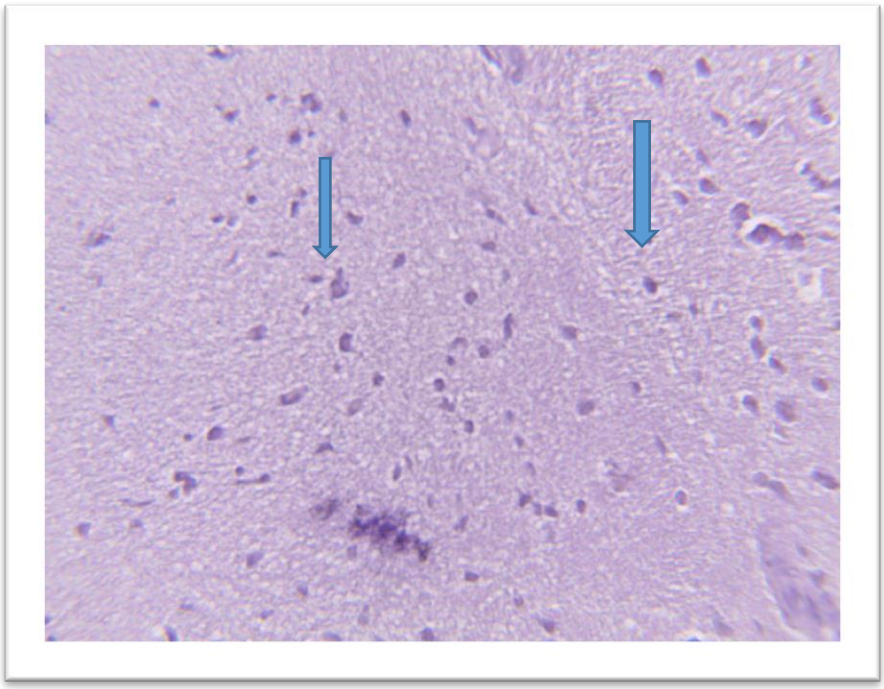


Figure 3.12. Rat brain, mild inflammation shows astrocytes with acute inflammatory cells infiltrate arrow (H&E× 400) (telmisartan+rosemary+AlCl3 group).

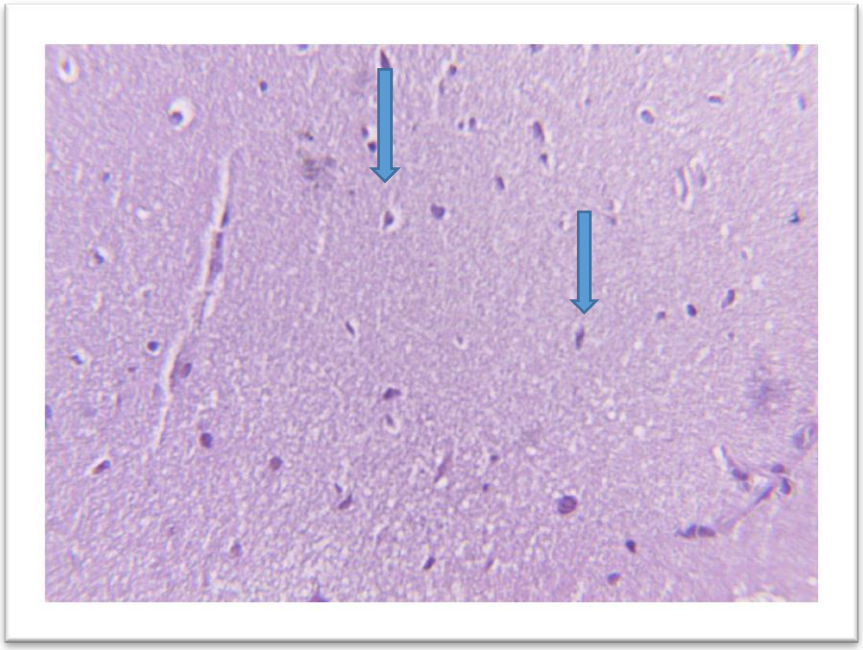


Figure3.13. Rat brain, normal to mild inflammation shows astrocytes with acute inflammatory cells infiltrate arrow (H&E× 400) (donepezil +AlCl3group)

Chapter four
Discussion

4.The Idea of the Current Study:

Recent studies are trying to find new stratigies in treating or smoothening the symptoms of Alzheimer's disease. Our study focused on the anti-hypertensive drug which belongs to the group' angiotensin receptor blocker' (telmisartan) and the plant (*Rosemarinus officinalis* L)

The results of this study demonstrate two things.First, it confirmed the previous findings about angiotensin II type I receptor blocker inhibitor (telmisartan), which save the integrity of blood brain barrier so will decrease the percolation of proinflammatory mediators that are found in AD disorder(So *et al*., 2015). It block the AngII receptor effect on oxidative stress by AT1 receptor that associated with increased reactive oxygen species like (superoxide) (Takane *et al.*, 2017).It also prevent the Ang II inhibition of acetylcholine release (Tota *et al*., 2013). So will decrease the reduction of it in Alzheimer's disease so that will reduce the cognitive impairment (Herholz, 2008). Second, the study found clear support for the previous studies about effectiveness of rosemary herbal to enhance the cognitive functions in patients with Alzheimer's disease(Balu *et al*.,2009). Futhermore, telmisartan with the plant rosemary as a combination was used based on its mutual mechanisim of interest.

4.1. Measurement of Spatial Memory in Rat's Groups

4.1.1. Y Maze Measurement

In Y maze spontaneous alteration task,that we have used to estimate the memory and spatial recognition learning abilities of the rats. In Y maze spontaneous alteration analysis, the three main output are the number of enteties and alteration also it present the percent alterations.The sequence of arm entries can be used to calculate the number of alterations,which can be defined as a successful entries into 3 different arms.This method can be used to measure or estimate the short term spatial memory in rats(Sarter *et*

al.,1988).In this research, the alterations were calculated and assume that one alteration is happened when entire rat fit into the arms (Ohno *et al*.,2004), the percent alterations calculated by using the equation

Alteration% = [(number of alternations) / (total arm entries − 2)] × 100

The Alzheimer's induced rats by AlCl3 showed a clear decrease in SAP when it was compared with rat group that received donepezil,telmisartan or rosemary extract alone or in combination.SAP for AlCl3 group was about 56% as compared with control group which was 80.60%.that indicated the decrease in spatial memory capacities of these rats in this group, as previous studies (Wagner *et al*.,2019) indicated that rats treated with AlCl3 show cognitive decline and histological changes in their brains as it cause neurodegeneration.

Rats group that received telmisartan,has enhanced SAP results (was about 74.60%) we have compared it with donepezil group result that was about (76.8%).The beneficial effects of telmisartan on memory showed agreement with previous study(Li *et al*., 2012), as telmisartan is a partial PPARγ agonist,has neuroprotective effects, that decrease cognitive decline and memory loss.The group that received rosemary extract has a SAP(74%) and that indicated the clear enhancement in SAP values.Rosemary received group showed that the herbal can enhance the spatial memory in rats after they received AlCl3 that cause the neurodegeneration,as it indicated in previous study (Munné-Bosch *et al*.,2001),carnosic acid is lipophilic antioxidant that prevents disruption of the biological membrane by scavenging free radicals,so it has an effect on hippocampus that has a magor role in spatial memory and is very sensitive to oxidative damage by these free radicals.

4.2.The Effect of Telmisartan with Rosemary Extract on Behavioural Results

When the combination of the two compounds was used together (telmisartan+rosemary), SAP was 76% ,that overcame the results of telmisartan alone or just rosemary extract, very near to the donepezil(SAP was 76.8%),and it was the nearest to the control group among the agents aside from donepezil.Donepezil was used to show the activity of telmisartan and rosemary as we have compared their values. The point of our research was about the combination group and we found that it has greater effects than each agent separately regarding the behavioural test results.

4.3.1.Neurodegeneration and Lipid Peroxidation

In our study,there was a significant increase in malondialdehyde level in the induced rats group by AlCl3 as compared to control group.AlCl3 that cause free radicals formation and neurodegeneration results in elevated MDA level as indicated in previous study(Newairy *et al*., 2009) that AlCl3 causes neurodegeneration and oxidative stress buildup, free radical-mediated cytotoxicity, lipid peroxidation, reduced cholinergic function, impact on gene expression and altered protein phosphorylation so it affected lipid peroxidation and MDA one of the two main products of omega-6 fatty acids lipid peroxidation. Lipid peroxidation, the process in which the oxidants like free radicals attack the polyunsaturated fatty acids (lipids that contain carbon-carbon double bond, that will result in abstraction of hydrogen from a carbon,then insertion of oxygen will result in hydroperoxides and peroxyl radicals.Phospholipids,cholesterol and glycolipids are the most important goals for damaging.It has a cytotoxic role and can cause promoting cell death and can be a cause for neural damage.

4.3.2. Neurodegeneration and TAOC

There was a decrease in total antioxidant activity in induced group as compared to control group,TAOC is a measure for antioxidant activity against oxidative stress and free radicals.This agreed with the previous studies(Ghiselli et al .,2000), AlCl3 acting as prooxidant agent at the same time it interacts with superoxide radicals and increase its harmful effects by the formation of peroxynitrite.Another strategy in neurotoxicity process is that aluminum showed to accumulate into the astrocytes and cause its apoptosis which causing the increasing in the free radicals formation ,the antioxidant capacity will be decreased as a consequence of that. (Mander and Brown.,2005).

4.3.3. Neurodegeneration and Reduced Glutathione

Glutathione level was clearly decreased after administration of aluminum chloride when we compared it to control group, glutathione is an important component acting as cofactor for signal transduction, and it is important for antioxidant defense, redux buffer activity.Our results showed comparable findings as indicated in the past (Murakami and Yoshino.,2004) that aluminium chloride inhibits the regeneration of glutathione from the oxidized state,as it inhibit NADP-isocitrate dehydrogenase which provide NADPH in mitochondria.NADPH supply,by malic NADP-isocitrate dehydrogenase and malic enzyme will be inhibited so there is an inhibition in glutathione regeneration.reduced glutathione is principal component that get rid of reactive oxygen species in mitochondria.Oxidative damage will appear in cellular biological processes All these actions result in neurodegeneration.

4.3.4. neurodegeneration and Proinflammatory Cytokines:

In our study, interleukin 1 was significantly high in induced group as compared to control group, That agreed with another previous study (Shi et

al.,2015) which indicated that neurodegeneration occurs by some chemicals like AlCl3, the astrocytes and microglia in central nervous system, plays an important role in pathogenesis.The proinflammatory cytokines are released by activation and proliferation of them after the neural damage process.

Other proinflammatory cytokines include interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), were significantly elevated , other studies indicated similar results, Microglia in CNS release interleukin-1β and it will cause the biological effects by IL-1 signaling pathway activation (Gabay *et al*., 2010),

Firstly, interleukin-1β will act on type1 IL-1 receptor and IL-1 receptor accessory protein,then the trimeric complex inducts two intracellular signaling components; myeloid differentiation primary response gene88(MyD88),and interleukin-1 receptor activated protein kinase(IRAK4) , IRAK4 autophosphorylation causes the phosphorylation of IRAK1/2,that will interact with tumor necrosis factor associated factor(TRAF6) that will translocate nuclear factor kappaB (NF-kB) to the nucleus, Inflammatory gene expressions including TNF-α, IL-1β and IL-6,is regulated by (NF-kB) to make the inflammatory lesions subsequently,neurotoxicity will appear. (Weber *et al*.,2010).

4.4.1. The Effects of Telmisartan on Proinflammatory Cytokines:

Our results indicated the decrease in proinflammatory cytokines in tissue samples after receiving telmisartan when we compared it to induced group, other study revealed similar results (Kelleni *et al*.,2016).They showed that telmisartan has antioxidant and anti-inflammatory activities, it has actions on some oxidative and antioxidant biomarkers but its mechanism of action is not well understood.

In the body, normally, there are the scavenger systems like CAT(catalase)and SOD(superoxide dismutase),(Demidchik *et al*.,2015) that control oxygen species and act as modulator for redox process.The

formation of free radicals of any cause, making multiple cellular molecules undergo oxidation.Studies indicated that telmisartan make changes in CAT and superoxide dismutase 2 gene expressions. After oxygen species free radicals formed,there is an modification in gene expression will occur like in CAT expression.CAT concentration and activity can be increased after administration of telmisartan. TLM in previous studies, has been shown to be selective PPARγ modulator. PPARγ belong to the nuclear hormone receptor superfamily that calles (orphan nuclear receptors) they can be bind with ligands that are naturally occurring or synthetic agents that acting as agonists or antagonists and form complex with them(Takada *et al*.,2020).All ARBs have some degree of PPARγ agonist activity,but telmisartan is the most potent one, the mechanism of partial agonist of telmisartan is not fully understood but it belongs to the structure of PPARγ and how it forms complex with telmisartan, it cause the weakening in hydrogen-bonding network found around H12 in PPARγstructure (Bruning *et al*.,2007). PPARγ isoforms all known to have anti-inflammatory in the body by different mechanisms.one mechanism include interaction with transcription factors of the nuclear factor kappa light chain enhancer of B cells (NF-kB) activation family, that considered the main regulator of inflammatory cascade.By interacting with p65 (that is important for NF-kB signaling) or by decreasing in proinflammatory gene inducers of NF-kB, inactivation of NF-kB will be enhanced by PPARγ (Schnegg *et al*.,2012).

4.4.2.Telmisartan and Glutathione

Glutathione is strong intracellular antioxidant fighter that maintains the redox process and get rid of harmful free radicals. ELISA test results have showed that, like previous study (Goyal *et al*.,2011) glutathione level was increased after administration of telmisartan as compared to induced group and that indicates the antioxidant activity of the drug and may refers

to the reduction in free radicals that are generated from the administration of AlCl3.

4.4.3.Telmisartan and MDA:

The results indicated the decreasing in MDA level after telmisartan administration as compared to induced group,and that was similar to previous studies(Kono *et al*.,2015),reactive oxygen species can react with different proteins like nucleic acids(cause DNA damage), cysteine residues and lipids(cause lipid peroxidation), telmisartan has antioxidant capacity,lipid peroxidation will be decreased and that lead to decreased MDA level as it was found in our reseach.That shows the antioxidant ability of the drug.

4.4.4.Effects of Telmisartan on Inflammatory Cytokines and TAOC:

After treating rats with telmisartan,there was a clear increasing in total antioxidant capacity and decreasing in inflammatory cytokines as compared to induced group.This agree with other studies (Goel *et al*., 2018), telmisartan is partial PPARγ agonist. PPARγ enhance antioxidant enzymes expression,consequently, oxygen species will be reduced.telmisartan decrease hippocampal amyloidogenic markers like phosphorylated tau protein and amyloid-beta level and amyloid precursor protein cleaving enzyme 1 expression so it has antioxidant capacity and prevent neural damage .There is a specific RAS in the brain.Angiotensin II participate in the process of AD ,it increase the cognitive impairment and memory deficits,as it cause the accomulation of amyloid beta1-42 and will induce tau phosphorylation that will lead to further amyloid neurotoxicity (Tian *et al.*,2012).

Impacts of telmisartan on RAS are mediated by several receptors including AT1 (AngII type 1 receptor),AT2(Ang II type2 receptor), AT4 and Mas (Singh and Karnik, 2016).It block the inflammation of the brain that is caused by AT1 stimulation. telmisartan reduce anxiety,enhance

spatial working memory by blocking the effect of ATI,so AT2 will be more available which has the neuroprotection effect (Inestrosa *et al*., 2005).So it has a good results both in Y maze task that was nearly resembling the influence of donepezil in this behavioural test. Due to its capacity to serve as both an angiotensin II receptor (AT1) antagonist and a partial agonist of PPARγ , telmisartan is classified as a selective modulator of PPARγ activity (SPPAR-M).

The primary function of PPARγ,a nuclear receptor superfamily transcription factor, is to regulate target gene expression in essential processes like lipid metabolism, differentiation, and inflammation. Since it has been shown to be effective as a therapeutic target in a wide range of brain disorders, including degenerative diseases, stroke, traumatic damage, and demyelinating diseases, PPAR- has recently drawn particular interest as it has antiinflammatory effects that prevenr brain damage (Glorieux *et al*.,2015), PPARγ regulate CAT gene expression by response element at -12kb from transcription initiation site of catalase gene. Excess nitrogen and oxygen species that caused by AlCl3 can cause negative effects on PPARγ and CAT gene expression ,then consequently,reduce in CAT and PPARγ proteins level.

After the neural damage caused by AlCl3 and inflammatory lesions and increased TNFα ,that cause downregulation of CAT gene expression(Glorieux *et al*.,2015). Telmisartan increase PPARγ protein and gene expression (Iqbal *et al*.,2008),so increase CAT gene expression. Increasing CAT proteins make the cells fight oxidative stress. All these actions make telmisartan has the ability to increase total antioxidant capacity,at the same time reduce the actions of cytokines.

4.5.Effects of Rosemary on Biomarkers

In regard to rosemary liquid extract, rats have been given the plant by oral route, there was a clear enhancement in the antioxidant state.Oxidative stress decreased as MDA level was decreased, interleukin 1 and interleukin 6 also decreased.Tumor necrosis factor (TNF-α) decreased, glutathione increased and total antioxidant capacity increased .

Studies have showed that one of the most important components of rosemary is carnosic acid.Researches had indicated that it has antioxidant and antiinflammtory abilities.it can suppress NF-kB P65 pathway.NF-kB is a transcription factor that is very important in the process of inflammation(Maione *et al*.,2017).NF-kB control the transcription process of proinflammatory cytokines promotors especially genes of interleukin6 and TNFα.NF-kB binding site is heterodimer that has two parts (P50 and P65),NF-kB in its activated form,has the most important part P65 that has the ability to combine with specific DNA sequence ,then lead to the transcription.the process of phosphorylation of inactive NF-kB is responsible for its activation since it will cause the dissociation of the transcription factor inactive dimers, then it will translocate to the nucleus and start the process of transcription and the production of proinflammatory cytokines,the inhibition of NF-KB lead to the reduction in proinflammatory cytokines Including interleukin 1b, TNFα, interleukin 6 (Maione *et al.*,2017).

4.5.1.Effects of Rosemay components Especially (Carnosic Acid) on Total Antioxidant Capacity

Our results showed a significant increase in total antioxidant capacity in group that received rosemary as compared to the induced group.We measured total antioxidant capacity and glutathione, the results came in agreement with previous studies (Abd El-Ghany *et al*.,2012).

Carnosic acid (the main component of rosemary), acts as cleanser that get rid of toxic oxygen reactive species.It is very reactive toward these free radicals as for example H2O2. It is already oxidized but it will be converted to metabolites.After giving rosemary liquid extract to the rats, the total antioxidant capacity has been increased. CA, which is lipophilic component,it prevent biological membrane disturbances and lipid peroxidation,it hunting free radicals (molecules that contain unpaired electrons) like superoxide radical anion, lipid peroxyl radicals , hydroxyl radicals, The results in our study came along with several studies about the cognitive effects of rosemary. (Munné - Bosch *et al*.,2001) .These free radicals are by-products of some endogenous processes that if built up will cause several cellular damage including neural damage (Kim *et al* .,2018).

Carnosic acid also activate (Keap1/Nrf2) pathway that protect cells from oxidative damage.The brain is so sensitive to the oxidative stress as it consume about 20% of body oxygen.neuronal cells are mainly post mitotic so the oxidative damage will be preserved in it.The hippocampus; which is the main region that responsible for cognition,learning and memory is very sensitive to the oxidative stress.generally,rosemary normalize the redox state in the hippocampus so will prevent the deterioration in cognitive functions .

4.5.2.Effects of Rosemary Components Especially Carnosic Acid on Lipid Peroxidation

Our results revealed the decreaseing in MDA level as compared to induced group.Our study showed similar results as previous studies(Romo *et al.*,2013)which stated that,carnosic acid has the ability to inhibit lipid peroxidation When the carnosic is oxidized it will give the metabolite(carnosol) it also found to have antioxidant capacity and lipid protection ability,so it will reduce the lipid peroxidation process in liposomal and microsomal systems.

Lipid radicals can react with carnosol so this metabolite will interfere with lipid peroxidation.In our study MDA level which is related to lipid peroxidation was clearly decreased

4.5.3.Effects of Rosemary on Glutathione

The results showed that rosemary essential oil has a positive impact on glutathione level as compared to induced group and it agreed with previous studies (Chen *et al.*,2011) that illustrated Nuclear factor erythroid2-related factor2(Nrf2) a transcription factor related factor that regulate glutathione synthesis.,it induce by electrophils , adminstration of the plant extract increases Nrf2 nuclear accumulation,GCLC/GCLM induction will occur, then Nrf2 antioxidant responsive element (ARE) reporter activity will be enhanced too,the rate limiting gene(GCL, then will start its responsibility in glutathione formation.

The administration of rosemary had the ability to increase the (GCL) expression so the level of glutathione which is tripeptide detoxifying agent was increased.

All these mechanisms were responsible for antioxidant and anti-inflammatory abilities of rosemary,so as we found. It enhanced the neurological behaviours of the rats as well as the antioxidant biomarkers

were increased and the proinflammatory cytokines that causing the neurodegeneration and damage were decreased.

4.6.The Combination Group Effect on Behavioural Parameters and Biomarkers

In this research,we focused on the combination group,the rats group that received both telmisartan doses with rosemary liquid extract at the same time.All biomarkers including the behavioural and biochemical were better than giving each agent alone.

In the y maze spontaneous alteration percentage calculation that indicates the spatial memory and learning which is a propery that mainly related to hippocampus that is the part most effected in Alzheimer's disease.We have found that SAP values for combination group was slightly better than results of each group alone.It was about 76%.The result of telmisartan alone SAP was about 74.60% and for rosemary liquid extract SAP was 74%. That indicated the better enhancement in cognitive functions in combination group.

In the biochemical analysis, the proinflammatory cytokines values also indicated the preference for the combination group. Several previous studies have indicated that rosemary dipertenoids components like carnosol and carnosic acid have anti-inflammatory and antioxidant characteristics.They interfere and inhibit the important inflammation pathways.As we have indicated in pervious section,the rosemary components interfere with NF-kB pathway and they inhibit it also inhibit the production of COX-2,IL1b,TNFα (Maione *et al*.,2017).

4.7.The Mutual Effect of Rosemary and Telmesartan on PPARγ

The property in which rosemary sharing with telmisartan is that rosemary also has been shown to have agonist activity on PPARγ,the ligand –activated transcription factor ,a member of neuclear receptor superfamily

that control the cytokines –mediated signal transduction and production(Rau *et al* .,2006).

The action of PPARs can occur either by prevention of binding between DNA to transcription factors of the inflammatory process, by direct mechanism (protein to protein interactions),or by interfering with cofactors that are important in the process.The first way,direct protein to protein interaction,prevent the transcripthion factors from interacting with response elements ,so the cytokines genes will be prevented from the transcription by these elements(Ricote *et al.*,2007), So the production of proinflammatory cytokines will be negatively effected and the actions of them will be prevented. CNS, which is the main part that is effected in Alzheimer's disease,will be protected from the harmful effects of these cytokines and the neurodegeneration will be decreased to some level.

Telmisartan is the most potent angiotensin receptor blocker in this property of partial agonist activity toward PPARγ (Schupp *et al.*,2004),as we mentioned before,so we have found that both agents can work on this point and their positive action will be douplicated in most biochemical test results also in behavioural results and that supports the main thought of this research.

Conclusion

and Recommendations

Conclusion:

1.Behavioural test demonstrated that Rosemarinus officinalis L. and telmisartan improve the cognition ability in male rats by enhancing the spatial memory that measured by Y maze.

2.Rosemary has anti-Alzheimer's disease effects on male rats as it has neuroprotective effects via its antioxidant and anti-inflammatory effects by increasing antioxidant biomarkers (glutathione,total antioxidant capacity,lipid peroxidation), and decreasing the pro-inflammatory cytokines (TNFα,IL-1,IL-6),in rat's brain after inducing neurodegeneration by administration of Alumnium chloride.

3.Telmisartan has showed its anti-Alzheimer's disease effects on male rats by its antioxidant and anti-inflammatory capacity, include decreasing in the oxidative stress biomarkers(glutathione,MDA,TAOC) ,and the proinflammatory cytokines (TNFα,IL-1 and IL-6) which were elevated after administration of AlCl3.

4.This study indicated that,the Co-Adminstration of telmisartan with rosemary liquid extract at the same time,has greater anti-Alzheimer's disease effects in male rats than the usage of only one of them,via enhancing behavioural test results and by increasing antioxidant biomarkers (GSH,TAOC,MDA), and reducing the proinflammatory biomarkers (TNFα,IL-1,IL-6.

Recommendations:

1.Larger sample size can be used,and rosemary can be tried to be used prophylactically even like aromatherapy in high risk groups like those with Alzheimer's disease family history or old ages, to see if it has prophylactic neuroptotective effects.

2.Telmisartan with rosemary can be tried on other groups including hypertensive group cases ,to illustrate the relationship between the agents and the blood pressure, like if their high blood pressure decreasing effects will cause greater effect since there are many studies indicated the relationship between high blood pressure and development of Alzheimer' disease.

3.Other oxidative stress parameters and inflammatory biomarkers can be used to get more precise results.

4.Other types of kits like tau proteins and beta amyloid can be used to measure their levels which have direct effects on Alzheimer's disease.

References

References:

- Abd El Aziz AE, Sayed RH, Sallam NA, El Sayed NS. Neuroprotective Effects of Telmisartan and Nifedipine Against Cuprizone-Induced Demyelination and Behavioral Dysfunction in Mice: Roles of NF-κB and Nrf2. Inflammation. 2021 Aug;44(4):1629-1642.

-Abd El-Ghany, Abd El-Ghany & Motawee, Mahmoud & Elkewawy, Hala. Biological effects of yoghurt with rosemary on injured liver rats. Australian Journal of Basic and Applied Sciences. 2012;6(3):525–532.

-Abo-Youssef AM, Khallaf WA, Khattab MM, Messiha BAS. The Anti-Alzheimer Effect of Telmisartan in a Hyperglycemic Ovariectomized Rat Model; Role of Central Angiotensin and Estrogen Receptors. Food Chem Toxicol. 2020; 142: 111441.

-Almad A, Maragakis N J. A stocked toolbox for understanding the role of astrocytes in disease. Nat. Rev. Neurol. 2018; 14: 351–362. doi: 10.1038/s41582-018-0010-2.

- Newairy AS, Salama AF, Hussien HM, Yousef MI. Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats. Food Chem Toxicol.2009;1093-1098.

-Atri A, Frölich L, Ballard C, Tariot PN, Molinuevo JL, Boneva N, Windfeld K, Raket LL, Cummings JL. Effect of Idalopirdine as Adjunct to Cholinesterase Inhibitors on Change in Cognition in Patients With Alzheimer Disease: Three Randomized Clinical Trials. JAMA. 2018;319:130–142.

- Balu DT, Lucki I. Adult hippocampal neurogenesis: regulation, functional implications, and contribution to disease pathology. Neurosci Biobehav Rev.2009;33(3):232–252.

-Barnes J, Bartlett JW, Wolk DA, van der Flier WM, Frost C. Disease Course Varies According to Age and Symptom Length in Alzheimer's Disease. J Alzheimers Dis. 2018;64(2):631-642.

-Bear MF, Connors BW, Paradiso MA. Neuroscience: Exploring the Brain, 4th Edn. Philadelphia, PA: Wolters Kluwer. 2016.

-Biancardi VC, Stern JE. Compromised blood–brain barrier permeability: novel mechanism by which circulating angiotensin II signals to sympathoexcitatory centres during hypertension. J. Physiol. 2016; 594: 1591–1600.

-Borges RS, Ortiz BLS, Pereira AC M, Keita H, Carvalho JCT. *Rosmarinus officinalis* essential oil: A review of its phytochemistry, anti-inflammatory activity, and mechanisms of action involved. J Ethnopharmacol. 2019;229:29–45.

-Brewer MS. Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. Compr Rev Food Sci Food Saf. 2011;10:221–247.

-Bruning JB, Chalmers M J, Prasad S, Busby S A, Kamenecka T M, He Y, Nettles K W, Griffin P R . Partial agonists activate PPAR [gamma] using a Helix 12 independent mechanism. Structure. 2007; 15(10): 1258-1271.

- Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol.1978; 52: 302-310.

-Chae IG, Yu MH, Im N-K, Jung YT, Lee J, Chun K-S. Effect of *Rosemarinus officinalis L*. on MMP-9, MCP-1 levels, and cell migration in RAW 264.7 and smooth muscle cells. J Med Food. 2012;15(10):879–886.

-Chandel NS. Mitochondria as signaling organelles. BMC Biol. 2014;12:1–7.

-Chen GF, Xu TH, Yan Y. Amyloid beta: structure, biology and structure based therapeutic development. Acta Pharmacol Sin. 2017;38:1205–1235.

- Chen CC, Chen HL, Hsieh CW, Yang YL, Wung BS. Upregulation of NF-E2-related factor-2-dependent glutathione by carnosol provokes a cytoprotective response and enhances cell survival. Acta Pharmacol Sin. 2011 Jan; 32(1): 62–69.

- Costa-Besada MA, Valenzuela R, Garrido-Gil P, Villar-Cheda B, Parga JA, Lanciego JL, Labandeira-Garcia JL. Paracrine and Intracrine Angiotensin 1-7/Mas Receptor Axis in the Substantia Nigra of Rodents, Monkeys, and Humans. Mol Neurobiol. 2018;55:5847–5867.

-Cummings J, Lee G, Ritter A, Sabbagh M, Zhong K. Alzheimer's disease drug development pipeline.Alzheimer's Dement Transl Res Clin Interv. 2020;6(1):e12050.

-De Kloet AD, Pitra S, Wang L, Hiller H, Pioquinto DJ, Smith JA. Angiotensin type-2 receptors influence the activity of vasopressin neurons in the paraventricular nucleus of the hypothalamus in male mice. Endocrinology. 2016;157(8):3167–3180.

-De Silva TM, Faraci FM. Effects of angiotensin II on the cerebral circulation: role of oxidative stress. Front Physiol. 2013;3:484.

-Demidchik V. Mechanisms of oxidative stress in plants: from classical chemistry to cell biology. Environmental and Experimental Botany. 2015;109:212–228.

-Diego MA, Jones NA, Field T, Hernandez-Reif M, Schanberg S, Kuhn C. Aromatherapy positively affects mood, EEG patterns of alertness and math computations. Int J Neurosci. 1998;96(3–4):217–224.

-Elhassan IA, Osman NM. New chemotype Rosmarinus officinalis L.(Rosemary): R. officinalis ct. bornyl acetate. Am J Res Commun. 2014;2(4):232–240.

-Elkahloun AG, Rodriguez Y, Alaiyed S, Wenzel E, Saavedra JM. Telmisartan protects a microglia cell line from LPS injury beyond AT1 receptor blockade or PPARγ activation. Mol Neurobiol. 2019;56:3193–3210.

-Esteras N, Abramov AY. Mitochondrial calcium deregulation in the mechanism of beta-amyloid and tau pathology. Cells. 2020;9(9):2135.

-Eyjolfsdottir H, Eriksdotter M, Linderoth B. Targeted delivery of nerve growth factor to the cholinergic basal forebrain of Alzheimer's disease patients: application of a second-generation encapsulated cell biodelivery device. Alzheimers Res Ther. 2016;8:30.

-Ferreira D, Westman E, Eyjolfsdottir H. Brain changes in Alzheimer's disease patients with implanted encapsulated cells releasing nerve growth factor. J Alzheimers Dis. 2015;43:1059–1072.

-Fogarty DJ, Matute C. Angiotensin receptor-like immunoreactivity in adult brain white matter astrocytes and oligodendrocytes. Glia. 2001;35(2):131–146.

-Foresti R, Bains SK, Pitchumony TS, de Castro Brás LE, Drago F, Dubois-Randé J-L. Small molecule activators of the Nrf2-HO-1 antioxidant axis modulate heme metabolism and inflammation in BV2 microglia cells. Pharmacol Res. 2013;76:132–148.

-Gabay C, Lamacchia C, Palmer G. IL-1 pathways in inflammation and human diseases. Nat Rev Rheumatol. 2010;6(4):232–241.

-Garrido-Gil P, Rodriguez-Pallares J, Dominguez-Meijide A, Guerra MJ, Labandeira-Garcia JL. Brain angiotensin regulates iron homeostasis in dopaminergic neurons and microglial cells. Exp Neurol. 2013;250:384–396.

-Gauthier S, Feldman HH, Schneider LS. Efficacy and safety of tau-aggregation inhibitor therapy in patients with mild or moderate Alzheimer's disease: a randomized, controlled, double-blind, parallel-arm, phase 3 trial. Lancet. 2016;388:2873–2884.

-Gebre AK, Altaye BM, Atey TM, Tuem KB, Berhe DF. Targeting renin–angiotensin system against Alzheimer's disease. Front Pharmacol. 2018;9:440.

-Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radic Biol Med. 2000;29:1106–1114.

- Glorieux C, Zamocky M, Sandoval JM, Verrax J, Calderon PB. Regulation of catalase expression in healthy and cancerous cells. Free Radic Biol Med. 2015;87:84–97.

-Goel R, Bhat SA, Hanif K, Nath C, Shukla R. Angiotensin II receptor blockers attenuate lipopolysaccharide-induced memory impairment by modulation of NF-κB-mediated BDNF/CREB expression and

apoptosis in spontaneously hypertensive rats. Mol Neurobiol. 2018;55:1725–1739.

-González-Trujano ME, Peña EI, Martínez AL, Moreno J, Guevara-Fefer P, Déciga-Campos M. Evaluation of the antinociceptive effect of *Rosmarinus officinalis* L. using three different experimental models in rodents. J Ethnopharmacol. 2007;111:476–482.

-Gowrisankar Y V, Clark MA. Angiotensin II induces interleukin-6 expression in astrocytes: role of reactive oxygen species and NF-κB. Mol Cell Endocrinol. 2016;437:130–141.

- Goyal BR, Parmar K, Goyal RK, Mehta AA. Beneficial role of telmisartan on cardiovascular complications associated with STZ-induced type 2 diabetes in rats. Pharmacol Rep. 2011; 63(4): 956-966.

-Guo Q, Shen Z, Yu H, Lu G, Yu Y, Liu X. Carnosic acid protects against acetaminophen-induced hepatotoxicity by potentiating Nrf2-mediated antioxidant capacity in mice. Korean J Physiol Pharmacol Off J Korean Physiol Soc Korean Soc Pharmacol. 2016;20(1):15

-Guo S, Som AT, Arai K, Lo EH. Effects of angiotensin-II on brain endothelial cell permeability via PPARalpha regulation of para-and trans-cellular pathways. Brain Res. 2019;1722:146353.

-Haapasalo A, Hiltunen M. A report from the 8th Kuopio Alzheimer Symposium. Neurodegener Dis Manag. 2018;8(5):289-299.

-Hadad N, Levy R. Combination of EPA with carotenoids and polyphenol synergistically attenuated the transformation of microglia to M1 phenotype via inhibition of NF-κB. Neuromolecular Med. 2017;19:436–451.

-Hager K, Baseman AS, Nye JS. Effects of galantamine in a 2-year, randomized, placebo-controlled study in Alzheimer's disease. Neuropsychiatr Dis Treat. 2014;10:391–401.

-Herholz K. Acetylcholine esterase activity in mild cognitive impairment and Alzheimer's disease. Eur J Nucl Med Mol Imaging. 2008;35:25–29.

-Ho JK, Nation DA. Cognitive benefits of angiotensin IV and angiotensin-(1–7): a systematic review of experimental studies. Neurosci Biobehav Rev. 2018;92:209–225.

-Ho MS. Microglia in Parkinson's disease. Neuroglia Neurodegener Dis. 2019;335–353.

-Huang X, Moir RD, Tanzi RE, Bush AI, Rogers JT. Redox-active metals, oxidative stress, and Alzheimer's disease pathology. Ann N Y Acad Sci. 2004;1012(1):153–163.

- Hussein W, Sağlık BN, Levent S, Korkut B, Ilgın S, Özkay Y, Kaplancıklı ZA. Synthesis and Biological Evaluation of New Cholinesterase Inhibitors for Alzheimer's Disease. Molecules. 2018 Aug ;(8)14-23.

-Inestrosa NC, Godoy JA, Quintanilla RA, Koenig CS, Bronfman M. Peroxisome proliferator-activated receptor γ is expressed in hippocampal neurons and its activation prevents β-amyloid neurodegeneration: role of Wnt signaling. Exp Cell Res. 2005;304(1):91–104.

-Iqbal M, Dubey K, Anwer T, Ashish A, Pillai KK. Protective effects of telmisartan against acute doxorubicin-induced cardiotoxicity in rats. Pharmacol reports. 2008;60(3):382.

-Isaacson RS, Ferris S, Velting DM. Cognitive efficacy (SIB) of 13.3 versus 4.6 mg/24 h rivastigmine patch in severe Alzheimer's disease. Am J Alzheimers Dis Other Dement. 2016;31:270–277.

-Jackson L, Wael E, Susan F, Adviye E. Within the Brain: The Renin Angiotensin System. Int J Mol Sci. 2018;19(3):876.

- Jack CR Jr, Bennett DA, Blennow K, Carrillo MC, Dunn B, Haeberlein SB, Holtzman DM, Jagust W, Jessen F, Karlawish J, Liu E, Molinuevo JL, Montine T, Phelps C, Rankin KP, Rowe CC, Scheltens P, Siemers E, Snyder HM, Sperling R; Contributors. NIA-AA Research Framework: Toward a biological definition of Alzheimer's disease. Alzheimers Dement. 2018;14(4):535–562.

-Kandalam U, Sarmiento N, Haspula D, Clark MA. Angiotensin III induces signal transducer and activator of transcription 3 and interleukin-6 mRNA levels in cultured rat astrocytes. J Renin-Angiotensin-Aldosterone Syst. 2015;16(4):758–767.

-Kehoe PG. The Coming of Age of the Angiotensin Hypothesis in Alzheimer's Disease: Progress Toward Disease Prevention and Treatment. J Alzheimers Dis. 2018;62(3):1443-1466.

-Kelleni MT, Ibrahim SA, Abdelrahman AM. Effect of captopril and telmisartan on methotrexate-induced hepatotoxicity in rats: impact of oxidative stress, inflammation and apoptosis. Toxicol Mech Methods. 2016;26(5):371–377.

-Kennedy DO, Scholey AB. The psychopharmacology of European herbs with cognition-enhancing properties. Curr Pharm Des. 2006;12(35):4613–4623.

-Khalifa M, Safar MM, Abdelsalam RM, Zaki HF. Telmisartan protects against aluminum-induced Alzheimer-like pathological changes in rats. Neurotoxicity research. 2020 Feb;37:275-285.

- Khan KA, Kumar N, Nayak PG, Nampoothiri M, Shenoy RR, Krishnadas N, Rao CM, Mudgal J. Impact of caffeic acid on aluminium chloride-induced dementia in rats. J Pharm Pharmacol.2013;65:1745-1752.

-Kim H. Detection of severity in Alzheimer's disease (AD) using computational modeling. Bioinformation. 2018;14(5):259-264.

- Kim J, Lee HJ, Park SK, Park JH, Jeong HR, Lee S, Lee H, Seol E, Hoe HS. Donepezil Regulates LPS and Aβ-Stimulated Neuroinflammation through MAPK/NLRP3 Inflammasome/STAT3 Signaling. Int J Mol Sci. 2021 Sep 30;22(19).

-Kono S, Kurata T, Sato K, Omote Y, Hishikawa N, Yamashita T, Deguchi K, Abe K. Neurovascular protection by telmisartan via reducing neuroinflammation in stroke-resistant spontaneously hypertensive rat brain after ischemic stroke. J Stroke Cerebrovasc Dis. 2015 Mar 1;24(3):537-547.

-Kulakowska A, Bierczynska-Krzysik A, Dylag T, Drabik A, Suder P, Noga M, Jarzebinska J, Silberring J. Methods for samples preparation in proteomic research. J Chromatogr B Analyt Technol Biomed Life Sci. 2007 Apr 15;849(1-2):1-31.

- Labandeira-Garcia JL, Rodríguez-Perez AI, Garrido-Gil P, Rodriguez-Pallares J, Lanciego JL, Guerra MJ. Brain Renin-Angiotensin System and Microglial Polarization: Implications for Aging and Neurodegeneration. Front Aging Neurosci. 2017 May 3;9:129.

-Labandeira-Garcia JL, Rodríguez-Perez AI, Villar-Cheda B, Borrajo A, Dominguez-Meijide A, Guerra MJ. Rho kinase and dopaminergic degeneration: a promising therapeutic target for Parkinson’s disease. The Neuroscientist. 2015 Dec;21(6):616-629.

-LaDonya J, Wael E, Susan F, Adviye E. Within the Brain: The Renin Angiotensin System. Int J Mol Sci. 2018;19(3):876.

- Li H, Horke S, Förstermann U. Oxidative stress in vascular disease and its pharmacological prevention. Trends Pharmacol Sci.2013 Jun 1;34(6):313-319.
- Li NC, Lee A, Whitmer RA, Kivipelto M, Lawler E, Kazis LE, Wolozin B. Use of angiotensin receptor blockers and risk of dementia in a predominantly male population: prospective cohort analysis. BMJ. 2010 Jan 13;340.
- Li W, Zhang JW, Lu F, Ma MM, Wang JQ, Suo AQ, Bai YY, Liu HQ. [Effects of telmisartan on the level of Aβ1-42, interleukin-1β, tumor necrosis factor α and cognition in hypertensive patients with Alzheimer's disease]. Zhonghua Yi Xue Za Zhi.2012 Oct 1;92(39):2743-2746.
- Li X, Zhao L, Han JJ, Zhang F, Liu S, Zhu L, Wang ZZ, Zhang GX, Zhang Y. Carnosol Modulates Th17 Cell Differentiation and Microglial Switch in Experimental Autoimmune Encephalomyelitis. Front Immunol. 2018 Aug 13;9:1807.
-Liljegren M, Landqvist Waldö M, Rydbeck R, Englund E. Police interactions among neuropathologically confirmed dementia patients: prevalence and cause. Alzheimer Dis Assoc Disord. 2018;32(4):346-350.
- Lin P, Sun J, Cheng Q, Yang Y, Cordato D, Gao J. The Development of Pharmacological Therapies for Alzheimer's Disease. Neurol Ther. 2021 Dec 1:1-8.
- Lipton SA, Rezaie T, Nutter A, Lopez KM, Parker J, Kosaka K, Satoh T, McKercher SR, Masliah E, Nakanishi N. Therapeutic advantage of pro-electrophilic drugs to activate the Nrf2/ARE pathway in Alzheimer's disease models. Cell Death Dis. 2016 Dec. 2016 Dec;7(12):e2499.

- Maccioni RB, González A, Andrade V, Cortés N, Tapia JP, Guzmán-Martínez L. Alzheimer´s Disease in the Perspective of Neuroimmunology. Open Neurol J. 2018;12:50-56.
- Maione F, Cantone V, Pace S, Chini MG, Bisio A, Romussi G, Pieretti S, Werz O, Koeberle A, Mascolo N, Bifulco G. Anti-inflammatory and analgesic activity of carnosol and carnosic acid in vivo and in vitro and in silico analysis of their target interactions. Br J Pharmacol. 2017 Jun;174(11):1497-1508.
-Malpas CB, Vivash L, Genc S. A phase IIa randomized control trial of VEL015 (Sodium Selenate) in mild-moderate Alzheimer's disease. J Alzheimers Dis. 2016;54:223–232.
- Mander P, Brown GC. Activation of microglial NADPH oxidase is synergistic with glial iNOS expression in inducing neuronal death: a dual-key mechanism of inflammatory neurodegeneration. J Neuroinflammation. 2005 Dec;2:1-5.
- Maynard ME, Underwood EL, Redell JB, Zhao J, Kobori N, Hood KN, Moore AN, Dash PK. Carnosic Acid Improves Outcome after Repetitive Mild Traumatic Brain Injury. J Neurotrauma. 2019 Jul 1;36(13):2147-2152.
- Moosavirad SA, Rabbani M, Sharifzadeh M, Hosseini-Sharifabad A. Protective effect of vitamin C, vitamin B12 and omega-3 on lead-induced memory impairment in rat. Res Pharm Sci.2016;11 (5):390-396.
-Morant AV, Vestergaard HT, Lassen AB, Navikas V. US, EU, and Japanese Regulatory Guidelines for Development of Drugs for Treatment of Alzheimer's Disease: Implications for Global Drug Development. Clin Transl Sci. 2020 Jul;13(4):652-664.
- Moschetti V, Boland K, Feifel U, Hoch A, Zimdahl-Gelling H, Sand M. First-in-human study assessing safety, tolerability and

pharmacokinetics of BI 409306, a selective phosphodiesterase 9A inhibitor, in healthy males. Br J Clin Pharmacol. 2016 Nov;82(5):1315-1324.

- Munné-Bosch S, Alegre L. Subcellular compartmentation of the diterpene carnosic acid and its derivatives in the leaves of rosemary. Plant Physiol. 2001;125(2):1094–1102.

-Murakami K, Yoshino M. Aluminum decreases the glutathione regeneration by the inhibition of NADP-isocitrate dehydrogenase in mitochondria. J Cell Biochem. 2004;93(6):1267–1271.

-Murphy MP, LeVine H 3rd. Alzheimer's disease and the amyloid-beta peptide. J Alzheimers Dis. 2010;19:311–323.

-Nelson TJ, Sun MK, Lim C. Bryostatin effects on cognitive function and PKCvarepsilon in Alzheimer's disease phase IIa and expanded access trials. J Alzheimers Dis. 2017;58:521–535.

-Nicolas G, Acuña-Hidalgo R, Keogh MJ, Quenez O, Steehouwer M, Lelieveld S, Rousseau S, Richard AC, Oud MS, Marguet F, Laquerrière A, Morris CM, Attems J, Smith C, Ansorge O, Al Sarraj S, Frebourg T, Campion D, Hannequin D, Wallon D, Gilissen C, Chinnery PF, Veltman JA, Hoischen A. Somatic variants in autosomal dominant genes are a rare cause of sporadic Alzheimer's disease. Alzheimers Dement. 2018 Dec;14(12):1632-1639.

-Novak P, Schmidt R, Kontsekova E. Safety and immunogenicity of the tau vaccine AADvac1 in patients with Alzheimer's disease: a randomised, double-blind, placebo-controlled, phase 1 trial. Lancet Neurol. 2017;16:123–134.

-Office of Research--Institutional Animal Care and Use program IACUC standard procedure 2021 Stanford Behavioral and functional Neuroscience Laboratory-maze spontaneous Alteration Test.

-Ohno M, Sametsky EA, Younkin LH, Oakley H, Younkin SG, Citron M, Vassar R, Disterhoft JF. BACE1 deficiency rescues memory deficits and cholinergic dysfunction in a mouse model of Alzheimer's disease. Neuron. 2004 Jan 8;41(1):27-33.

-Ou Z, Jiang T, Gao Q, Tian YY, Zhou JS, Wu L, Shi JQ, Zhang YD. Mitochondrial-dependent mechanisms are involved in angiotensin II-induced apoptosis in dopaminergic neurons. J Renin Angiotensin Aldosterone Syst. 2016 Oct;17(4):1470320316672349.

-Ozarowski M, Mikolajczak PL, Bogacz A, Gryszczynska A, Kujawska M, Jodynis-Liebert J. Rosmarinus officinalis L. leaf extract improves memory impairment and affects acetylcholinesterase and butyrylcholinesterase activities in rat brain. Fitoterapia. 2013;91:261–271.

-Park KW, Kim EJ, Han HJ. Efficacy and tolerability of rivastigmine patch therapy in patients with mild-to-moderate Alzheimer’s dementia associated with minimal and moderate ischemic white matter hyperintensities: a multicenter prospective open-label clinical trial. PLoS ONE. 2017;12: e0182123.

-Pérez-Fons L, Garzón MT, Micol V. Relationship between the antioxidant capacity and effect of rosemary (*Rosmarinus officinalis* L.) polyphenols on membrane phospholipid order. J Agric Food Chem. 2010;58:161–171.

-Petersen RC. How early can we diagnose Alzheimer disease (and is it sufficient)? The 2017 Wartenberg lecture. Neurology. 2018 Aug 28;91(9):395-402.

-Rašković A, Milanović I, Pavlović N, Ćebović T, Vukmirović S, Mikov M. Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil

and its hepatoprotective potential. BMC Complement Altern Med. 2014;14:225.

- Rau O, Wurglics M, Paulke A, Zitzkowski J, Meindl N, Bock A, Dingermann T, Abdel-Tawab M, Schubert-Zsilavecz M. Carnosic acid and carnosol, phenolic diterpene compounds of the labiate herbs rosemary and sage, are activators of the human peroxisome proliferator-activated receptor gamma. Planta Med.2006;72:881–887.

-Ricote M, Glass CK. PPARs and molecular mechanisms of transrepression. Biochimica et Biophysica Acta. 2007;1771(8):926–935.

-Rietdijk CD, Van Wezel RJ, Garssen J, Kraneveld AD. Neuronal toll-like receptors and neuro-immunity in Parkinson's disease, Alzheimer's disease and stroke. Neuroimmunology and neuroinflammation. 2016 Feb 15;3:27-37.

-Romo Vaquero M, Garcia Villalba R, Larrosa M, Yáñez-Gascón MJ, Fromentin E, Flanagan J, Roller M, Tomás-Barberán FA, Espín JC, García-Conesa MT. Bioavailability of the major bioactive diterpenoids in a rosemary extract: metabolic profile in the intestine, liver, plasma, and brain of Zucker rats. Molecular nutrition & food research. 2013 Oct;57(10):1834-1846.

-Royea J, Zhang L, Tong XK, Hamel E. Angiotensin IV receptors mediate the cognitive and cerebrovascular benefits of losartan in a mouse model of Alzheimer's disease. Journal of Neuroscience. 2017 May 31;37(22):5562-5573.

- Ruginsk S, Vechiato F, Elias L, Antunes-Rodrigues ,J. and Cruz, J. Angiotensin II reduces mRNA expression for glutamate transporters

and glutamine synthetase in cultured hypothalamic astrocytes. The FASEB Journal. 2015 Apr; *29*:968-19.

- Saavedra JM. Beneficial effects of Angiotensin II receptor blockers in brain disorders. Pharmacol Res. 2017 Nov 1;125:91-103.

-Sarter M, Bodewitz G, Stephens DN. Attenuation of scopolamine-induced impairment of spontaneous alternation behaviour by antagonist but not inverse agonist and agonist β-carbolines. Psychopharmacology. 1988 Apr;94:491-495.

- Schnegg CI, Kooshki M, Hsu FC, Sui G, Robbins ME. PPARδ prevents radiation-induced proinflammatory responses in microglia via transrepression of NF-κB and inhibition of the PKCα/MEK1/2/ERK1/2/AP-1 pathway. Free Radic Biol Med. 2012 May 1;52(9):1734-1743.

-Schupp M, Janke J, Clasen R, Unger T, Kintscher U . Angiotensin type 1 receptor blockers induce peroxisome proliferator-activated receptor-gamma activity. Circulation 2004; 109: 2054–2057.

- Shi X, Li D, Deng Q, Li Y, Sun G, Yuan X, Song Y, Wang Z, Li X, Li X, Liu G. NEFAs activate the oxidative stress-mediated NF-κB signaling pathway to induce inflammatory response in calf hepatocytes. J Steroid Biochem Mol Biol. 2015 Jan 1;145:103-112.

- Singh KD, Karnik SS. Angiotensin Receptors: Structure, Function, Signaling and Clinical Applications. J Cell Signal. 2016 Jun;1(2).

-So G, Nakagawa S, Morofuji Y, Hiu T, Hayashi K, Tanaka K. Candesartan improves ischemia-induced impairment of the blood-brain barrier in vitro. Cell. Mol. Neurobiol. 2015; 35: 563–572.

-Sturm S, Forsberg A, Nave S. Positron emission tomography measurement of brain MAO-B inhibition in patients with Alzheimer’s disease and elderly controls after oral administration of sembragiline. Eur J Nucl Med Mol Imaging. 2017;44:382–391.

- Tahami Monfared AA, Byrnes MJ, White LA, Zhang Q. Alzheimer's Disease: Epidemiology and Clinical Progression. Neurol Ther. 2022;11(2):553-569.

-Tai SY, Chen CH, Chien CY. Cilostazol as an add-on therapy for patients with Alzheimer’s disease in Taiwan: a case control study. BMC Neurol. 2017;17:40 .

-Takada I, Makishima M. Peroxisome proliferator-activated receptor agonists and antagonists: A patent review (2014-present). Expert opinion on therapeutic patents. 2020 Jan 2;30(1):1-3.

- Takane K, Hasegawa Y, Lin B, Koibuchi N, Cao C, Yokoo T, Kim-Mitsuyama S. Detrimental Effects of Centrally Administered Angiotensin II are Enhanced in a Mouse Model of Alzheimer Disease Independently of Blood Pressure. J Am Heart Assoc. 2017 Apr 20;6(4):e004897.

- Tang Y, Lutz MW, Xing Y. A systems-based model of Alzheimer's disease. Alzheimers Dement. 2019 Jan;15(1):168-171.

-Tian M, Zhu D, Xie W, Shi J. Central angiotensin II-induced Alzheimer-like tau phosphorylation in normal rat brains. FEBS letters. 2012 Oct 19;586(20):3737-3745.

-Tong BC, Wu AJ, Li M, Cheung KH. Calcium signaling in Alzheimer's disease & therapies. Biochim Biophys Acta Mol Cell Res. 2018 Nov;1865(11 Pt B):1745-1760.

- Tota S, Goel R, Pachauri SD, Rajasekar N, Najmi AK, Hanif K, Nath C. Effect of angiotensin II on spatial memory, cerebral blood flow, cholinergic neurotransmission, and brain derived neurotrophic factor in rats. Psychopharmacology (Berl). 2013 Mar;226:357-369.

- Trudler D, Nazor KL, Eisele YS, Grabauskas T, Dolatabadi N, Parker J, Sultan A, Zhong Z, Goodwin MS, Levites Y, Golde TE, Kelly JW, Sierks MR, Schork NJ, Karin M, Ambasudhan R, Lipton SA. Soluble α-synuclein-antibody complexes activate the NLRP3 inflammasome in hiPSC-derived microglia. Proc Natl Acad Sci U S A. 2021 Apr 13;118(15) :e2025847118

-Tuszynski MH, Yang JH, Barba D. Nerve growth factor gene therapy: activation of neuronal responses in Alzheimer disease. JAMA Neurol. 2015;72:1139–1147.

- Valenzuela R, Costa-Besada MA, Iglesias-Gonzalez J, Perez-Costas E, Villar-Cheda B, Garrido-Gil P, Melendez-Ferro M, Soto-Otero R, Lanciego JL, Henrion D, Franco R, Labandeira-Garcia JL. Mitochondrial angiotensin receptors in dopaminergic neurons. Role in cell protection and aging-related vulnerability to neurodegeneration. Cell Death Dis. 2016 Oct;7(10):e2427-.

-Verma M, Wills Z, Chu CT. Excitatory Dendritic Mitochondrial Calcium Toxicity: Implications for Parkinson's and Other Neurodegenerative Diseases. Front Neurosci. 2018;12:523.

-Vik-Mo AO, Bencze J, Ballard C, Hortobágyi T, Aarsland D. Advanced cerebral amyloid angiopathy and small vessel disease are associated with psychosis in Alzheimer's disease. J Neurol Neurosurg Psychiatry. 2019 Jun;90(6):728-730.

- Villapol S. Roles of Peroxisome Proliferator-Activated Receptor Gamma on Brain and Peripheral Inflammation. Cell Mol Neurobiol. 2018 Jan;38(1):121-132.

- Villar-Cheda B, Costa-Besada MA, Valenzuela R, Perez-Costas E, Melendez-Ferro M, Labandeira-Garcia JL. The intracellular angiotensin system buffers deleterious effects of the extracellular paracrine system. Cell Death Dis. 2017 Sep;8(9):e3044.

- Villar-Cheda B, Rodríguez-Pallares J, Valenzuela R, Muñoz A, Guerra MJ, Baltatu OC, Labandeira-Garcia JL. Nigral and striatal regulation of angiotensin receptor expression by dopamine and angiotensin in rodents: implications for progression of Parkinson's disease. Eur J Neurosci. 2010 Nov;32(10):1695-1706.

- Wagner JM, Sichler ME, Schleicher EM, Franke TN, Irwin C, Löw MJ, Beindorff N, Bouter C, Bayer TA, Bouter Y. Analysis of Motor Function in the Tg4-42 Mouse Model of Alzheimer's Disease. Front Behav Neurosci. 2019;13(May):1-13.

-Wallace L, Theou O, Rockwood K, Andrew MK. Relationship between frailty and Alzheimer's disease biomarkers: A scoping review. Alzheimers Dement (Amst) 2018;10:394-401.

-Wang K, Sun QI, Zhong X, Zeng M, Zeng H, Shi X, Li Z, Wang Y, Zhao Q, Shao F, Ding J. Structural mechanism for GSDMD targeting by autoprocessed caspases in pyroptosis. Cell. 2020 Mar 5;180(5):941-955.

-Weber A, Wasiliew P, Kracht M. Interleukin-1 (IL-1) pathway. Science signaling. 2010 Jan 19;3(105): 1-1.

- Winklewski PJ, Radkowski M, Wszedybyl-Winklewska M, Demkow U. Brain inflammation and hypertension: the chicken or the egg? J Neuroinflammation. 2015 Dec;12:1-7.

- Wischik CM, Staff RT, Wischik DJ, Bentham P, Murray AD, Storey JM, Kook KA, Harrington CR. Tau aggregation inhibitor therapy: an exploratory phase 2 study in mild or moderate Alzheimer's disease. J Alzheimers Dis. 2015 Jan 1;44(2):705-720.

-Yu JW, Lee MS. Mitochondria and the NLRP3 inflammasome: physiological and pathological relevance. Archives of pharmacal research. 2016 Nov;39:1503-1518.

- Zhang XX, Tian Y, Wang ZT, Ma YH, Tan L, Yu JT. The Epidemiology of Alzheimer's Disease Modifiable Risk Factors and Prevention. J Prev Alzheimers Dis.2021 April;8,313-321.

-Zaky A, Mohammad B, Moftah M, Kandeel KM, Bassiouny AR Apurinic/apyrimidinic endonuclease 1 is a key modulator of aluminum-induced neuroinflammation. BMC Neurosci. 2013; 11(14):26.

-Zawada WM, Mrak RE, Biedermann J, Palmer QD, Gentleman SM, Aboud O, Griffin WS. Loss of angiotensin II receptor expression in dopamine neurons in Parkinson’s disease correlates with pathological progression and is accompanied by increases in Nox4-and 8-OH guanosine-related nucleic acid oxidation and caspase-3 activation. Acta neuropathologica communications. 2015 Dec;3:1-6.

-Zilberzwige-Tal S, Gazit E. Go with the Flow-Microfluidics Approaches for Amyloid Research. Chem Asian J. 2018 Nov 16;13(22):3437-3447.

الخلاصة:

مرض الزهايمر هو مرض تحلل عصبي يمتاز ببداية وتقدم تدريجي وضعف الوظائف الذهنية والسلوك من ضمنها الذاكرة,طريقة التفكير,الادراك,اللغة والانتباه.حتى الان لم يعرف السبب الاساسي للمرض اوالالات الفسيولوجية وكيف يبدأ. هو مسؤول عن ثلثي حالات فقدان الذاكرة بالمرضى بعمر 65 سنة فما فوق.توجد العديد من النظريات حول نشأته لكن الصفة الاساسية للمرض هي وجود تجمعات غير طبيعية للتشابكات الليفية العصبية واللوحات العصبية في الدماغ. وحتى هذا الوقت لايوجد علاج يوقف تقدمه او يشفيه.لكن توجد بعض العلاجات المستخدمة لأبطاءه ولتقليل اعراضه الى الشكل الذي يسمح للمريض بممارسة حياته.جميع العلاجات السابقة المستخدمة تحتوي الكثير من الاعراض الجانبية كما انها غير فعالة لدرجة كافية.في الاونة الاخيرة يتم التركيز على استخدام الأعشاب في الطب لكونها مواد طبيعية قليلة التأثيرات الجانبية.توجد بعض البحوث السابقة حول استخدام مضادات مستقبلات الانجيوتينسين كذلك توجد بحوث منفصلة عن فوائد نبتة أكليل الجبل على الذاكرة.في هذا البحث قمنا باختيار (التيلميزارتان) لانه يتميز عن مجموعته بانه يمتلك صفة كونه ناهض جزئي للمستقبلات المنشطة بمكاثر البيروكسيسوم(PPARs) وهي عبارة عن مستقبلات نووية تقوم بالعديد من الوظائف من ضمنها التحكم بالسايتوكينات كذلك تتوسط عملية نقل وانتاج الاشارات.ولان البحوث السابقة وجدت علاقة بين الاجهاد التأكسدي والالتهاب مع التنكس العصبي,فيمكن ان يكون لهذا العلاج تاثير ايجابي على المرض.أكليل الجبل او الروزماري (*Rosemarinus officinalis* L) هي عشب خشبي وعطري دائم الخضرة,استخدم منذ العصور القديمة لتعزيز وتقوية الذاكرة. يتميز هذا النبات بفعالية مشابهه للتيلميزارتان فيما يخص(PPARs) .في هذا البحث حاولنا أعطاء المادتين لجرذان التجارب بنفس الوقت لمعرفة التأثير الجمعي وكيف انه يختلف عن تأثير المادتين على حدة.كذلك قمنا بمقارنة التأثير مع العلاج الحاصل على موافقة ادارة الاغذية والعقاقير(دونبزيل).قمنا بتقسيم الجرذان الى ست مجموعات,مجموعة السيطرة التي لم تتسلم شيئ غير النورمل سلاين (normal saline) يوميا عن طريق الحقن داخل الصفاق لمدة 60 يوم.المجموعة الثانية تشمل مجموعة الزهايمر الذي حفزناه فيها عن طريق اعطاء كلوريد الالمنيوم بالحقن تحت الصفاق يوميا بجرعة (10 mg/kg) يوميا لمدة 60 يوم. المجموعة الثالثة هي مجموعة التلميزارتان تسلمت(10 mg/kg) يوميا عن طريق الفم لمدة 60 يومزالمجموعة الرابعة كانت مجموعة مستخلص نبتة اكليل الجبل تسلمت (300 mg/kg) يوميا عن طريق الفم يوميا لمدة 60 يوم.المجموعة الخامسة كانت المجموعة المركبة المكونة من التلميزارتان والمستخلص العشبي بنفس الوقت بشكل فموي بنفس الجرع أعلاه لمدة 60 يوم.والمجموعة الاخيرة

هي مجموعة الدونبزيل الذي استخدمناه لمقارنة النتائج بجرعة (5mg/kg) عن طريق الفم يوميا لمدة 60 يوم. بعد حوالي ٢٤ ساعة من اخر جرعة, قمنا بدراسة الجوانب السلوكية للجرذان.المتاهة Y (Y maze) كانت قد استخدمت لقياس الادراك المكاني والذاكره لدى الجرذان. في المجموعة الثانية التي هي مجموعة التحفيز,الجرذان ذو الذاكرة المنخفضة أظهروا انخفاض في (SAP) في Y maze بشكل كبير($p < 0.01$) مقارنة مع مجموعة التحكم الاولى وبقية المجموعات. من المجموعة 3 الى المجموعة 6 كان هناك ارتفاع بشكل كبير ($p < 0.01$) لنسبة التغيير العفوي (SAP) على متاهة Y. بعد أكمال الدراسة السلوكية, قمنا بقطع رؤوس الجرذان واستخرجنا الأدمغة والهيبوكامبص (قرن امون) كي ندرس تأثير هذه العناصر على مجموعة من المؤشرات الحيوية من ضمنها مالونداياالديهايد (MDA),المؤشرات الالتهابية (IL6, IL 1) ,عامل نخر الورم الفا (TNFα) ,النشاط الكلي لمضادات الاكسدة (TAOC) وكذلك الكلوتاثيون (GSH) عن طريق استخدام تقنية المقايسة الامتصاصية المناعية للانزيم المرتبط او الأيلايزا(ELISA). كذلك فيما يخص النتائج الكيميائية الحيوية, المجموعة الثانية انخفض (GSH) و(TAOC) بشكل كبير ($p < 0.01$) بالمقارنة مع مجموعة التحكم الاولى, بينما كان هنالك زيادة بشكل كبير($p<0.001$) في (IL1,IL6,MDA,TNFα) .في المجموعة 3 كان هناك زيادة بشكل ملحوظ في الجلوتاثيون ($p<0.05$) بينما في المجموعة 4 الى المجموعة 6 كان هناك زيادة بشكل كبير في الجلوتاثيون والنشاط الكلي لمضادات الأكسدة ($p < 0.01$) بينما الزيادة في النشاط الكلي لمضادات الأكسدة في المجموعة 3 كان بشكل ملحوظ ($p<0.05$) وانخفاض بشكل كبير($p < 0.01$) في (IL1,IL6,MDA,TNFα) في المجموعة 3 الى االمجموعة 6 مقارنة مع المجموعة الثانية (مجموعة التحفيز).أما فيما يخص نتائج تشريح الانسجة, مجموعة الجرذان التي تسلمت كلوريد الالمنيوم لتحفيز الزهايمر,اظهرت تغيرات شكلية واضحة في انسجة الدماغ كذلك التهاب متوسط مقارنة مع مجموعة التحكم الاولى.اما فيما يخص مجموعات التلميزارتان والروزماري ومجموعة المزيج كان هناك تحسن لحالة الالتهاب في انسجة الدماغ.اما فيما يخص مجموعة الدونبزيل,كانت اقرب الى مجموعة التحكم الاولى فيما يخص حالة التهاب انسجة الدماغ.

في الاستنتاج, التلميزارتان والروزماري يمتلكان خواص مضادة للاكسدة والالتهاب كما جاء موضح في الاختبارات السلوكية والكيميائية الحيوية حيث انهما يعملان على زيادة المؤشرات الحيوية المضادة للاكسدة ويقللان من العوامل الالتهابية وهذا له تاثير ايجابي على الاعصاب حيث يقومان بحماية الاعصاب من التاثيرات السلبية للعوامل الالتهابية والجذور الحرة.وجمعهما بنفس الوقت كان له تاثير اكبر من اعطاء كل مادة على حدة.لهذا يمكن استعمالهم لتقليل اعراض مرض الزهايمر.

جمهورية العراق
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جامعة بابل/ كلية الطب
قسم الأدوية

تأثيرات التلميزارتان مع أكليل الجبل على نموذج الجرذان المستحث بالزهايمر

رســـالـــــــة

مقدمـــــة إلـــى مجلس كليـــة الطـــب / جامعـــة بابـــل

كجزء من متطلبـــات نيـل درجـــة الماجستيـر في علم الأدوية

من قبل

صبا عبد الرحيم مخيف حسن

بكالوريوس صيدلة (2016)

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