

Neuroprotective Effects of β -Caryophyllene, Cinnamic Acid, and *p*-Coumaric Acid against Chemical-Induced Dementia in Mice

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By

Harsimran Singh

Registration Number: 41500120

Supervised By

Dr. Navneet Khurana (18252)

Department of Pharmacology (Professor)

Lovely Professional University

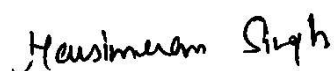


LOVELY PROFESSIONAL UNIVERSITY, PUNJAB

2026

DECLARATION

I hereby declare that the presented work in the thesis entitled “**Neuroprotective Effects of β -Caryophyllene, Cinnamic Acid, and *p*-Coumaric Acid against Chemical-Induced Dementia in Mice**” in fulfillment of the degree of **Doctor of Philosophy (Ph.D.)** is the outcome of research work carried out by me under the supervision of Dr. Navneet Khurana, working as Professor, in the Department of Pharmacology of Lovely Professional University, Punjab, India. In keeping with the general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on the findings of other investigators. This work has not been submitted in part or full to any other university or institute for the award of any degree.



Harsimran Singh

Registration No.: 41500120

School of Pharmaceutical Sciences

Lovely Professional University,

Punjab, India

CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled “**Neuroprotective Effects of β -Caryophyllene, Cinnamic Acid, and *p*-Coumaric Acid against Chemical-Induced Dementia in Mice,**” submitted in fulfillment of the requirement for the award of the degree of **Doctor of Philosophy (Ph.D.)** in the Department of Pharmacology, is a research work carried out by Mr. Harsimran Singh, 41500120, and is a bonafide record of his original work carried out under my supervision and that no part of the thesis has been submitted for any other degree, diploma, or equivalent course.



Dr. Navneet Khurana

Professor

School of Pharmaceutical Sciences

Lovely Professional University,

Punjab, India

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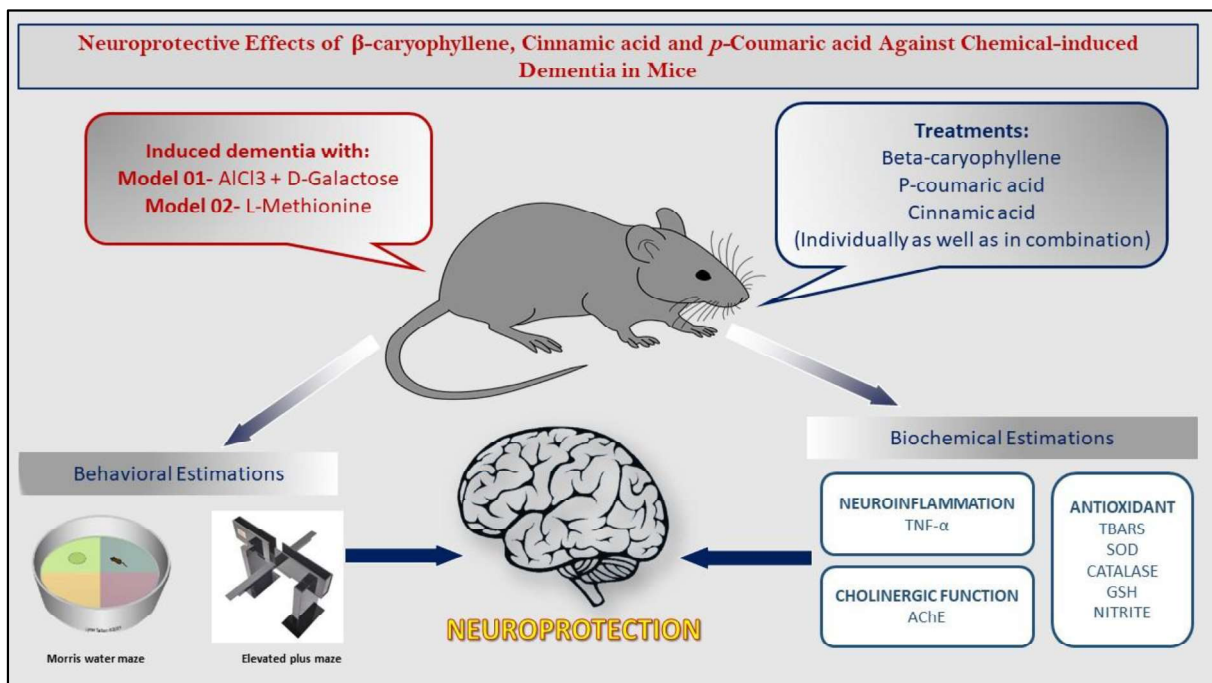
My most sincere and heartfelt gratitude towards all the animals who sacrificed their invaluable lives for the sake of advancement in the field of medicine and pharmacotherapy of Dementia.

Harsimran Singh

ABSTRACT

Dementia is a neurological disease that becomes worse over time. One of its most common signs is the inability to do everyday chores on your own. Many different diseases can cause memory loss and cognitive decline, but dementia has a wide range of symptoms and neuropathological causes. Alzheimer's disease (AD) is the most commonest type of dementia, after AD is vascular dementia. It can be hard to tell the difference between types since the underlying mechanisms are hard to tell apart and the symptoms tend to overlap. The exact mechanism is still not clear; however, there are a few ideas that could explain it. One of these possibilities is the amyloid beta theory, which says that the formation of amyloid beta plaques causes inflammation, disrupts neural processes, and leads to cognitive impairments. An alternate theory elucidates the role of AChE in the initiation of cognitive decline. An alternative idea posits that energy supplies are exhausted due to mitochondrial dysfunction. Oxidative stress can cause neurodegeneration and cognitive impairment by changing the amounts of antioxidants and free radicals. Dementia is caused by neurodegenerative illnesses or the gradual death of brain cells. Research continues to investigate potential treatments, while preventative techniques emphasize lifestyle adjustments, cognitive engagement, and general brain health, resembling symptomatic alleviation. Donepezil is one of the most commonly utilized treatments right now. It is an AChE inhibitor that seeks to boost cholinergic functioning, which may help the disease get better. Rivastigmine and galantamine are two other medicines that stop AChE. NMDA receptor antagonists are another type of medication that is now being employed. Memantine is the most common NMDA receptor antagonist. Based on prior studies, it was understood that interoceptive model 01, which entails the blend of AlCl₃ and D-galactose, can provoke oxidative trauma, neuroinflammation, variations in cholinesterase enzyme activity, and pathogenic pathways linked to Alzheimer's disease (AD). Thus, the abovementioned factors make cognitive dysfunction and memory problems worse. Additionally, disease model 02, marked by the high multiple doses of L-methionine, may induce vascular dysfunction, neurodegeneration, neuroinflammation, oxidative stress, and Alzheimer's disease-like pathological alterations. No previous studies have used both interoceptive models in the same investigation. As a result, both animal models provide a more comprehensive view of the disease by focusing on more pathogenic processes and allowing for a thorough examination of the test interventions individually or in various combinations. These test drugs, either individually or in combination, have shown possible advantages for cholinergic functioning, oxidative stress, neuroinflammation, and better vascular health, which means they could help with diseases connected to AD. Beta-caryophyllene has been shown to

be an antioxidant. It may also stimulate the cannabinoid receptor type 2, which can aid with cognitive problems. It is also said to stop excitotoxicity caused by glutamate. P-coumaric acid and cinnamic acid are said to be powerful antioxidants, as well as anticholinesterase and anti-inflammatory agents. It was postulated that these three phytoconstituents could mitigate the cognitive deficits induced by the interoceptive agents. The administration of beta-caryophyllene, p-coumaric acid, and cinnamic acid has not only shown efficacy in treating dementia but also identified prospective therapeutic targets for the condition. They focus on fixing the imbalance between free radicals and antioxidants that was caused by interoceptive models. This makes things better. They also focused on neuroinflammation and greatly reduced it by lowering the levels of certain pro-inflammatory proteins. This was also the cause of the memory and learning problems. They also made the cholinergic activities that are important for memory and learning a lot better by lowering the influence of AChE in the brain. The study also looked at the synergistic effect of these phytoconstituents when they were combined. The outcomes of the phytoconstituent combinations were noteworthy, indicating the potential for combinational pharmacological therapy in dementia care by addressing many pathogenic causes, hence expanding the therapeutic spectrum.



Keywords: Dementia, neurodegeneration, beta-caryophyllene, p-coumaric acid, cinnamic acid, neuroprotection.

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List of Abbreviations

Abbreviation	Full Form
AChE	Acetylcholinesterase
AD	Alzheimer's Disease
AGE	Advanced glycation end products
ALS	Amyotrophic lateral sclerosis
AMDA	Anti-NMDA
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of Variance
APP	Amyloid- β protein precursor
ATChI	Acetylthiocholine iodide
AXT	Astaxanthin
A β	Amyloid Beta
BACE1	A β PP cleaving enzyme 1
BBB	Blood brain barrier
BChE	Butyrylcholinesterase
BCP	Beta-caryophyllene
BCPHD	Beta-caryophyllene high dose
BCPLD	Beta-caryophyllene low dose
BDNF	Brain-Derived Neurotrophic Factor
CA	Cinnamic Acid
CAD	Cinnamic acid derivatives
CAF	Caffeic acid
CAHD	Cinnamic acid high dose
CALD	Cinnamic acid low dose
CAT	Catalase
CB2R	Cannabinoid type 2 receptor
CBD	Cannabidiol
CBF	Cerebral blood flow
CCD	Canine cognitive dysfunction
CE	Chromatography and capillary electrophoresis
CGA	Chlorogenic acid
ChEI	Cholinesterase inhibitors

COM01	Combination 01
COM02	Combination 02
COM03	Combination 03
CoQ10	Coenzyme Q10
COX	Cyclooxygenase
CTME	Caralluma tuberculata methanolic extract
CYP1A2	Cytochrome P450 1A2
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
DTNB	5,5'-Dithiobis(2-nitrobenzoic acid)
EGCG	Epigallocatechin gallate
ELT	Escape latency time
EPM	Elevated plus maze
ER	Endoplasmic reticulum
GLP1	Glucagon-like peptide 1
GSH	Glutathione
GSH-Px	Glutathione peroxidase
HCA	Hydroxycinnamic acid
HPT	Hisperetin
HP β CD/BCP	β -caryophyllene and β -hydroxypropyl- β -cyclodextrin
I/HPC	Ischemic/Hypoxic preconditioning
IL	Interleukin
IL-6	Interleukin-6
JAK2/STAT1	Janus kinase 2/signal transducer and activator of transcription 1
L-MET	L-methionine
LOX	Lipoxygenase
LPS	Lipopolysaccharide
M01	Disease model 01
M02	Disease model 02
MAO	Monoamine oxidase
MAP	Mitogen-activated protein
MAPK	Mitogen-activated protein kinase
MAPK	Mitogen-activated protein kinase
MCAO	Middle cerebral artery occlusion
MCI	Mild cognitive impairment

MDA	Malondialdehyde
MPP+	1-methyl-4-phenylpyridinium
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MS	Mass Spectroscopy
mTOR	Mechanistic target of rapamycin
MWM	Morris water maze
NADPH	Nicotinamide adenine dinucleotide phosphate
NBP	3-N-butylphthalide
NBT	Nitro-blue tetrazolium
NEP	Nepilysin
NF- κ B	Nuclear factor- κ B
NFT	Neurofibrillary tangles
NLRP3	NOD-, LRR- and pyrin domain-containing protein 3
NMDA	N-methyl-D-aspartate
NO	Nitric Oxide
NOS	Nitric oxide synthase
NQO1	NADPH quinone oxidoreductase
PCA	P-Coumaric acid
PCAHD	P-coumaric acid high dose
PCALD	P-Coumaric acid low dose
PD	Parkinson's disease
PON2	Paraoxonase 2
PPAR γ	Peroxisome proliferator-activated receptor- γ
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
ROT	Rotenone
RV	Resveratrol
SA	Sinapic acid
SAM	S-adenosyl methionine
SD	Sandhoff disease
SGLT2	Sodium glucose cotransporter 2
SGLT2i	Sodium glucose cotransporter 2 inhibitor
SIRT1	Silent information regulator 1
SN	Substantia nigra

SNpc	Substantia nigra pars compacta
SOD	Superoxide dismutase
SOD	Superoxide dismutase
STZ	Streptozotocin
TLT	Transfer latency time
TNF- α	Tumor necrosis factor- alpha
TSD	Tay-Sachs disease
TSTQ	Time spent in target quadrant

Chapter 01
INTRODUCTION

1. INTRODUCTION:

The brain controls our thinking, feeling, perceiving, learning and memory, curiosity, and behavior (Parle et al., 2006). Memory is the brain's most complicated job. It includes many parts, such as perception/sensation, registration, consolidation, storage, retrieval, and decay/forgetting (Lindeboom & Weinstein, 2004). Among the cognitive functions, first comes learning, in which we educate ourselves about something, which can be a language, a process, an activity, a task, etc., and second is memory, which enables us to redo something that we have learned (Okano et al., 2000). These two cognitive functions let us do things around something that we know from our past experiences (Liu et al., 2022). Memory can be sensory (registration), short-term, or long-term. Consolidation is the process of turning short-term memory into long-term memory (Robbins & Murphy, 2006), Life without memory is not life at all. Our memories shape who we are, how we think, how we feel, and even what we do. Without it, we are nothing.

As people get older, they start to lose their ability for learning and memory and become unable to memorize new things and recall already memorized things. They also start to forget with their growing age. This does not affect their daily activities. People who have memory problems often use adaptive strategies like making lists and using sticky notes to stay safe and independent (Dementia and Cognitive Impairment guidelines). There are four main types of memory: episodic memory, semantic memory, procedural memory, and working memory. Dementia, Alzheimer's disease, Parkinson's disease, multiple sclerosis, Korsakoff syndrome, and other neurodegenerative diseases are all part of these systems. Among all these disorders, the most prevalent form of neurodegenerative disorder is dementia. Dementia is a condition marked by significant impairments in memory and at least one additional cognitive function—such as learning, reasoning, language, spatial awareness, orientation, or the management of complex tasks—that disrupt daily living activities (Dementia & Cognitive Impairment, Guideline). Changes in the brain that produce dementia may not last long, but they usually do and get worse over time, making people more disabled and shortening their lives. According to a report published by WHO in 2025, in 2021 the total number of people affected by dementia is 57 million, and the number is increasing by 10 million every year (Dementia, n.d.). According to the very same report, dementia stands seventh leading cause of death. According to a report published by Alzheimer's Disease International, the number of cases dementia is doubling every 20 years, the number will reach 78 million by 2030 and by 139 million by 2050 (Alzheimer's disease, 2020). Alzheimer's disease (a problem with brain proteins) is the most

common cause of dementia, followed by vascular dementia (Chun, 2005). This disorder frequently commences with memory loss or with nuanced deficits in other cognitive functions. These alterations might first show up as ordinary forgetfulness or absentmindedness, or as small issues with language, judgment, or vision. As dementia gets worse, memory loss and cognitive impairment get worse too, until the person can't remember fundamental social and survival skills or do things on their own. Language, spatial or temporal orientation, judgment, perception, and other cognitive abilities deteriorate, accompanied by potential alterations in personality (Parle & Singh, 2007).

It is disclosed that Alzheimer's patients exhibit diminished amounts of acetylcholine in their brains. Other significant elements, such as NMDA and AMPA receptors, also significantly influence neural plasticity, including long-term potentiation and long-term depression, which presumably elucidates their involvement in learning and memory. Glutamate is very harmful to neurons because it activates NMDA, AMPA, and metabotropic receptors, which depolarize the cell even more, open NMDA channels, and let more calcium in. This raises the level of glutamate outside the cell, which activates proteases and lipases, nitric oxide synthase, and ROS, which harm many important biomolecules, proteins, and DNA (Z. R. Chen et al., 2022).

Reactive oxygen species (ROS) are responsible for damage to neurons. Reactive oxygen species (ROS) include hydrogen peroxide (H_2O_2), nitric oxide (NO), superoxide anions, and the extremely reactive hydroxyl and monoxide radicals ($OH\cdot$, $NO\cdot$). Damaged mitochondria and activated microglia operate as storage spaces for reactive oxygen species (ROS). At first, it was considered that reactive oxygen species (ROS) were only created when there was an imbalance between their production and elimination, together with reactive nitrogen species (RNS). Recent advancements in chemistry and molecular biology have elucidated several mechanisms that regulate reactive oxygen species (ROS), which are vital for the modulation of important cellular activities (Rueggsegger et al., 2019). The production of free radicals and reactive oxygen species (ROS) initiates the mitogen-activated protein (MAP) kinase cascade, which transports calcium into excited cells, ultimately leading to cell death via apoptosis (Schipper, 2004). Free radicals are the leading cause of various neurological disorders like Parkinson's disease, neurological ischemia, epilepsy, bipolar disorder, and AD (Ionescu-Tucker & Cotman, 2021).

For more than a decade, the mainstay of Alzheimer's disease treatment development has been methodologies that improve central cholinergic function. The methodology is based on the cholinergic hypothesis of Alzheimer's disease, which says that memory performance

gets worse when cholinergic neurotransmission in the hippocampus and cortex goes down (Bergmann et al., 1978). Pharmacological interventions designed to improve this pathophysiology, leading to enhancements in the fundamental cognitive impairments of the disorder, have understandably sparked interest in the application of acetylcholinesterase inhibitors such as rivastigmine, tacrine, donepezil, and galantamine for dementia treatment (Holden & Kelly, 2002). Nonetheless, NMDA receptor antagonists such as memantine may also be utilized in the therapeutic regimen.

β -caryophyllene (BCP) is a naturally found sesquiterpene and which is found as a foremost plant volatile in large amounts in the essential oils of several spices and plants used as food, like oregano, cinnamon, and black pepper. BCP has been reported to be a selective agonist of cannabinoid receptor of type 2 (CB₂) (Al Mansouri et al., 2014; Gertsch et al., 2008); which is used in a previous study to prevent Alzheimer's disease (AD) (Cheng et al., 2014). Some studies revealed that BCP exhibits protective effect against central glutamate-induced excitotoxicity (Assis et al., 2014). BCP previously used in ischemic models (Choi et al., 2013). BCP also has already proven antioxidant activity (Calleja et al., 2013); hence, it can be estimated that BCP can be incorporated into memory disorders. Still very little is known about the direct role of BCP in dementia. Therefore, we examined the effects and mechanisms of BCP against dementia.

P-Coumaric acid (PCA) is a hydroxyderivative of cinnamic acid which is present in fruits, vegetables, and plant products. Propolis, cranberry, tomatoes, grapes, rice, and apple cider are the commonest and daily used herbals which contains PCA (Zhao & Liu, 2016), it can also be biologically metabolised from phenolic acids such as rosmarinic acid and chlorogenic acid (Yang et al., 2013). PCA previously has been challenged for its potential as, anti-platelet agent (Luceri et al., 2007), antioxidant (Shaheen, 2011), against protective effect in anti-Alzheimer's disease (J. H. Yoon et al., 2014), anti-metabolic disorder (S. Yoon et al., n.d.), and for its anti-inflammatory activities (Pragasam et al., 2013). Plants containing p-coumaric acid also been reported to lower the brain AChE levels (Szwajgier, 2013). Hence, it can be used against memory disorders.

Cinnamic acid and its derivatives (CADs) are widely present various naturally fruits, vegetables, and flowers. Because of its presence in various eatables, it is possibly consumed day to day. Cinnamic acid and its derivatives are used commercially in various products in pharmaceutical and cosmetic industry. In this study, cinnamic acid is used on the basis of its previously performed activities like antioxidant (Ahn et al., 2007), hepatoprotective (Kaur & Kaur, 2022), anxiolytic (Reis et al., 2014), antidiabetic (Hafizur et al., 2015), and

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anticholesterolemic (Coelho-e-Silva et al., 2013), etc. Different derivatives derived from the cinnamic acid like, m-hydroxy or p-methoxy residue on cinnamic acid (CA) had explored for its potential as insulin-releasing agent in animal model of diabetes (Ohnishi et al., 2004), other derivative 3, 4-dihydroxycinnamic acid i.e., caffeic acid had explored for its hepatoprotective activity (Yang et al., 2013). Various previous studies proves that cinnamic acid and its derivative tends to lower the brain AChE levels in various animal models (J. Chen et al., 2008).

Using beta-caryophyllene, p-coumaric acid, and cinnamic acid individually as well as together can help fight dementia in animal models in several ways. The test substances can target several pathogenic events, such as oxidative stress, neuroinflammation, and changes in cholinergic activities caused by AChE individually as well as in combinations. This could be very helpful for treatment of dementia. The selected intervention has not been assessed in the specified animal models, either individually or in combination, rendering this study unique.

In this study, we have investigated the antioxidant property of BCP and AChE-lowering activity and the antioxidant activity of cinnamic acid and *p*-coumaric acid against chemically induced dementia in mice.

Chapter 02
REVIEW OF LITERATURE

02. REVIEW OF LITERATURE

(Karvandi et al., 2023) Lots of different diseases can impact the CNS, but the most common ones are neurodegenerative ones, such as ALS, Alzheimer's disease, Parkinson's disease, and Huntington's disease. The degeneration of neurons is a hallmark of these diseases. Different diseases have different symptoms and signs, but they all have the same ways of making people sick. Patients with increasing regional neuronal loss are more likely to have problems with memory, motor skills, and thinking, as well as a higher risk of death. Neurodegenerative disorders can result in neuronal cell loss through various pathways and conditions. Most neurodegenerative diseases are characterized by protein misfolding and aggregation, mitochondrial dysfunction, the generation of reactive oxygen species (ROS), and the activation of the innate immune response. Consequently, the primary pathogenic mechanisms responsible for neuronal cell death are oxidative stress, neuroinflammation, and endoplasmic reticulum (ER) stress. It is widely recognized that the aforementioned components substantially facilitate neuronal death, despite the precise mechanisms remaining inadequately elucidated. Researchers are looking into the neuroprotective effects of targeting underlying pathways in the hopes of finding a way to treat diseases that works. This article talks about how ER stress, oxidative stress, and neuroinflammation can kill neurons and focuses on the neuroprotective benefits of targeting these pathways or parts.

(Angeloni et al., 2023) There is a wide range of symptoms that are associated with neurodegenerative disorders. These symptoms show as a slow degeneration of brain cells, connections throughout the nervous system, and ultimately the death of cells. These disorders make it easier for users to experience a decline in key cognitive and motor functions, such as the ability to move, learn, and feel. As of right now, there is no comprehensive treatment available for neurodegeneration, which is a condition that affects millions of people all over the world. It has been established that bioactive compounds have a variety of beneficial effects as a consequence of their amazing bioactivity; hence, these molecules may be great candidates for the development of innovative neuroprotective strategies. Recent research has demonstrated that plant by-products, particularly pulp, can have a significantly higher phenolic content than the edible sections of the plant themselves. This is despite the fact that it is true that plants can contain phenolic bioactives through their edible components. Therefore, one of the potential ways to repurpose these

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agricultural byproducts (those that are obtained from the processing of plants) is to use them as a novel neuroprotective method. To provide a concise overview of the key factors that can cause neurodegeneration, to update readers on the current research on the use of plant extracts to prevent neurodegeneration, and to discuss the potential neuroprotective benefits of the primary phenolic compounds that are found in these extracts, the purpose of this review is to provide an overview at a high level. To achieve this objective, thorough literature research was carried out, which resulted in the discovery of a great number of papers that provided information regarding the neuroprotective effects of phenolic compounds that were produced from by-products. Last but not least, phenolics continue to be interesting for research into new multifactorial anti-neurodegenerative nutraceuticals, despite the fact that their surprising lack of exploration substantially restricts their application.

(Evans et al., 2022) Neurological ailments impact around 50 million Americans annually, and an aging population is linked to a higher risk of these illnesses. Amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Alzheimer's disease all have two hallmarks: neuroinflammation and oxidative stress. These variables play a role in the onset, progression, and worsening of these neurodegenerative illnesses. Many naturally occurring chemicals have been the subject of extensive research due to their antioxidant, anti-inflammatory, and neuroprotective capabilities. Extensive research in animal models of neurodegenerative diseases has shown that the flavonoid hesperetin (HPT), an aglycone of hesperidin present in citrus fruits like mandarins, oranges, and lemons, possesses neuroprotective characteristics. Several studies have used high-performance tissue engineering (HPT) in in vivo or in vitro scenarios to acquire a deeper knowledge of neurodegeneration. These findings are included in this review. Investigating how hesperetin influences the secretion of mediators that protect cells from oxidative and inflammatory stress was our principal goal. Also covered in this work is the process via which hesperetin prevents neurodegeneration. To achieve this, hesperetin controls autophagy and keeps miRNA levels stable. In addition, the evidence that HPT can penetrate the blood-brain barrier is examined in this review. Additionally, this work aims to offer experimental evidence that hesperetin may have neuroprotective properties. The available research suggests that hesperetin can help postpone the development of neurodegenerative diseases.

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(Xu et al., 2022) Biologically active macromolecular complexes include polysaccharides. Multiple lines of evidence from studies in both live animals and controlled lab environments point to polysaccharides' protective effects on neurons. Thus, these polysaccharides may have therapeutic and preventative potential against neurodegenerative diseases. The recent developments in our knowledge of the neuroprotective effects of bioactive polysaccharides are the focus of this investigation. Articles published between 2015 and 2020 are also summarized in the study. Fifteen bioactive polysaccharides derived from plants and fungi are going to be reviewed in this article. The primary mechanism by which these polysaccharides mitigate neurotoxic effects of excitatory amino acids, neuroinflammation, oxidative stress, and apoptosis is by modulation of nuclear factor. mitochondrial protein kinase, kappa-B, nuclear factor-E2-related factor, phosphatidylinositol-3-kinase/protein kinase B. Many routes involve leucine. There are a number of signaling pathways that are engaged in multiple pathways, including rich repeats and pyrin domains. The use of naturally occurring bioactive polysaccharides in the treatment and prevention of neurodegenerative disorders is being considered. This is due to their ability to target multiple locations simultaneously, minimal toxicity, and synergistic effects. However, up until now, the great majority of studies in this area have relied on animal and cell models. Confirmation of the therapeutic advantages of these neuroprotective polysaccharides in patients suffering from neurodegenerative disorders will require large-scale randomized clinical trials in the future.

(Mendes et al., 2022) From mild cognitive impairment (MCI) to moderate and severe clinical stages of Alzheimer's disease (AD) dementia, this neurodegenerative disease progressively worsens over time. The underlying causes of Alzheimer's disease are thought to be complex and multifactorial. Tacrine, donepezil, rivastigmine, and galantamine are some examples of the most commonly used cholinesterase inhibitors (ChEIs) for the treatment of Alzheimer's disease (AD). Good news for those with Alzheimer's disease: there's a surprise chance that ChEI also has neuroprotective properties. Researchers have looked into the ways ChEI works to prevent Alzheimer's disease. Neuronal function and neuroprotection are both affected by the PI3K/AKT signaling cascade, which activates cell survival pathways. One important signaling cascade that ChEI can affect is this one. Despite this, neuroprotection remains a difficult procedure. New opportunities for the development of more efficient medicines that target many proteins simultaneously have emerged, however, within the framework of comprehending the PI3K/AKT pathway.

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(Yu et al., 2023) Due to the fact that it is the organ that has the highest oxygen need, the brain has a low oxygen tolerance as well as a tolerance to ischemia and hypoxia. Even while severe ischemia and hypoxia can induce a multitude of illnesses that affect the central nervous system (CNS), sublethal injuries have the potential to develop tolerance and, as a result, offer a significant degree of protection against more fatal injuries. In order to cure disorders that are linked with the central nervous system and are caused by ischemia or hypoxia, it is essential to look for strategies to ameliorate circumstances that are characterized by ischemia or hypoxia in the brain. Ischemic/hypoxic preconditioning (I/HPC) is a strategy that involves exposing the body to moderate ischemic/hypoxic circumstances for a short period of time. The goal of this strategy is to raise the body's tolerance for potentially more severe ischemia/hypoxia occurrences in the future. It is believed to be a potential therapy option for illnesses that impact the central nervous system, and it has been the subject of a significant amount of research. Inducing autophagy, activating hypoxia signaling pathways, reducing inflammation, and reducing oxidative stress were some of the different techniques that it utilized in order to protect itself. One of the most fascinating new areas of research in the field of neurotherapy is the I/HPC approach, which is a technique that tries to produce endogenous neuroprotection. Within the scope of this, we provide a concise summary of the workings of I/HPC and discuss its progress in both basic and clinical research on central nervous system disorders. In addition to this, we discuss the challenges and limitations that are associated with transporting them from the laboratory to the clinic.

(Pawlos et al., 2021) In people with diabetes, cognitive impairment and cardiovascular disease are more prevalent than in other patients. SGLT2 inhibitors, which include sotagliflozin, empagliflozin, canagliflozin, dapagliflozin, and ertugliflozin, are the most recent class of hypoglycemic treatments. These medications have a wide range of positive side effects. During this review, we will discuss the neuroprotective capabilities that they possess. When it comes to lipid-soluble SGLT2 inhibitors (SGLT2i), the ratio of brain to serum might range anywhere from 0.3 to 0.5. There are SGLT receptors found in the central nervous system (CNS). Because they bind to the SGLT1 receptor, flozins protect the brain from ischemia/reperfusion injury. However, flozins are not totally selective for the SGLT2 receptor. The reduction of proinflammatory cytokines, the polarization of M2 macrophages, the suppression of the JAK2/STAT1 and NLRP3 inflammasomes, and the regression of cIMT are all instances of the anti-inflammatory and anti-atherosclerotic actions that are exhibited by SGLT2i. As a result, they lessen the impact that free

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radicals have on cells. Protected by SGLT2i are the neurovascular unit, pericytes, astrocytes, microglia, and oligodendrocytes, as well as the blood-brain barrier. Endothelial function is enhanced and remodeling is decreased by SGLT2i. One component of flozins' ability to improve cognition is their ability to suppress AChE. An increase in brain BDNF, which controls neurotransmission and guarantees the longevity, adaptability, and growth of neurons, is one of the most notable negative side effects of empagliflozin. Beyond that, they may be able to return the circadian regularity of mTOR activation, which is a huge step forward in the study of metabolic diseases and cognitive impairment. Patients with type 2 diabetes mellitus may greatly benefit from SGLT2i due to its ability to prevent atherosclerosis and cognitive deterioration.

(Griñán-Ferré et al., 2021) The prevalence of neurological illnesses is soaring at the same time that the percentage of the population that is over the age of 65 continues to increase. A person's rate of aging can be affected by a variety of factors, including their food, the amount of physical activity they engage in, the level of education they have, and other aspects of their lifestyle. The neurological disorders that are due to neuronal damage, like AD and Parkinson's disease, can be managed with the help of day-to-day nutritional and dietary interventions. For example, resveratrol can be a potent neuroprotective agent due to its antioxidant and cytoprotective properties. The accumulation of reactive oxygen species and other free radicals due to their overproduction and mismanagement can speed up the process of aging. One of the most significant effects of reactive oxygen species (ROS) within the cell is the damage they cause to the genome. According to the knowledge that is currently available, OS has the potential to induce additional molecular alterations. These alterations include abnormalities in mitochondrial function, inflammation, apoptosis, and epigenetic changes. That the dietary polyphenol RV has pleiotropic properties, which means that it exerts its antioxidant efficacy through a number of different molecular mechanisms, is something that should be taken into consideration. Modern research has also shown that RV is responsible for epigenetic changes that affect aging and CNS function and are passed down through generations. These abnormalities are inherited from one generation to the next. Research has also shown that RV changes the types of bacteria that live in the intestines, which leads to good results. It has been demonstrated through a variety of experimental models, including clinical trials, that RV displays neuroprotective properties. We will provide a summary of these findings and an explanation of how the interaction of these chemical events may be responsible for these effects in this review post.

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(Mohd Sairazi & Sirajudeen, 2020) Extensive research has recently focused on the therapeutic potential of natural products derived from plants, animals, and fungi, as well as their bioactive components, for a wide range of diseases, including cardiovascular, diabetes, hypertension, reproductive, cancer, and neurodegenerative disorders. Studies on these all-natural substances have been conducted. Progressive malfunction and loss of neuronal structure and function leading to neuronal cell death characterize neurodegenerative disorders such as Alzheimer's disease, Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis. One possible approach to preventing and treating neurodegenerative diseases is a neuroprotection strategy that aims to restore function to damaged neurons while preventing cell death. This is due to the fact that numerous factors contribute to the development of neurodegenerative illnesses, which in turn are associated with numerous pathogenic pathways. New evidence suggests that certain naturally occurring compounds may have neuroprotective properties that might provide hope for the eventual treatment of neurodegenerative illnesses. The major goal of this review was to examine the neuroprotective effects of natural products and the bioactive compounds contained within them in relation to the pathologies of neurodegenerative illnesses.

(W. Chen et al., 2020) Molecular hydrogen (H_2) gas does not have any effect on the body's physiological processes. However, over the course of the last ten years, an increasing amount of evidence has surfaced that demonstrates its biological activities in sick environments. Hydrogen dioxide (H_2) has the potential to alleviate symptoms of a variety of diseases and conditions; however, it is most successful in treating conditions that impact the nervous system. Ischemia/reperfusion injury, trauma, subarachnoid hemorrhage, neuropathic pain, neurodegenerative disorders, postoperative cognitive impairment, anxiety, depression, and hypoxia are some of the conditions that fall within this category. Furthermore, hydrogen also maintains the operation of mitochondria and the blood-brain barrier, controls autophagy, and functions as an antioxidant, anti-inflammatory, anti-apoptotic, and regulator of cell death. One of the most promising therapy approaches in clinical settings is the administration of hydrogen peroxide (H_2O_2) because it is simple to use, possesses neuroprotective qualities, and does not have any major adverse effects. In this section, a summary of the pharmacokinetics and H_2 donors is provided. During this interim period, we investigate the safety and effectiveness of hydrogen peroxide (H_2O_2) in both animal and human studies. Our findings lead us to the conclusion that H_2 has the potential to be a straightforward and efficient clinical treatment for diseases that affect the

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central nervous system, such as ischemic-reperfusion brain injury, Parkinson's disease, and disorders that are characterized by cognitive dysfunction. It is being investigated whether there are any pathways that could be responsible for the neuroprotective benefits of hydrogen.

(C. H. Yang et al., 2021) An excessive amount of lipids, a low antioxidant capacity, and a high energy requirement make the brain an easy target for oxidative stress. Reactive oxygen species (ROS) cause oxidative alterations in the brain that increase the vulnerability of neurons to damage and functional impairments; this, in turn, contributes to neurodegenerative disorders. When there is an aberrant or excessive amount of reactive oxygen species (ROS) or metal overload in the brain, the cellular defense mechanisms, intracellular signaling, and antioxidant physiological processes handle it. Numerous single and complex antioxidant medications that target oxidative stress, redox metals, and neuronal cell death have been investigated in various preclinical and clinical trials with the aim of combating the oxidative stress linked to neurodegenerative disorders. To help safeguard the brain from oxidative damage, we provide antioxidant treatment regimens and give a broad review and analysis of antioxidants in this article. Lastly, we go over what our findings could mean. This review delves into the various enzymatic and non-enzymatic antioxidant processes found in the brain. Also included are the pros and cons of using various methods to assess antioxidant chemicals for the treatment of neurodegenerative diseases.

(Rehman et al., 2019) The abundance of land and marine natural resources is a gift from Mother Nature. In order to stay healthy and avoid getting sick, natural commodities are crucial, and this is true for both humans and animals. Experimental evidence suggests that these naturally occurring compounds have multiple biological functions, including anti-inflammatory, free radical scavenging, and apoptosis inhibition. Natural products have shown promise in many preclinical models of neurodegenerative diseases, according to in vitro and in vivo research. Polyphenolic antioxidants are one type of phytoconstituent found in natural products. Produce from both land and sea, as well as herbs, fruits, nuts, and vegetables, are all part of this category of natural goods. In addition to reducing the rate of neurodegeneration, these phytoconstituents can enhance memory and other cognitive functions. In addition, their importance in the management and avoidance of neurological disorders like epilepsy, Parkinson's, Alzheimer's, and similar ailments has been well documented. Natural compounds can block inflammatory processes, up-regulate certain cell survival proteins, or use both strategies to produce their large-scale neuropharmacological effects.

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In light of the dearth of human studies examining the neuroprotective effects of natural products, this review delves into the various actions of these items in in vitro and in vivo preclinical models and their possible uses in neurotherapy, utilizing the current corpus of research.

(Yaribeygi et al., 2018) Neuroprotection, or the practice of preventing the loss of neurons, is a complex biochemical process regulated by multiple cellular pathways. There has been a surge of literature detailing the neuroprotective effects of various pharmacological drugs in the last several decades. This class of neuroprotective chemicals works by inhibiting inflammatory and apoptotic pathways while simultaneously reducing free radical and oxidative stress levels. The regulation of this damaging molecular process, which is vital for decreasing neuronal damage, is associated with the improvement of functional outcomes and recovery rates for patients who have been brought to the critical care unit. The purpose of this research is to examine neuroprotective chemicals and the various methods in which they shield the central nervous system from potential damage.

(Lalkovičová & Danielisová, 2016) The neurodegenerative disease known as ischemia, which is a catastrophic condition, comprises multiple changes in the nervous system. Reperfusion injury is also a component of ischemia. There are a number of biochemical reactions and key processes that are responsible for the bulk of neuronal damage. These include protein aggregation, free radical reactions, inadequate blood flow, glutamate excitotoxicity, and oxidative stress. There is a possibility that these processes will result in the death of cells, either by apoptosis or necrosis, as well as lasting damage. Therefore, research into neuroprotection and the prevention of neurodegeneration is extremely important. Damage caused by ischemia can be avoided in a variety of different ways. Through the utilization of numerous modern therapeutic methods and the addition of various substances to patients' diets, it is possible to achieve the goals of improving their quality of life and stimulating their endogenous defensive mechanisms.

(Jiménez-Arroyo et al., 2018) Even though the number of people suffering from neurodegenerative diseases is on the rise, there is yet no neuroprotective medication that is effective. Nutrition is essential for neuroprotection, as indicated by the findings of recent epidemiological and biochemical research, which discovered that components of food have the potential to constitute therapeutic agents. In order to provide neuroprotection, a number of different bioactive substances are synthesized and released, alterations are made to the activity of enzymatic neurons, and certain receptors are activated. Every one of these systems has as its major objective the prevention of

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damage to neurons and the mitigation of the consequences of widespread cell death. Understanding the neurochemical and structural characteristics of the neuropathological diseases that affect particular populations of neurons is essential for the development of effective treatments. This is because these diseases affect distinct populations of neurons. The effects of these therapies would be felt throughout the nervous system, despite the fact that they would be directed towards particular sections of neurons that are susceptible to damage. The difficulty of breaking through the blood-brain barrier has been a barrier to the development of efficient drugs for the prevention or protection of diseases. In addition to its role as a physical and enzymatic barrier, this structure also performs the function of an influx barrier, which serves to protect the brain from substances that are foreign to it. For this reason, in order to provide a more effective treatment for conditions that affect the central nervous system (CNS), it is necessary to develop new methods, such as nanocarriers, that will improve the brain's access to neuroprotective substances. To determine the concentration of target biomolecules at trace levels and to follow the impact of these nanoplatforms on the release of the bioactive cargo in the central nervous system, analytical chemistry and, more specifically, instrumental separation methods are essential. Both of these procedures are essential. With the help of these approaches, it is now possible to quantify and identify naturally occurring neuroprotective chemicals that are present in complex matrices at various quantities. Chromatography and capillary electrophoresis (CE) are two methods of separation that make use of detectors that are either optical or mass spectrometry (MS). The quantitative and qualitative investigation of bioactive analytes in biological samples can be carried out using these approaches in a wide variety of combinations, regardless of whether the analytes are present at lower concentrations or much greater concentrations. In light of this, the production of neuroprotective chemicals that can be found in food and that can be used as therapeutic agents in the brain is a complex endeavor that requires close collaboration and the participation of a variety of areas. As a result, this research draws attention to prospective strategies for brain neuroprotection and provides a summary of recent developments in the field in order to shed light on the current state of the art.

(Grimmig et al., 2017) The carotenoid known as astaxanthin (AXT) is believed to have a number of beneficial effects on one's health. The fact that it is currently being marketed as a dietary supplement is a direct result of its reputation as an antioxidant. Evidence has lately revealed that a wide array of biological tasks are capable of being performed. As a result of the compound's

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growing popularity over the past few years, there has been a stratospheric rise in the number of studies that have utilized this molecule across a wide variety of disease models. An increasing amount of evidence points to neuroprotective characteristics, including antioxidant, anti-inflammatory, and anti-apoptotic effects, and the ability to enhance or maintain brain plasticity. The newly discovered action pathways suggest that AXT may be a useful therapy for neurodegenerative diseases. This aims to bolster the case for AXT's ability to alleviate neuropathy in both healthy aging and neurodegenerative diseases by analyzing and drawing conclusions from the most current research in the field.

(Elufioye et al., 2017) Protecting neurons from neurodegenerative diseases (NDs) and other toxins that can damage their cells is what neuroprotection is all about. Neurodegenerative diseases (NDs) impact millions of people globally, and advancing age is a major risk factor for many of these conditions. The list of these disorders includes amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, and Parkinson's disease. There is a wide variety of neurodegenerative illnesses, each with its own unique set of symptoms, chemical profile, and impact on brain tissue and cells. Neurodegenerative disorders (NDs) and their potential treatments have been the focus of extensive research using a wide variety of in vitro and in vivo models tailored to specific diseases. The accumulation of proteins, the manifestation of inflammation in the brain, and the role of deficits in specific neurotransmitters like dopamine and acetylcholine in this process have been the focus of many of the most common drug development strategies employed in the last several decades. This article aims to showcase well-known instances of plant-based neuroprotective drugs. By emphasizing their structural class and unique action modes, these examples are brought to light. The various modes of action discussed earlier seem to be the means by which a large proportion of the naturally occurring compounds with medicinal value execute their actions.

(Wąsik & Antkiewicz-Michaluk, 2017) All human neurodegenerative illnesses, including Alzheimer's and Parkinson's, originate from an imbalance in the brain's redox balance. It is evident from the previous studies that oxidative overload can lead to cell death. DNA, lipids, and proteins can all be damaged when there are large quantities of reactive oxygen and nitrogen species. Although synthetic drugs have demonstrated some promising benefits in treating neurodegenerative illnesses, their usage is often associated with a variety of unwanted side effects.

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It would appear that interest in plant-based natural chemicals has been on the rise in recent years. The neuroprotective potential of a number of natural substances has been established in clinical trials conducted on humans as well as in laboratory settings. These substances include 1MeTIQ, resveratrol, curcumin, vitamin C, and ginkgo biloba. It is important to note that 1MeTIQ is an endogenous amine, which means that it is created in the brains of both humans and animals. This gives it a significant edge over the other compounds. There is evidence from scientific research that reveals the natural chemicals indicated above all have a similar protective mechanism. This mechanism is the ability to diminish or even eliminate free radicals that are formed in brain nerve cells as a result of oxidative and neurotoxin-induced responses. The processes of apoptosis, activation of caspase-3, and suppression of mitochondrial complex activities Reactive oxygen species are responsible for activating a number of other molecular pathways that are involved with neurotoxicity; however, these mechanisms take place at a later stage. Furthermore, these all-natural compounds have the ability to halt the progression of neurotoxic-induced cell death in the brain during its progression.

(Kolahdouzan & Hamadeh, 2017) Coffee, which has several positive effects like lowering inflammation and preventing cell death, is the most popular psychostimulant in Western countries. Caffeine may have positive benefits on both sexes and animals when it comes to Alzheimer's disease (AD). Caffeine has similar effects on male and female Parkinson's disease patients. However, this is due to the fact that caffeine competes with estrogen for the enzyme CYP1A2, which is responsible for the metabolism of estrogen. As a result, the validity of this specific information is called into question. Blocking A2A R has been proven to have protective effects, according to research conducted utilizing animal models of amyotrophic lateral sclerosis (ALS). Caffeine use (more than 190 milligrams per day) has been related to an increased chance of acquiring Huntington's disease at a younger age, according to research conducted on humans. However, the findings of studies conducted on animals have been inconsistent. Caffeine in doses ranging from three to five milligrams per kilogram protects against Alzheimer's disease and Parkinson's disease. However, additional research is needed to determine caffeine's impact on Parkinson's disease in females. In order to better understand how caffeine affects conditions including Alzheimer's, Parkinson's, and Machado-Joseph syndrome, more research is required. Further research is required to fully comprehend the mechanisms of caffeine's effects in connection to neurodegenerative diseases.

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(A. Singh et al., 2019) Canine cognitive dysfunction (CCD) is a degenerative neurological disorder that worsens with age and is characterized by impaired learning and memory consolidation abilities. It mimics the neurodegenerative signs of Alzheimer's disease (AD) in both animals and people, and it manifests in aging cats and dogs. An important challenge in the battle against Alzheimer's disease and chronic myelogenous leukemia is the creation of disease-modifying medications with neuroprotective properties. In chronic myelogenous dementia and Alzheimer's disease, the oxidative stress and production of amyloid beta plaques play the lead role in development. It is evident from the previous findings that there is a direct relationship between oxidative damage caused by free radicals and age. Consuming antioxidants from natural sources on a daily basis is one possible way to avoid the onset of CCD and AD. *Withania somnifera*, also known as "Indian ginseng" or ashwagandha, is an Ayurvedic tonic herb that has been used for a long time to improve memory. However, not much is known about how this plant protects the brain. The main objective of this study was to explore the effect of *Withania somnifera* extract on the A β plaque formation in brain. We showed that the human neuroblastoma cell line SK-N-SH was considerably protected from the effects of A peptide and acrolein when treated with WS extract. Multiple cell survival assays confirmed this. Reactive oxygen species generation in SK-N-SH cells was considerably decreased after WS extract treatment, which is an additional positive observation. Finally, we found that WS extract significantly inhibited acetylcholinesterase activity. Our first data suggests that WS extract may have antioxidant and cholinergic modulatory effects, which may have therapeutic applications in the management of chronic diseases and Alzheimer's disease.

(M. Singh & Ramassamy, 2017) Canine cognitive dysfunction (CCD) is a degenerative neurological disorder that worsens with age and is characterized by impaired learning and memory consolidation abilities. It mimics the neurodegenerative signs of Alzheimer's disease (AD) in both animals and people, and it manifests in aging cats and dogs. An important challenge in the battle against Alzheimer's disease and chronic myelogenous leukemia is the creation of disease-modifying medications with neuroprotective properties. Oxidative stress and the production of amyloid β peptides are implicated in the development of chronic myelogenous dementia (CCD) and Alzheimer's disease (AD), according to ample evidence. A linear association between age and the degree of oxidative damage in both human and canine brains is observed. One possible strategy that could delay the onset of CCD and AD is the consumption of antioxidants derived from natural

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sources. Although the Ayurvedic tonic herb *Withania somnifera* (WS), also called "Indian ginseng" or ashwagandha, has a long history of use in memory-enhancing treatments, very little is known about the neuroprotective properties of this plant. Finding out if WS extract can inhibit the harmful effects of A β peptides and acrolein was the main aim of this research. We showed that the human neuroblastoma cell line SK-N-SH was considerably protected from the effects of A peptide and acrolein when treated with WS extract. Another promising result was the substantial decrease in reactive oxygen species generation in SK-N-SH cells following treatment with WS extract, which was shown utilizing a battery of cell survival assays. Finally, we found that WS extract significantly inhibited acetylcholinesterase activity. Early results suggest that WS extract may have antioxidant and cholinergic modulatory effects, which may be useful in treating chronic conditions including Alzheimer's disease.

(Talarek et al., 2017) It is becoming increasingly common for people in industrialized nations to suffer from neurological diseases, which coincides with the rise in the average lifespan. It is of the utmost importance to discover novel therapeutic compounds because there is currently no treatment available for neurodegenerative illnesses. Natural items, particularly those that are used in traditional medicine, have shown promise as a potential alternative to pharmaceuticals in the treatment of neurodegenerative illnesses. There are large amounts of honokiol in the bark of the magnolia tree. This is a naturally occurring bioactive phenylpropanoid molecule that is a member of the neolignan class. It possesses a variety of pharmacological actions, including neuroprotective benefits. Due to its ability to cross both the blood-brain and blood-cerebrospinal fluid barriers, honokiol can be made more bioavailable in neurological tissues. Several studies have shown that honokiol has a neuroprotective effect on the central nervous system due to its strength as an antioxidant, its capacity to alleviate excitotoxicity (a condition primarily associated with glutamate receptor blockage), and its capacity to decrease neuroinflammation. Furthermore, new research indicates that honokiol may mitigate the neurotoxicity caused by the unwarranted buildup of A β in Alzheimer's conditions. Here we provide the data that point to honokiol as a potential drug for the management and treatment of neurodegenerative diseases. The neuroprotective benefits of honokiol and its likely molecular mechanisms of action are the reasons behind this.

(Lalkovičová & Danielisová, 2016) The neurodegenerative disease known as ischemia, which is a catastrophic condition, comprises multiple changes in the nervous system. Reperfusion injury is

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also a component of ischemia. There are a number of biochemical reactions and key processes that are responsible for the bulk of neuronal damage. These include protein aggregation, free radical reactions, inadequate blood flow, glutamate excitotoxicity, and oxidative stress. There is a possibility that these processes will result in the death of cells, either by apoptosis or necrosis, as well as lasting damage. Therefore, research into neuroprotection and the prevention of neurodegeneration is extremely important. Damage caused by ischemia can be avoided in a variety of different ways. Through the utilization of numerous modern therapeutic methods and the addition of various substances to patients' diets, it is possible to achieve the goals of improving their quality of life and stimulating their endogenous defensive mechanisms.

(Bagli et al., 2016) Glaucoma and Alzheimer's disease are two examples of pathologic diseases that are marked by neurodegeneration. These diseases have devastating effects on both the economy and society. Protein misfolding, oxidative stress, mitochondrial dysfunction, excitotoxicity, and inflammation are all components of this intricate process. One option for treating multifactorial diseases is the use of natural substances. The reason being, there is a vast array of biological and pharmacological effects exhibited by naturally occurring chemicals. Regardless, their insufficient brain transport and low bioavailability have kept their therapeutic potential against neurodegenerative illnesses in the laboratory. Here we can see the big picture of the chemical mechanisms by which natural substances protect neurons. It is possible to increase the neuro-availability of natural substances by using innovative delivery devices that are filled with these chemicals.

(Sarkar et al., 2016) In the US, the progressive motor disease known as Parkinsonism is second only in frequency to Alzheimer's disease among neurodegenerative disorders. Roughly 1.5 million Americans live with Parkinson's disease. One characteristic pathological event in PD, is the degeneration of dopaminergic neurons in particular areas of brain i.e., substantia nigra's pars compacta. By way of the nigrostriatal pathway, these neurons convey impulses to the striatum. Another typical neuropathological feature is the buildup of Lewy bodies, which are cytoplasmic fibrillary inclusions containing ubiquitin and α -synuclein. In Parkinson's disease, motor symptoms present as tremors, rigidity, bradykinesia, inability to maintain posture, and the characteristic Parkinsonian walking. These features primarily differentiate Parkinson's disease from other illnesses. We must not fail to address the serious problem of non-motor symptoms, which

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encompass not only motor symptoms but also impairments in autonomic function, mental functioning, and cognitive functioning. As a result of neuroinflammation, dopaminergic neuronal loss in the midbrain's substantia nigra, and stress-induced protein unfolding in the endoplasmic reticulum (ER), Lewy bodies form in Parkinson's disease. It is already difficult to model Parkinson's disease (PD) and assess potential treatments for this debilitating condition due to the wide variety of risk factors. In this section, we will explore various processes linked to Parkinson's disease (PD), explore neuroprotective and therapeutic approaches that are either being developed or are in clinical trials, and share our ideas on approaches that could lessen the pathology PD causes.

(Sevigny et al., 2016) Modern medical research has shown that Alzheimer's disease (AD) is actually a mosaic of many brain disorders. Some of the diseases that can develop include cholinergic neuronal loss, oxidative stress, inflammation of the nervous system, metal imbalances, neurofibrillary tangles inside cells, plaques containing amyloid β peptides outside of cells, and elevated MAO enzyme activity. One possible explanation for the current consensus that multifunctional drugs are the best option for treating Alzheimer's disease is their ability to target multiple brain regions that are thought to be involved in the disease's pathology. However, the exact rationale behind this consensus remains unclear. The main goal of this research is to learn more about Youdim and his colleagues' new multimodal neuroprotective brain-permeable pharmaceuticals and how they could help combat Alzheimer's disease. This paper highlights the pharmacological and neuroprotective effects of these multitarget-directed ligands, as well as their potential therapeutic role in aging and cognitive functions associated with Alzheimer's disease (AD). The cholinergic system, iron accumulation, formation and aggregation of amyloid β peptides, and MAO enzymes are the specific targets of these ligands.

(Costa et al., 2016) Research into the possible neuroprotective effects of a class of natural chemicals known as nutraceuticals in the neurological systems of adults, children, and the elderly has recently garnered a lot of attention. This research has been conducted in the United States. The majority of the study that has been done in this field has been on the polyphenol quercetin, which is common in the natural world. Various studies conducted among animals, human and other mediums have evaluated the protective effect of quercetin against neuronal inflammation and degeneration. The results of these investigations lend credence to quercetin's neuroprotective

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benefits. We still don't know what processes are accountable for the reported protective benefits, even though many theories have been put out. Apart from its direct antioxidant effect it also helps in reinforcing the defense mechanisms of cell to fight against the free radicals. Right now, this may happen. The activation of the Nrf2-ARE, and the antioxidant activity and activation of anti-inflammatory enzyme paraoxonase 2 (PON2) are two examples of such pathways. There is mounting evidence that quercetin may exert its neuroprotective benefits by triggering autophagy, activating sirtuins (SIRT1), and functioning as a phytoestrogen. All of these are explanations that have been put up to account for its neuroprotective effects.

(Abdoulaye & Guo, 2016) Since the beginning of time, neurology has placed a significant emphasis on the investigation of potential non-pharmaceutical approaches to the treatment of ischemic stroke and other degenerative diseases. The class of compounds known as 3-N-butylphthalide (NBP), which was discovered for the first time in the seeds of *Apium graveolens* Linn., has been demonstrated to be remarkable in its ability to safeguard neurons against injury. NBP has been proven to enhance outcomes following a stroke by working on many pathways simultaneously. These pathways include oxidative stress, mitochondrial dysfunction, apoptosis, and inflammation. Extensive study has demonstrated that NBP is effective in improving outcomes. New research on a wide range of neurological conditions has further proved that the therapeutic benefits of neurobehavioral therapy (NBP) extend beyond the treatment of stroke. Even though there have been many studies aimed at improving our understanding and many new treatment options developed recently, the fact that dl-3-N-butylphthalide, which is a synthetic version of l-3-N-butylphthalide, is the only anti-ischemic agent with a clinical approval in China highlights the difficulties that are involved in making a successful leap from the laboratory to the clinic. As a result of their multitargeted action and their structural flexibility, neurobiopharmaceutical products (NBP) have the potential to transform the treatment of a wide range of neurological illnesses, according to what researchers have discovered so far. In this review, we discuss recent advancements in our understanding of the neuroprotective processes of compounds formed from NBP, as well as the prospective therapeutic use of these compounds in the treatment of a variety of neurological illnesses [28].

(Ahmad et al., 2020) Neurodegenerative disorders, such as Alzheimer's and Parkinson's, have far-reaching effects on people's lives and the economy. Inflammation, oxidative stress, excitotoxicity,

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and altered signal transduction pathways are some of the secondary biological effects brought about by their principal feature, the buildup of altered proteins. Throughout the last decade, scientists have invested much in studying these diseases in the hopes of finding a treatment. A lot of hope has been shown by the symptom-oriented remedies they have developed. Regardless, no treatment has been found so far that can be deemed conclusive. Finding a class of drugs that can fix brain injury and stop additional brain death from happening is, therefore, of the highest significance. Therefore, this work aims to evaluate the neuroprotective effects of epigallocatechin gallate (EGCG) by investigating its antioxidant, pharmacological, biological, and metal chelation characteristics. Scientists are particularly curious about the chemicals' potential to stimulate several biological pathways in the brain. The paper continues by talking about how nanotechnology could be used to make EGCG even more neuroprotective.

(X. Yang et al., 2015) Additionally, coenzyme Q10 (CoQ10), which is a component of the electron transport chain, possesses antioxidant capabilities. A further benefit is that it shields neurons from the damaging effects of oxidative stress and mitochondrial malfunction. Because of this, coenzyme Q10, often known as CoQ10, is becoming increasingly popular as a possible therapy and prevention method for neurodegenerative illnesses. The structure and function of CoQ10, as well as the connection between neurodegenerative illnesses, oxidative stress, and mitochondrial dysfunction, are the key topics that are discussed in this review paper. In addition to that, the influence that CoQ10 has on neurological illnesses such as Alzheimer's, Parkinson's, and Huntington's is also discussed. In conclusion, a few recommendations are offered for the future. These recommendations pertain to the development of effective treatments for neurodegenerative diseases.

(Balez & Ooi, 2016) Alzheimer's disease (AD) is a neurological disorder that causes memory loss and behavioral issues. It happens when brain neurons break down. Currently, no cure or prevention of Alzheimer's disease is present. Neuroinflammation is a hallmark of Alzheimer's disease syndrome and can cause neurotoxic insults such as nitric oxide (NO). However, as research into the earliest stages, including those that occur before cognitive symptoms emerge, progresses, this paradigm is beginning to evolve. As a result of the findings, it has been demonstrated that NO possesses a compensatory and neuroprotective effect, which involves increasing neuronal excitability in order to protect synapses. Because of its effects on voltage-gated potassium channels

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(Kv7 and Kv2), NO may increase excitability. Identifying the particular ionic processes and signaling pathways that are responsible for mediating this protection is the next critically important step for the medical industry. Utilizing the protective effect of NO and the accompanying signaling pathways is one of the prospective therapy options that could be utilized to prevent the loss of synapses in the early stages of the disease.

(Javed et al., 2016) Among those sixty and up, 1% suffer from Parkinson's disease (PD), a degenerative disorder that impairs motor skills. A rising amount of evidence indicates that neuroinflammation, mitochondrial abnormalities, and oxidative stress play important roles in the disease, even though the exact cause is still unknown. Currently present Parkinson's disease treatments can only manage symptoms; they do not halt the illness's advancement. Although there are a variety of existing treatments for Parkinson's disease (PD), new medications are desperately needed. Rotenone (ROT) was given intraperitoneally to male Wistar rats at a dose of 2.5 mg/kg body weight once a day for four weeks as part of this study. This research set out to test the hypothesis that nerolidol, a sesquiterpene alcohol, would protect neurons in a rotenone-induced Parkinson's disease model. Our study provides the first proof that nerolidol's antioxidant and anti-inflammatory properties mediate its neuroprotective effects. This provides more evidence that nerolidol may have therapeutic potential in the treatment of Parkinson's disease.

(Taram et al., 2016) While the number of people diagnosed with neurodegenerative disorders is on the rise, there are no medicines that can halt the underlying process—the loss of neuronal cells—that causes these diseases. Coffee contains large levels of the polyphenolic chemical chlorogenic acid (CGA), which can scavenge free radicals and functions as an antioxidant. Utilizing primary cultures of rat cerebellar granule neurons, this study set out to examine the neuroprotective effects of CGA and its principal metabolites. We show that coffee and CGA significantly reduced the toxic effects of sodium nitroprusside, a nitric oxide donor, within the parameters of this study. As a countermeasure to this nitrosative stress, ferulic acid and quinic acid were completely useless. While caffeic acid and ferulic acid protected nerve cells from excitotoxicity, neither chlorogenic acid nor quinic acid protected nerve cells from glutamate-induced cell death. Retina stress, proteasome inhibition, hydrogen peroxide, and caspase-dependent intrinsic apoptosis were all significantly mitigated by caffeic acid, the sole chemical to exhibit such a protective effect. That doesn't mean it was the sole molecule to show this behavior, though. All things considered, these

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studies show that caffeic acid, unlike its parent polyphenol CGA and the other main metabolites, ferulic acid and quinic acid, exhibits a far more extensive profile of neuroprotection against a broad range of stresses. We found that caffeic acid, based on preclinical models of neurodegeneration, shows promise as a potential therapeutic approach.

(Shah et al., 2017) Cognitive decline and oxidative stress are both linked to age-related diseases such as Alzheimer's. This group's reputation has grown substantially. This study is targeted to find out the ameliorating effect of methanolic extract of *Caralluma tuberculata* (CTME) against D-galactose induced dementia in mice. The goal of this research was to determine if CTME could alleviate cognitive impairment in d-galactose-induced mice. The results of this study were derived from evaluations of both cognitive and behavioral abilities. To assess the potential role of CTME in safeguarding against oxidative damage induced by d-galactose, various biochemical parameters were examined. Cognitive function and oxidative defense were considerably diminished after seven weeks of chronic subcutaneous administration of d-galactose at a dose of 150 mg/kg compared to the control group. This was noted in activities such as active avoidance, step-through passive/active avoidance, Hole-Board, and Morris water maze. Memory associated functions were shown to improve due to improvement in the oxidative profile after the treatment with CTME at three dose levels i.e., 100, 200, and 300 mg/kg orally. It showed significant improvements in the values obtained via Morris water maze, step-through passive and active avoidance, hole board, and novel item identification test. In addition to this, CTME at 300 and 200 mg/kg orally, exhibited a significant improvement in brain levels of SOD, GSH-Px, NEP and β -site A β PP cleaving enzyme 1 (BACE1). Alternatively, there was a decrease in the concentrations of nitric oxide (NO), nitric oxide synthase (NOS) activity, malondialdehyde (MDA), and presenilin 1 (PS1). This study found that CTME improved cognitive performance and corrected metabolic abnormalities in mice. Hence, it significantly reduces the effects of d-galactose on learning, memory, and spontaneous behavior in a mouse model.

(Campos et al., 2016) CBD, also known as cannabidiol, is a phytocannabinoid that is produced from the cannabis plant. It does not generate symptoms that are similar to those of intoxication. There is a wide range of neuropsychiatric conditions that may react favorably to treatment with this medication. In patients suffering from neurodegenerative and ischemic disorders, cannabidiol (CBD) lessens the severity of brain damage. As an additional benefit, it is effective in alleviating

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symptoms of psychosis, sadness, and anxiety. Additionally, cannabidiol (CBD) has been shown to have an effect on synaptic plasticity and stimulation of neurogenesis. While not all details are known at this time, it seems that several different pharmacological targets are involved in the mechanisms that cause these effects. This study brings together the key molecular and pharmacological processes linked to CBD's therapeutic effects, with a focus on the ways CBD affects neuroprotection, neuropsychiatric diseases, and brain function in general.

(Nabavi et al., 2018) Within the next forty years, the percentage of the world's population that is sixty years old or older is expected to increase from 11 percent to 22 percent, as predicted by the World Health Organization (WHO). With this in mind, the rates of death and disability that are brought on by illnesses associated with aging will continue to climb. Mental diseases are at the top of the list when it comes to the health challenges faced by the aging population. Neuroprotective drugs that are effective and have few adverse effects have been the subject of a significant amount of research and development. It is becoming increasingly clear, based on research conducted in both in vivo and in vitro settings, that phytochemicals have the ability to safeguard neurons and mitigate the consequences of neurodegeneration. Polyphenolic compounds, and flavonoids in particular, are among the most abundant phytochemicals that have been discovered to benefit health. There are several phytochemicals that have positive effects on health. In addition to the numerous bioactive components that can be discovered in fruits, vegetables, and even mushrooms, chrysin, which belongs to the flavone class, is also present. There are neuroprotective and anti-inflammatory activities associated with chrysin. Additionally, chrysin has the ability to alleviate cognitive impairment, in addition to its significant anti-amyloidogenic and neurotrophic effects. Moreover, it has been claimed that chrysin has been beneficial for people who suffer from epilepsy and depression. This research was conducted with the intention of conducting a comprehensive literature review on the neuroprotective characteristics, chemistry, sources, and bioavailability of chrysin.

(Pani, 2015) From the most basic model organisms to mammals and non-human primates, dietary restriction (DR)—whether it's through lower calorie intake or alternating fasting with overall normal energy supply—extends lifespan by evoking cell protective responses in practically all tissues and organs, including the brain. This is in addition to the fact that DR can be accomplished through alternate fasting. Research on DR offers the potential to identify novel strategies to

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postpone the onset of age-related diseases and improve the average lifetime of humans. This is an extremely important benefit of this research. This review focuses on the neuroprotective benefits of DR, which draws on intriguing but so far little clinical and epidemiological evidence. The review draws on the findings of previous studies. At the outset, the essay will conduct a comprehensive analysis of the most compelling evidence indicating that DR is effective in treating neurodegenerative illnesses, brain aging, and damage in both animals and humans. After that, it will talk about the primary mechanisms that are thought to be involved in these impacts, both at the cellular and tissue levels. These mechanisms include autophagy and mitochondrial biogenesis, among others. After that, it will "zoom in" on the molecular circuits (AMPK/mTOR, sirtuins, and CREB/Sirt1) that enable neuronal cells to recognize when there is a lack of nutrition and react defensively. For the purpose of delving more into this matter, we will investigate the connection between chromatin remodeling and cell metabolism, as well as the ways in which this connection is connected to our understanding of the impacts of nutrition on the expression of genes in neurons and the quality of the brain.

(Patel et al., 2015) A growth factor that falls into this category is glucagon-like peptide 1 (GLP-1). As well-tolerated medications, GLP-1 mimetics are now available for the treatment of type 2 diabetes. Scientific studies employing animal models of neurodegenerative diseases have demonstrated the neuroprotective characteristics of these medications. Another GLP-1 copycat, exendin-4, has demonstrated promising early results in a human trial including people with Parkinson's disease (PD) and protective effects in animal models of the disease. Two more modern GLP-1 mimics, liraglutide and lixisenatide, outlive exendin-4 by a greater margin. Previously, we used an animal model of Alzheimer's disease to prove that these medications have neuroprotective effects. We show that the neuroprotective effects are effective using a mouse model of Parkinson's disease called 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Every day for seven days, an intraperitoneal injection of 20 mg/kg of MPTP was administered. Afterwards, the drugs were administered intraperitoneally once daily for a duration of fourteen days. The results showed that at the chosen dose, exendin-4 did not show any protective benefits when compared to lixisenatide (10 nmol/kg), liraglutide (25 nmol/kg), and liraglutide. In addition to reducing TH levels (dopamine production) in the basal ganglia and substantia nigra, they both increased B-cell lymphoma-2, a substance that inhibits cell death, and inhibited MPTP-induced motor impairment (Rotarod, open-field locomotion, and catalepsy test). Researchers discovered that exenatide and

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liraglutide effectively prevented catalepsy. The results of this clinical research demonstrated that lixisenatide and liraglutide were superior to exendin-4. These two medications show promise as future Parkinson's disease (PD) treatments.

(Pischiutta et al., 2018) Neuroprotective strategies have been developed through significant study employing animal models of cerebral lesions (ischemic, hemorrhagic, traumatic, and nontraumatic). These approaches have been found to minimize subsequent tissue loss and boost functional outcomes. The implementation of these potential treatments in randomized controlled trials involving human participants, on the other hand, has often resulted in unsatisfactory outcomes. Neuroprotective treatments for people with acute brain trauma are an area of active research, and this paper aims to summarize their current state. Both the early and late stages of brain injury can be protected using these strategies. Furthermore, we consider the prospect of creating novel approaches and point out some future paths for research to follow.

(Nabavi et al., 2018) According to projections made by the World Health Organization, by the year 2050, there will be two billion people who are sixty years old or older. A significant number of neurodegenerative disorders contain aging as one of their primary risk factors. These ailments that are associated with aging are among the most serious and challenging health concerns that are prevalent in the modern world. Because of this, a significant amount of work has been put into developing neuroprotective medicines that are derived from natural components. These phytochemicals have been demonstrated to be extremely effective with a minimal number of adverse effects, according to a number of studies conducted both *in vitro* and *in vivo*. There are a variety of fruits and vegetables that include phytochemicals, the most prominent of which are dietary flavonoids, which belong to a special category of bioactive molecules. Products derived from plants such as broccoli, pepper, thyme, and celery are examples of those that contain luteolin, which is an essential flavone. Numerous *in vitro* and *in vivo* studies have established the neuroprotective benefits of luteolin. Despite this, luteolin's neuroprotective effects have not been conducted in a comprehensive study. A literature review on luteolin's neuroprotective properties and the molecular mechanisms linking its action is, hence, the goal of this study. In order to complete our research, we also looked for information on luteolin's chemical composition, herbal sources, and bioavailability as a pharmaceutical agent for the treatment of age-related neurological diseases.

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(Subash et al., 2014) The results of recent clinical research indicate that berry fruits have the potential to improve cognitive and motor functions, as well as protect against neurodegenerative diseases that are associated with aging. Berry fruits can also improve neuroplasticity, control signalling pathways that are linked to inflammation, cell survival, and neurotransmission, and speed up neurotransmission. Phytochemicals as anthocyanin, caffeic acid, catechin, quercetin, kaempferol, and tannin have been linked to the neuroprotective benefits of berry fruits on mental and neurological illnesses. Our goal during the experiment was to give as full an explanation as possible of how different types of berries can protect the brain.

(Rege et al., 2014) Deterioration of cognitive and behavioral abilities is a hallmark of Alzheimer's disease, a degenerative neurological disorder that progresses over time. This deterioration happens gradually. The hallmarks of Alzheimer's disease are intracellular neurofibrillary tangles and extracellular senile plaques. Although there are not enough treatments for Alzheimer's disease at the moment, researchers are still trying to figure out what causes it and the molecular pathways that play a role. Resveratrol (3, 5, 4'-trihydroxy-trans-stilbene) is a polyphenolic molecule that has been demonstrated in much recent research to possess neuroprotective characteristics when administered to in vitro and in vivo models of Alzheimer's disease. Several intriguing results have been uncovered by these investigations. Resveratrol has a wide variety of potential modes of action; however, this review will concentrate on its antioxidant and neuroprotective effects on Alzheimer's disease. Even worse, resveratrol in its natural form has a very short plasma half-life. So, we also detail possible analogues that aim to increase plasma bioavailability.

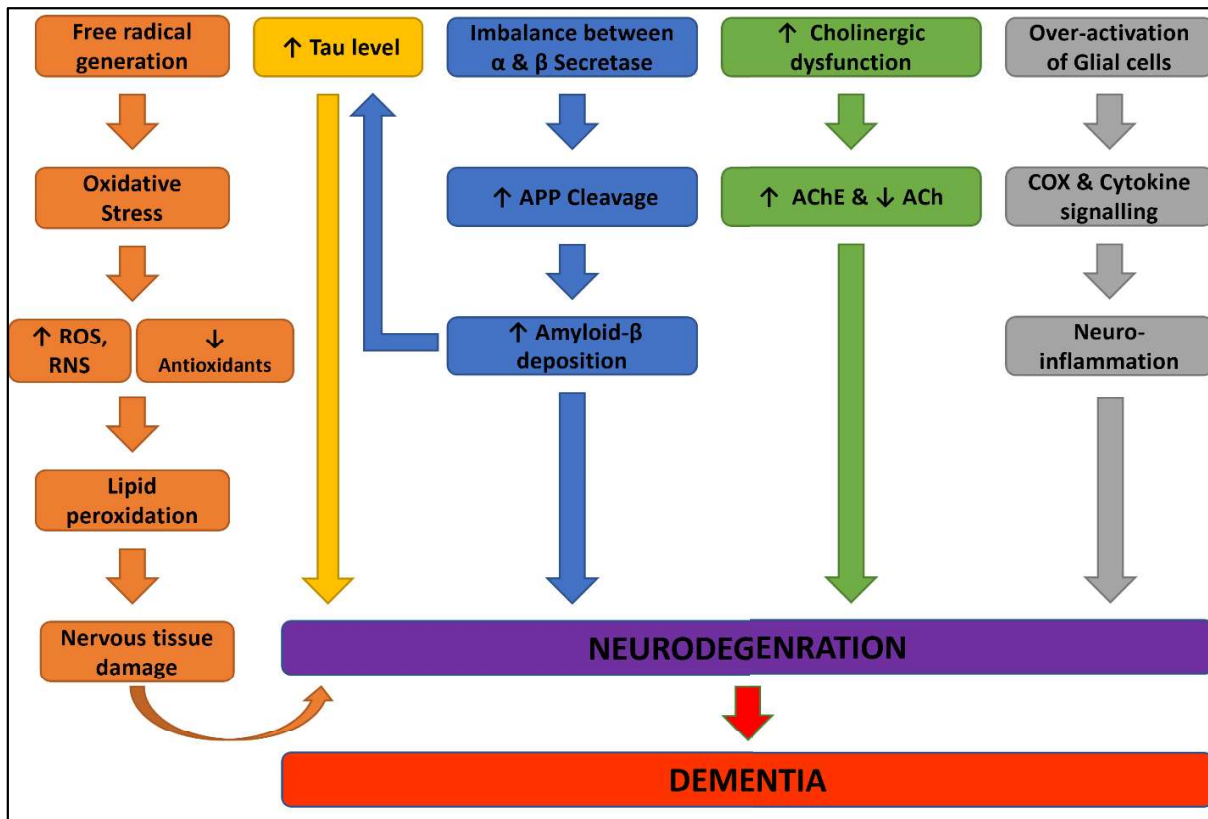


Figure No. 2.1: Pathogenesis of Dementia.

(Viveros-Paredes et al., 2017) The degeneration of dopaminergic neurons in the substantia nigra (SN) is a defining characteristic of Parkinson's disease (PD). Researchers don't know exactly what causes Parkinson's disease (PD), but they have shown that inflammation and oxidative stress may play a role in the disease's development. People may be protected from Parkinson's disease by the natural bicyclic sesquiterpene β -caryophyllene (BCP), according to recent research. BCP is a selective agonist of the cannabinoid receptor of type 2 (CB2R). Evidence from previous research suggests that BCP could be useful in the management or prevention of inflammatory bowel disease. We have come to this conclusion since we are aware of BCP's antioxidative and anti-inflammatory characteristics. Here in this study the neurological protective effect of BCP was found in a Parkinson disease model. Here the disease was induced by using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice. A significant improvement in Motor dysfunction, prevention in dopaminergic neuron loss in striatum and SN, dopaminergic neuron loss in the striatum and SN was prevented, and also reduction in neuroinflammation by glial cell activation. Also there is significant decrease in the cytokine levels in NS by BCP. The involvement of CB2R

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was evidenced by the fact that treatment with the CB2R-specific antagonist AM630 negated the observed neuroprotection and reduced glial activation. Given that our mouse model shows that BCP acts through many neuroprotective pathways, the possibility of BCP being used as a therapeutic or preventative medicine for Parkinson's disease arises.

(Lee et al., 2012) Sinapic acid (SA) is an intriguing chemical that is classified as a phenylpropanoid and possesses anti-inflammatory and neuroprotective effects and characteristics. This study set out to examine how SA, in a mouse model of Alzheimer's disease (AD) generated by the amyloid β ($A\beta$) (1-42) peptide, protected the neurons of the animal. To evaluate the effect of SA, $A\beta$ (1-42) protein was administered to mice directly into the hippocampus of mice brain. The sinapic acid SA was administered at a dose of 10 mg/kg a day orally for 07 days. The SA was administered immediately after injecting $A\beta$ protein. Following the administration of the final dose of SA, the mice were put through an acquisition trial of the passive avoidance task one hour later. Afterwards, the animals were sacrificed for the estimation of immunohistochemistry. In the CA1 region of the hippocampus, SA was able to prevent the death of neuronal cells and diminish the effects of the $A\beta$ (1-42) protein. These effects included an increase in the production of iNOS, activation of glial cells, and nitrotyrosine expressions. Memory impairment was significantly reduced by SA when it was tested using the passive avoidance method. On the basis of these findings, it is possible that SA could be an effective treatment for Alzheimer's disease (AD) due to its ability to reduce pathology connected to the $A\beta$ (1-42) protein. This pathology includes neuronal cell death and cognitive dysfunction. SA's antioxidative and anti-inflammatory effects were responsible for this reduction.

(Küpelı Akkol et al., 2021) Epilepsy, headaches, essential tremors, stroke, Alzheimer's disease, Parkinson's disease, and many more neurological disorders are often linked with psychiatric issues. When it comes to neurological problems, the most prevalent co-occurring diagnoses are cognitive impairment, depressive disorders, and anxiety disorders. Comorbidities are typically overlooked by individuals, even when they may be contributing factors. For example, a reduction in functionality or shared neurochemical pathways are examples of comorbidities that are usually overlooked. Potential therapeutic alternatives include pharmacological therapies, cognitive-behavioral therapy, somatic interventions, and electroconvulsive therapy. Other potential choices include somatic interventions. Oral antidepressant medication has the potential to offer a multitude

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of advantages; however, it is not without its drawbacks, which include the possibility of adverse drug interactions, unpleasant side effects, and ineffectiveness. There is a significant amount of research being conducted on natural compounds that are derived from plants in an attempt to seek out an alternative treatment that is both more effective and safer. The phytochemicals alkaloids, terpenes, flavonoids, phenolic acids, and lipids have shown promising outcomes in animal and laboratory models of mental illnesses. These results have been demonstrated in both animal and laboratory models. In this review, the neurotherapeutic potential of natural products is examined. The review examines the roles of natural products in the treatment of psychiatric disorders and the many *in vitro* and *in vivo* research that have evidenced their effectiveness.

(Zhang et al., 2022) Even though there has been a significant amount of research conducted on Alzheimer's disease (AD), there are still difficulties in diagnosing and treating the condition, and there are no medications that are known to be effective. In Alzheimer's disease (AD), intracellular neurofibrillary tangles and amyloid β ($A\beta$) extracellular plaques are histopathological symptoms. These characteristics have been associated with neuropathological events, including cell death in the nervous system and synapses. The main goal of our research was to determine if the "JAK2-STAT3-BACE1" pathway is significantly involved in the neuroprotective effects of the food flavoring ingredient β -caryophyllene, or BCP. The objective of dividing PC-12 cells with overexpressed amyloid- β protein precursor (APP) into four groups was to establish an *in vitro* model of Alzheimer's disease (AD). These groups included a control group, an empty vector group, an APP overexpression group, and a battery of BCP (5, 10, and 20 μ M). Cell viability was assessed using CCK-8, synaptic morphology was examined by immunofluorescence, and gene and protein expression levels were analyzed via quantitative real-time polymerase chain reaction and western blotting. The expression levels of JAK2, STAT3, and BACE1 mRNA were dramatically elevated in transfected PC-12 cells. Furthermore, at 72 hours post-transfection, the cells demonstrated a significant morphological alteration, adopting a rounder form and exhibiting a reduced number of nuclei. By conserving the morphology of the cell, BCP exhibited the capacity to significantly enhance the viability of PC-12 cells. BCP suppressed all of these processes, including the overexpression of BACE1 mRNA and protein, the hyperphosphorylation of JAK2 and STAT3, and APP. For the purpose of simulating the binding of BCP to JAK2, STAT3, BACE1, and CB2, molecular docking was utilized. The JAK2 protein was found to be the most stable of all the

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proteins that were examined. By inhibiting the "JAK2-STAT3-BACE1" signalling pathway, BCP may have the capacity to protect neurons and mitigate the neurotoxicity induced by A β .

(Ueda et al., 2019) One neurodegenerative disease that causes the slow loss of motor neurons is amyotrophic lateral sclerosis (ALS). Prior research by our group demonstrated that a Brazilian green propolis (EBGP) ethanol extract could mitigate neuronal damage caused by a mutant copper-zinc superoxide dismutase 1 (SOD1mut). In this demonstration, we will show how p-coumaric acid (p-CA), an active component of EBGP, protects neurons from damage caused by SOD1 mutations. Our research revealed that p-CA protected against the neurotoxicity caused by SOD1mut by reducing the formation of subcellular aggregation. Furthermore, p-CA alleviated oxidative stress and endoplasmic reticulum stress, two features of the pathogenesis of SOD1-mutated amyotrophic lateral sclerosis (ALS). We found that this molecule stimulated autophagy, which was the primary focus of our inquiry into the neuroprotective effects of p-CA. Autophagy was the neuroprotective benefit that we were looking into. In addition, chloroquine, which is itself an inhibitor of autophagy, was able to prevent the neuroprotective effects of p-CA from occurring. Therefore, the findings of this research offer a glimmer of hope for a therapy for amyotrophic lateral sclerosis (ALS) by indicating that p-CA reduces SOD1mut-induced neurotoxicity via activating autophagy.

(Iorio et al., 2022) Neurodegeneration, diabetes, cardiovascular disease, and cancer are only a few chronic disorders that involve inflammation and oxidative stress. These processes are interdependent. Consequently, targeting inflammatory pathways could be a potential treatment method that could be taken. Several phytochemicals that are found in edible plants have been the subject of recent research, which suggests that they may help relieve disease symptoms. In this study, it was shown that BCP and CA, two types of sesquiterpenes, have antioxidant, anti-inflammatory, neuroprotective, and mitoprotective effects in various in vitro and in vivo experiments. In order to significantly increase the effects of the cannabinoid type-2 receptor (CB2R), BCP selectively agonizes and allosterically modifies the receptor. As soon as it comes into contact with oxygen, CA changes from a pro-electrophilic to an electrophilic state. This may interact with the Keap1/Nrf2/ARE transcription pathway, leading to an upregulation of endogenous antioxidant "phase 2" enzyme production. Aside from that, CA's chemical makeup gives it direct antioxidant qualities. Accumulating in brain regions and easily crossing the blood-

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brain barrier (BBB) are molecular mechanisms linked to mitochondrial quality control and pro-survival signaling pathways, such as those that regulate apoptosis and autophagy. By blocking activated microglia and avoiding mitochondrial malfunction, this has neuroprotective effects. The results of many *in vitro* and *in vivo* models of neurodegenerative illnesses suggest that these chemicals could be a useful tool in the management of these conditions. The improvement of glia-mediated neuroinflammation and maintenance of mitochondrial homeostasis are evidence of this.

(Mohd Sairazi & Sirajudeen, 2020) Extensive research has recently focused on the therapeutic potential of natural products derived from plants, animals, and fungi, as well as their bioactive components, for a wide range of diseases, including cardiovascular, diabetes, hypertension, reproductive, cancer, and neurodegenerative disorders. Studies on these all-natural substances have been conducted. Progressive malfunction and loss of neuronal structure and function leading to neuronal cell death characterize neurodegenerative disorders such as Alzheimer's disease, Huntington's disease, Parkinson's disease, and amyotrophic lateral sclerosis. One possible approach to preventing and treating neurodegenerative diseases is a neuroprotection strategy that aims to restore function to damaged neurons while preventing cell death. This is due to the fact that numerous factors contribute to the development of neurodegenerative illnesses, which in turn are associated with numerous pathogenic pathways. New evidence suggests that certain naturally occurring compounds may have neuroprotective properties that might provide hope for the eventual treatment of neurodegenerative illnesses. This review set out to do two things: (1) provide a general outline of the bioactive components present in natural products and (2) explore the potential therapeutic utility of these components in the treatment of neurodegenerative disease patterns.

(Marino et al., 2022) Antioxidants that are found in nature include things like vitamins, polyphenols, and other substances that occur naturally. It is possible to classify these molecules according to their chemical structure, chemical-physical properties (such as hydrophilicity or lipophilicity), and activity (whether it be enzymatic or nonenzymatic). Finding the natural antioxidant is the biggest obstacle for the research scientists and entrepreneurs, so that it can be employed in the pharmaceutical, cosmetics and nutraceutical products. From the previous studies it is evident that the reactive oxygen species also can serve as the promising treatment for many neurodegenerative diseases like ALS, AD, Parkinson's disease, huntington's disease and many

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others. The ability of ROS to scavenge free radicals is the foundation of this potential. A variety of natural extracts comprising a complex combination of antioxidants and anti-inflammatory agents have demonstrated efficacy, alongside the application of pure phytochemical substances, in ameliorating preclinical models of various illnesses. Ginkgo biloba, grape, oregano, curcumin, tea, and ginseng extracts may provide multifaceted therapeutic advantages due to their synergistic interactions across several biochemical pathways. Moreover, the incidence of undesirable effects has diminished because of the reduced toxicity of several of these chemicals. Employing nanotechnology to enhance cerebral transport, modulate release, and decelerate degradation and elimination of these compounds is essential. This paper showcases the most promising outcomes from clinical studies, in vivo models, and in vitro systems utilizing antioxidant chemicals and extracts from natural sources, either independently or encapsulated in nanocarriers. The use of antioxidant chemicals and extracts resulted in these outcomes.

(Ferreira et al., 2019) The phenolic compound p-coumaric acid (p-CA), also called 4-hydroxycinnamic acid, has been the subject of much study since it provides protection against several diseases and is present in many different plant species. The chemical structure dictates both its bioavailability and the metabolic processes it undergoes because this phenolic component can exist free or conjugated with other substances. P-CA exhibits a wide range of biological activities such as antioxidant, anti-inflammatory, anticancer and antibacterial, which may contribute to its therapeutic effect. This is why choosing the best analytical method for p-CA in different matrices (e.g., drug delivery systems, plant extracts, plasma, and urine) is crucial for both quantitative and qualitative evaluations. One of the most often mentioned analytical procedures for this specific goal is high-performance liquid chromatography, which is often used in conjunction with detectors like ultraviolet/visible detectors. Conversely, a number of analytical procedures are used to ascertain this chemical as well. Examining its chemical and pharmacokinetic properties, drug delivery modes, pharmacological effects, and analytical methods for quantifying it, this study aims to give an overview of p-CA.

(Kanojia et al., 2021) Cognitive decline is an indicator of the neurological disorder dementia. In spite of the wide variety of dementias, two common etiological mechanisms are neuroinflammation and aging-induced apoptosis. Neuroprotective benefits in cerebral ischemia and neuro-inflammation have been attributed to β -caryophyllene, a cannabinoid type-2 receptor

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agonist. The results have been hailed as encouraging. In this study the dementia was induced using AlCl_3 , which mimics the exact pathology of dementia to evaluate the effect of BCP. Morris water maze was used to assess the potential protective effect of two different doses of BCP. The dosages that were examined were 50 and 100 milligrams per kilogram of body weight. Additionally to Morris water maze, new object recognition test and chemobrain paradigm was used to assess the effect of doxorubicin on episodic memory. A model of dementia caused by a mitochondrial dysfunction caused by D-galactose was also used to examine its effects. BCP significantly improved the TSTQ, ELT and path efficiency in the Morris water maze test. BCP, at a dose level of 100 mg/kg was found to be significantly decreasing acetylcholinesterase activity and improving the catalase levels. When compared to the healthy control group, this happened. A significant improvement in recognition index and discrimination index was observed in the novel object recognition task in mice that were treated with 100 mg/kg of β -caryophyllene. There was a marked decrease in lipid peroxidation and a rise in catalase in the frontal brain and hippocampus, respectively, suggesting this enhancement. The animals' performance on the recognition index also improved significantly. When examined in the D-galactose model of mitochondrial dysfunction, β -caryophyllene showed no positive benefits on spatial memory. Furthermore, it failed to reverse the effects of D-galactose on mitochondrial complex I and II activity.

(Machado et al., 2018) Beta (β)-caryophyllene, often known as BCAR, is a crucial sesquiterpene that may be discovered in numerous essential oils derived from plants. Researchers have discovered that it possesses a wide range of major pharmacological benefits, including the ability to shield the immune system, the heart, the liver, the intestines, and the kidneys against potentially hazardous bacteria. There is evidence from recent studies that suggests it may also possess neuroprotective effects. A compilation and analysis of past studies on the neuropharmacological effects of BCAR is presented in this study. We conducted a search across multiple databases, such as PubMed, Scopus, MedLine Plus, and Google Scholar, using the phrase "beta (β)-caryophyllene" and other neurological terms that are cognate with it. In order to collect data, we read articles that provided specifications regarding the dosage, concentration, and administration method, as well as the testing setup, findings, and discussion, and the predicted mechanism of action. Through the application of an exclusion criterion, this review was able to reduce the total number of research articles from 545 to 41 experimental investigations. This study indicates that several disorders impacting the neurological system, such as pain, anxiety, spasms, convulsions, depression,

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alcoholism, and Alzheimer's disease, seem to give a protective effect for BCAR. In addition to its potential as an immunomodulatory drug and its action similar to that of a local anesthetic, BCAR may also protect the nervous system from the damaging effects of oxidative stress and inflammation during nervous system development. Cannabinoid receptors (CBRs), and more specifically the cannabinoid receptor 2 (CB2R), have been linked to the majority of the neurological effects that are connected with this natural substance. According to the findings of this investigation, BCAR might possess neuroprotective qualities.

(Chang et al., 2013) In addition, many plants contain the non-toxic bicyclic sesquiterpene β -caryophyllene, which goes by several names: trans-4,11,11-trimethyl-8-methylenebicyclo[7,2,0]undec-4-ene. From this study it is concluded that BCP at a dose of 10 mg/kg i.p. can reduce the volume of cortical infarct by 68% after the middle cerebral artery occlusion (MCAO). The neurological defects caused by MCAO were considerably reduced by β -caryophyllene, according to one investigation. At a specific dosage, β -caryophyllene showed a dose-dependent protective effect on cortical cells that had been exposed to oxygen-glucose deprivation. BCP do not produce any significant effect against oxidative stress as well as NMDA induced excitotoxicity in cultured cortical cells. Furthermore, oxidative stress was not significantly reduced. The levels of nitric oxide and prostaglandin E2 were shown to decrease at a dose of 100 μ M after β -caryophyllene treatments were administered. Additionally, BCP can express the mRNA for the production of inducible nitric oxide synthase, cyclooxygenase 2, interleukin (IL)-1 β , and IL-6 in C6 microglial cells. Based on the results of this study it can be concluded that BCP can control the neuroinflammation by modulating various inflammatory mediators.

(Cheng et al., 2014) Beta-caryophyllene can activate the peroxisome proliferator-activated receptor- γ (PPAR γ) pathway and improve the neurological inflammation by activating the cannabinoid receptor 2 (CB2) in the treatment of Alzheimer's disease (AD). It is also known that AD symptoms are exacerbated by agonists of both receptors. From the previous studies it is uncovered that the BCP and its metabolites can specifically bind to the CB2 receptor and antagonize the receptor. Our main goal in conducting this study was to determine if CB2 and PPAR γ were involved in the process by which β -caryophyllene's anti-inflammatory actions were mediated in a transgenic APP/PS1 Alzheimer's disease mouse. Oral administration of β -caryophyllene protected APP/PS1 mice from cognitive impairment. The good effect on cognition

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was linked to a decrease in the amount of β -amyloid in the brain's hippocampus and cerebral cortex. Additionally, it was shown that the levels of proinflammatory cytokines in the cerebral cortex were decreased when β -caryophyllene was present. Astrogliosis, microglial activation, COX-2 protein levels, interleukin- 1β , and tumor necrosis factor- α messenger RNA levels were all cytokines that were included. Administration of CB2 selective antagonist like AM630 and selective antagonist of PPAR γ receptors like GW9662, the protective effects of BCP can be diminished. The sesquiterpene β -caryophyllene was shown to activate CB2 receptors and the PPAR γ pathway, which in turn reduced inflammation, according to the study's results. The results indicate that β -caryophyllene could be a molecule with great promise for the creation of new and effective medications to treat Alzheimer's disease [55].

(Flores-Soto et al., 2021) A neurological disorder known as Parkinson's disease (PD) develops when neurons in the part of the brain that processes dopamine begin to die off. The phytocannabinoid β -caryophyllene (BCP) has several pharmacological effects, along with its anti-inflammatory and antihypertensive capabilities. MPTP can induce the degeneration of dopaminergic neurons, or it might be due to BCP's antioxidant activity. So far, no one has provided a response to this question. In order to find out, this study used mice that had been treated with MPTP to investigate how BCP affected the expression and activity of NADPH quinone oxidoreductase (NQO1). In the control group intraperitoneal injection of normal saline was given to four male C57BL/6J mice. Animals were given MPTP at a dose of 5 mg/kg for seven days in the second group. The third group received 10 mg/kg of BCP orally over the course of seven days. The fourth and the last group received MPTP for 07 days and 10 mg/kg of BCP for seven days, it start at the fourth day of MPTP administration. The observation exhibited that the BCP can prevent the neurodegeneration caused by MPTP induced oxidative stress. At the same time that this was achieved, NQO1 expression and activity were both increased. Treatment with BCP not only improved motor dysfunction but also protected the SNpc's dopaminergic cells from MPTP's harmful effects. Therefore, it would appear that BCP shields cells from the toxicity of MPTP in part by boosting the activity of NQO1, which is an antioxidant in and of itself. By protecting dopaminergic neurons and putting a stop to Parkinson's disease in its tracks, this phytocannabinoid has the potential to be an excellent pharmaceutical option.

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(Lou et al., 2017) This study aimed to create an inclusion complex of β -caryophyllene and β -hydroxypropyl- β -cyclodextrin (HP β CD/BCP) and to examine its various effects on mitigating cognitive impairments in rats diagnosed with vascular dementia (VD). An initial step involved the creation, fine-tuning, description, and evaluation of the HP β CD/BCP. Subsequently, the expression of cannabinoid receptor type 2 (CB2) in VD rats was changed, either elevated or diminished, through the administration of HP β CD/BCP and AM630. The findings of the study indicated that the utilization of HP β CD/BCP has the potential to significantly boost the bioavailability of BCP. Through the utilization of the Morris water maze test, HP β CD/BCP has the capability to alleviate learning and memory deficits in rats. The results obtained from the assessment of cerebral blood flow (CBF) demonstrated that HP β CD/BCP has the potential to significantly contribute to the restoration of CBF. This research made it evident that the expression of CB2, PI3K, and Akt can be increased in hippocampus and white matter containing parts of brain. It can be concluded from this study that the combination of both the test drugs can produce a protective effect against cerebral ischemia. Furthermore, the findings suggested that the utilization of HP β CD/BCP could be a potential future treatment for vascular dementia.

(Chávez-Hurtado et al., 2020) Dietary treatments may have an effect on many of these changes, despite the fact that aging is connected to negative cellular and cognitive alterations. This is a significant public health problem. Through the activation of cannabinoid type-2 receptors, the naturally occurring sesquiterpene β -caryophyllene (BCP) is able to boost its anti-inflammatory and antioxidant properties in a variety of animal models, including cognitive impairment. This activation ultimately results in neuroprotection. In this study the effect of BCP is evaluated against long term use of D-Gal in aged mice. The cognitive flexibility and long-term memory capability of male BALB/c mice were assessed using the Morris water maze test. The rats were given either GAL (300 mg/kg intraperitoneally) or BCP (10 mg/kg orally) or a combination of the two. Furthermore, the histological study was conducted on the slices of prefrontal and hippocampal brain areas, and was examined for the glial cell activities and DNA damage. There is a reduction in the cognitive functionality after the administration of D- galactose (P=.0308), the administration of BCP was unable to revert the effects caused by D-galactose. The administration of D-gal can intensify the DNA reduction in the prefrontal cortex, and increase the number of astrocytes and intercellular connection in the hippocampus was compromised. Even while it failed to improve

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cognitive flexibility, BCP had a neuroprotective effect at the cellular and molecular levels in the GAL model of aging.

(Ullah et al., 2021) Adenosine triphosphate (ATP) synthesis, reactive oxygen species (ROS) generation, proteostasis, and cellular quality management can all be negatively impacted by mitochondrial malfunction. Microtubule DNA and electron transport chain components are vulnerable to damage when reactive oxygen species (ROS) production increases. This damage can result in a breakdown in the electrochemical gradient that is present across the inner membrane of the mitochondria, which can then lead to the termination of energy production by the mitochondria. Neurons are more susceptible to harm caused by mitochondrial dysfunction because of the increased oxygen and ATP requirements that they have of their cells. The pathological changes that are characteristic of neurodegenerative illnesses include the dysfunction of mitochondria, the damage of electron transport chains, the modification of membrane permeability and Ca^{2+} homeostasis, and the weakening of mitochondrial defense systems as a result of oxidative stress. According to an increasing body of research, the utilization of antioxidants may be appropriate for the purpose of minimizing neuronal loss since antioxidants contribute to the stabilization of mitochondria. Researchers have not yet discovered a medication that is useful for neurodegenerative illnesses, despite the fact that a number of natural substances have shown promise in addressing mitochondrial dysfunction and oxidative stress. The bicyclic sesquiterpene known as β -caryophyllene is the active component that is found in essential oils that are derived from a variety of spices and culinary herbs. Multiple medical investigations have shown that this molecule's role as a selective cannabinoid receptor 2 (CB2) agonist causes it to display a broad range of pharmacological effects. Among the effects encompassed by these actions are those that fight infections, inflammation, cancer, neurodegeneration, and pain. Throughout this review, the primary focus is placed on the potential role that β -caryophyllene plays in neuroprotection, as well as its capacity to reduce oxidative stress and mitochondrial dysfunction.

(Zhang et al., 2022) Much research has gone into Alzheimer's disease (AD), yet the disease is still hard to diagnose and treat, and no drug has been found to be successful. In Alzheimer's disease (AD), intracellular neurofibrillary tangles and amyloid β ($\text{A}\beta$) extracellular plaques are histopathological symptoms. These characteristics have been associated with neuropathological events, including cell death in the nervous system and synapses. The main goal of our research

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was to determine if the "JAK2-STAT3-BACE1" pathway is significantly involved in the neuroprotective effects of the food flavoring ingredient β -caryophyllene, or BCP. The objective of dividing PC-12 cells with overexpressed amyloid- β protein precursor (APP) into four groups was to establish an in vitro model of Alzheimer's disease (AD). These groups included a control group, an empty vector group, an APP overexpression group, and a battery of BCP (5, 10, and 20 μ M). Cell viability was assessed using CCK-8, synaptic morphology was examined by immunofluorescence, and the expression of genes and proteins levels were analyzed via quantitative real-time polymerase chain reaction and western blotting. The expression levels of JAK2, STAT3, and BACE1 mRNA were dramatically elevated in transfected PC-12 cells. Furthermore, at 72 hours post-transfection, the cells had a significant morphological alteration, becoming more spherical and exhibiting a reduced number of nuclei. By retaining the form of the cell, BCP demonstrated that it has the potential to dramatically improve the vitality of PC-12 cells. All of these processes were inhibited by BCP, including the overexpression of BACE1 mRNA and protein, the hyperphosphorylation of JAK2 and STAT3, and APP. For the purpose of simulating the binding of BCP to JAK2, STAT3, BACE1, and CB2, molecular docking was utilized. The JAK2 protein was found to be the most stable of all the proteins that were examined. By blocking the "JAK2-STAT3-BACE1" signaling pathway, it is possible that BCP possesses the ability to safeguard neurons and reverse the neurotoxicity caused by $A\beta$.

(Dorri et al., 2018) Cinnamon, which belongs to the Lauraceae family and is known as *Cinnamomum zeylanicum*, is regularly utilized in the culinary arts as a flavor enhancer. The medical benefits of this plant, on the other hand, have just recently brought it to the forefront of attention. There are a number of health benefits that are associated with the components of cinnamon. These components include cinnamaldehyde and cinnamic acid, which are antioxidants and free radical scavengers. Cinnamon also helps to reduce blood glucose levels, alleviate pain, fight against microbes and inflammation, inhibit the growth of yeast and secretagogues, and protect against gastric ulcers. Cinnamon has been found to be protective against environmental as well as man-made contaminants, according to the findings of a number of various investigations conducted on animals and in vitro, which are compiled in this paper. The purpose of these studies is to investigate the antidotal and protective properties of cinnamon and its primary components against toxicities that are caused by both natural conditions and chemical agents. The basic components of cinnamon have been discovered to have the ability to lessen the detrimental impact

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that chemical poisons have on a variety of organs and systems. This is achieved in part by their antioxidant capabilities, radical scavenging, anti-inflammatory activities, fungistatic and fungicidal actions, and modulation of numerous signaling pathways such as CK-MB, LDH, TNF- α , IL-6, mitogen-activated protein kinase (MAPK), and nuclear factor- κ B (NF- κ B).

(Neelam et al., 2020) There are a wide variety of plant foods that include phenylpropanoids or compounds of these compounds as secondary metabolites. These plant foods include fruits, vegetables, cereals, beverages, spices, and herbs. The antimicrobial, antioxidant, anti-inflammatory, antidiabetic, anticancer, renoprotective, neuroprotective, cardioprotective, and hepatoprotective qualities that they possess are only a few of the many functions that have been thoroughly established. The photoprotective, antibacterial, and antioxidant properties of these compounds make them helpful in a wide variety of industries, such as the food, pharmaceutical, cosmetic, and dye industries, as well as in the domains of biofuel, sensors, and the detection of biologically relevant chemicals. Phenylpropanoids are found in dietary supplements and skin care products that are offered at retail establishments. For the purpose of this review, we have assembled the most recent information on phenylpropanoids and their derivatives. This material includes what is known about their biosynthesis, occurrence, biological functions, action mechanism, and potential industrial applications.

(Ruegsegger et al., 2019) Despite the fact that there appears to be a connection between diabetes and dementia, the manner in which insulin deficiency has a detrimental effect on brain activity is not thoroughly understood. In STZ-induced diabetes in mice, we show that neurons in the brain, hypothalamus, and hippocampus were less active when insulin was insufficient due to administration of STZ. This was the case in all three regions of the brain. Changes in mitochondrial function were likely caused by a concurrent reduction in mitochondrial fusion proteins and an elevation in fission proteins in certain areas of the brain. Insulin deficiency did not lead to an elevation in reactive oxygen species (ROS) production; rather, the inhibition of monocarboxylate transporters resulted in an increase in ROS production and a subsequent reduction in ATP synthesis. Consequently, this indicates that increased levels of ketones and lactate have a role in reducing oxidative stress and providing energy for the generation of ATP in situations where insulin levels are insufficient. In addition, insulin has a direct regulatory influence on the activity of mitochondria in the brain; positive intranasal insulin injections increased the amount of ATP

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that was produced by mitochondria in animals that were otherwise healthy. A proteomics investigation of the brain found that insulin treatment increased neuronal development and neurotransmission pathways. This was the case despite the fact that insulin deficiency produced neurofibrillary degeneration and tau phosphorylation by oxidative post-translational modification of a large number of proteins. When considered as a whole, these findings provide support for the essential role that insulin plays in controlling mitochondrial homeostasis in the brain and shed light on the potential therapeutic benefits that intranasal insulin may potentially offer.

(Carregosa et al., 2021) There are growing worries regarding health, society, and the economy in relation to the increasing prevalence of neurodegenerative diseases in a population that is getting older. A diet that is rich in polyphenols has been shown to protect against cognitive decline and neurodegenerative illnesses, according to research that was conducted through intervention and epidemiological studies. At the same time, hundreds of low molecular weight (poly)phenol metabolites have been found in plasma and urine. This data makes it increasingly evident that gut bacteria play a role in controlling the breakdown of (poly)phenols during this process. There is a greater possibility that these byproducts may reach quantities in the bloodstream, and their half-life is significantly longer than that of the parent (poly)phenols. This is despite the fact that research on their potential effects on the brain is still in its early stages. In order to acquire valuable knowledge on the potential of low molecular weight (poly)phenol metabolites to alleviate the primary symptoms of neurodegeneration, we will discuss the numerous model species that have been utilized in the research that has been conducted on the effects that these metabolites have on neuronal-related physiological processes.

(C. Chen, 2015) Sinapic acid, also known as 3,5-dimethoxy-4-hydroxycinnamic acid, is a phytochemical that can be taken orally and has a number of beneficial effects. Some of these effects include reducing inflammation, protecting neurons from damage, preventing cancer, and lowering blood sugar levels. Sinapic acid can be found in abundance in spices, berries, vegetables, cereals, and oilseed crops. According to research, sinapic acid, which is a bioactive phenolic acid, has the potential to reduce the effects of a variety of substances that cause toxicity. The objective of this concise review is to offer a summary of the research that has been conducted on the pharmacokinetics, therapeutics, and protective effects of this versatile molecule.

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(Khatoon et al., 2018) There are around 600 neurological disorders that have been recognized. Some examples of these disorders include Alzheimer's disease (AD) and Parkinson's disease (PD). Natural goods make up a significant portion of the pharmaceutical options available for the treatment of neurological conditions. According to the findings of the researchers, natural product synthesis is responsible for approximately half of the medications that have been approved for use since 1994. This article provides a summary of the natural substances that have been shown to be effective in the treatment of Alzheimer's disease and Parkinson's disease, as well as a list of some of the most recent pharmacological advancements that have been made in the fight against both diseases.

(Iqbal et al., 2023) Several spices that are often used in the kitchen have been recognized by complementary and alternative medicine (CAM) for the possible health advantages they may offer. Ginger, fenugreek, cloves, saffron, cardamom, garlic, cumin, cinnamon, turmeric, and lemongrass are some of the spices that fall under this category. A sense of humor is imparted upon them by them. It is common knowledge that garlic's antibacterial properties contribute to the maintenance of a healthy heart and provide protection against illness. Clove is a great herb for pain management since it has qualities that include being analgesic and anti-inflammatory and being beneficial to nerve health. As a result of its antioxidant properties, cumin can be beneficial for both the management of one's weight and digestion. Cinnamon possesses qualities that are both anti-inflammatory and anti-diabetic, giving it the ability to support cardiovascular health and maintain blood sugar balance. It is effective in treating a wide range of disorders in addition to relieving pain in the joints. As a result of its anti-inflammatory and digestive properties, ginger can be of assistance to individuals who are experiencing constipation conditions. Fenugreek has a wide range of applications, including helping to maintain blood sugar levels and providing support to parents who are breastfeeding their children. A number of health benefits, including the reduction of inflammation and dyspepsia, are associated with celery. Lemongrass has a number of useful applications in addition to its antibacterial properties. These applications include alleviating stress and improving digestion. Through the use of cardamom, you can improve your oral health and make digestion easier. There is a well-deserved reputation for saffron's ability to improve mental health, elevate spirits, and alleviate symptoms of depression. These spices have a wide variety of therapeutic properties, which contribute to the fact that the medicinal value of food in rituals is increased.

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(Hemmati et al., 2018) This study set out to examine how cinnamic acid affected oxidative stress, cholinergic dysfunction, memory impairment, and a streptozotocin (STZ)-induced diabetes paradigm in mice. The 42 male mice used in the study were randomly assigned to one of six groups: control, diabetes, cinnamic acid (20 mg/kg/day, intraperitoneal), and cinnamic acid-treated diabetic (10, 20, and 40 mg/kg/day, intraperitoneal). The mice were all from the Naval Medical Research Institute (NMRI) and could weigh between 35 and 38 grams. When 50 mg/kg of STZ is given intraperitoneally, memory is negatively affected. Injecting cinnamic acid for forty days, starting on the 21st day after STZ-induced dementia was confirmed, allowed us to assess the substance's therapeutic efficacy. The subjects underwent a battery of memory assessments, including the Morris Water Maze, the Passive Avoidance Test, and the Cross-Arm Maze. Neurochemical markers of oxidative stress and cholinergic activity were assessed after the infant was delivered. New findings suggest that while cinnamic acid treatment substantially enhanced memory in a dose-dependent manner, STZ production substantially diminished memory. Brain homogenate analysis in the diabetes group showed lower glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activity; increased levels of reactive oxygen species (ROS); and lipid peroxidation when compared to the control group. Cinnamic acid treatment, however, improved these metrics in diabetic mice. Cinnamic acid alleviates diabetic mice's oxidative stress and cholinergic dysfunction, as shown in the present work. This leads to enhanced memory function.

(Prorok et al., 2019) The second most prevalent and tragic neurodegenerative illness in humans, Parkinson's disease (PD), has no known cure at this time. Despite a lot of research on the topic, this remains the case. Cinnamic acid is an essential building block in the production of many different plant-based compounds. It is an aromatic fatty acid that occurs naturally and has few negative consequences. This study examines the neuroprotective effects of cinnamic acid using the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mice model of Parkinson's disease. Oral administration of cinnamic acid retained dopaminergic neurons and fibers in mice that had been insulated with MPTP, including the substantia nigra pars compacta (SNpc) and striatal tyrosine hydroxylase (TH)-positive neurons. Mice injected with MPTP showed a comparable response to oral cinnamic acid administration, with normalized striatal neurotransmitter levels and improved locomotor activity restored. Our investigation into the processes led us to the surprising finding that cinnamic acid activated PPAR α in primary mouse astrocytes but did not activate PPAR β . In this model of Parkinson's disease, cinnamic acid enhances locomotor activity in the

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nigrostriatal pathway, protecting dopaminergic neurons in both wild-type and PPAR β (-/-) mice. Unfortunately, animals with Parkinson's disease who lack the protein PPAR α do not experience this protective effect. This research shows that cinnamic acid can protect dopaminergic neurons through the PPAR α pathway in a special way. The field of Parkinson's disease (PD) may benefit from this finding.

(Chainoglou et al., 2020) A hybridization approach involving cinnamic acid derivatives and curcumin analogues was employed for the manufacture of the novel hybrids. The hybridization technique was conducted in dry DMF or thionyl chloride and specifically involved hydroxy-benzotriazole (HOBT) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. To verify the new hybrids' structures, we used infrared, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, LC/MS ESI+, and elemental analysis. To make sure the hybrids weren't dangerous, this was done. In both theoretical and experimental contexts, the reversed chromatography method was used to determine the RM values, which are representations of the lipophilicity of substances. Researchers examined the new compounds' antioxidant, cyclooxygenase-2, acetylcholinesterase, and lipoxygenase inhibitory properties in vitro. All of the compounds were effective in preventing 2,2'-azobis(2-amidinopropane) hydrochloride-induced lipid peroxidation of linoleic acid. Among the pleiotropic derivatives, the hybrid 3e had the greatest impact, followed closely by the hybrid 3a. All hybrids were expected to diffuse and absorb through the blood-brain barrier (BBB) with relative ease. They had a very fast absorption rate and great oral bioavailability overall, with the exception of the initial three hours. We found that none of the CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 enzymes showed inhibition when we tested them. With the exception of the third generation, all hybrids tested positive for mutagenicity in the Ames test. First, we tried to figure out how the in vitro results relate to the most basic physical properties of the molecular building blocks. After that, we tried to figure out how the behaviors of these parts were related to one another. Hydrophobic interactions with amino acids were shown in docking experiments of soybean lipoxygenase (LOX). Hydrophobic interactions were found with TRP281, LEU282, TYR332, PHE333, and TYR336 in docking studies performed on acetylcholinesterase (AChE). In contrast, another investigation found π -stacking interactions with TYR336.

(Ghafary et al., 2020) It is thought that β -amyloid buildup, an excess of β -secretase, and cholinergic deficits in the hippocampus and cortex are the causes of Alzheimer's disease. Both AChE and

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BChE inhibitory activity IC₅₀ values were determined using the colorimetric Ellman's method. Researchers conducted a docking study, assessed its inhibitory efficiency on β -amyloid (A β) aggregations produced by AChE, conducted a series of kinetic studies to anticipate its functioning, and evaluated its neuroprotective and β -secretase inhibitory effects. Our group developed, synthesized, and tested a novel class of dual cholinesterase inhibitors that are combinations of cinnamic acid and tryptamine. When studied *in vitro*, these substances were able to inhibit both acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). An example of a synthetic molecule that fits this description is ϵ -N-(2-(1H-indol-3-yl)ethyl). The AChE inhibitory activity was most strongly demonstrated by 5-(3,4-dimethoxyphenyl)acrylamide (5q), which had an IC₅₀ value of 11.51 μ M. The second chemical with the second-highest IC₅₀ value was (E)-N-(2-(1H-indol-3-yl)ethyl), after this one. The compound that was found to be the most efficient in inhibiting BChE was 5-(2-chlorophenyl)acrylamide (5b), which had an IC₅₀ value of 1.95 μ M. Kinetic testing and molecular modeling also showed that 5q and 5b are mixed-type inhibitors that bind to both the PAS and CAS of AChE and BChE, respectively. Each of these inhibitors is a mixed-type inhibitor. A small number of neuroprotective effects and a modest β -secretase inhibitory activity were observed in the PC12 cell line when compound 5q was administered. On the occasion of the self-induced peptide aggregation test, this particular chemical was also effective in preventing the β -amyloid (A β) from aggregating together at a concentration of 10 μ M. The molecular modeling and kinetic studies, exhibited that 5q and 5b cooperated with AChE and BChE's catalytic active site and peripheral anionic site at the same time. Keep in mind that these interactions manifested at the same time.

(Sood et al., 2024) Memory impairments are marked by high levels of acetylcholinesterase (AChE) activity and oxidative stress, which are two major pathogenic aspects. As a result of this, researchers are investigating the possibility of using drugs that target oxidative stress and AChE as potential therapies for cognitive impairment. Numerous studies have demonstrated that *Morus alba* fruits possess both antioxidant and AChE inhibitory properties. Because of the high nutritional value that they have, these fruits are frequently consumed. On the other hand, nobody has yet mentioned how *Morus alba* fruits can help with memory issues. The objective of this research was to identify the constituents of *Morus alba* fruit extracts that were accountable for the antioxidant and AChE inhibitory effects that were seen *in vitro*. Additionally, the bioactive component that was created was studied for its ability to improve memory and alleviate the symptoms of dementia

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that were caused by streptozotocin (STZ). Through the use of guided fractionation, as well as in vitro DPPH and AChE assays, the bioactive component was successfully extracted. For the purpose of gaining an understanding of the mechanism of action, we conducted an analysis of the biochemical parameters of the brain and utilized the Morris Water Maze test to quantify the memory functions of mice. After the results of the in vitro testing demonstrated that the methanol extract (ME) possessed a significant inhibitory power for AChE and DPPH, the ME was subjected to further separation. When compared to the other fractions that were created, the ethyl-acetate fraction (EAF) demonstrated the most potent inhibitory effects on acetylcholinesterase (AChE) and diphenhydramine (DPPH). It was found that the bioactivity of the sub-fractions that were formed as a result of further fractionation of EAF was lower than the bioactivity of EAF itself. In addition, the endogenous antioxidant status was restored (superoxide dismutase and reduced glutathione), and thiobarbituric acid reactive species, nitric oxide, and brain AChE and myeloperoxidase activity were reduced (through ME and EAF). This resulted in an improvement in the cognitive dysfunction that was induced by STZ (intracerebroventricular) in animals. According to the findings of TLC densitometric tests, phenolic acids and quercetin were discovered in large quantities in both the EAF and their ME counterparts. By influencing the cholinergic and oxidative systems, the extract from *Morus alba* fruit may be able to assist in the treatment of memory issues. This is due to the high concentration of phenolic and flavonoid components that it contains.

(Pozdnyakov, 2021) Ischemic stroke is characterized by a complex pathophysiology, which makes it imperative that pharmaceutical therapies be utilized in a timely and acceptable manner. A neuroprotective therapy that aims to improve mitochondrial function is one of the potential treatment options that could be considered for this medical condition. Replicating the effects of cerebral ischemia in male Wistar rats was accomplished through the application of a permanent blockage of the middle cerebral artery. Through intraperitoneal injection, doses of 4-hydroxy-3,5-di-tertbutyl cinnamic acid ranging from 25 mg/kg to 100 mg/kg were delivered to the subjects. Finally, the administration took place three days after the ischemia modeling had been completed. In addition, we examined the mitochondrial activity of the hippocampus of the rats and used the Morris water maze test to see whether or not there were any deviations in the rodents' cognitive ability. There was a dose-dependent improvement in mitochondrial function in the rat hippocampus after administration of the drug under study. An increase in citrate synthase activity

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of 55.1% ($P < 0.05$) and a rise in ATP content of 25.7% ($P < 0.05$) and 23.9% ($P < 0.05$), respectively, were observed after administering the test material at doses of 20 mg/kg and 50 mg/kg, respectively. Also, oxidative stress (an increase in antioxidant enzyme activity at the same time that TBARS concentrations decreased) and apoptosis (a decrease in calcium content, concentration of apoptosis-inducing factor, and caspase-3 activity) were both reduced after the test compound was administered. Doses of 100 mg/kg demonstrated a decrease in the tested chemical's efficacy. In a model of chronic brain ischemia, 4-hydroxy-3,5-di-tertbutyl cinnamic acid protected hippocampus cells from damage when given at doses of 25 and 50 mg/kg, respectively.

(Elkharsawy et al., 2024) The application of microbial transformation is widespread because it allows for the production of novel, highly active, and selective metabolites in considerable quantities. Because of the significant pharmacological effects that are known to be associated with cinnamic acid, microbial transformation was utilized in order to develop new derivatives of cinnamic acid that demonstrated enhanced biological activity. Through the modification of the biotransformation two-stage fermentation process, cinnamic acid was transformed into five distinct metabolites. Two new chemicals, namely 2-methyl heptyl benzoate and N-propyl cinnamamide, were produced by the *Alternaria alternata* fungus. The other three chemicals, p-hydroxybenzoic acid, cinnamyl alcohol, and methyl cinnamate, were produced by *Rhizopus* species, *Rhodotorula rubra*, and *Penicillium chrysogenum*, respectively. Cinnamic acid and its byproducts were studied for their potential to inhibit cyclooxygenase (COX) and acetylcholinesterase (AChE). Furthermore, the capacity of human neuroblastoma (SH-SY5Y) cells to shield neurons from the neurotoxic effects of hydrogen peroxide (H_2O_2) and adenosine monophosphate ($A\beta_{1-42}$) was tested. The metabolite showed more potency than the original medicine as a COX-2 inhibitor, with an IC_{50} value of $1.85 \pm 0.07 \mu M$. Out of all the molecules that were evaluated, the metabolite molecule was the only one that exhibited an AChE inhibitory effect, as indicated by its IC_{50} value of $8.27 \mu M$. Further evidence that these findings are accurate was provided by an in-silico examination of the binding interactions that occurred between the active metabolites and the active sites of the enzymes that were being investigated. When compared to catechin and epigallocatechin-3-gallate, which were employed as positive controls, the metabolite demonstrated a greater capacity to act as a neuroprotective agent against the neurotoxicity caused by H_2O_2 and $A\beta_{1-42}$. As a result of these findings, the two new metabolites and the metabolite have been identified as potentially fruitful routes for further investigation into

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neurodegenerative diseases that are associated with neurotoxicity, neuroinflammation, or cholinergic insufficiency.

(Huang et al., 2021) one of the most prominent targets of AD is increased levels of cytokines which promotes the inflammatory responses in brain. In this paper, we reported on our efforts to investigate cinnamic acid derivatives with the purpose of determining whether or not they have the ability to reduce inflammation in the nervous system. After significant structural changes in the compound 4f, it becomes able to show the anti-inflammatory response against experimental settings. This compound 4f is capable of restoring the normal glucose uptake and its cellular metabolism, also the memory impairment cause by the lipopolysaccharide (LPS), when it was administered orally. The studies have confirmed that the compound 4f has capability to bind to the mitogen activated protein kinase (MAPK) signaling pathway. This in turn, inhibits the downstream signaling pathway and halts the advancement of neuroinflammatory processes. It is clear from the docking experiments that the compound 4f has ability to reach to the active storage sites of interleukin-1 β (IL-1). Furthermore, it was uncovered that the hydrogen bond between the 4f and SER84 tends to increase the binding affinity of the compound. These findings from the study direct us towards the future perspective of the research for treatment of Alzheimer's disease, including CAD.

(Chainoglou et al., 2020) For the purpose of synthesizing the novel hybrids, a hybridization method that included cinnamic acid derivatives and curcumin analogues was utilized. Specifically, hydroxy-benzotriazole (HOBT) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide were utilized in the hybridization process, which was carried out in dry DMF or thionyl chloride. IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, LC/MS ESI+, and elemental analysis were utilized in order to validate the structures of the new hybrids. This was done in order to ensure that the hybrids were safe to use. The reversed chromatography method was utilized in both experimental and theoretical settings in order to ascertain the RM values, which are a representation of the lipophilicity of compounds. In vitro research was conducted to study the antioxidant and cyclooxygenase-2 inhibitory actions of the novel compounds, as well as the acetylcholinesterase and lipoxygenase inhibitory activities. Each of the compounds demonstrated sufficient protection against the lipid peroxidation of linoleic acid that was caused by 2,2'-azobis(2-amidinopropane) hydrochloride. The hybrid 3e was the most significant of the pleiotropic derivatives, with the hybrid 3a coming as a close second. With a

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reasonable amount of ease, it was hoped that all hybrids would be able to diffuse and absorb via the blood-brain barrier (BBB). They exhibited exceptional oral bioavailability and a very rapid absorption rate, with the exception of the first three hours. When CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 were examined, it was discovered that none of them exhibited any inhibition. According to the results of the Ames test, every single hybrid, with the exception of the third generation, produced mutagenicity. In the first place, we endeavored to determine the manner in which the in vitro findings are connected to the most fundamental physicochemical features of the structural components of the molecules. After that, we made an effort to determine the ways in which the behaviors of these components are connected to one another. Docking studies conducted on soybean lipoxygenase (LOX) demonstrated the presence of hydrophobic interactions with amino acids. In docking tests conducted on acetylcholinesterase (AChE), it was revealed that hydrophobic contacts were observed with TRP281, LEU282, TYR332, PHE333, and TYR336. On the other hand, π -stacking interactions with TYR336 were observed in another study.

(Raha et al., 2024) An excessive accumulation of GM2 ganglioside, primarily in the lysosomes of nerve cells, is a common sign of Tay-Sachs disease (TSD), which is an autosomal recessive lysosomal storage metabolic illness, and Sandhoff disease (SD), which is a severe version of TSD. Despite the fact that they appear to be normal at birth, persons who have these illnesses eventually pass away as a result of an accumulation of GM2 gangliosides in their neurons that have not been destroyed. This accumulation leads to issues with motor skills and a gradual decline in their behavioral capacities. The lack of a viable treatment for TSD/SD is deeply disheartening. Focusing on halting disease progression in a transgenic mouse model of sickle cell disease (SD), the present work brought attention to the importance of cinnamic acid (CA), an aromatic fatty acid found naturally in several plants. Brain cortical GM2 ganglioside and glycoconjugate accumulation, inflammation, and glial activation were all significantly reduced in CA-treated Sandhoff mice after oral dosing. The behavioral abilities of Sandhoff mice were enhanced by oral CA, which also increased the survival rate of the mice. The oral treatment of CA increased the levels of peroxisome proliferator-activated receptor α (PPAR α) in the brains of Sandhoff mice, something we discovered. Nevertheless, no reduction of glycoconjugates, improvement of behavior, or augmentation of survival was observed when CA was orally administered to Sandhoff mice deficient in PPAR α . We have found that CA significantly slows down the progression of SD and increases the longevity of Sandhoff mice through a PPAR α -dependent mechanism.

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(Bae et al., 2018) Researchers have shown that cinnamaldehyde (CA), a key component of cinnamon essential oil's taste, possesses antioxidant, anti-inflammatory, and anti-angiogenic properties. All of these characteristics have been found. Multiple neurodegenerative diseases, notably Parkinson's disease (PD), have shown that CA has neuroprotective properties, which have only lately come to light. Autophagy in neurons is so tightly controlled that disruptions in this process may lead to the onset of neurodegenerative diseases. Here, we show that CA can block 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced selective death of dopaminergic neurons in rat substantia nigra cells. Following treatment with MPTP, CA was able to suppress the activation of LC3 puncta, which are microtubule-associated proteins. After being treated with CA, the substantia nigra of MPTP mice displayed an upregulation of p62 expression. Our results showed that autophagy inhibitors could stop 1-methyl-4-phenylpyridinium (MPP⁺) from killing BE(2)-M17 cells. Considered collectively, our results suggest that CA has neuroprotective benefits in a PD model, and they raise the possibility that blocking autophagy could be a viable therapeutic strategy for AD.

(Chavarria et al., 2015) New lipophilic hydroxycinnamic acid derivatives were created and manufactured by us, and then we put them through a series of tests to determine whether or not they have neuroprotective and antioxidant properties. By chemically altering the cinnamic acid scaffold, it is possible to develop compounds that have a greater lipophilicity and, in general, a higher antioxidant activity in comparison to natural models (caffeic and ferulic acids). Without causing any cytotoxicity, the compounds demonstrated a significantly protective effect against the damage that was caused by 6-OH-DA to SH-SY5Y cells. The effectiveness of compound 6 as a radical scavenger and iron(II) chelator ensures that it possesses properties that are similar to those of a conventional medication. Furthermore, it was observed that even at a moderate level of 1 μ M, neuroprotection against oxidative damage was seen. As a consequence of this, compound 6, which was produced through the application of a method that is focused on biology, possesses a number of desirable features that can be improved in order to produce a novel antioxidant that can be utilized therapeutically for the treatment of neurodegenerative diseases.

(Muronetz et al., 2020) This study possibly uncovers the potential neuroprotective effects of hydroxycinnamic acid against neurodegenerative diseases. Efficiently preventing amyloidogenic protein modification by these chemicals is one of the areas of research being pursued. We will start

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by outlining the key events that led to the rise of interest in natural chemicals and other derivatives of HCA. Another half of the study, covers the synthesis part of HCA and about its characteristics. Afterwards, thorough research is carried out on the outcomes of the molecular modelling evaluation of HCA derivatives in relation to prion protein and α -synuclein sheets. The next thing to do is to thoroughly examine all of the experiments that were run to find out how different chemicals, including phenylacetic acid and benzoic acid derivatives, which are structurally similar to HCA, affect the pathological alteration of prion protein and α -synuclein. The HCA has potential to inhibit the process of the amyloid beta formation and its conversion into the amyloid beta plaques, hence causing neuroprotection. This is because they exist as natural metabolites in foods and in human tissues and blood.

(Moon et al., 2020) Chronic, persistent, and inflammatory skin reactions are known as atopic dermatitis (AD). One of the main components of Panax ginseng Meyer, a member of the Araliaceae family, is p-coumaric acid (pCA). The levels of thymic stromal lymphopoietin (TSLP), tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1 β) in HMC-1 cells were found to decrease after pCA was administered. After administering PCA to HMC-1 cells, reduced levels of RIP2, caspase-1, phosphorylated (p)p38/pJNK/pERK, and pIKK β /pI κ B α /NF- κ B were noted. Also, pCA reduced the levels of TSLP, TNF- α , IL-6, IL-4, and IFN- γ in the activated splenic cells' supernatant. There was less pathological change in AD-like lesions in the pCA group compared to the DNFB-sensitized control group. After applying PCA to skin lesions, researchers found that TSLP, IL-6, and IL-4 protein and mRNA expression levels were lower. Additionally, in lesions that resembled Alzheimer's disease, pCA therapy was discovered to decrease caspase-1 activity. Histamine, IgE, TSLP, TNF- α , IL-6, and IL-4 serum levels decreased after pCA treatment.

(Peng et al., 2018) One possible strategy for preventing cataracts is to shield the lens epithelial cells from oxidative stress, which can cause their cell death. This study set out to look at how p-coumaric acid (p-CA) prevented hydrogen peroxide (H₂O₂)-induced cell death in human lens epithelial (HLE) cells (SRA 01-04). The cells were pretreated with p-CA at dosages of 3, 10, and 30 μ M before being treated with H₂O₂ at a concentration of 275 μ M. After pretreatment with p-CA, HLE cells showed a dose-dependent protection against cell death caused by H₂O₂ and a downregulation of the expressions of cleaved caspase-3 and cleaved caspase-9. Furthermore, p-

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CA significantly decreased intracellular reactive oxygen species (ROS) production and mitochondrial membrane potential loss in H₂O₂-treated cells. Contrarily, GSH-Px, CAT, and T-SOD activities were all upgraded. P-38, ERK, and JNK phosphorylation in HLE cells was inhibited by p-CA, according to in vitro studies. This was in response to H₂O₂. The results suggest that p-CA could be utilized as a treatment to prevent cataracts by reducing H₂O₂-induced cell death and by regulating MAPK signaling pathways.

(Reina et al., 2021) The molecule known as p-coumaric acid may be found in virtually every plant, and it is also a typical component of the food that people consume. For the purpose of conducting a methodical and reasonable search for novel p-coumaric acid derivatives, a computer-assisted methodology that was based on chemical features of the compounds was utilized. The fundamental structure was improved by the addition of functional groups, and 156 derivatives were produced by employing a selection score (SS) that was specifically devised for the purpose. A selection of ten p-coumaric acid derivatives was selected with the intention of lowering the level of toxicity while preserving the drug-like behavior of the candidate compounds. The reactivity indices, pKa values, and bond dissociation energies were computed in order to arrive at a measurement of the antioxidant capacity. Based on the findings, it was determined that three p-coumaric acid derivatives have the greatest potential to perform the role of chemical antioxidants. According to the mechanics of electron transfer and/or hydrogen transfer, it appears that these derivatives will be more effective than p-coumaric acid itself in terms of accomplishing that particular goal. By acting as new scaffolds of COMT inhibitors that do not contain nitrocatechol, they have the potential to treat Parkinson's disease without the undesirable side effects that are associated with the inhibitors that are now in use. In light of the discoveries that have been published, we believe that additional theoretical and experimental investigations into these substances will be prompted.

(He et al., 2021) Traditional Chinese medicine prescribes the plant *Alpinia oxyphylla* Miq. (AOM) to stroke patients; however, little is understood about the herb's effects or mechanism of action. It is estimated that the AOM can prevent the drastic effects of the stroke like cognitive impairment by uplifting the activities of the brain-derived neurotrophic factor (BDNF) signaling pathway. The result would be an upsurge in adult hippocampal neurogenesis. For proving the hypothesis, the research used two models, in the first model MCAO was induced in rats and in the second model involve a neural stem cell (NSC) model. To start, rats with MCAO ischemia showed better

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cognitive function and spatial learning/memory after getting AOM treatment in vivo. Along with that, it increased p-AKT, tropomyosin receptor kinase B (TrkB) expression, and adult hippocampal neurogenesis. Rodents showed signs of all these consequences. In-vitro studies have confirmed that the p-coumaric acid is the key phytoconstituent present in the AOM extract. It activate the neural stem cell proliferation and activate the BDNF/TrkB/AKT signaling pathway. The BDNF/TrkB specific inhibitor ANA12 can counteract the effect of PCA. We can conclude from the study that treatment with P-CA can uplift the neuronal proliferation in hippocampus, thus improve the spatial learning and memory skills, hence decrease the anxiety in the rats with MCAO induced ischemia.

(Zang et al., 2000) P-coumaric acid (CA) is known to have antioxidant properties, but we do not yet have a complete understanding of how it works. This study aims to establish whether or not CA has the ability to directly scavenge reactive oxygen species (ROS) and whether or not it can diminish the oxidation of LDL. Animals were given low doses of CA in water at 29 mg/day for ten, twenty-one, and thirty days, and high doses at 317 mg/day for the same amount of time. We monitored the levels of 8-epiprostaglandin F2 α in the bloodstream in order to determine the extent of LDL oxidation that was taking place. Oral administration of 317 mg of CA for a period of thirty days resulted in a significant reduction in LDL oxidation. In spite of the fact that CA contributed to a reduction in serum levels of low-density lipoprotein cholesterol, it had no effect whatsoever on HDL cholesterol levels. Through the utilization of electron spin resonance in conjunction with spin trapping techniques, researchers investigated CA's ability to scavenge reactive oxygen species (ROS) and change the oxidation of LDL in vitro. During the process of scavenging, OH, CA demonstrated dose-dependent effectiveness. Assuming a rate constant of $1.8 \times 10^{11} \text{ M}^{-1} \cdot \text{s}^{-1}$, the maximal velocity for the scavenging of CA by $\bullet\text{OH}$ was 1.2 $\mu\text{M/s}$, and the median inhibitory concentration (IC50) was 4.72 μM . According to the findings of our research, one of the ways in which CA is able to exercise its antioxidant effects is by directly eliminating reactive oxygen species (ROS) like OH.

(Roychoudhury et al., 2021) The phenolic compound known as para-coumaric acid (p-CA) is an example of a different kind of secondary metabolite that can be found in plants. In addition to grains, fruits, and vegetables, which are its principal sources of nutrition, it can also be found in a wide variety of other plant-based foods. It is clear from the previous studies that PCA has free

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radical scavenging properties, hence in this study its antioxidant property and anti-inflammatory were put to test. These findings were obtained through both in vitro and in vivo examinations. A comprehensive investigation into the potential therapeutic and protective activities of p-CA is presented in this evidence-based review. p-CA and its conjugates have been shown to exhibit a variety of bioactivities, including antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, and anti-melanogenic properties. PCA has very potent antioxidant property, that is why it can be employed in various illnesses like arthritis, neurodegenerative disorders as well as heart related complications. Newer findings points towards beneficial effects of PCA against reproductive system complications like erectile dysfunction due to its capability to reduce the oxidative stress.

(Sevindik et al., 2021) This study explored the anti-oxidant, antibacterial, and antigenotoxic properties of *Octaviania asterosperma* due to presence of its phenolic content. The catechin, epicatechin, caffeic acid, p-hydroxybenzoic acid, gallic acid, coumaric acid, and chlorogenic acid were the components that were found in the total phenolic content (ppm) of *O. asterosperma*. The measurements for these components were as follows: 54.73 ± 4.68 , 123.90 ± 8.52 , 4.23 ± 0.97 , 37.72 ± 3.84 , 58.07 ± 5.40 , 56.64 ± 6.39 , 80.76 ± 4.92 , and 2.45 ± 0.15 , respectively. The total antioxidant status (TAS) was determined to be 3.410 ± 0.099 mmol/L, the total oxidant status (TOS) was assessed at 7.548 ± 0.147 μ mol/L, and the oxidative stress index (OSI) was calculated to be 0.221 ± 0.005 . The fact that *O. asterosperma* possessed antibacterial properties was very encouraging. Furthermore, the extract did not demonstrate any genotoxic potential and was able to minimize the oxidative DNA damage that was generated in neurons by hydrogen peroxide (H_2O_2). To preserve mitochondrial function, reduce cleaved-caspase-3 and apoptosis-inducing factor expression levels, and modulate protein kinase B (Akt), the mammalian target of rapamycin (mTOR), and the phosphatase and tensin homolog on chromosome ten (PTEN), pretreatment with *O. asterosperma* was found to be effective at pathophysiological concentrations of GLU (25 mM). For the therapy or management of neurodegenerative illnesses, *O. asterosperma* is an essential meal because of its phenolic content, strong antioxidant, and anti-excitotoxic effects.

(K. J. Kim et al., 2022) The changes in one's mental and physical state that are brought on by depression have an effect on the quality of life that one experiences. The pathophysiology of depression is still unknown, and treatment does not provide sufficient protection. As a result, many people feel that depression may soon become the second most common disease in the world. This

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is despite the fact that depression is quite prevalent. P-Coumaric acid, also known as p-CA, is a type of phenolic acid that may be found in food. It possesses a number of characteristics that are advantageous, such as anti-inflammatory, anti-melanogenic, anti-fungal, anti-HIV, and antioxidant effects. We investigated the possibility that p-CA could ameliorate depressive symptoms in mice that had been treated with LPS by reducing the levels of inflammatory cytokines. This was done with the intention of minimizing the unfavorable effects that were observed. We monitored the changes that occurred in synaptic activity, inflammatory cytokines, neurotrophic factors, and behaviors that are traditionally associated with feelings of depression. When p-CA was given to the mice that were examined, there was a noticeable improvement in the behavioral indicators that were associated with feelings of hopelessness. The forced swim test (FST), the tail suspension test (TST), and the sucrose splash test (SST) all revealed the presence of these symptoms after being administered. In addition, the presence of p-CA prevented the inflammatory cytokines, such as cyclooxygenase-2 and tumor necrosis factor- α , from being enhanced in the hippocampus, which was caused by the presence of LPS. It also prevented the capacity of LPS to reduce brain-derived neurotrophic factor (BDNF), which was at a lower level. Electrophysiologically, p-CA was able to prevent the reduction of long-term depression in organotypic tissue slices that had been exposed to LPS. After conducting behavioral, biochemical, and electrophysiological tests, researchers came to the conclusion that p-CA protected rats from the depression that was caused by LPS. According to these findings, p-CA has the potential to be an effective medicine for the treatment and prevention of depression.

(Gao et al., 2020) The perennial shrub *Lycium ruthenicum* Murr. (*L. ruthenicum* Murr.) finds widespread application in both conventional medicine and dietary supplements. During this examination of *Lycium ruthenicum*, eight distinct polyphenols were found. Among these polyphenols were syringic acid, quercetin, ferulic acid, rutin, p-coumaric acid, and protocatechuic acid. This study is aimed to explore the protective effects of *L. ruthenicum* Murr. polyphenols (LRP) on hydrogen peroxide induced oxidative stress model. The study uncover the protective effect LRP against the toxic effect of H₂O₂ on PC12 cells. During cell death caused by oxidative stress, LRP pretreatment inhibited the activation of various caspases like 3, 8, and 9 on PC12 cells. In addition to reducing ROS generation and restoring mitochondrial membrane potential, this was achieved. More proof shows that LRP might be a safe and efficient strategy to slow down the progression of neurodegenerative illnesses that are linked to oxidative stress.

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(Caruso et al., 2022) Cognitive impairment, often called cognitive decline, can present itself in two ways: gradually or abruptly, and it can be transient or permanent. The prevalence of this problem, which can be caused by aging or other neurological diseases like Alzheimer's disease (AD), is a growing issue in the field of public health. It is now widely acknowledged that dietary habits and other lifestyle factors play a major role in promoting good aging and warding off cognitive decline as we become older. A number of naturally occurring substances, such as phenolic acids and other dietary polyphenols, have recently attracted considerable attention. When consumed in supplement form, these polyphenols have been linked to improved cognitive function and protection against cognitive decline. It is surprising that there is a lack of human studies investigating the link between a phenolic acid diet and cognitive outcomes, given the possible therapeutic benefit of phenolic acids. This review aims to provide preclinical evidence that certain dietary polyphenols, including cinnamic aldehyde, rosmarinic acid, and ellagic acid, can modulate inflammatory status, antioxidant machinery, and other molecular pathways to promote neuroprotection and cognition. To back up the promising results from preclinical trials, more *in vivo* research is required. Future research should focus on identifying the specific metabolites produced by phenolic acid consumption that may have neuroprotective effects. This subgroup of polyphenols has excellent pharmacokinetic properties and can accumulate in the brain at pharmacologically relevant levels.

(H. Kim et al., 2021) p-Coumaric acid (p-CA) is an anti-inflammatory and antioxidant with a reputation for having protective qualities. To know the content of PCA available in the human plasma, liquid chromatography mass spectrometry (LC-MS) method was employed. This method was created using UPLC-MS/MS, which stands for ultra-performance liquid chromatography-tandem mass spectrometry. This study examines the effects of p-CA derived from *Bambusaecaulis* in Taeniam (BC) on the treatment levels of IGF-1, osteocalcin, and p-CA in children using a pharmacokinetics/pharmacodynamics (PK/PD) model. These three factors are crucial in determining how children's height develops. Positive ion detection in Multiple Reaction Monitoring (MRM) scan mode allowed us to identify p-CA and an internal standard in a plasma sample. Analyzing the material allowed us to do this. There were 34 people in the research. 17 got a placebo and 16 got the therapy. The research participants were randomly divided into two groups: the control group and the BC group. Each of the three capsules containing 258 milligrams of the investigational drug was given orally to the trial participants twice daily, with a gap of two meals in between each dose. By injecting the material into the UPLC-MS/MS system, we were able to

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construct standard calibration curves (reproducibility) and ascertain that the p-CA LLOQ was 0.2 ng/mL. We were able to confirm that reproducibility was attained in this way. The results showed that p-CA had an intra-accuracy of 99.6 to 108.4 percent and an inter-accuracy of 1.3 to 6.4 percent in terms of precision. Previous studies on the pharmacokinetics and pharmacodynamics of p-CA in human plasma have made good use of this methodology. The fact that the Taeniam extract caused a change in height relative to the baseline and an increase in IGF-1 and osteocalcin levels implies that the p-CA may play a significant role in the development of longitudinal bone. Therefore, p-CA, which is made from BC in Taeniam, could be a good substitute for GH treatment.

(Kaur & Kaur, 2022) There are three possible isomers of coumaric acid, a hydroxy derivative of cinnamic acid. P-coumaric acid is a highly popular form of this compound. 4-hydroxycinnamic acid a powerful phenolic compound is present in variety of cereals, fruits and vegetables. It is found in lignocellulosic materials and plays an important role in the phenolic polymer lignin. Numerous p-coumaric acid derivatives, both synthetic and naturally occurring, have been identified in scientific literature. Scientists have looked at a variety of extraction methods to learn more about the quantitative and qualitative properties of plant materials. Acidification and solvent extraction are two examples of more traditional procedures; clip-off, sugaring out, and soft microwave extraction are examples of more modern approaches. All sorts of p-coumaric acid derivatives, including amides, esters, aldehydes, polymers, and copolymers, are within the purview of this study. Among the many bioactive properties exhibited by p-coumaric acid and its derivatives are the following: antioxidant, antimicrobial, anticancer, antiarthritic, anti-inflammatory, gout prevention, anti-diabetic, anti-melanogenic, skin regeneration, gastroprotective, anti-ulcer, cardioprotective, hepatoprotective, reno-protective, bone formation, anti-angiogenic, anti-platelet, and a seemingly endless list of other attributes. Cosmetics, consumable foods, pharmaceuticals, and many more goods might incorporate p-coumaric acid due to its high bioactive potential. Finding out if they are compatible with these goods requires additional investigation.

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Table No. 2.1: Pharmacological activities of Beta-caryophyllene, Cinnamic acid and p-Coumaric acid.

Compound	Pharmacological activity	Mechanism / Key actions	Reported relevance
β-Caryophyllene	Anti-inflammatory	Selective CB ₂ receptor agonist; inhibits NF-κB, COX-2, iNOS	Neuroinflammation, arthritis, colitis
	Antioxidant	Scavenges ROS; enhances SOD, CAT, GPx	Oxidative stress-related disorders
	Neuroprotective	Reduces microglial activation; protects against Aβ-induced toxicity	Alzheimer's disease, Parkinson's disease
	Analgesic	CB ₂ -mediated modulation of pain signaling	Neuropathic and inflammatory pain
	Anxiolytic & antidepressant	Modulates endocannabinoid and dopaminergic pathways	Anxiety, depression
	Hepatoprotective	Reduces lipid peroxidation and inflammatory mediators	Drug- and toxin-induced liver injury
	Anticancer	Induces apoptosis; enhances chemosensitivity	Breast, colon, and prostate cancer
Cinnamic acid	Antioxidant	Free radical scavenging; inhibits lipid peroxidation	Oxidative stress-mediated diseases
	Anti-inflammatory	Suppresses NO, TNF-α, IL-6; inhibits NF-κB	Inflammatory and autoimmune disorders
	Neuroprotective	Inhibits Aβ aggregation; reduces neuronal apoptosis	Alzheimer's disease
	Antidiabetic	Improves insulin sensitivity; inhibits α-glucosidase	Type 2 diabetes mellitus
	Antimicrobial	Disrupts microbial cell membrane	Bacterial and fungal infections

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	Cardioprotective	Prevents LDL oxidation; improves endothelial function	Atherosclerosis
	Anticancer	Cell cycle arrest; apoptosis induction	Colon, liver, lung cancers
p-Coumaric acid	Antioxidant	ROS scavenging; increases endogenous antioxidant enzymes	Neurodegenerative disorders
	Anti-inflammatory	Inhibits COX-2, LOX, NF- κ B signaling	Chronic inflammatory diseases
	Neuroprotective	Protects neurons from oxidative and excitotoxic injury	Alzheimer's and Parkinson's disease
	Hepatoprotective	Reduces liver enzymes and oxidative stress	Chemical-induced hepatotoxicity
	Cardioprotective	Inhibits LDL oxidation; anti-platelet activity	Cardiovascular diseases
	Antimicrobial	Inhibits bacterial growth and biofilm formation	Foodborne and pathogenic bacteria
	Anticancer	Suppresses tumor growth; induces apoptosis	Breast, colon, gastric cancers

Interoceptive Models:

Disease Model 01 (AlCl₃ + D-galactose):

Aluminum is known to have neurotoxic effects that impede memory and cognitive function and contribute to neurodegeneration. According to research, aluminum chloride (AlCl₃) after crossing the blood brain barrier can reach to certain areas of brain like hippocampus and cerebral cortex, where it can increase the levels of inflammatory markers, can disturb the mitochondrial functionality, also can increase the formation of amyloid-β plaques by increasing the availability of NFT & Amyloid-β proteins and can also increase the levels of free radicals, by doing so it will promote the linked to neuronal degeneration (K. Kaur et al., 2022).

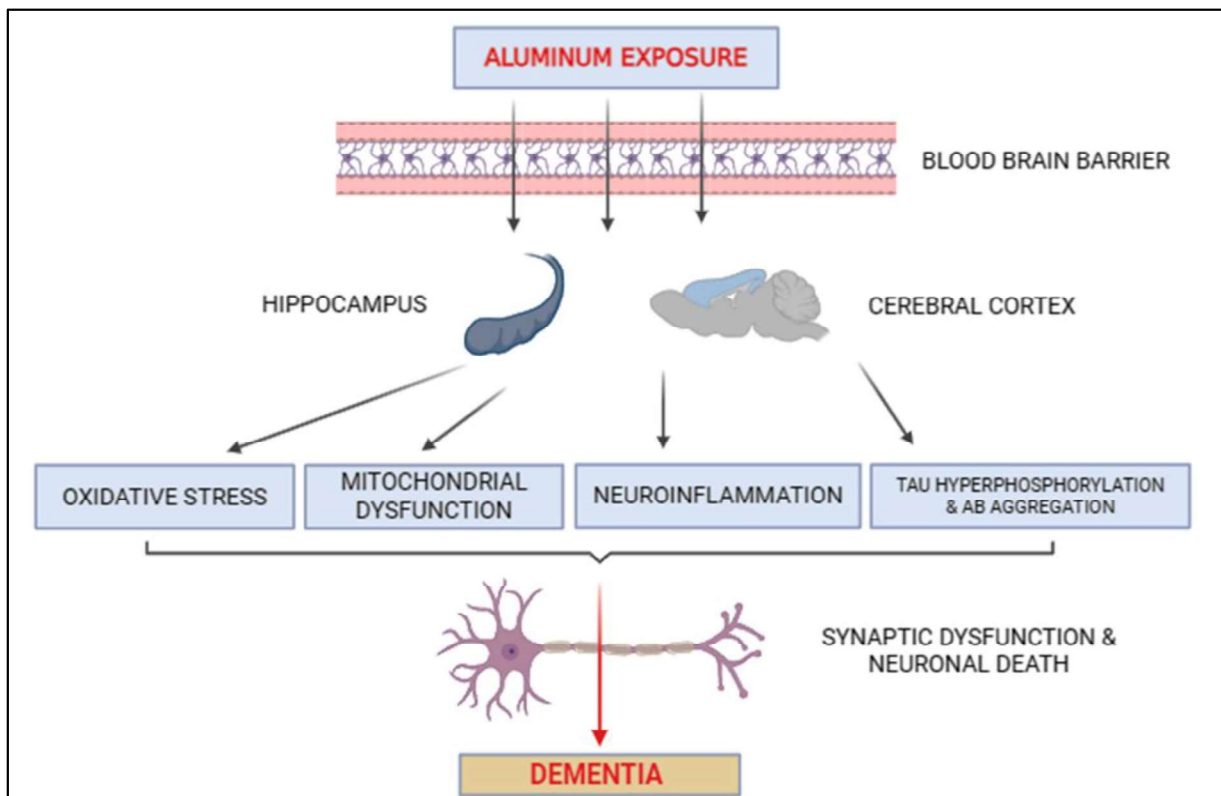


Figure No. 2.2: Role of Aluminium in inducing dementia.

Another monosaccharide that has been connected to brain aging is D-galactose, which is produced when lactose is hydrolyzed in the intestine. Research has shown that D-galactose causes a decrease in brain-derived neurotrophic factors and a number of other processes that contribute

to cognitive decline, such as mitochondrial dysfunction, elevated oxidative stress, inflammation, and apoptosis (Shwe et al., 2018).

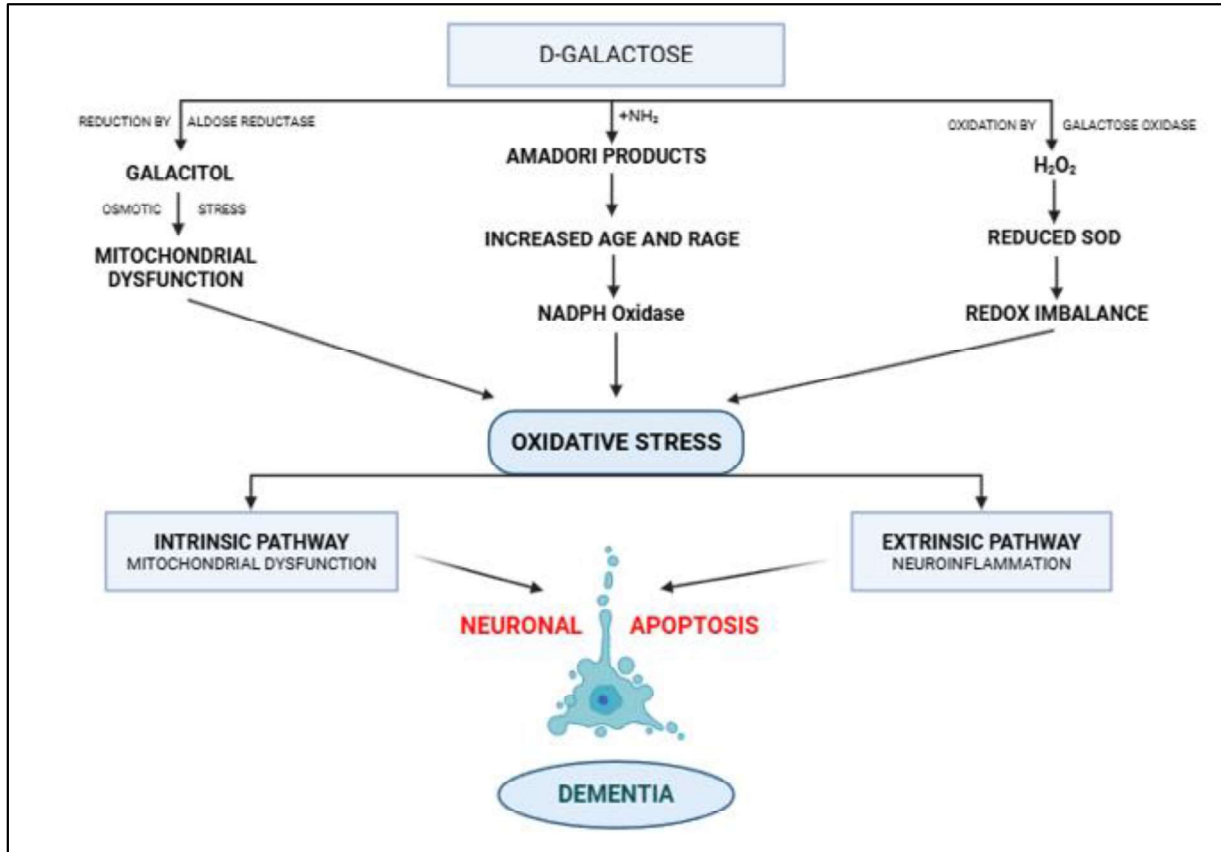


Figure No. 2.3: Role of D-galactose in inducing dementia.

From the previous studies it is understood that co-administration of $AlCl_3$ at a dose of 5 mg/kg p.o. and 60 mg/kg i.p. of D-galactose for a period of 90 days effectively induce the dementia in mice (Kumar et al., 2021).

Disease Model 02 (L-methionine)

The production of S-adenosyl methionine (SAM), a fundamental methyl donor for a range of biological methylation events involving RNA, proteins, carbohydrates, phospholipids, and neurotransmitters, depends on methionine, a crucial human amino acid. Furthermore, glutathione, a powerful antioxidant that guards against oxidative stress, and homocysteine, a neurotoxic

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intermediary in the methylation process, are both precursors of L-methionine (L-MET) (Loenen, 2010; Sharma et al., 2017; Trivedi & Deth, 2012).

Disturbances in methionine metabolism have been linked to mental and neurological disorders. Changes in L-MET and other metabolites in the one-carbon (C1) cycle have been connected to age-related neurodegenerative illnesses, including Alzheimer's disease (AD) and vascular dementia (Fayez et al., 2019; Y. Liu et al., 2022), as well as neurodevelopmental disorders like autism and schizophrenia (Indika et al., 2021).

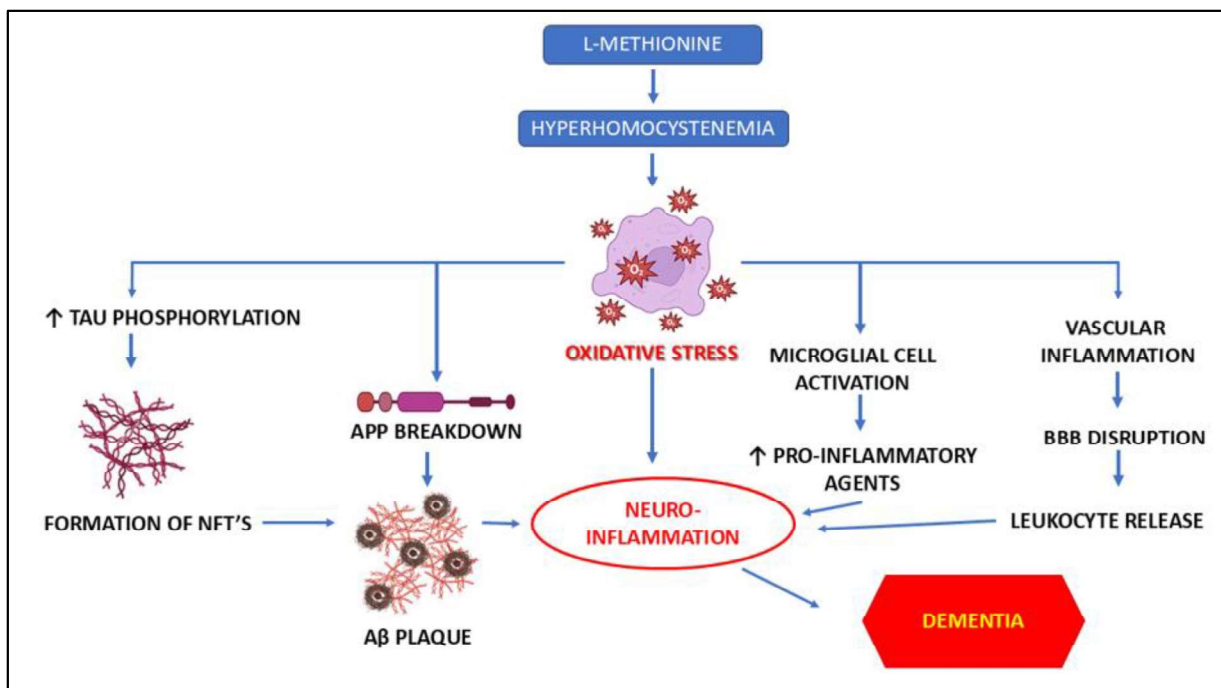


Figure No. 2.4: Role of L-methionine in inducing Dementia.

According to research, giving L-methionine intraperitoneally twice a day for seven days at doses of 750 mg/kg at 9:00 am and 15 mg/kg at 3:00 pm can raise the brain's amyloid beta levels, which can lead to cognitive impairments similar to those observed in Alzheimer's disease (W. Liu et al., 2019). Through glial cell activation and an increase in oxidative stress (Tapia-Rojas et al., 2015), this administration has also been connected to neuroinflammation (Grimble, 2006). These results demonstrate the fine balance needed for methionine metabolism and its consequences for brain function.

Chapter 03

RESEARCH ENVISAGED AND RESEARCH METHODOLOGY

3. RESEARCH ENVISAGED AND PLAN OF WORK

3.1 RATIONALE OF THE STUDY:

Dementia is an advanced condition, which can be categorized by a decay in cognitive functions, eventually impairing the ability to perform daily activities independently (Jeyabalan et al., 2022). While dementia is a broad term used for symptoms of memory and cognitive deterioration, it arises from various underlying diseases, each with distinct signs, symptoms, and neuropathological causes. The principal types of dementia are Alzheimer's disease (AD) and vascular dementia (Wei et al., 2017). Distinguishing between different types can be puzzling due to overlapping symptoms. However, most forms of dementia are progressive, irreversible, and incurable. Age is the primary risk factor. Dementia affects approximately 2% of individuals aged 65–69, rising to 20% in those 85 and older. Globally, an estimated 30 million people live with dementia, with 5-6 million new cases diagnosed annually. Projections suggest the number of cases could double every 20 years, surpassing eighty million by 2045 (Husband & Worsley, 2006).

Donepezil, an AChE inhibitor, is one of the most often utilized medications right now (Birks & Harvey, 2018). It seeks to improve cholinergic functioning, which may help with the disease. Rivastigmine and galantamine are two other medicines that stop AChE (Marucci et al., 2021). NMDA receptor antagonists are another type of medication that is now being employed. Memantine is the most common NMDA receptor antagonist (Olivares et al., 2012).

Based on what we found before, it is already proved that aluminum can quickly get into the brain and affect sluggish axonal transit, produce inflammation, and cause structural and synaptic problems that eventually lead to dementia (Kandimalla et al., 2015). D-galactose is a reduced form of sugar, which can produce a reaction with freely available amines to make various products of Advanced Glycation. When given for a long time, D-galactose causes changes in animals that are similar to natural aging, such as cognitive loss, oxidative stress, a weaker immune system, and genetic alterations (Shwe et al., 2018). It also causes the mitochondria to not work properly and raises the level of acetylcholinesterase in the brain (Cui et al., 2006). When both, $AlCl_3$ and D-galactose were to administered for a long period of 90 days, the combination can induce the dementia by causing production of free radicals, neuro-inflammation, causing an increase in cholinesterase enzyme, and other biochemical events that can lead to cause Alzheimer's disease (AD) (Kumar et al., 2021). These things make cognitive dysfunction and memory loss worse. Disease model 02, which involves giving high doses of L-methionine, can also cause problems with blood vessels, neurodegeneration,

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neuroinflammation, oxidative overload, and changes in the brain that are similar to those seen in Alzheimer's disease (Alachkar et al., 2022). There are other animal models like scopolamine induced dementia, LPS induced dementia, and other chemical induced dementia models, but they all have limitations, that is why we have employed these two models. Both interoceptive models had never been employed in the same study before. Both animal models give a much broader view of the disease by focusing on more pathogenic processes.

In this study, the individual and combined effects of beta-caryophyllene, p-coumaric acid, and cinnamic acid have been evaluated against the above-mentioned two different interoceptive models of dementia in mice.

β -caryophyllene is a sesquiterpene found naturally in the essential oils of various food plants and spices, including cinnamon and pepper (Oliveira et al., 2018). A prior study on BCP revealed that it functions as a selective agonist for CB2 receptors, which are cannabinoid receptors in the body, by specifically targeting these receptors (Gertsch et al., 2008; Mallmann et al., 2022; Rava et al., 2025). BCP is very important for keeping Alzheimer's disease from happening (Cheng et al., 2014). Some research showed that BCP can protect neurons from damage caused by too much glutamate (Assis et al., 2014). BCP had also shown that it could protect against ischemia models (Liu et al., 2021). BCP is a strong antioxidant (Al-Taeie et al., 2019). Hence, it may positively influence memory impairments.

P-Coumaric Acid (PCA) is a hydroxyl derivative of cinnamic acid, widely found in fruits and vegetables such as pectin, rice, cranberry syrup, white jelly mushrooms, and tomatoes, among others. It also exists in different forms, such as a metabolite of phenolic acid. PCA has demonstrated antioxidant (Shaheen, 2011), anti-inflammatory (Pragasam et al., 2013), and antiplatelet properties (Luceri et al., 2007); notably, its dose-dependent antioxidant efficacy surpasses that of vitamin C (Luo et al., 2007). PCA has also been shown to be beneficial in Alzheimer's disease (Yoon et al., 2014) and in many metabolic illnesses (Kang et al., 2013). Plants containing PCA have demonstrated a reduction in AChE levels in the brain (Szwajgier, 2013), suggesting their potential utility in memory disorders.

Dietary phenolic compounds include cinnamic acid and its derivatives (CADs) from fruits, vegetables, and flowers. These are essential for forming commercially important intermediate compounds for pharmaceutical component synthesis. Cinnamic acid and its derivatives are studied for their antioxidant, hepatoprotective, anxiolytic, antidiabetic, and anticholesterolemic properties in this review. The m-hydroxy or p-methoxy residue on cinnamic acid (CA) is an

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effective insulin release agent (Ohnishi et al., 2004), while 3, 4-Dihydroxycinnamic acid (caffeic acid, CAF) has hepatoprotective activity. Cinnamic acid and its derivative reduce brain AChE (Chen et al., 2008). Thus, this phytoconstituent may help memory issues.

As per reported endpoints in the literature, escape latency time using the Morris water maze paradigm and transfer latency time using the elevated plus maze paradigm were used to assess the memory-improving and disease-preventing effects of all three phytoconstituents individually and in combination. Furthermore, to evaluate the antioxidant profile of these test compounds alone and in combination, the effect on biochemical parameters like TBARS, SOD, GSH, catalase, and nitrite levels of the brain was assessed. Also, to understand the effect of these interventions on other parameters responsible for dementia, their inhibitory effect on brain AChE levels and TNF- α was also observed.

The use of combinations of beta-caryophyllene, p-coumaric acid, and cinnamic acid can provide a multifactorial approach against animal models of dementia. Various pathological episodes like oxidative stress, neuroinflammation, and altered cholinergic functions due to AChE can be targeted with the combination of test compounds, which may provide significant benefit in therapeutic terms. The chosen intervention has not been evaluated in the chosen animal models either alone or in combination, which makes this study novel.

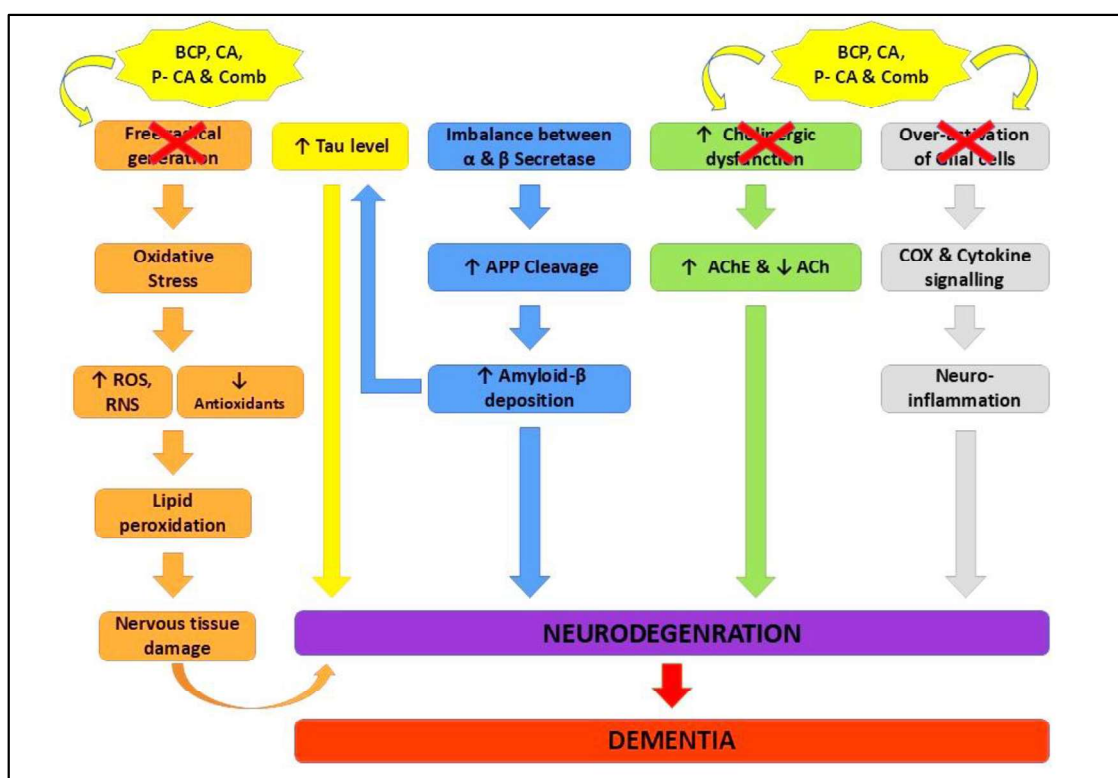


Figure No. 3.1: Purposed mechanism of action.

RESEARCH ENVISAGED AND PLAN OF WORK

3.2 AIM

In this study, β -caryophyllene, cinnamic acid, and *p*-coumaric acid were evaluated against chemically induced mice models of dementia.

3.3 OBJECTIVES

- β -caryophyllene, cinnamic acid, and *p*-coumaric acid were evaluated against chemically induced dementia in mice individually as well as in combination.
- All three phytoconstituents were explored for their potential against brain AChE, TBARS, SOD, catalase, GSH, nitrite, and TNF- α levels.
- All phytoconstituents were evaluated for various behavioral parameters.

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3.4 PLAN OF WORK

The plan of work is illustrated in figure no. 3.1, as follows:

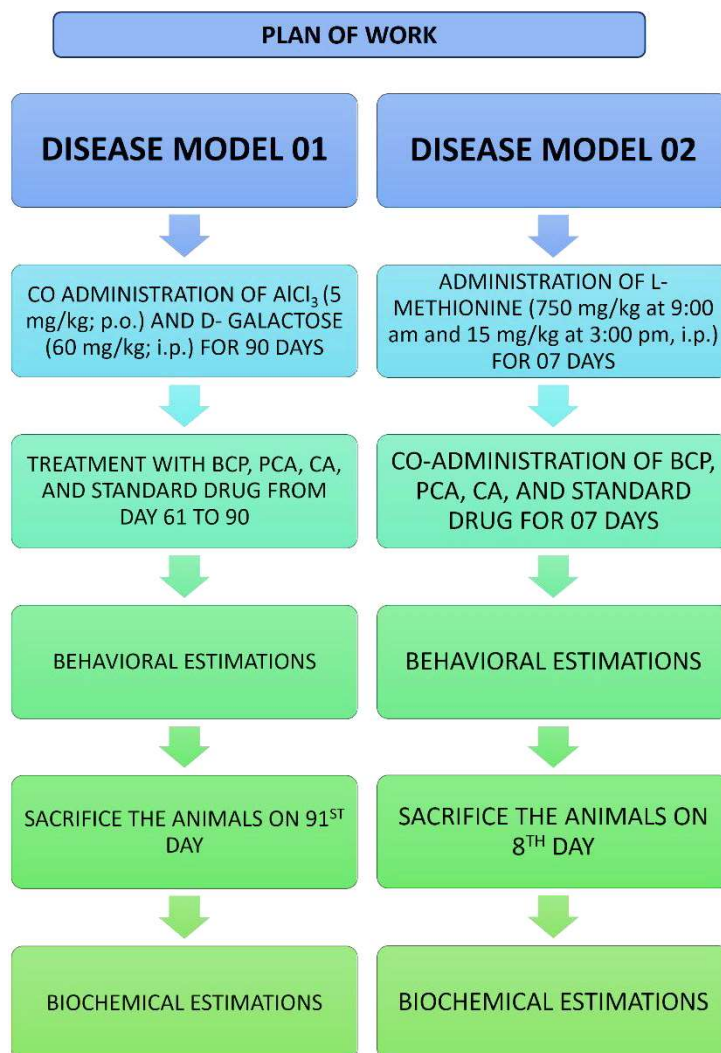


Figure No. 3.2: Plan of work.

Chapter 04
MATERIALS & METHODS

4. MATERIALS & METHODS

4.1 MATERIALS

4.1.1 Equipment

The equipment used for the experimental work has been mentioned in Table 4.1.

Table 4.1: Equipment used for experimental work.

S. No.	Equipment	Maker
1	UV-Visible spectrophotometer	Shimadzu Corp., Japan
2	Centrifuge	Remi Instruments, India
3	Cooling centrifuge	Remi Instruments, India
4	Hot air oven	Navyug Udyog, India
5	Digital weighing balance	Contech Instruments Ltd., India
6	Deep freezer	Voltas Ltd., India
7	Refrigerator	LG, India
8	iMark™ Microplate Reader	Bio-Rad

4.1.2 Chemicals and kits

The chemicals used for the experimental work have been listed in Table 4.2.

Table 4.2: Chemicals used for experimental work.

S. No.	Chemical	Manufacturer
1	Aluminium chloride (AlCl ₃)	Loba Chemie
2	D-Galactose	Loba Chemie
3	L- Methionine	Sigma Aldrich
4	Beta-caryophyllene	Sigma Aldrich
5	P-coumaric acid	Sigma Aldrich
6	Cinnamic acid	Sigma Aldrich
7	Donepezil hydrochloride	Sigma Aldrich

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4.1.3 ANIMALS

Adult Swiss albino mice of either sex, weighing 25–30 gms of body weight were used. Animals were allowed to acclimate for one week prior to the initiation of the study under standard animal laboratory conditions. Mice were housed in standard polycarbonate cages with a diet of mouse chow and water supplied *ad libitum*. Animals were kept at $25 \pm 2^\circ\text{C}$, and humidity conditions were kept between $65 \pm 10\%$. Photoperiod was 12-hr. light and dark cycle. The study protocol was approved by the Institutional Animal Ethics Committee and assigned the protocol number PBRI/IAEC/PN-22113.

4.2 METHODS

4.2.1 EXPERIMENTAL DESIGN

In this study, a few interoceptive agents (L-methionine, AlCl_3 , and D-Galactose) were administered for the induction of dementia.

In Model 01, AlCl_3 5 mg/kg p.o. and 60 mg/kg i.p. of D-galactose were administered to mice for a period of ninety days (Kumar et al., 2021). Animals were treated with the standard drug Donepezil HCl (2 mg/kg; i.p.) and low & high doses of beta-caryophyllene, p-coumaric acid, and cinnamic acid for a month (from day sixty-one to day ninety). Afterwards, animals were subjected to behavioral tests on day 91, following which they were euthanized for biochemical parameter analysis.

In Model 02, L-methionine (750 mg/kg at 9:00 am and 15 mg/kg at 3:00 pm, i.p.) was given for 7 days to induce dementia (Alachkar et al., 2022). Animals were treated with the standard drug Donepezil HCl (2 mg/kg; i.p.) and low & high doses of beta-caryophyllene, p-coumaric acid, and cinnamic acid for the same seven days. On the eighth day, animals underwent behavioral testing methods and were sacrificed for various biochemical analyses.

4.2.2 ANIMAL GROUPING & DOSAGE REGIME

Animals were randomly allocated into 23 groups; each group contained 6 animals ($n=6$). The dosage of the various interventions according to each group is mentioned in Table No. 4.3, given as below:

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Table No. 4.3: Animal grouping and dosage regime.

S. No.	Group Name	Description	No.
1	Normal Control	All the animals will be treated with normal saline.	06
2	M01	Model 01 (AlCl ₃ (5 mg/kg; p. o.), + D-galactose (60 mg/kg; i. p.)	06
3	M01 + Standard	AlCl ₃ (5 mg/kg; p. o.) + D-galactose (60 mg/kg; i. p.) & Donepezil HCL (2 mg/kg i.p.)	06
4	M01 + BCPLD	AlCl ₃ (5 mg/kg; p. o.) + D-galactose (60 mg/kg; i. p.) & Beta-Caryophyllene (100 mg/kg p.o.)	06
5	M01 + BCPHD	AlCl ₃ (5 mg/kg; p. o.) + D-galactose (60 mg/kg; i. p.) & Beta-Caryophyllene (400 mg/kg p.o.)	06
6	M01 + PCALD	AlCl ₃ (5 mg/kg; p. o.) + D-galactose (60 mg/kg; i. p.) & <i>p</i> -Coumaric Acid (100 mg/kg p.o.)	06
7	M01 + PCAHD	AlCl ₃ (5 mg/kg; p. o.) + D-galactose (60 mg/kg; i. p.) & <i>p</i> -Coumaric Acid (400 mg/kg p.o.)	06
8	M01 + CALD	AlCl ₃ (5 mg/kg; p. o.) + D-galactose (60 mg/kg; i. p.) & Cinnamic Acid (100 mg/kg p.o.)	06
9	M01 + CAHD	AlCl ₃ (5 mg/kg; p. o.) + D-galactose (60 mg/kg; i. p.) & Cinnamic Acid (200 mg/kg p.o.)	06
10	M02	Model 02 (L-Methionine (750 mg/kg and 15 mg/kg i.p.)	06
11	M02 + Standard	L-Methionine (750 mg/kg and 15 mg/kg i.p.) & Donepezil HCL (2 mg/kg i.p.)	06
12	M02 + BCPLD	L-Methionine (750 mg/kg and 15 mg/kg i.p.) & Beta-Caryophyllene (100 mg/kg p.o.)	06
13	M02 + BCPHD	L-Methionine (750 mg/kg and 15 mg/kg i.p.) & Beta-Caryophyllene (400 mg/kg p.o.)	06
14	M02 + PCALD	L-Methionine (750 mg/kg and 15 mg/kg i.p.) & <i>p</i> -Coumaric Acid (100 mg/kg p.o.)	06
15	M02 + PCAHD	L-Methionine (750 mg/kg and 15 mg/kg i.p.) & <i>p</i> -Coumaric Acid (400 mg/kg p.o.)	06

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16	M02 + CALD	L-Methionine (750 mg/kg and 15 mg/kg i.p.) & Cinnamic Acid (100 mg/kg p.o.)	06
17	M02 + CAHD	L-Methionine (750 mg/kg and 15 mg/kg i.p.) & Cinnamic Acid (200 mg/kg p.o.)	06
18	M01 + COM01	AlCl ₃ (5 mg/kg; p.o.) & D-galactose (60 mg/kg; i.p.) & BCPHD (400 mg/kg; p.o.) + PCAHD (400 mg/kg; p.o.)	06
19	M01 + COM02	AlCl ₃ (5 mg/kg; p.o.) & D-galactose (60 mg/kg; i.p.) & BCPHD (400 mg/kg; p.o.) & CAHD (200 mg/kg; p.o.)	06
20	M01 + COM03	AlCl ₃ (5 mg/kg; p.o.) & D-galactose (60 mg/kg; i.p.) & PCAHD (400 mg/kg; p.o.) & CAHD (200 mg/kg; p.o.)	06
21	M02 + COM01	L-Methionine (750 mg/kg and 15 mg/kg i.p.) & BCPHD (400 mg/kg; p.o.) & PCAHD (400 mg/kg; p.o.)	06
22	M02 + COM02	L-Methionine (750 mg/kg and 15 mg/kg i.p.) & BCPHD (400 mg/kg; p.o.) & CAHD (200 mg/kg; p.o.)	06
23	M02 + COM03	L-Methionine (750 mg/kg and 15 mg/kg i.p.) & PCAHD (400 mg/kg; p.o.) & CAHD (200 mg/kg; p.o.)	06

After inducing the disease, animals were included into the study after assessing the effects of interoceptive models on the memory and learning by using the behavioral parameters like Morris water maze, EPM. The doses of all three test compounds were selected on the basis of studies carried out previously (da Costa Sobral et al., 2022; Prorok et al., 2019; Sakamula & Thong-asa, 2018). Aluminium chloride, donepezil hydrochloride was formulated with distilled water. D-galactose was administered intraperitoneally after formulating into normal saline. Test compounds BCP, Cinnamic acid, and p-coumaric acid was given with normal saline.

4.2.3 BEHAVIORAL PROCEDURES:

4.2.3.1 MORRIS WATER MAZE:

The Morris water maze investigates the spatial learning and memory of rodents through the use of a submerged platform in a water-filled pool. This procedure explores the capability of animals to memorize the learned tasks for a longer period of time by means of repeated trials. In this test, animals are positioned in a circular pool of water, and the goal is to escape from the water by finding an invisible platform that's submerged just below the surface of the water. The task is based on the rodents' ability to use spatial cues (e.g., visual landmarks) present in the environment to swim around in the pool and find the escape platform.

Method: A circular pool (circumference 90 cm, height 45 cm) was filled with water to a depth of 30 cm and a temperature of $28\pm 1^\circ\text{C}$. To make the water opaque, a white dye was added. Two threads perpendicular to each other were attached to the edge of the pool, segmenting it into four quadrants. A white platform (10 cm^2) was positioned ectopically in the target quadrant of the pool at 1 cm below the water surface. The position of the platform did not change during the training. The mice were then gently dipped in the water in the basin between the quadrants, oriented toward the wall. For each test, the place where the animals were dropped was changed, and they had two minutes to locate the submerged platform. Platform After locating the platform, the animal was permitted to stay on the platform for an additional 20 s. If the mice were unable to locate the plate within 120 s, they were gently positioned on the platform and remained there for 20 s. Escape latency time (ELT) is one measure of learning or acquisition. On all four days, two trials were conducted. The first trial was the acquisition trial, which was conducted in the morning, and the second trial was the retrieval trial, which was conducted in the evening.

After the platform was removed on the day of the fifth, each mouse was placed in the pool and given 120 seconds to explore. The average time spent in each of the three quadrants, Q1, Q2, and Q3, of the maze was recorded, and the search index was defined as the time spent in the target quadrant (TSTQ) to locate the missing platform (Parle et al., 2007).

4.2.3.2 ELEVATED PLUS MAZE:

The basic purpose of the elevated plus maze is to explore the anxiety from the height in animals, but when modified, it can be used to explore the short-term memory, like remembering the last choices of arms. The duration for the animals to traverse from the open arm to the closed arm functions as the evaluative criterion. Improvement in the memory will be estimated only by the mean of improvement in the transfer latency time (the duration it took for the animal to return from the open arms to the closed arms). According to this metric, it was shown that once an animal entered the open arms, the transfer latency was drastically decreased. Memory utilization was also slower the shorter the transmission delays. Consequently, in the case that animals were subjected to the plus maze on day 2, the latency for retrieval was shorter than that of day 1 (Itoh et al., 1990).

Procedure: Two open arms (16 x 5 x 12 cm) of the elevated plus maze were connected to the upper surface of the central platform (5 x 5 cm). The elevated maze was raised 25 centimeters from the ground. Each mouse was positioned at the end of an open arm, facing away from the central platform, on day 01. This was followed by an observation of transfer latency. Transfer latency (TL) is the time (in seconds) for an animal to relocate all four paws from one open arm into a covered arm. Ninety seconds was the time limit for getting to the closed arm. If the mouse did not find the closed arm within 90 seconds, it was kindly guided into one. The mouse was rested after exploring the maze for 20 seconds. Memory retention for this learned task was measured 24 hours after the learning trial. A significant drop in the TL value indicates better memory (Dhingra & Kumar, 2012).

4.2.4 BIOCHEMICAL TESTS:

4.2.4.1 Estimation of AChE Activity

The method employs adrenocorticotrophic hormone iodide as a synthetic substrate for AChE. AChE decomposition of adrenocorticotrophic hormone iodide yields thiocholine and acetate. The thiocholine produced reacts with DTNB to produce the yellow-coloured compound.

Materials: Phosphate buffer 0.1 M (pH 8 and pH 7)

Substrate: 30 mM ATChI

DTNB: 10 mM

Method: The brain tissue was weighed and then freshly purified as a 5 percent homogenate (50 mg per ml) in 0.1 M phosphate buffer at pH 7.0. Samples were centrifuged at 5,000 rpm at 4°C for 10 min. Both the serum secretion test and enzyme test were conducted. Then all samples were stored at -20 degrees Celsius or in ice. Test tubes were labeled as blank and test (Ellman et al., 1961).

4.2.4.2 Thiobarbituric acid reactive substances

The TBARS test uses thiobarbituric acid as a reagent for the detection of thiobarbituric acid-reactive substances (TBARS), by-products of lipolysis. Reactive oxygen species like TBARS can be quantified as oxidation end-products of oxidative stress because they are one of the products with a very short lifetime whose direct detection is impossible. The TBARS method allows for the quantification of both malondialdehyde (MDA) in the sample and MDA produced by the hydrolysis of lipids under reaction conditions.

Methods: Tissue homogenate (0.1 ml) from the reaction mixture, 0.2 ml of sodium dodecyl sulfate (SDS; 8.1 mmol), 20 mmol, and 0.8 mmol thiobarbituric acid solutions in 1.5 ml of acetic acid. NaOH (1 mmol) was used to adjust the pH to 3.5, after which 20 mmol acetic acid was added. The mixture was completed with distilled water to 4.0 ml and heated for 60 minutes on an oil burner at 95 degrees Celsius. Once cooled with running water, 5.0 ml of the 15:1 phosphoric acid extract of n-butanol:pyridine mixture was added, along with 1.0 ml of distilled water, and shaken strongly in a centrifugal pump. The absorbance of the solvent (upper layer) was then scanned promptly by UV spectrophotometer at 532 nm immediately following centrifugation for five minutes at 3000 rpm (Ohkawa et al., 1979).

4.2.4.2 GSH in the brain

Glutathione donates electrons to break the disulfide bonds formed between cysteines in cytoplasmic proteins. This reduces glutathione into glutathione disulfide (GSSG), the oxidized version of glutathione. Through NADPH as an electron donor, the oxidation of glutathione can be re-reduced by glutathione reductase.

Procedure: The determination of GSH serum was performed off differential testing, for which the standard Ellman method (1959) was used to differentiate the serum isolated from the

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standardized daily allowance. After that, 0.2 ml of a buffer consisting of a previously homogenized sample was added on top of 2.3 ml of potassium phosphate buffer [0.2 M, pH 7.6] inside a cell (or cup). Next, 0.5 ml of [DTNB] (0.001M) was added to tissue homogenate in buffer. Absorbance of the reaction product was determined 5 min after initiation of the enzymatic reaction by a UV or visible spectrophotometer at 412 nm. A standard curve of diminishing GSH was used to determine daily allowance in the calculation of the average daily allowance (Ellman, 1959).

4.2.4.3 Nitrite estimation

Nitric oxide is a critical biochemical mediator of several biological processes, including vasodilatation, inflammation, immunity, and neurological transmissions. Nitrite is estimated using the Griess diazotization reaction and also by spectrophotometry and the impulsive oxidation of nitric oxide in normal biological conditions. Using nitric acid will give a quantitative conversion of succinic acid into the diazonium salt in an acidic solution. A diazonium salt is then coupled to N-(1-naphthyl) ethylenediamine to form an azo dye.

Procedure: The chromophore was generated as follows: the supernatant from the brain homogenate was mixed with the same volume of the Griess reagent (1.1 mmol sodium dihydrochloride or 2.5 mmol phosphoric acid) and allowed to stand for 10 min at room temperature. Absorbance was determined using a spectrophotometer at 543 nm. Nitrite concentrations were derived from the standard curve and reported as μ molar nitrite per milliliter of clarified alcohol using sodium nitrite as a standard (Bories et al., 1999).

4.2.4.4 Superoxide Dismutase Activity

Superoxide dismutase is the front line of protection against superoxide radicals in the body because it catalyzes superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2) and oxygen (O_2). Superoxide ions (O_2^-) generated from the oxidation of xanthine to uric acid and hydrogen peroxide via the XOD process in the test convert NBT to NBT-diformazan via a process that absorbs light at 560 nm. SOD slows down the synthesis of NBT-diformazione through reducing the concentrations of superoxide anions. The amount of SOD activity present in a test sample is reflected in the extent to which the appearance of NBT-diformazan has been diminished. The activity of the sample is determined in terms of relative activity based on the percentage of inhibition of NBT-diformazan formation by SOD (Kono, 1978).

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Procedure: Kono (1978) technique was used to determine the SOD activity. 250 μ l (0.8 ml) of Solution A (0.1 mM EDTA and 50 mM sodium carbonate, pH 10) was supplemented with 0.5 ml (96 mM nitro-blue tetrazolium (NBT) solution-B) and 0.2 ml (96 mM nitro-blue tetrazolium and 0.6 mM Triton X-100, pH 10) (solution-C) in solution-A (20 mM, pH 6.0) and 0.1 ml of morphine was added to the above test mixture. Auto-oxidation of the hydroxylamine amine was monitored by measuring absorbance at 560 nm. All of the components except hydroxylamine were present in the control. SOD activity of the protein was assessed in U/mg.

4.2.4.5 Catalase activity

The method by H. Luck in 1971, which was used to determine catalase activity, was based on the measurement of hydrogen peroxide (H_2O_2) decomposition at 240 nm. The final test mixture included 0.05 ml of 10% tissue homogenate supernatant, 3 ml of phosphate buffer, and H_2O_2 . Absorbance changes at 240 nm were recorded. The results were expressed as micromoles of hydrogen peroxide decomposed per milligram of protein per minute (H, 1971).

4.2.4.6 Estimation of TNF- α

Homotrimeric 17kDa protein, alias tumor necrosis factor-alpha (TNF-alpha), is a powerful modulator of metabolic and inflammatory pathways. The potent antitumor activity of TNF-alpha was initially described when the cytokine was found to be a highly cytotoxic cytokine with in vitro cytotoxic activity against tumor cells and in vivo antitumor necrosis. The cytokine TNF is a major mediator of many inflammatory and immunological processes.

Procedure: Measurement of TNF-alpha levels in the brain: TNF-alpha levels were measured by ELISA kits, and results were expressed as pg/g wet tissue (den Blaauwen et al., 1983).

4.2.5 STATISTICAL ANALYSIS

All results are expressed as average Mean \pm SEM. Data of each group was analyzed using parametric tests, one-way and two-way ANOVA followed by a Tukey's T-test utilizing Prism GraphPad 8.0 (GraphPad Software, Boston, MA, USA). The statistical significance was considered at the p-values lesser than 0.05, 0.01 and 0.001.

Chapter 05
RESULTS & DISCUSSION

5. RESULTS

Here in the results, ***, **, and * have been used to represent $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively. Here we have used “A” to represent the comparison with the control group, “B” represents the comparison with the disease model group, and “C” represents the comparison with the standard treated group.

5.1. DISEASE MODEL 01 (ALCL₃ + D-GALACTOSE)

5.1.1. EFFECT ON ESCAPE LATENCY TIME USING MORRIS WATER MAZE

The escape latency time was observed through two trials, i.e., the acquisition trials and retrieval trials. These trials were conducted on days 87, 88, 89, and 90. The purpose of the acquisition trial was to provide the animals with the learning of the task, and retrieval trials were conducted to assess the learned tasks. The acquisition trials were conducted during the morning, and retrieval trials were conducted in the evening.

5.1.1.1. ACQUISITION TRIAL

On the day 87, first day of the trial it was observed that the mean Escape Latency Time of Disease Model 01 group was observed to be significantly higher than the mean ELT of Control Group with a p-value of lesser than 0.001. The treatment groups i.e., M01 + STD, M01 + BCPLD, M01 + BCPHD, M01 + CAHD, and M01 + PCAHD, showed significant reduction in the Mean ELT when compared with the Disease Model 01 group, with a p-value lesser than 0.001. On the following day, i.e. day 88, the Mean ELT of the Disease Model 01 group was found significantly higher ($p < 0.001$) when it was compared with the value of Control group. Among the treatment groups, M01 + STD, M01 + BCPLD, M01 + showed significant reduction in the ELT ($p < 0.001$) upon comparison with the Disease Model 01 group. Other treatment groups i.e., M01 + BCPHD, M01 + CALD, M01 + CAHD, and M01 + PCAHD significantly lower the ELT value with $p < 0.001$, they also exhibit significant reduction in ELT as compared to the value of same group on day 87, M01 + CALD, M01 + CAHD, and M01 + PCAHD showed significant reduction with $p < 0.001$ but M01 + BCPHD showed significant reduction with $p < 0.01$.

On 88th day, we have observed a significant reduction in the mean ELT of Control group as compared to the ELT value obtained in control group on the 87th day with a significance value $p < 0.01$. Mean ELT value of Disease Model 01 group is significantly ($p < 0.001$) higher compared to Control group; the mean ELT was also observed to be significantly lesser than the

value of same group on day 87 with a significance value of $p < 0.001$. the treatment groups i.e., M01 + STD, M01 + BCPLD, M01 + BCPHD, M01 + CALD, M01 + CAHD, and M01 + PCAHD significantly ($p < 0.001$) reduce the mean ELT value compared to the Disease Model 01 group and also with the value of the same groups on 87th day. On the other hand we observed the reduction in the ELT of M01 + PCALD group as compared to the Control group but the reduction was not significant, the value of ELT of this group is significantly ($p < 0.001$) lesser than the value of same group on the day 87.

On the final day i.e., Day 90, the value of mean ELT of control group was significantly ($p < 0.001$) lesser than the value of same group on day 87. The mean ELT of Disease Model 01 group was observed to be significantly higher as compared to the Control group with $p < 0.001$. The value of ELT of Disease Model 01 was also significantly lesser than the value of same group on the day 87 with a significance value of $p < 0.001$. All the treatment group i.e., M01 + STD, M01 + BCPLD, M01 + BCPHD, M01 + CALD, M01 + CAHD, M01 + PCALD, and M01 + PCAHD, significantly improved the mean ELT as compared to the value of Disease Model 01 group with a significance value of $p < 0.001$, all the treatment groups also showed the significant ($p < 0.001$) reduction as compared to the values of strictly same groups on day 87.

The results are depicted in the following figure no. 5.1.

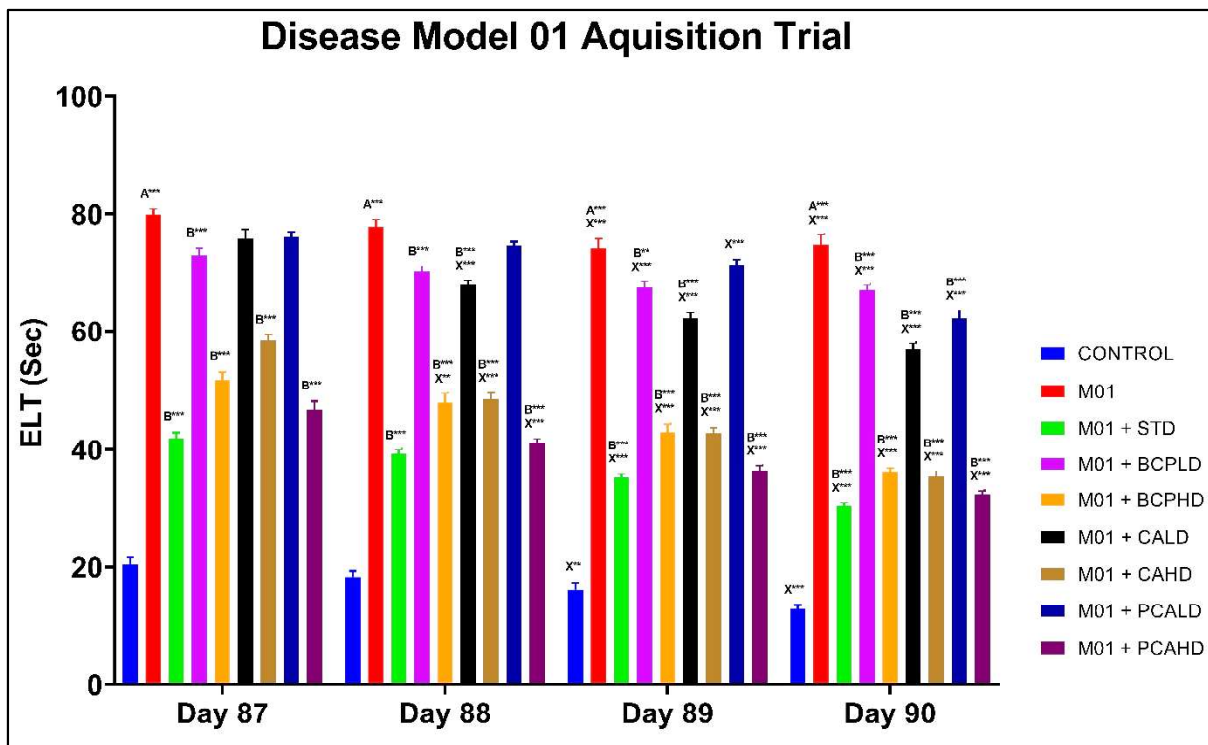


Figure No. 5.1: Effect of various interventions on Escape Latency Time (ELT) during the Acquisition trial on day 87,88, 89, and 90.

Here A represent the comparison with the Control group, B represents the comparison with the Disease Model 01 group and X represent the comparison with the same group on day 87 and ***, **, and * have been used to represent $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

5.1.1.2. RETRIEVAL TRIAL

The retrieval trials were conducted in the evening timings on day 87, 88, 89, and 90.

On the day 87 during the retrieval trial, it was observed that the mean ELT value of Disease Model 01 was observed to be significantly higher than the ELT value of Control group with a significant value of $p < 0.001$. among the treatment groups, M01 + STD, M01 + BCPHD, M01 + CAHD, and M01 + PCAHD, significantly improve the mean ELT value with a significance value of $p < 0.001$, another treatment group i.e., M01 + BCPLD observed to lower the ELT value but with a lesser significant level ($p < 0.01$).

On the day 88, it was observed that the mean ELT value of Disease Model 01 group was significantly higher than the values obtained from the Control group with a significance value of $p < 0.001$. Among the treatment groups, M01 + STD, M01 + BCPLD, M01 + BCPHD, M01 + CALD, M01 + CAHD, and M01 + PCAHD successfully produce the significant improvements in the ELT with $p < 0.001$ when compared against the Disease Model 01 group. Among treatment groups M01 + BCPHD, M01 + CALD, M01 + CAHD, and M01 + PCAHD exhibited significant reduction in ELT values against the value of same respective groups on Day 87 with $p < 0.001$, other than M01 + STD group which exhibited reduction in the ELT with a significance value of $p < 0.01$.

On day 89 it was observed that the ELT value of Control group was significantly ($p < 0.001$) better than the value obtained from the same group on day 87. The mean ELT value of Disease Model 01 group was again significantly ($p < 0.001$) higher than the values of Control group, but was significantly better than the value of same group observed on the 87th day with $p < 0.01$. Among the treatment groups, M01 + STD, M01 + BCPLD, M01 + BCPHD, M01 + CALD, M01 + CAHD, and M01 + PCAHD, exhibited significant ($p < 0.001$) improvement in the mean ELT value than Disease Model 01 group and whereas, M01 + PCALD group showed significant reduction in ELT but with a lesser significant value i.e., $p < 0.01$. Whereas, all the

treatment groups exhibited significant ($p < 0.001$) reduction in the mean ELT value than the values of same groups on day 87.

On the final day of the study, it was observed that the mean ELT of the Control group was significantly ($p < 0.001$) improved than the value of Control group observed on the 87th day. The ELT of the Disease Model 01 group was observed to be significantly higher than the values obtained from the Control group with a significance value of $p < 0.001$ and the values obtained on the 90th day were significantly ($p < 0.001$) lesser than the value of same group observed on 87th day. All the treatment groups exhibited significant reduction in the mean ELT when compared to the Disease Model 01 group with a significant value of ($p < 0.001$), also it was observed that there was a significant improvement in the ELT of all treatment groups on day 90 than the ELT observed from the very same groups on day 87.

The results of retrieval trials are depicted in figure no. 5.2, as follows:

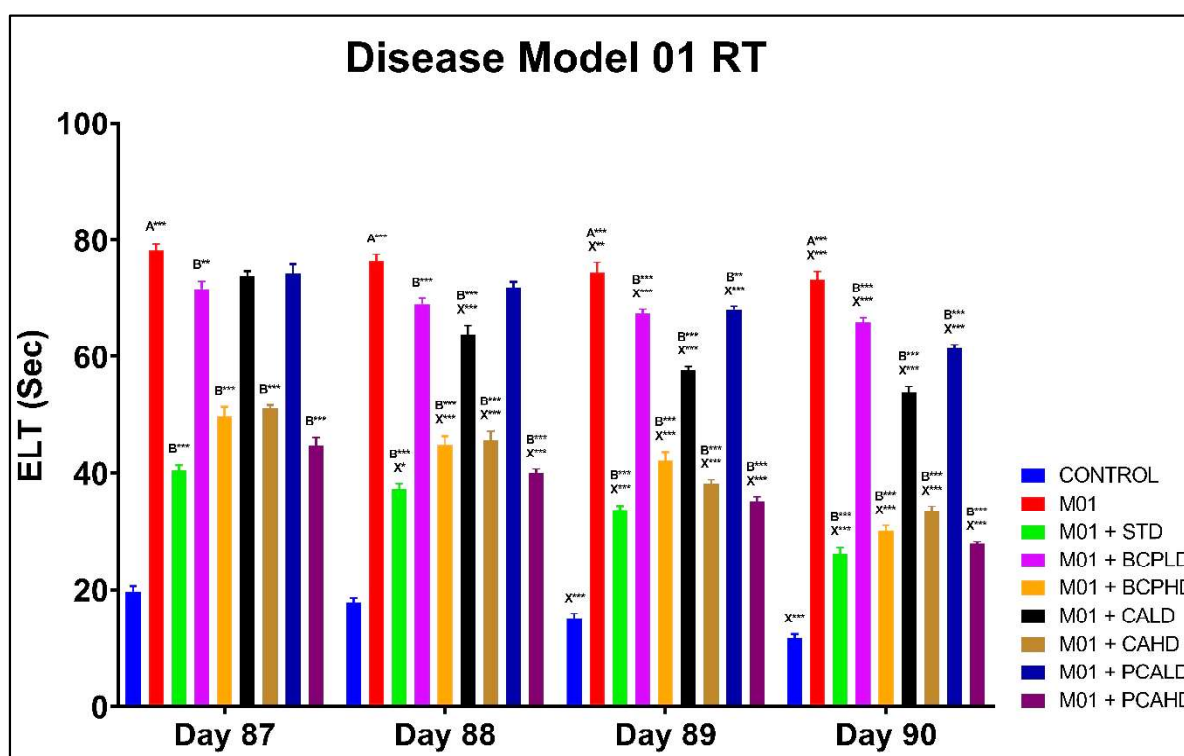


Figure No. 5.2: Effect of various interventions on Escape Latency Time (ELT) during the Retrieval trial on day 87,88, 89, and 90.

Here A represent the comparison with the Control group, B represents the comparison with the Disease Model 01 group and X represent the comparison with the same group on day 87 and ***, **, and * have been used to represent $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

5.1.1.3. EFFECT ON TSTQ

Time spent in the target quadrant is a marker of long-term memory. In this, we particularly observe the time spent by the animal in the target quadrant after removal of the platform. It was observed on the 91st day of the study.

It was observed that the TSTQ was significantly reduced in the disease model 01 group when compared with the control group, with a significance value of $p < 0.001$. TSTQ improved significantly in the M01 + STD, M01 + BCPHD, M01 + CAHD, and M01 + PCAHD groups with a significance value of $p < 0.001$ when compared with the values of the disease model 01 group. The treatment groups M01 + PCALD showed significant improvement with a p-value less than 0.01 and M01 + BCPLD with p less than 0.05. The effects on values of TSTQ are depicted in Figure 5.3, as follows:

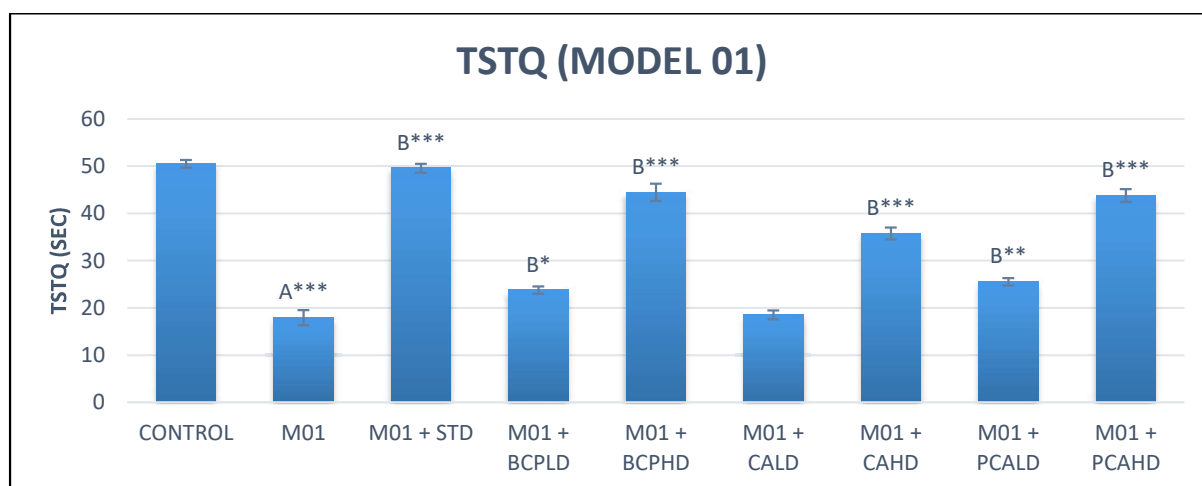


Figure 5.3: Effect of test compounds and other interventions on TSTQ on day 91.

5.1.2. EFFECT ON TRANSFER LATENCY TIME USING ELEVATED PLUS MAZE

Transfer latency time is the time taken by the animals on the Elevated Plus Maze when they move all their paws into the closed arm. The transfer latency time was observed on the 91st day of the study.

It was observed that the transfer latency time increased with the progression of the disease. It was clearly observed with the value of the Disease Model 01 group when they were compared with the TLT values of the control group. It showed a significant jump in the TLT value with a significance value of $p < 0.001$. Among the treatment groups, the M01 + STD, M01 + BCPHD, M01 + CAHD, and M01 + PCAHD groups showed significant improvement in the TLT with a significance value of $p < 0.001$ when compared with the Disease Model 01 group.

The treatment group M01 + CALD showed a significant decrease in the TLT with $p < 0.01$, and treatment groups M01 + BCPLD and M01 + PCALD showed significant improvement in TLT with $p < 0.05$ when compared with the Disease Model 01 group. The results are depicted in Figure 5.4, as follows:

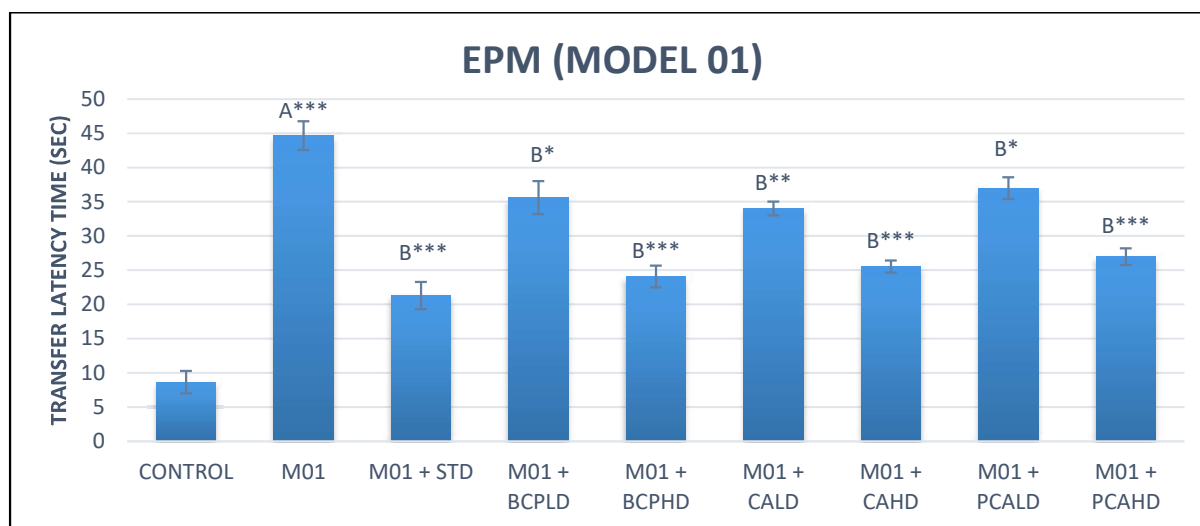


Figure 5.4: Effect of test compounds and other interventions on TLT on day 91 using the Elevated Plus Maze.

5.1.3. EFFECT ON BRAIN AChE

Levels of brain AChE explains the cholinergic activities of the brain, hence the memory and learning capabilities of the brain. Levels of brain AChE increase in memory-associated disorders.

From the study, it was observed that the brain AChE levels significantly increased in the Disease Model 01 group with a significance value of $p < 0.001$ upon comparison with the control group, which remarks about the diseased condition of animals. Among the treatment groups, the M01 + STD, M01 + BCPHD, M01 + CAHD, and M01 + PCAHD groups showed a significant decrease in the brain levels of AChE with $p < 0.001$ when compared with the disease model 01 group. The results are depicted in figure no. 5.5 as follows:

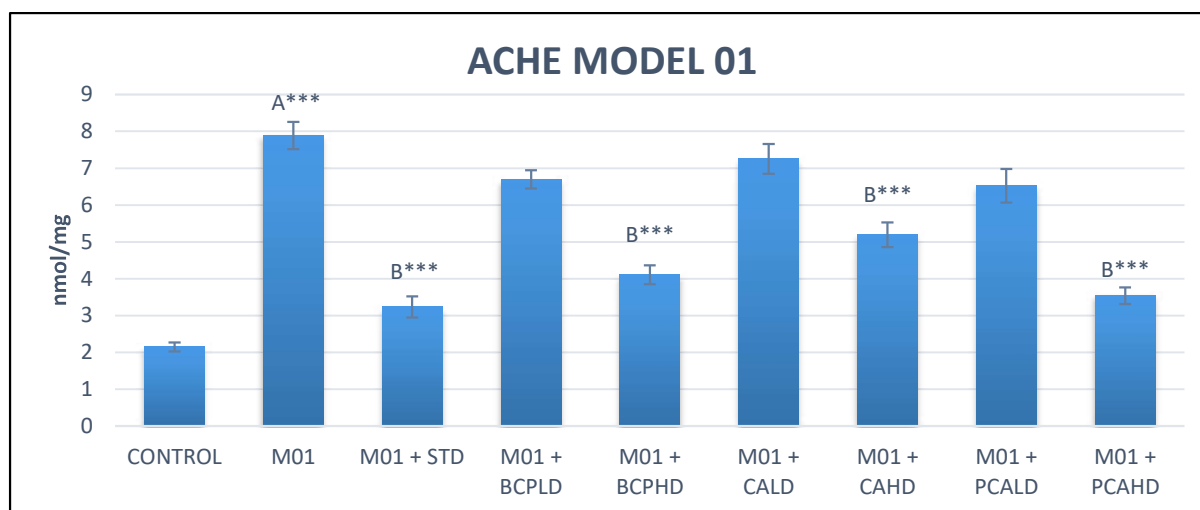


Figure 5.5: Effect of test compounds and other interventions on brain AChE levels in Disease Model 01.

5.1.4. EFFECT ON BRAIN TNF- α

The levels of brain TNF- α denote the presence of neuroinflammation. In some cases of the memory- and learning-associated disorders, it had been observed previously that there are chances of neuroinflammation.

From the study it was observed that the brain TNF- α levels of the Disease Model 01 group were significantly higher when compared with the control group with a significance level of $p < 0.001$. It denoted the presence of neuroinflammation in the disease model 01 group. The treatment groups M01 + STD and M01 + PCAHD showed improvement in the brain TNF- α levels with $p < 0.001$, the M01 + CAHD group showed significant improvement with $p < 0.01$, and M01 + BCPHD showed a significant decrease with $p < 0.05$ upon their respective comparison with the disease model 01 group. The results are presented in figure no. 5.6, as follows:

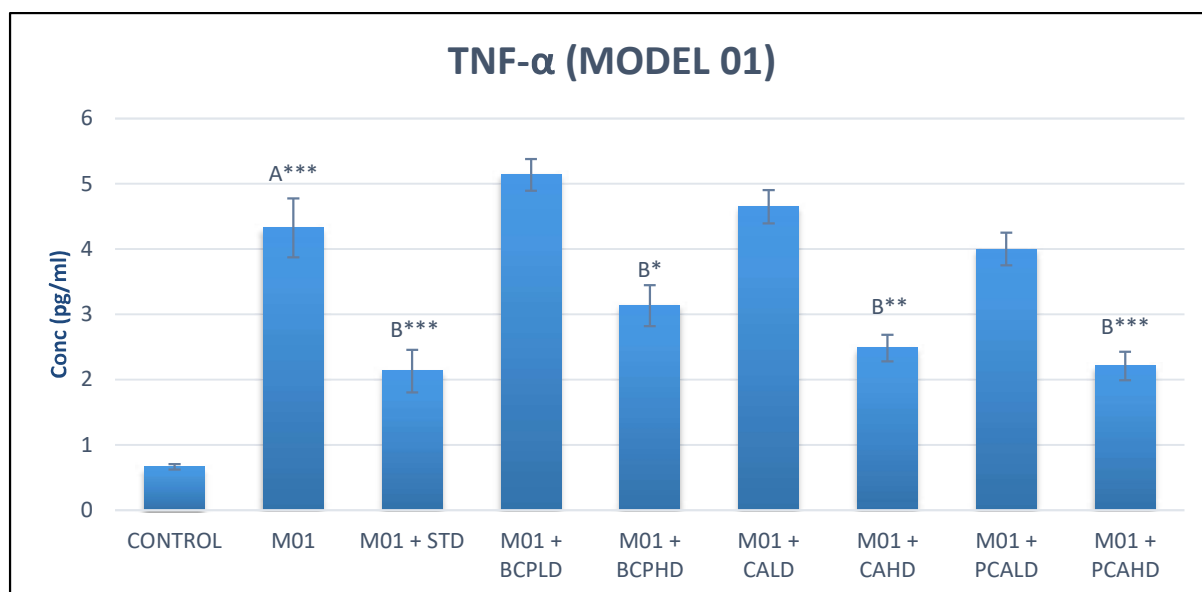


Figure 5.6: Effect of test compounds and other interventions on brain TNF- α levels in Disease Model 01.

5.1.5. EFFECTS ON BRAIN TBARS

The brain TBARS levels are markers of oxidative stress in the brain. In some cases of memory-associated disorders, oxidative stress can be observed.

In the study it was observed that the brain TBARS levels increased in the disease model 01 group significantly with $p < 0.001$ upon comparison with the control group. Treatment groups M01 + STD, M01 + BCPHD, M01 + CAHD, and M01 + PCAHD showed a significant decrease in the brain TBARS levels with $p < 0.001$, and M01 + CALD showed a significant decrease with $p < 0.05$ when compared with the disease model 01 group. As depicted in figure no. 5.7, as follows:

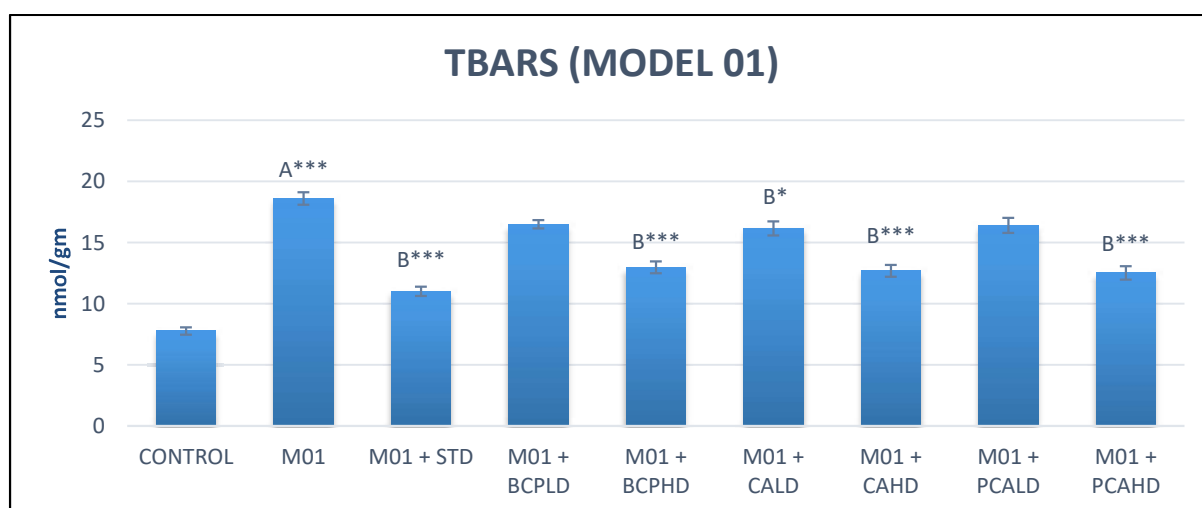


Figure 5.7: Effect of test compounds and other interventions on brain TBARS levels in Disease Model 01.

5.1.6. EFFECTS ON BRAIN GSH

The brain GSH levels are markers of oxidative stress in the brain. From the study it was observed that the animals in the disease model 01 group had significantly lower levels of brain GSH as compared to the control group, with a significance value of $p < 0.001$. It can be concluded from it that oxidative stress had been induced. The treatment groups M01 + STD, M01 + BCPHD, M01 + CAHD, and M01 + PCAHD had increased the brain GSH levels with a significant value of $p < 0.001$. No other treatment groups had shown any significant improvements in brain GSH levels. The results are presented in figure no. 5.8, as follows:

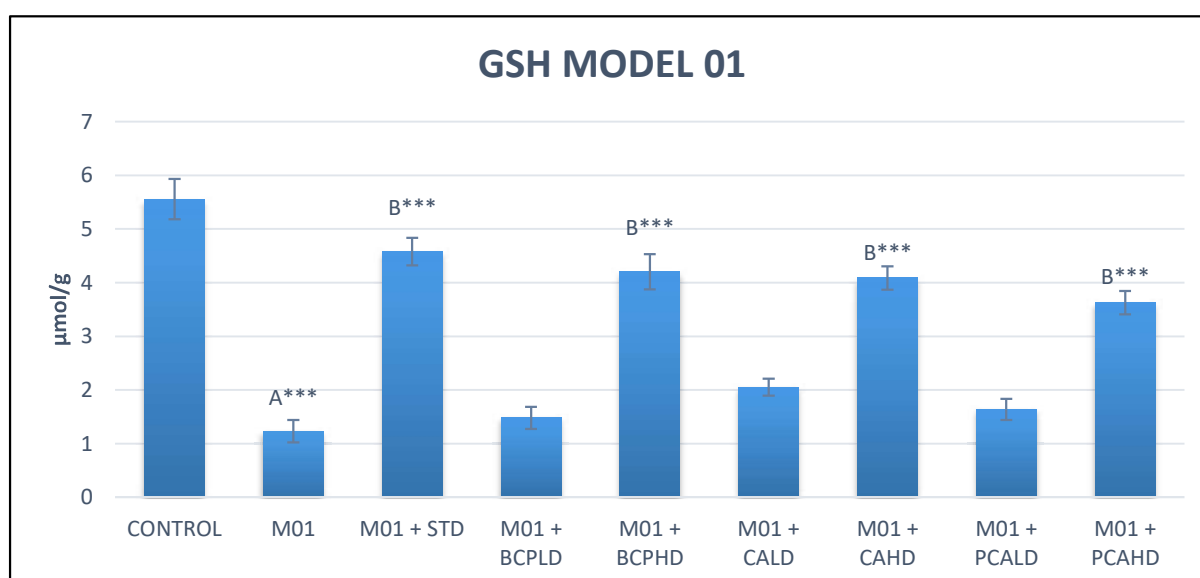


Figure 5.8: Effect of test compounds and other interventions on brain GSH levels in Disease Model 01.

5.1.7. EFFECT ON BRAIN SUPEROXIDE DISMUTASE

Superoxide dismutase is another parameter to evaluate the antioxidant property of treatments. In this study, the levels of brain SOD significantly fell in disease model 01 when they were compared with the values of the control group with a significance value of $p < 0.001$. The treatment groups M01 + STD, M01 + BCPHD, M01 + CAHD, M01 + PCALD, and M01 + PCAHD showed a significant increase in the levels of brain SOD with $p < 0.001$ when compared with the disease model 01. Among other treatment groups, M01 + BCPLD showed a significant improvement with $p < 0.01$ when compared with the disease model 01. The results are depicted in Figure No. 5.9, as follows:

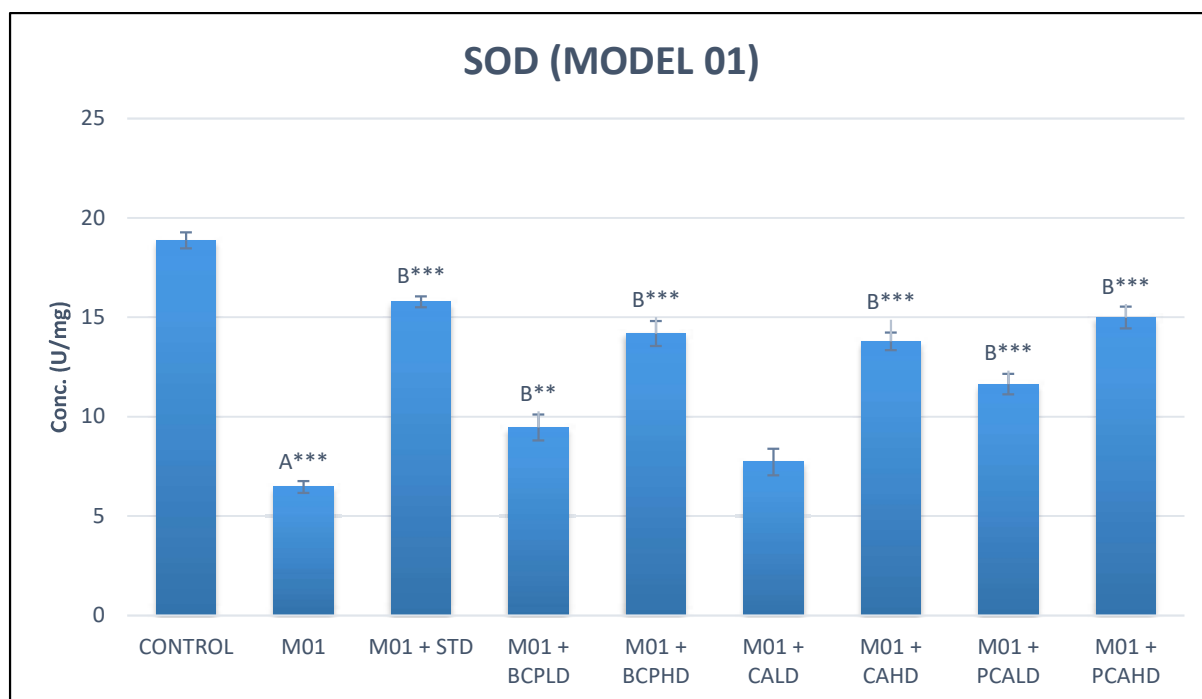


Figure 5.9: Effect of test compounds and other interventions on brain SOD levels in Disease Model 01.

5.1.8. EFFECT ON BRAIN CATALASE

Brain catalase levels remark on the oxidative stress levels in the brain. In this study, it was observed that the levels of brain catalase got significantly lower when they were compared with the levels of the control group, with a significant value of $p < 0.001$. Among all the treatment groups, only one group, i.e., M01 + PCAHD, showed the significant increase in the brain levels of catalase, with a significant value of $p < 0.001$. Other treatment groups, M01 + STD and M01 + BCPHD, showed significant improvements in the brain levels of catalase, with a significant value of $p < 0.01$. The M01 + CAHD group showed a significant increase in the catalase levels with $p < 0.05$. The results are presented in Figure No. 5.10, as follows:

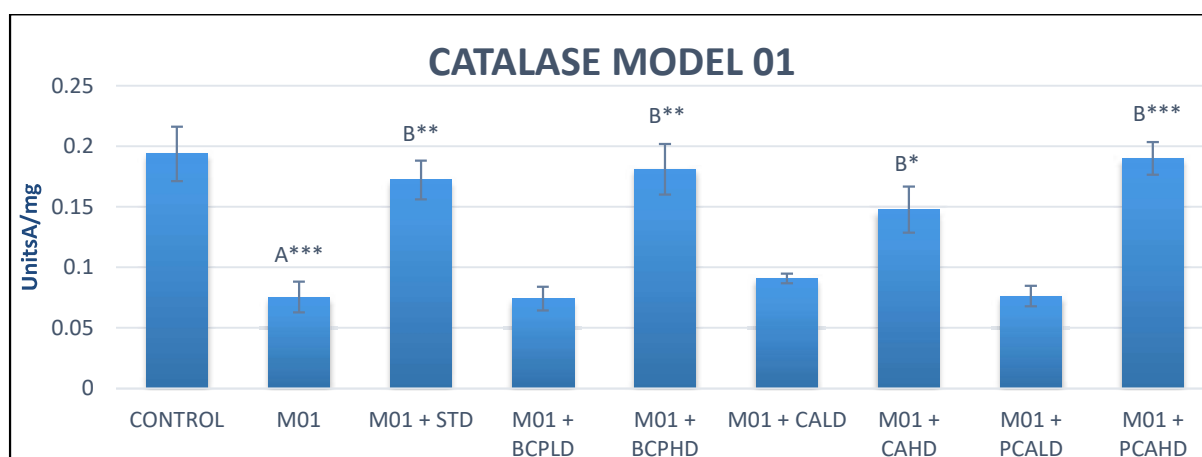


Figure 5.10: Effect of test compounds and other interventions on brain CATALASE levels in Disease Model 01.

5.1.9. EFFECT ON BRAIN NITRITE

The nitrite levels also represent the free radical stress in the brain. In this study, it was observed that the levels of NITRITE in the brain significantly rise in disease model 01 group when the value was compared with the values of control groups with a significance value of $p < 0.001$. Among the treatment groups, M01 + STD, M01 + BCPHD, M01 + CAHD, and M01 + PCAHD showed the significant decrease in the brain nitrite levels with $p < 0.001$, as compared to the disease model 01 group. M01 + BCPLD showed significant improvement with $p < 0.05$, as compared to the disease model 01 group. The results are graphically presented in Figure No. 5.11, as follows:

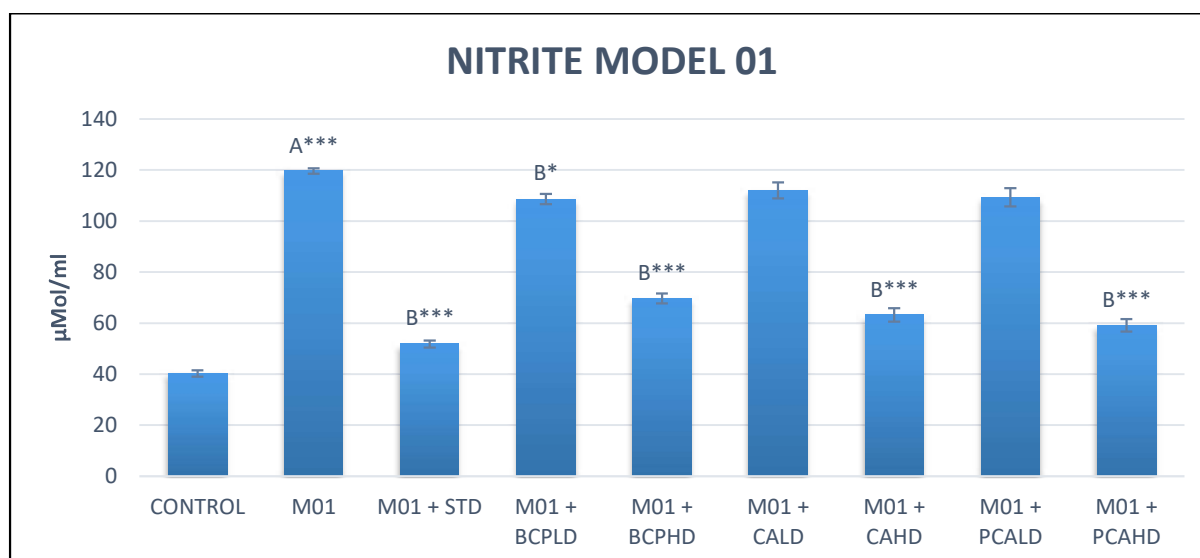


Figure 5.11: Effect of test compounds and other interventions on brain NITRITE levels in Disease Model 01.

5.2. DISEASE MODEL 02 (L-METHIONINE)

5.2.1. EFFECT ON ESCAPE LATENCY TIME USING MORRIS WATER MAZE

Similarly, as in Disease Model 01, here in Disease Model 02, the escape latency time was observed through two trials, i.e., the acquisition trials and retrieval trials. Here in Disease Model 02, these trials were conducted on days 04, 05, 06, and 07. The purpose of both the trials remained the same. The acquisition trials were conducted during the morning, and retrieval trials were conducted in the evening.

5.2.1.1. ACQUISITION TRIAL

During the acquisition trial, on day 04, it was observed that the mean ELT of Disease model 02 group was found to be significantly higher than the control group with $p < 0.001$. Furthermore, the treatment groups i.e. M02 + STD, M02 + BCPHD, M02 + CAHD, and M02 + PCAHD, significantly ($p < 0.001$) lower the ELT as compared to the Disease Model 02 group, whereas M02 + BCPLD showed significant reduction in ELT with $p < 0.01$ and M02 + CALD showed significant reduction in mean ELT with $p < 0.05$.

On day 05, it was observed that the mean ELT of Disease Model 02 was again found to be significantly higher than the Control group with a significance level of $p < 0.001$. Whereas among the treatment groups, it was observed that M02 + STD along with M02 + BCPLD, M02 + BCPHD, M02 + CALD, M02 + CAHD, and M02 + PCAHD significantly ($p < 0.001$) improved the mean ELT as compared to the Disease Model 02 group. The treatment groups i.e., M02 + STD, M02 + BCPLD, M02 + BCPHD, M02 + CAHD, and M02 + PCAHD also showed significant reduction in ELT when it was compared with the values of same group on day 04, also M02 + PCALD showed reduction as compared to the same group on day 04 with a significance value of $p < 0.01$, but this group failed to produce any significant difference in ELT against the Disease Model 02 group. Also, M02 + CALD observed to produce significant reduction in ELT as compared to the values of same group on day 04.

On day 06, we observed that there is significant ($p < 0.01$) reduction in the ELT value of Control group against the value of same group on day 04. It was observed that the mean ELT of Disease Model 02 group was significantly ($p < 0.001$) higher than the Control group's values and also against the values of same group on day 04. All the treatment groups other than M02 + PCALD produced significant reduction in the ELT against Disease Model 02 group with a significance value of $p < 0.001$, only M02 + PCALD showed reduction with significance value of $p < 0.01$.

All the treatment groups also produced significant ($p < 0.001$) improvement in the ELT against the values of very same groups on day 04.

On the final day, day 07, the mean ELT of the Control group kept significantly improving than day 04 with a significance value of $p < 0.001$. ELT value of Disease Model 02 group was still significantly ($p < 0.001$) higher than the Control group and also was significantly lower than the value of same group observed on day 04 with a significance value of $p < 0.001$. All the treatment groups showed significant reduction in the mean ELT values as compared to the Diseases Model 02 group with a significance value of $p < 0.001$. Also, all the treatment groups produced significant reduction in ELT against the values of same groups on day 04 with significance of $p < 0.001$.

The results of acquisition trials are depicted in figure no. 5.12, which is given below:

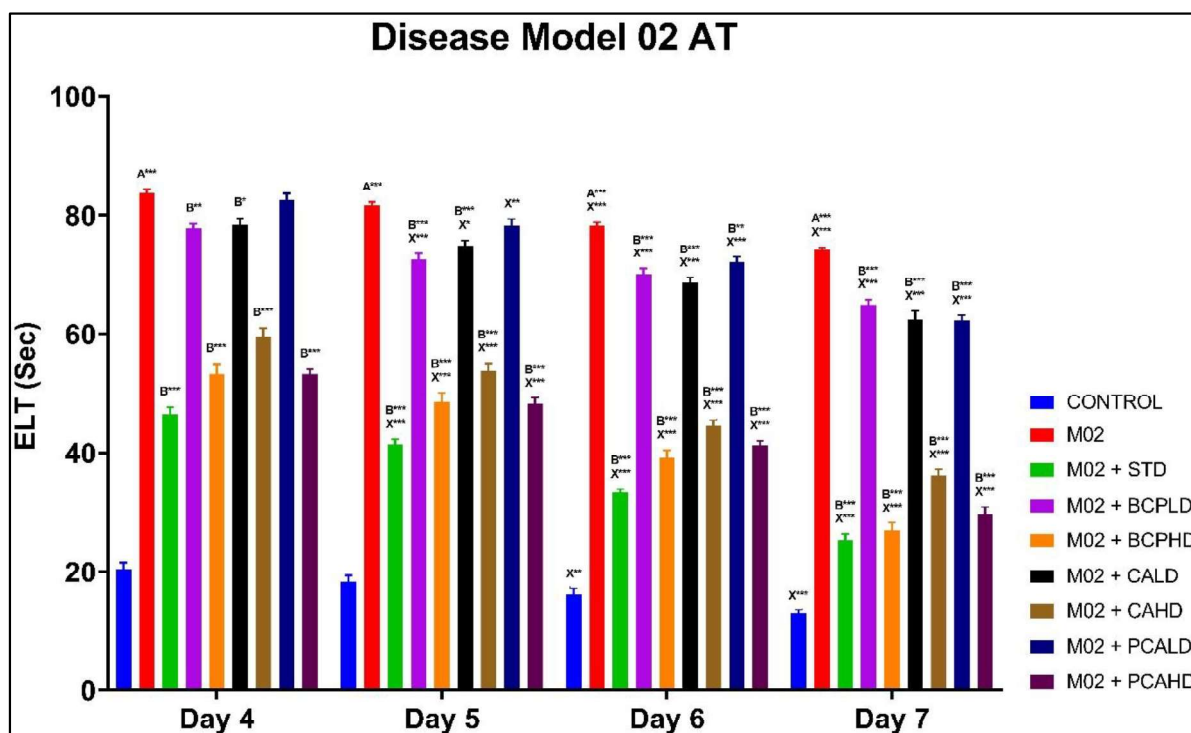


Figure No. 5.12: Effect of various interventions on Escape Latency Time (ELT) during the Acquisition trial on day 04, 05, 06, and 07.

Here A represent the comparison with the Control group, B represents the comparison with the Disease Model 01 group and X represent the comparison with the same group on day 04 and ***, **, and * have been used to represent $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

5.2.1.2. RETRIEVAL TRIAL

During the retrieval trial, on day 04 it was observed that mean ELT value Disease Model 02 group was significantly ($p < 0.001$) higher than the Control group. Among the treatment groups, it was observed that along with the M02 + STD group, other groups i.e., M02 + BCPLD, M02 + BCPHD, M02 + CAHD, and M02 + PCAHD showed significant reduction in the mean ELT with a significance level of $p < 0.001$. among other treatment groups only M02 + CAHD had shown significant reduction in ELT with $p < 0.01$.

On the 2nd day, mean ELT of the Disease Model 02 group was significantly ($p < 0.001$) higher against the Control group. Treatment groups i.e., M02 + STD, M02 + BCPLD, M02 + BCPHD, M02 + CALD, M02 + CAHD, and M02 + PCAHD, lowered the ELT values with a significance value of $p < 0.001$ as compared to the Disease Model 02 group. Along with that treatment groups i.e., M02 + STD, M02 + BCPHD, M02 + CAHD, and M02 + PCAHD also showed significant reduction in the mean ELT against the values of the same group on day 04 with a significance value of p lesser than 0.001, M02 + PCALD also showed lesser significant reduction in ELT with $p < 0.05$.

On day 06 of the study, it was found that the value of ELT of Control group significantly fell against the mean ELT values of same group on 4th day of the study with a significance level of $p < 0.01$. As we move further, we found out that ELT of Disease Model 02 group was again significantly ($p < 0.001$) higher than the Control group and significantly lesser than the ELT of same group on 4th day of the study. Among the treatment groups, M02 + STD group along with all the test drug treated group exhibited the significant reduction in the ELT against Disease Model 02 group with a significance value of $p < 0.001$. Along with that all the treatment groups also showed significant ($p < 0.001$) lowered the ELT against the very same groups on 4th day of the study.

On the final day of the study, the Control group exhibited significant improvement in the ELT as compared to the values exhibited by same group on 4th day. Furthermore, mean ELT of Disease Model 02 group was found to be significantly higher than the Control group with a significance value of $p < 0.001$ and we also observed the significant ($p < 0.001$) reduction in the ELT against this group's value of ELT on day 04. All the treatment groups along with standard treated group showed marked reduction in ELT with respect to the Disease Model 02 group as well as the very same group's ELT on day 04 with a significance level of $p < 0.001$.

The results of retrieval trials are depicted in figure no. 5.13, which is given below:

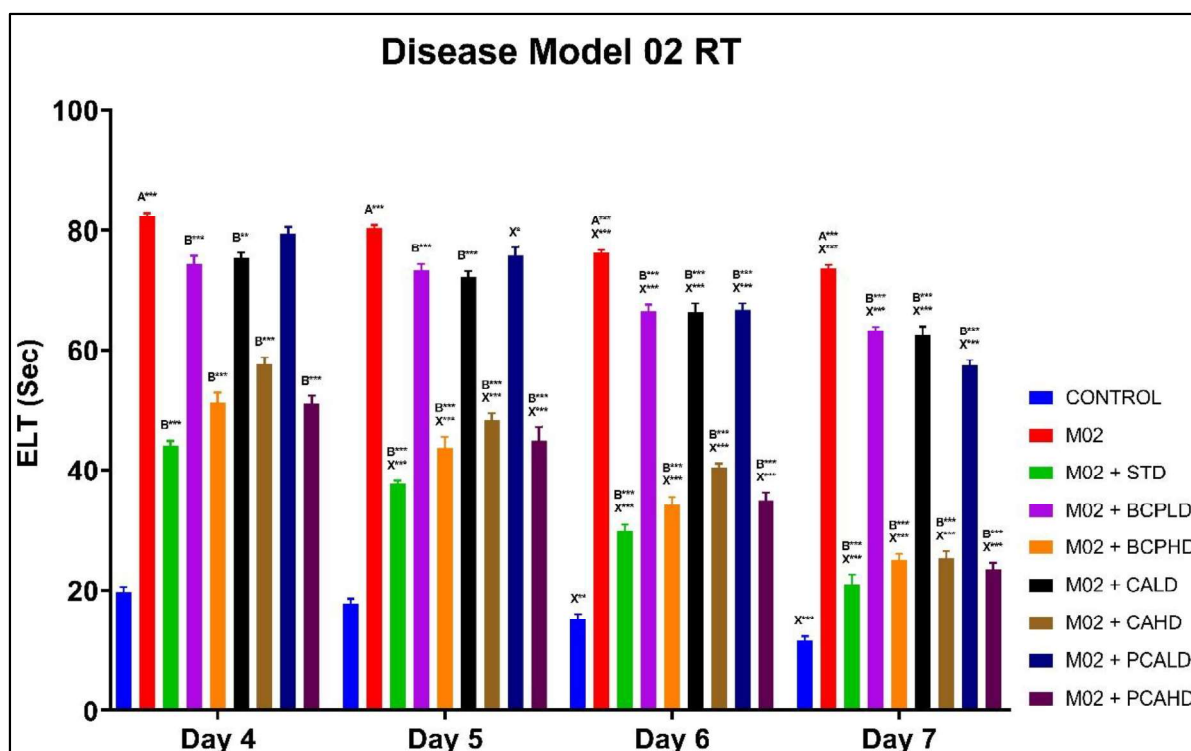


Figure No. 5.13: Effect of various interventions on Escape Latency Time (ELT) during the Retrieval trial on day 04, 05, 06, and 07.

Here A represent the comparison with the Control group, B represents the comparison with the Disease Model 01 group and X represent the comparison with the same group on day 04 and ***, **, and * have been used to represent $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

5.2.1.3. EFFECTS ON TSTQ

The TSTQ estimation was done on the 8th day of the study, and the time spent by the animals in the target quadrant was observed. It is estimated that the mean TSTQ of animals in the disease model 02 group was significantly less than the animals in the control group, with $p < 0.001$. Among all the treatment groups, M02 + STD, M02 + BCPLD, M02 + BCPHD, M02 + CAHD, and M02 + PCAHD had shown the significant improvements in TSTQ values with $p < 0.001$ upon their comparison with disease model 02 group. Among the remaining groups, only the M02 + PCALD group had shown significant improvements with $p < 0.01$ against the disease model 02 group. The results are depicted in Figure 5.14, as follows:

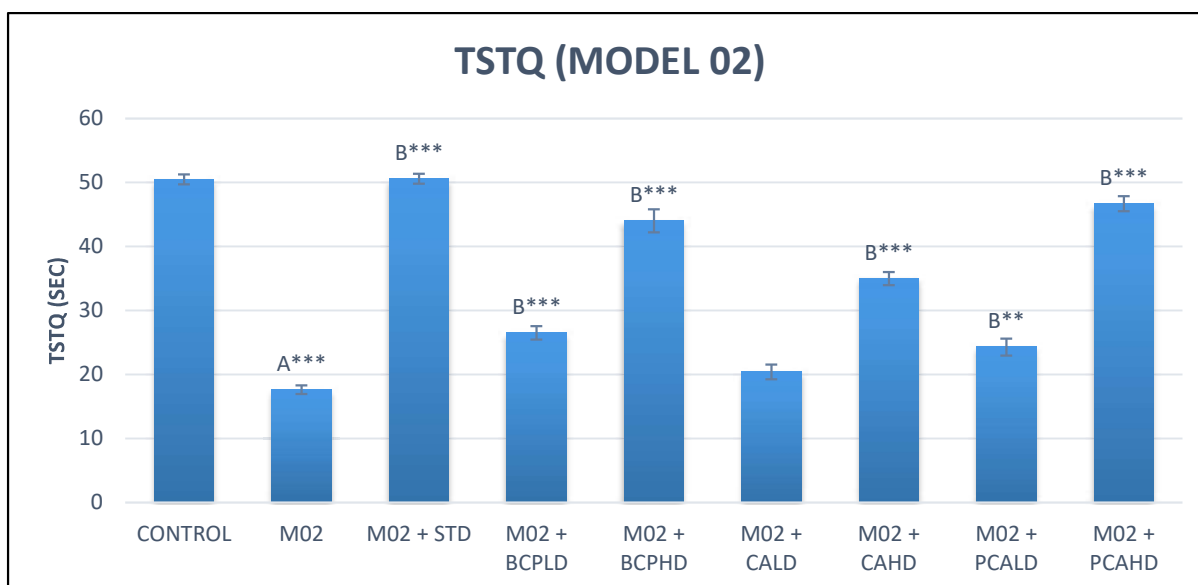


Figure 5.14: Effect of test compounds and other interventions on TSTQ on day 08.

5.2.2. EFFECT ON TRANSFER LATENCY TIME USING ELEVATED PLUS MAZE

Transfer latency time is recorded by using the elevated plus maze apparatus. From the study, it was observed that the mean TLT taken by the animals of the disease model 02 group was significantly higher than the mean TLT of animals in the control group with a significance value of $p < 0.001$. The treatment groups M02 + STD, M02 + BCPHD, M02 + CAHD, and M02 + PCAHD showed a significant decrease in the mean TLT values when compared with the values of disease model 02 group with a significance value of $p < 0.001$, as depicted in figure no. 5.15.

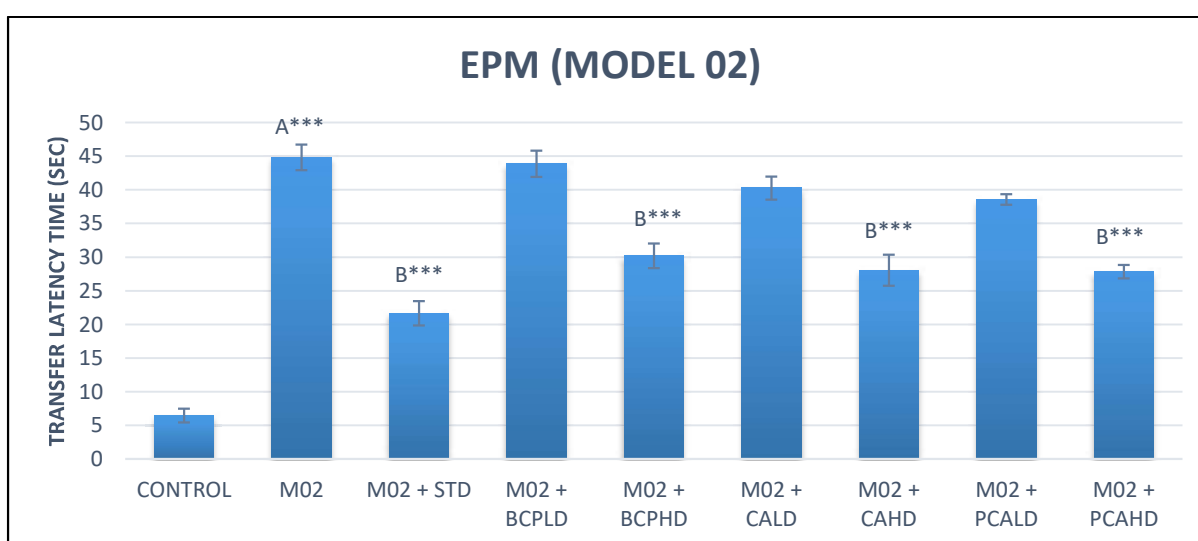


Figure 5.15: Effect of test compounds and other interventions on TLT on day 08 using the Elevated Plus Maze.

5.2.3. EFFECT ON BRAIN AChE

The brain AChE levels are the markers of cholinergic functions of the brain. As the cholinergic system plays a very important role in cognitive functions. From this study it was observed that the brain AChE levels of animals under disease model 02 group were significantly higher than the animals under the control group with a significance value of $p < 0.001$. Further, among the treatment groups, M02 + STD and M02 + PCAHD produced a significant reduction in the brain AChE levels with $p < 0.001$, and the M02 + BCPHD and M02 + CAHD groups reduced the brain AChE levels with $p < 0.01$ as compared to the disease model 02 group. The graphical presentation of the results is depicted in figure no. 5.16 as given below:

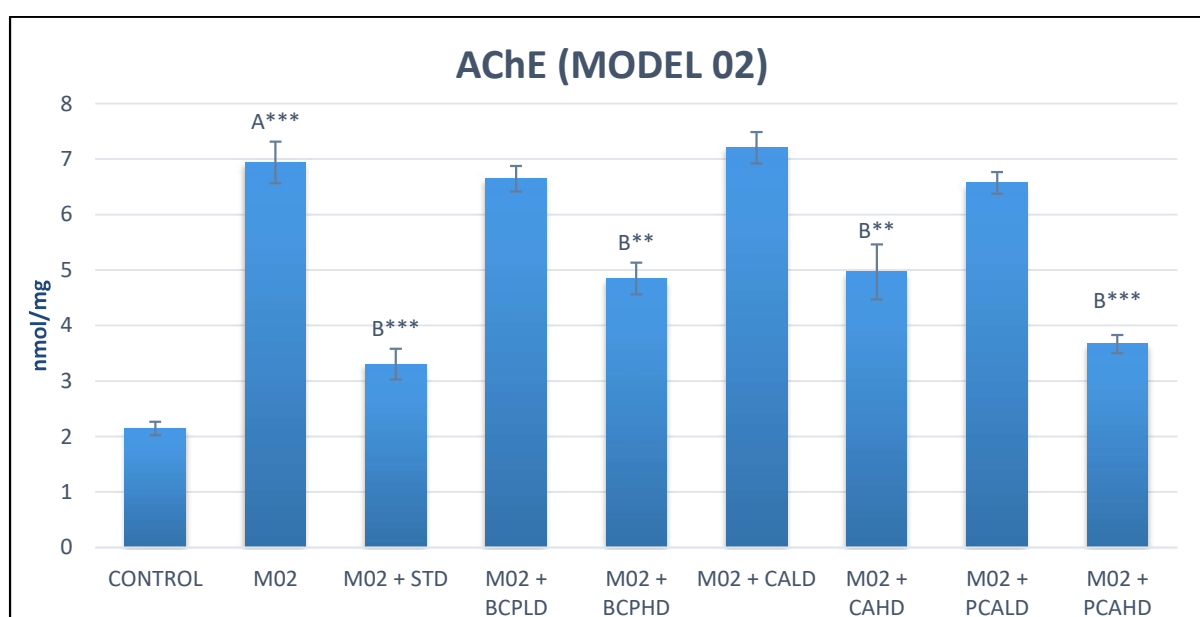


Figure 5.16: Effect of test compounds and other interventions on brain AChE levels in Disease Model 02.

5.2.4. EFFECT ON BRAIN TNF- α

The TNF- α levels represent the presence of neuroinflammation. In this study it was observed that in the disease model 02 group there was a significant increase in the brain levels of TNF- α when the values are compared with the values of the control group, with a significance value of $p < 0.001$, which denotes the presence of inflammation in the brain. The treatment groups, M02 + STD, M02 + CAHD, and M02 + PCAHD, showed a significant decrease in the brain TNF- α levels with $p < 0.001$, and M02 + BCPHD and M02 + CALD showed a decrease in the levels of TNF- α with $p < 0.01$ when they were compared with the values of disease model 02 group. The graphical presentation of the results is given in Figure No. 5.17 as follows:

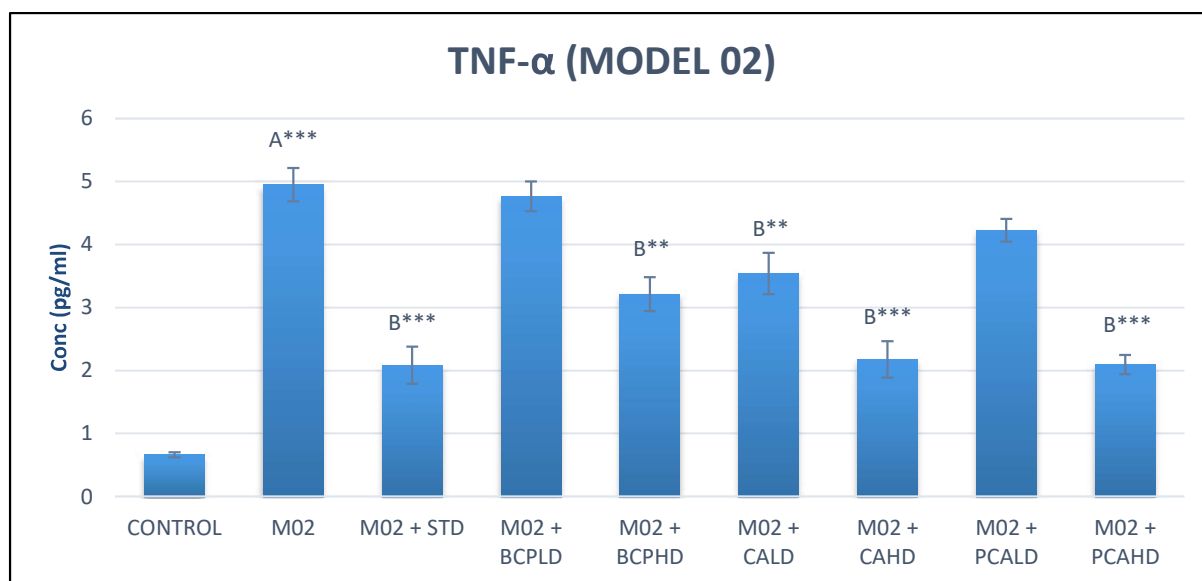


Figure 5.17: Effect of test compounds and other interventions on brain TNF- α levels in Disease Model 02.

5.2.5. EFFECT ON BRAIN TBARS

Brain TBARS levels are assessed for the purpose of evaluating the oxidative stress levels. In this study, the levels of brain TBARS of disease model 02 group were significantly higher than the control group with a significance value of $p < 0.001$, which directed towards the damage caused by the oxidative stress in the brain. Among the treatment groups, M02 + STD, M02 + BCPHD, M02 + CAHD, and M02 + PCAHD had shown a significant decrease in the brain TBARS levels with a significant value of $p < 0.001$ upon their comparison with the disease model 02 group. No other treatment group produced any significant changes in brain TBARS. The results are depicted in figure no. 5.18, which is given below:

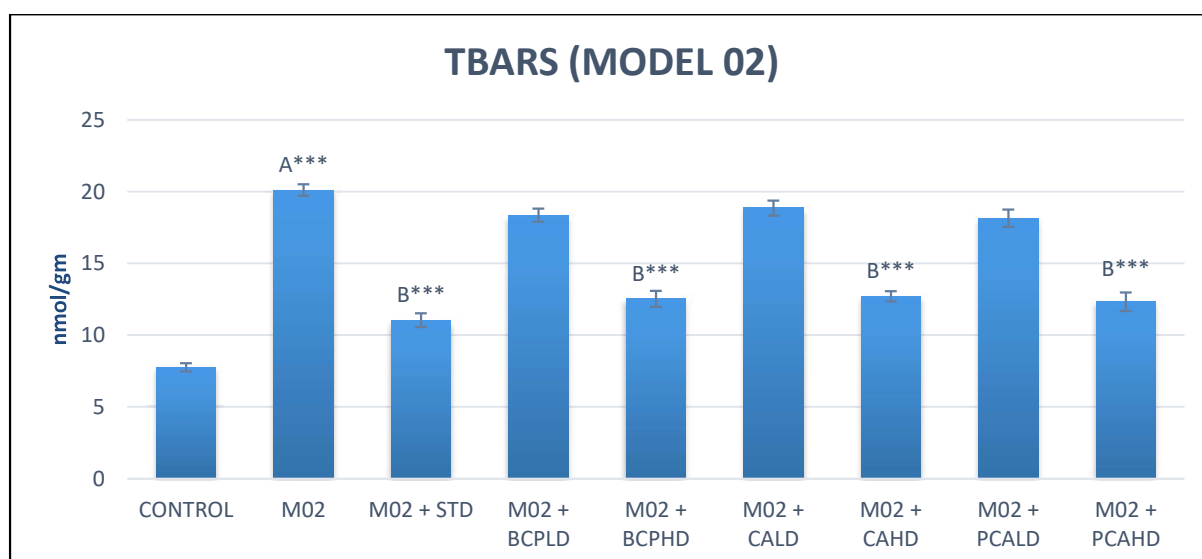


Figure 5.18: Effect of test compounds and other interventions on brain TBARS levels in Disease Model 02.

5.2.6. EFFECT ON BRAIN GSH

The GSH levels of the brain represent the oxidative stress in the brain. Here in this study, it was observed that the disease model 02 group had higher values of brain GSH when it was compared with the control group's values with a significance value of $p < 0.001$. Treatment groups M02 + STD, M02 + BCPHD, M02 + CAHD, and M02 + PCAHD imparted the significant increase in the brain levels of GSH with $p < 0.001$ when compared with the disease model 02 group. The results are depicted in Figure No. 5.19, which is given below:

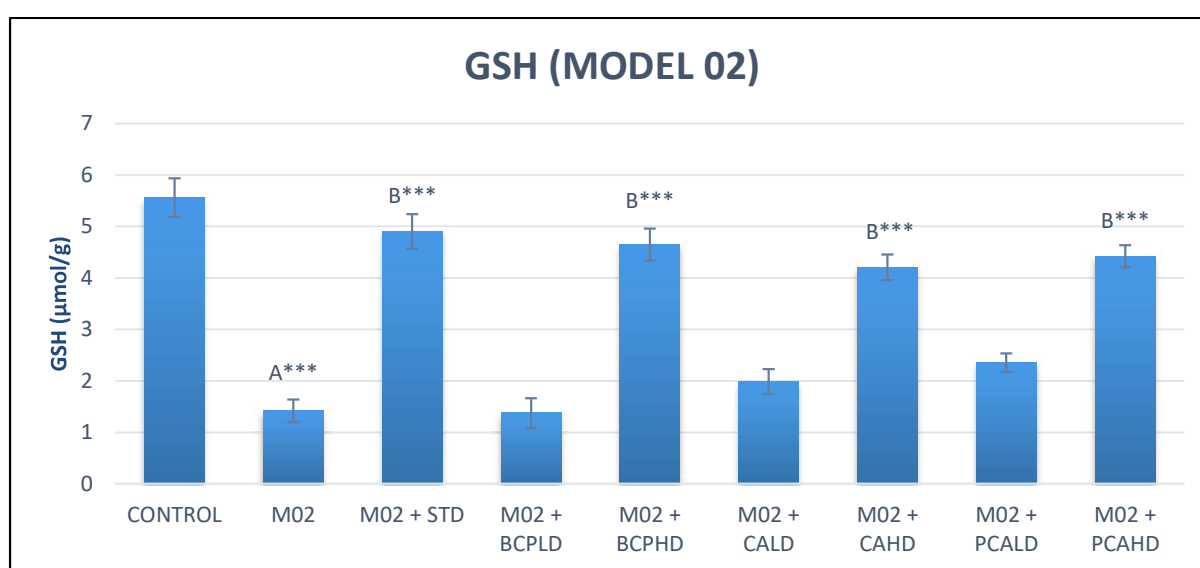


Figure 5.19: Effect of test compounds and other interventions on brain GSH levels in Disease Model 02.

5.2.7. EFFECTS ON BRAIN SUPEROXIDE DISMUTASE

Brain levels of superoxide dismutase also tell us about the levels of oxidative stress in the brain. Here in this study, it was observed that due to induction of disease, the levels of brain SOD decreased significantly in the disease model 02 group with a significance value of $p < 0.001$ when it was compared with the values obtained from the control group. Later, with the treatments, it was observed that the brain SOD levels were again increased. Among treatment groups, M02 + STD, M02 + BCPHD, M02 + CAHD, M02 + PCALD, and M02 + PCAHD imparted the significant increase in the brain SOD levels with $p < 0.001$; other than that, M02 + BCPLD showed significant improvements in brain SOD levels with $p < 0.01$, and M02 + CALD significantly increased brain SOD levels with $p < 0.05$ when they were respectively

compared with the disease model 02 group. The results are depicted in figure no. 5.20, as follows:

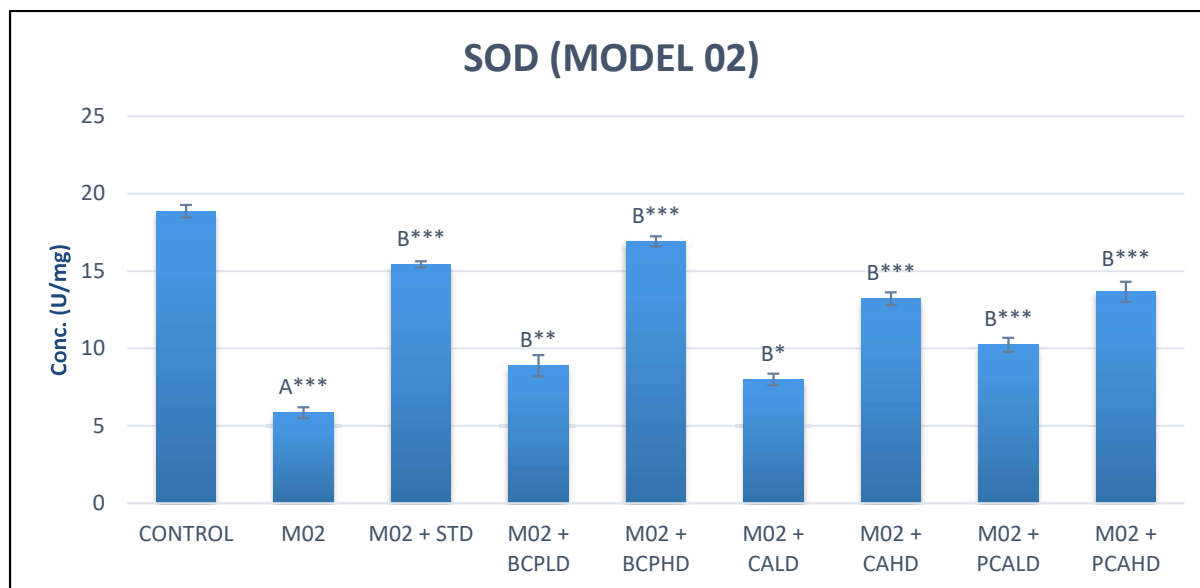


Figure 5.20: Effect of test compounds and other interventions on brain SOD levels in Disease Model 02.

5.2.8. EFFECTS ON BRAIN CATALASE

Brain catalase levels again talk about the levels of oxidative stress in the brain. Here in the study, it was estimated that the levels of brain catalase significantly got exhausted in the disease model 02 group with $p < 0.01$ when they were compared with the brain levels of catalase of the control group, which indicated the presence of oxidative stress in the brain. Among the treatment groups, M02 + PCAHD showed significant improvement with $p < 0.001$, and M02 + STD and M02 + BCPHD showed a significant rise in the brain levels of catalase with $p < 0.01$, as compared with the disease model 02 group. The effect of all test compounds and other interventions is presented in figure no. 5.21, as follows:

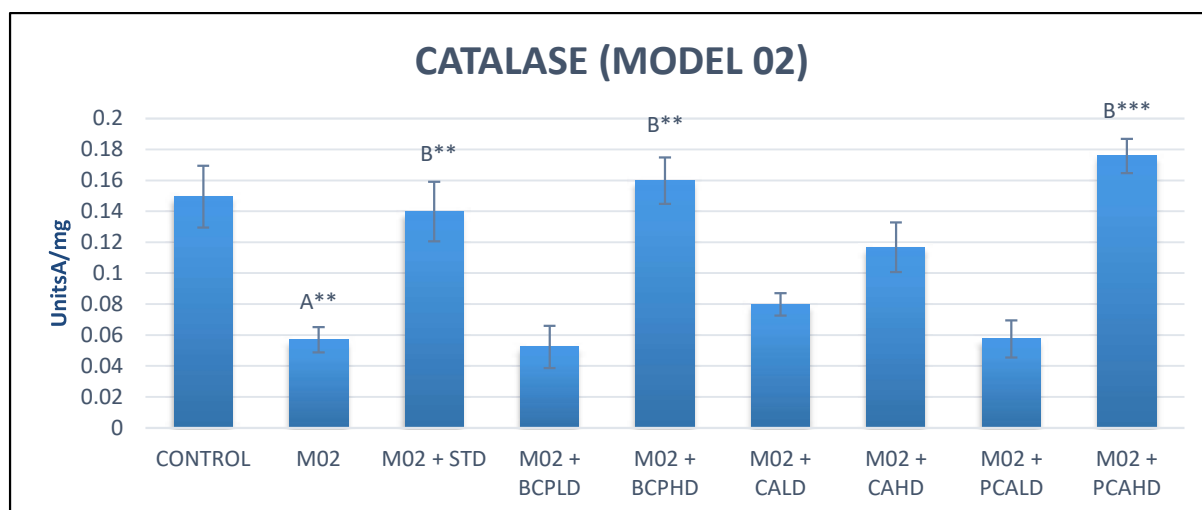


Figure 5.21: Effect of test compounds and other interventions on brain CATALASE levels in Disease Model 02.

5.2.9. EFFECT ON BRAIN NITRITE

In this study, it was observed that the levels of brain nitrites significantly increased in the disease model 02 groups with a significance value of $p < 0.001$ when the values were compared with the values of the control group. It signifies that there is free radical stress and damage present in the brain. The treatment groups, M02 + STD, M02 + BCPHD, M02 + CAHD, M02 + PCALD, and M02 + PCAHD, showed a significant reduction in the brain nitrite levels with $p < 0.001$, and treatment group M02 + BCPLD showed significant improvements with $p < 0.01$ when compared with the disease model 02 group. The results are presented in graphical form in figure no. 5.22 as follows:

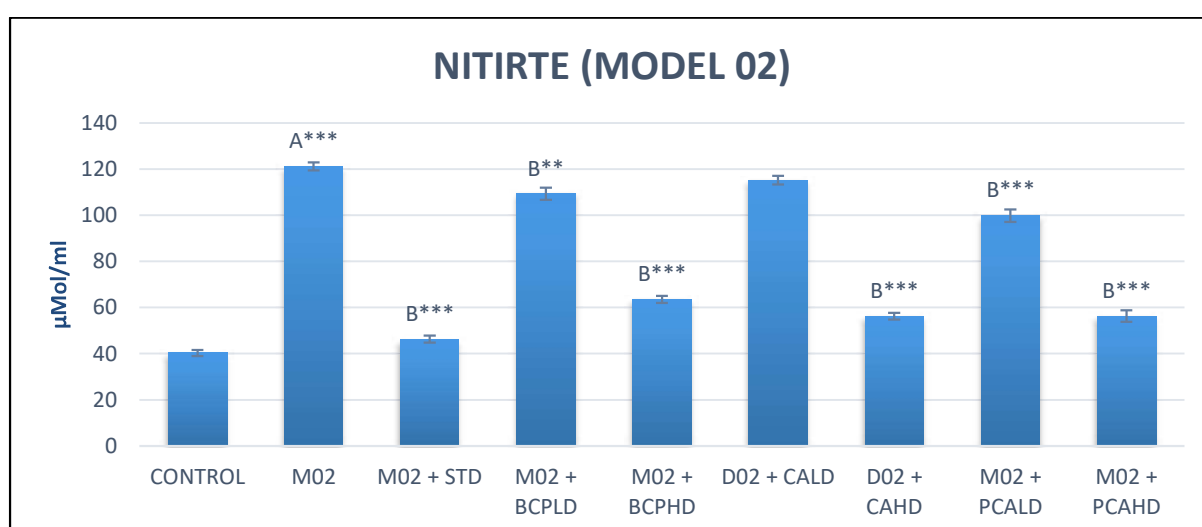


Figure 5.22: Effect of test compounds and other interventions on brain NITRITE levels in Disease Model 02.

5.3. TEST DRUG COMBINATION GROUPS

In the test drug combination groups, the 03 test compounds were randomly assigned to the 3 different groups to test the combinatorial effects of the test compounds. Each group had 02 test compounds. The dose of the test compound to be used was selected on the basis of the best results produced as an individual test compound. In Combination 01, a high dose of beta-caryophyllene and a high dose of cinnamic acid were used; in Combination 02, a high dose of cinnamic acid and a high dose of p-coumaric acid were used; and in Combination 03, a high dose of beta-caryophyllene with a high dose of p-coumaric acid was used. These combinations were later tested against both of the disease models.

5.3.1 DISEASE MODEL 01 (AICL₃ + D-GALACTOSE)

5.3.1.1 EFFECT ON ESCAPE LATENCY TIME USING MORRIS WATER MAZE

In this study the escape latency time was again assessed by the means of acquisition trials and retrieval trials, as follows:

5.3.1.1.a. ACQUISITION TRIAL

The acquisition trials were conducted on days 87, 88, 89, and 90 in the morning timings.

On day 87, it was observed that the escape latency time is significantly higher in the disease model 01 group with a p-value lesser than 0.001. In the treatment groups, M01 + STD, M01 + COM01, M01 + COM02, and M01 + COM03 showed significant improvements in the ELT with $p < 0.001$ when compared with the disease model 01 group.

On day 88, it was observed that the mean ELT of the Disease Model 01 was significantly ($p < 0.001$) higher than the control group. All the treatment groups significantly lower the ELT as compared to the Disease Model 01 group with a significance value of $p < 0.001$. Other than that, it was observed that the mean ELT of the treatment groups other than M02 + STD were significantly improved that the values obtained on day 87 with a significance of $p < 0.001$.

On day 89 and 90, it was found out that the mean ELT of control groups was significantly lesser than the ELT observed on day 87, on 89th day it was significantly lesser with a significance value of $p < 0.01$ and on 90th day it was significantly lesser than 87 day with significance value of $p < 0.001$, also the ELT of Disease Model 01 was observed to be significantly lesser than the values observed on the day 87th with a significance value of $p < 0.001$. All the treatment groups including standard drug treated group the mean ELT was found

to be significantly ($p < 0.001$) improved than the Disease Model 01 group as well as the values obtained from the same group on 87th day.

The results for all days are depicted in Figure No. 5.23, as follows:

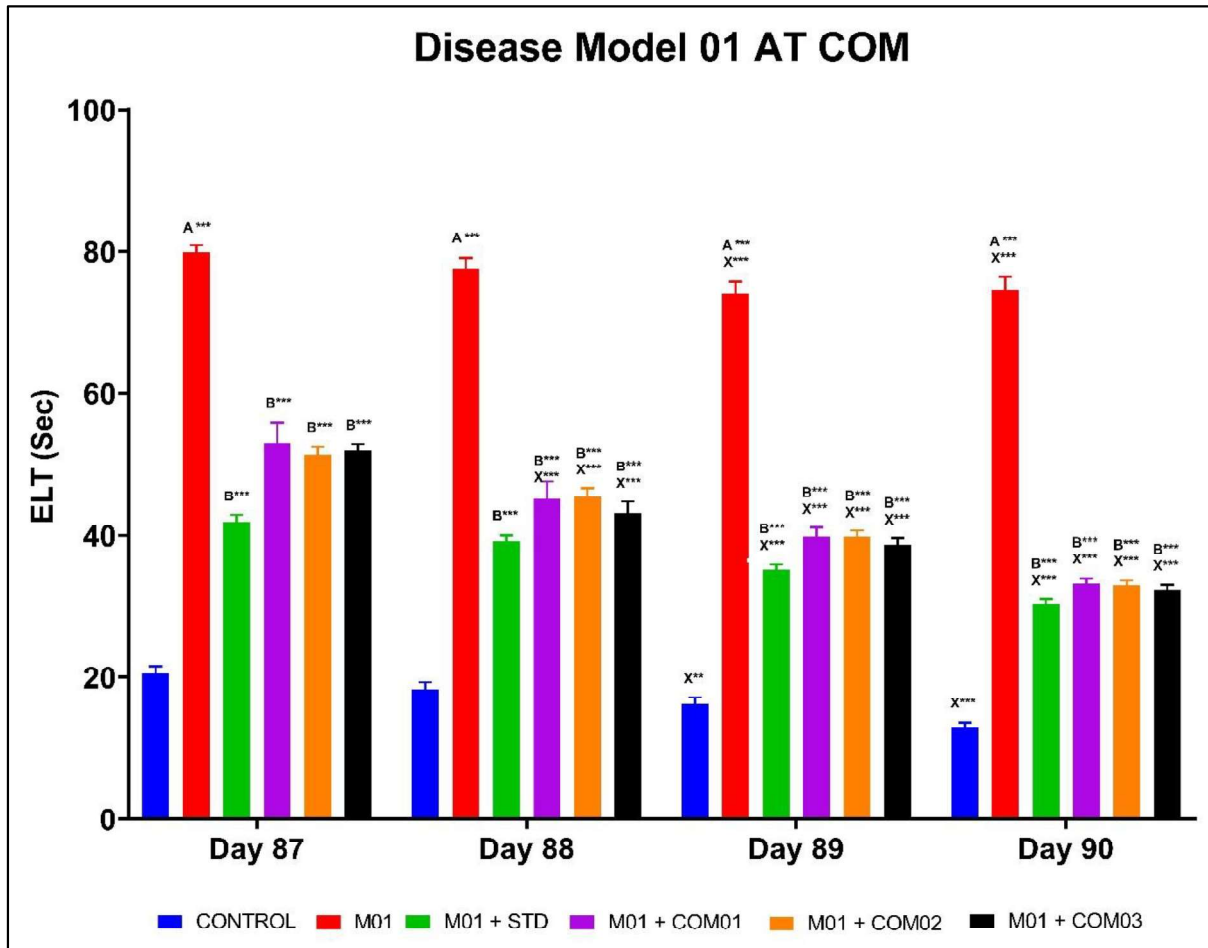


Figure No. 5.23: Effect on escape latency time (ELT) on days 87, 88, 89, and 90 during the acquisition trial.

Here A represent the comparison with the Control group, B represents the comparison with the Disease Model 01 group and X represent the comparison with the same group on day 87 and ***, **, and * have been used to represent $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

5.3.1.1.b. RETRIEVAL TRIAL

Retrieval trials were conducted on days 87, 88, 89, and 90 in the evening.

From this study, on day 87, it was observed that the mean ELT of the disease model 01 group was significantly increased with $p < 0.001$ as compared with the control group. The treatment groups, M01 + STD, M01 + COM01, M01 + COM02, and M01 + COM03, showed a

significant decrease in the mean ELT when compared with the disease model 01 group with $p < 0.001$.

From the observations of day 88, it was observed that the ELT of Disease Model 01 group was again significantly ($p < 0.001$) higher than the values of Control group. Treatment groups i.e., M01 + STD, M01 + COM01, M01 + COM02, and M01 + COM03, showed significant ($p < 0.001$) reduction in the mean ELT as compared to the Disease Model 01 group. Among treatment groups we also observed the reduction in the mean ELT as compared to the values of the same groups on day 87, M01 + COM01 showed significant reduction with $p < 0.001$, other groups, M01 + COM02 and M01 + COM03, showed significant reduction with significance value of $p < 0.05$.

On the following two days i.e., day 89 and 90, the nature of the results was found to be same. The mean ELT of Control group on 90th day was found to be significantly ($p < 0.05$) lesser than the values obtained in same group on but on 87th day. On both days i.e., 89th and 90th, it was observed that the mean ELT of Disease Model 01 group was significantly ($p < 0.001$) higher than the Control group, also the value of ELT of Disease Model 01 group on 90th day was found to be significantly lesser than the values obtained on the 87th day with a significance value $p < 0.05$. All the treatment groups i.e., M01 + STD, M01 + COM01, M01 + COM02, and M01 + COM03, found to significantly ($p < 0.001$) improved than the Disease Model 01 group on both days. Also, it was observed that the ELT value of each individual group was significantly improved than the value of same group on 87th day with a significance value of $p < 0.001$.

The results of the retrieval trial are depicted in figure no. 5.24, which is given below:

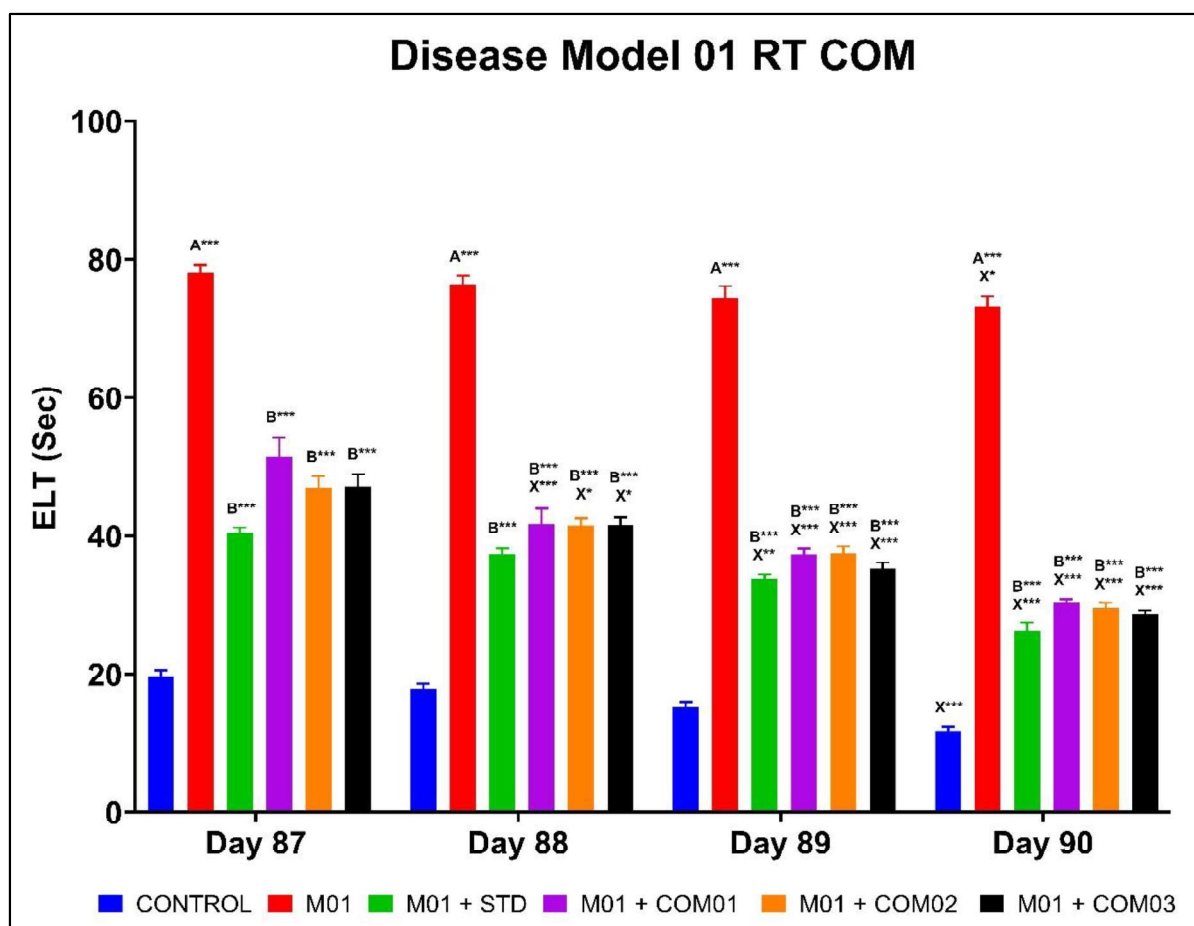


Figure No. 5.24: Effect on escape latency time (ELT) on days 87, 88, 89, and 90 during the retrieval trial.

Here A represent the comparison with the Control group, B represents the comparison with the Disease Model 01 group and X represent the comparison with the same group on day 87 and ***, **, and * have been used to represent $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

5.3.1.1.c. EFFECT ON TSTQ

In this study the effect of the combination of test drugs was observed on the TSTQ. It was observed that the TSTQ value significantly decreased in the disease model 01 group when it was compared with the control group with a significance value of $p < 0.001$. Among the treatment groups, it was estimated that M01 + STD, M01 + COM01, M01 + COM02, and M01 + COM03 had significantly improved the TSTQ levels with a significant value of $p < 0.001$ when compared with the disease model 01 group. The results are depicted in figure no. 5.25, as follows:

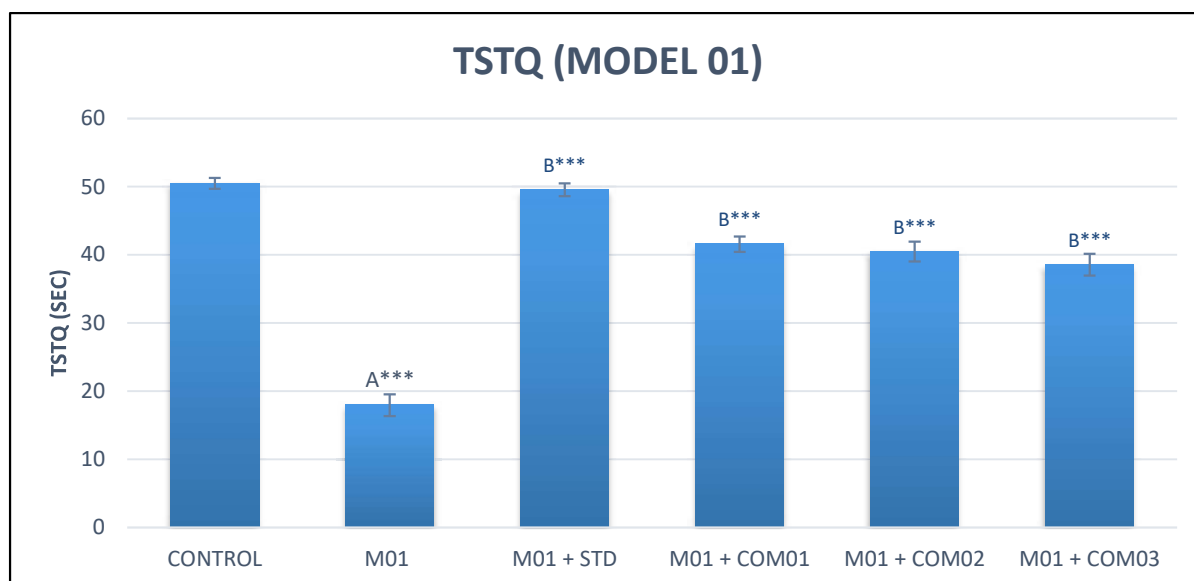


Figure 5.25: Effect of combination of test compounds and other interventions on TSTQ on day 91.

5.3.1.2. EFFECT ON TRANSFER LATENCY TIME USING ELEVATED PLUS MAZE

In this study, it was observed that the transfer latency time taken by the animals of disease model 01 group was significantly higher than the TLT of the control group with a significance value of $p < 0.001$. All the treatment groups, i.e., M01 + STD, M01 + COM01, M01 + COM02, and M01 + COM03, had shown the significant improvements with transfer latency time when compared with the disease model 01 group with a significance value of $p < 0.001$, as depicted in figure no. 5.26, as follows:

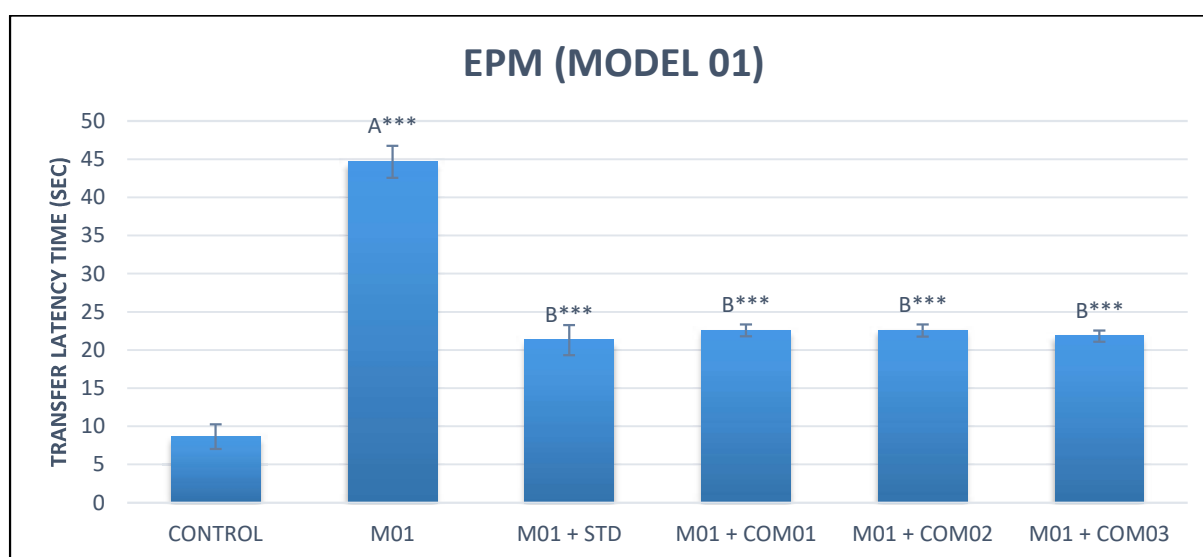


Figure 5.26: Effect of combination of test compounds and other interventions on transfer latency time using elevated plus maze.

5.3.1.3. EFFECT ON BRAIN AChE

It was estimated that the brain levels of AChE were significantly raised in the disease model 01 group due to induction of disease as compared to the control group with a significance value of $p < 0.001$. The treatment groups, M01 + STD, M01 + COM01, M01 + COM02, and M01 + COM03, showed significant reduction in the brain AChE levels with $p < 0.001$ as compared to the disease model 01 group. The results are depicted in Figure No. 5.27, as follows:

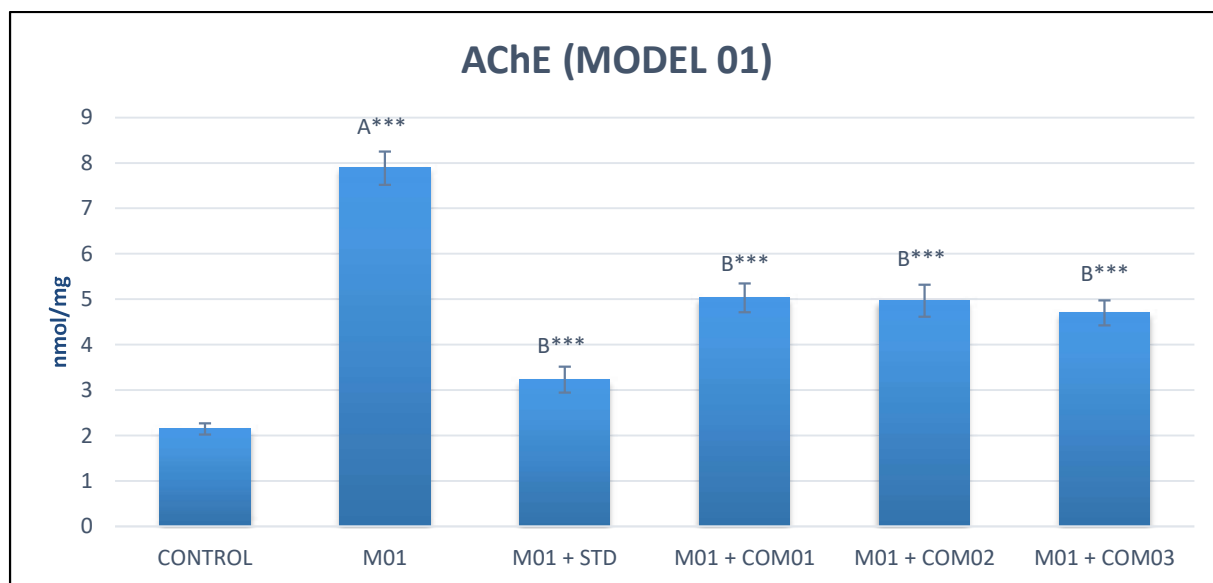


Figure 5.27: Effect of combination of test compounds and other interventions on brain AChE levels.

5.3.1.4. EFFECT ON BRAIN TNF- α

TNF- α levels of the brain talk about the inflammatory levels of the brain. Here in this study, it was evaluated that the brain TNF- α levels significantly increased in the disease model 01 group with a significance value of $p < 0.001$ when it was compared with the control group. Among the treatment groups, M01 + STD and M01 + COM01 showed the significant reduction with $p < 0.001$; on the other hand, M01 + COM02 and M01 + COM03 showed significant improvements in the brain TNF- α levels with $p < 0.01$ upon their respective comparisons with the disease model 01 group. The graphical presentation of the results is depicted in figure no. 5.28, given below:

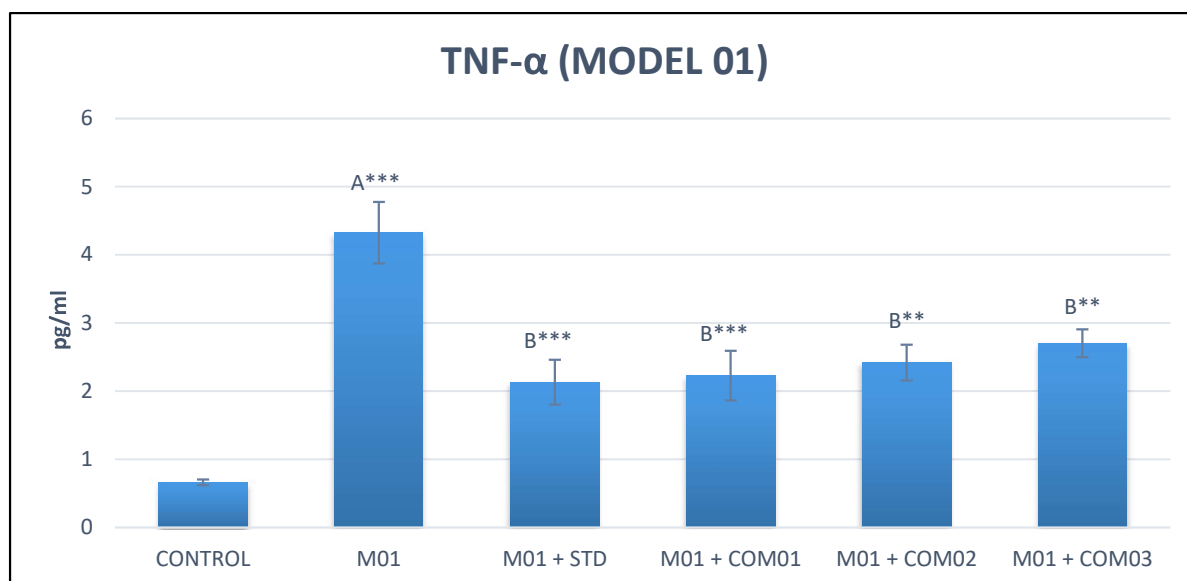


Figure 5.28: Effect of combination of test compounds and other interventions on brain TNF- α levels.

5.3.1.5. EFFECT ON BRAIN TBARS

From this study it was evaluated that the brain levels of TBARS significantly raised in the disease model 01 group as compared to the TBARS value obtained from the control group with a significant value of $p < 0.001$. The treatment groups, M01 + STD, M01 + COM01, M01 + COM02, and M01 + COM03, showed a significant decrease in the brain levels of TBARS with $p < 0.001$ as compared to the disease model 01 group. The results are depicted in figure no. 5.29, as follows:

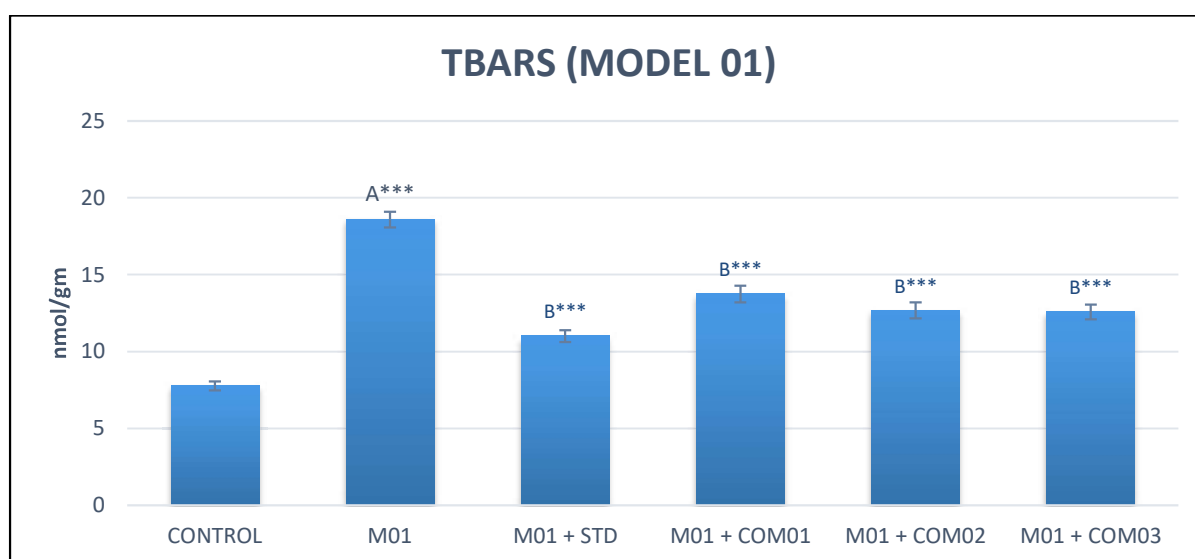


Figure 5.29: Effect of combination of test compounds and other interventions on brain TNF- α levels.

5.3.1.6. EFFECT ON BRAIN GSH

It is evaluated from this study that the level of brain GSH decreased as the disease progressed. It was observed that the brain levels of GSH significantly decreased in the disease model 01 group with a significance value of $p < 0.001$ when it was compared with the value obtained from the control group. Among the treatment groups, all the groups, i.e., M01 + STD, M01 + COM01, M01 + COM02, and M01 + COM03, produced significant improvement in the brain GSH level with $p < 0.001$ upon their respective comparison with disease model 01 group. The results are depicted in figure no. 5.30, given below:

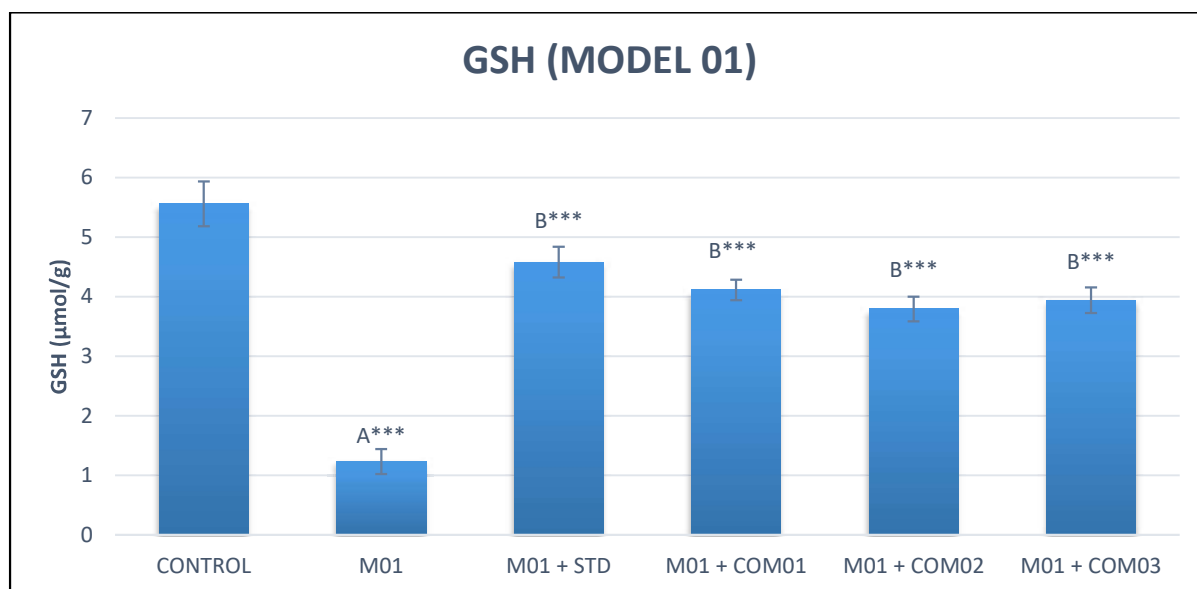


Figure 5.30: Effect of combination of test compounds and other interventions on brain GSH levels.

5.3.1.7. EFFECT ON BRAIN SUPEROXIDE DISMUTASE

From this estimation, it was observed that the levels of SOD in the brain decreased significantly in disease model 01 group as it was compared with the value obtained from the control group with a significance value of $p < 0.001$. It improved with the treatments; the treatment groups such as M01 + STD, M01 + COM01, M01 + COM02, and M01 + COM03 showed the significant increase in the brain SOD levels with $p < 0.001$, as depicted in figure no. 5.31, as given below:

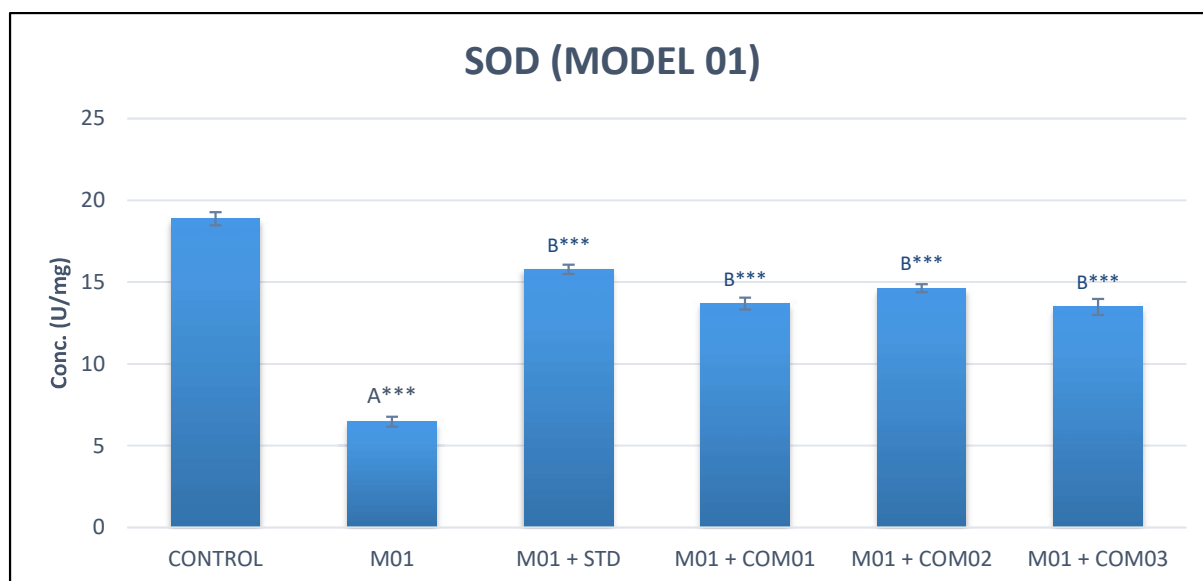


Figure 5.31: Effect of combination of test compounds and other interventions on brain SOD levels.

5.3.1.8. EFFECT ON BRAIN CATALASE

From the study it was estimated that the brain catalase levels fell as the disease progressed. It was estimated that the brain levels of catalase in the disease model 01 group significantly decreased as compared to the control group with a significance value of $p < 0.001$. Among the treatment groups, the M01 + STD group improved the brain catalase level with $p < 0.01$, and M01 + COM03 improved it with $p < 0.05$, upon their respective comparisons with the disease model 01 group. The results are depicted in figure no. 5.32, as follows:

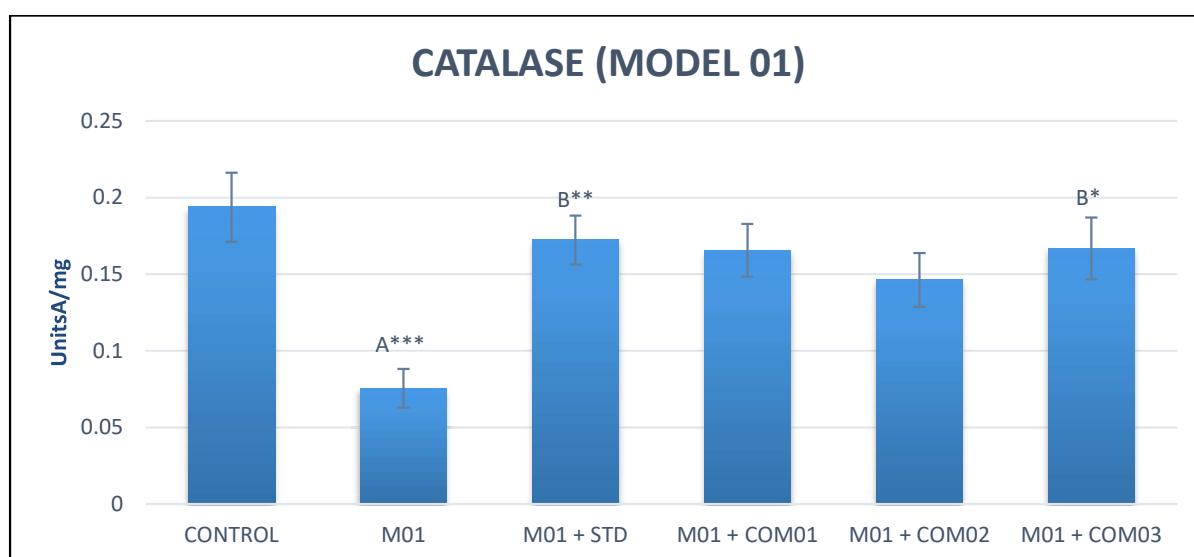


Figure 5.32: Effect of combination of test compounds and other interventions on brain CATALASE levels.

5.3.1.9. EFFECT ON BRAIN NITRITE

From this study it was estimated that the brain levels of nitrite were significantly higher in the disease model 01 group as compared to the control group with a significance value of $p < 0.001$. Among the treatment groups, all groups, i.e., M01 + STD, M01 + COM01, M01 + COM02, and M01 + COM03, showed a significant decrease in the brain nitrite levels upon their respective comparison with the disease model 01 group with a significance value of $p < 0.001$. The results are depicted in figure no. 5.33 as follows:

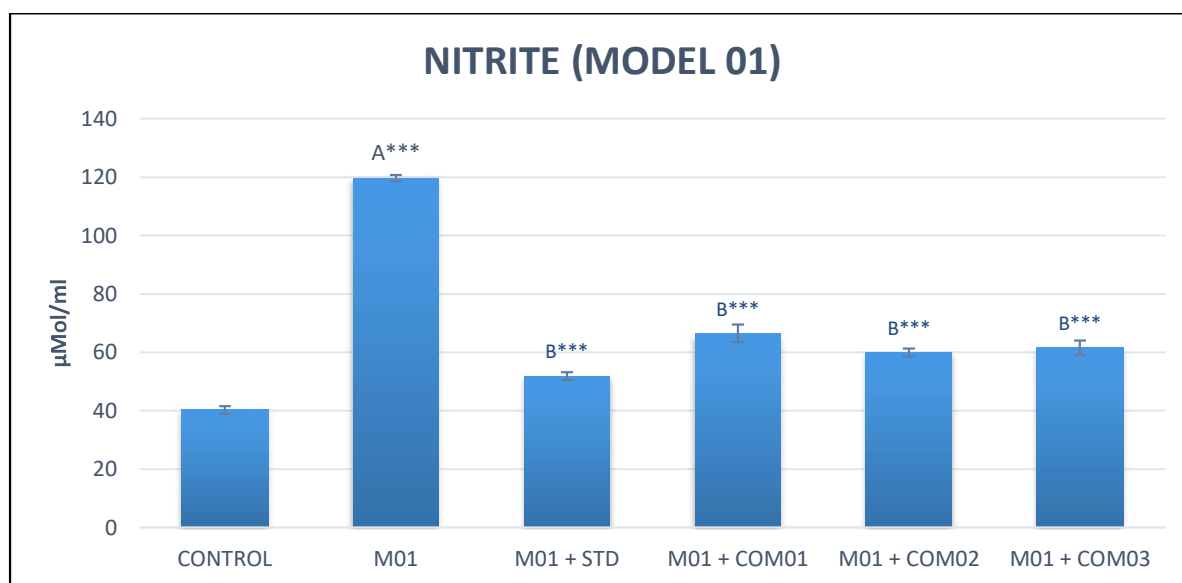


Figure 5.33: Effect of combination of test compounds and other interventions on brain NITRITE levels.

5.3.2 DISEASE MODEL 02 (L-Methionine)

5.3.2.1 EFFECT ON ESCAPE LATENCY TIME USING MORRIS WATER MAZE

The escape latency time was estimated with the help of the acquisition trial and retrieval trial.

5.3.2.1.a ACQUISITION TRIAL

The acquisition trials were conducted on days 04, 05, 06, and 07 of the study.

On day 04, It was observed that the escape latency time was significantly high in the disease model 02 group as compared to the mean escape latency time of the control group with a significance value of $p < 0.001$. The treatment groups, i.e., M02 + STD, M02 + COM01, M02 + COM02, and M02 + COM03, produced significant improvement in the escape latency time as compared with the disease model 02 group with a significance value of $p < 0.001$ on all four days of study.

On day 06 and 07, it was observed that the ELT of Control group was significantly improved than the value of same group on day 04 with a significance value of $p < 0.001$. on day 05, 06, and 07, the Disease Model 02 group was significantly higher than the Control group with a significance level of $p < 0.001$. Also, on day 06 and 07, the value of ELT of Disease Model 02 group was found to be significantly ($p < 0.001$) better than the values obtained on day 04 from same group. Treatment groups, i.e., M02 + STD, M02 + COM01, M01 + COM02, and M02 + COM03 were found to be significantly improved than the Disease Model 02 group with a significance level of $p < 0.001$. The ELT of these treatment groups were also found to be significantly ($p < 0.001$) improving on day 05, 06, and 07 than the values obtained on 4th day.

The results are depicted in the following figure, no. 5.34:

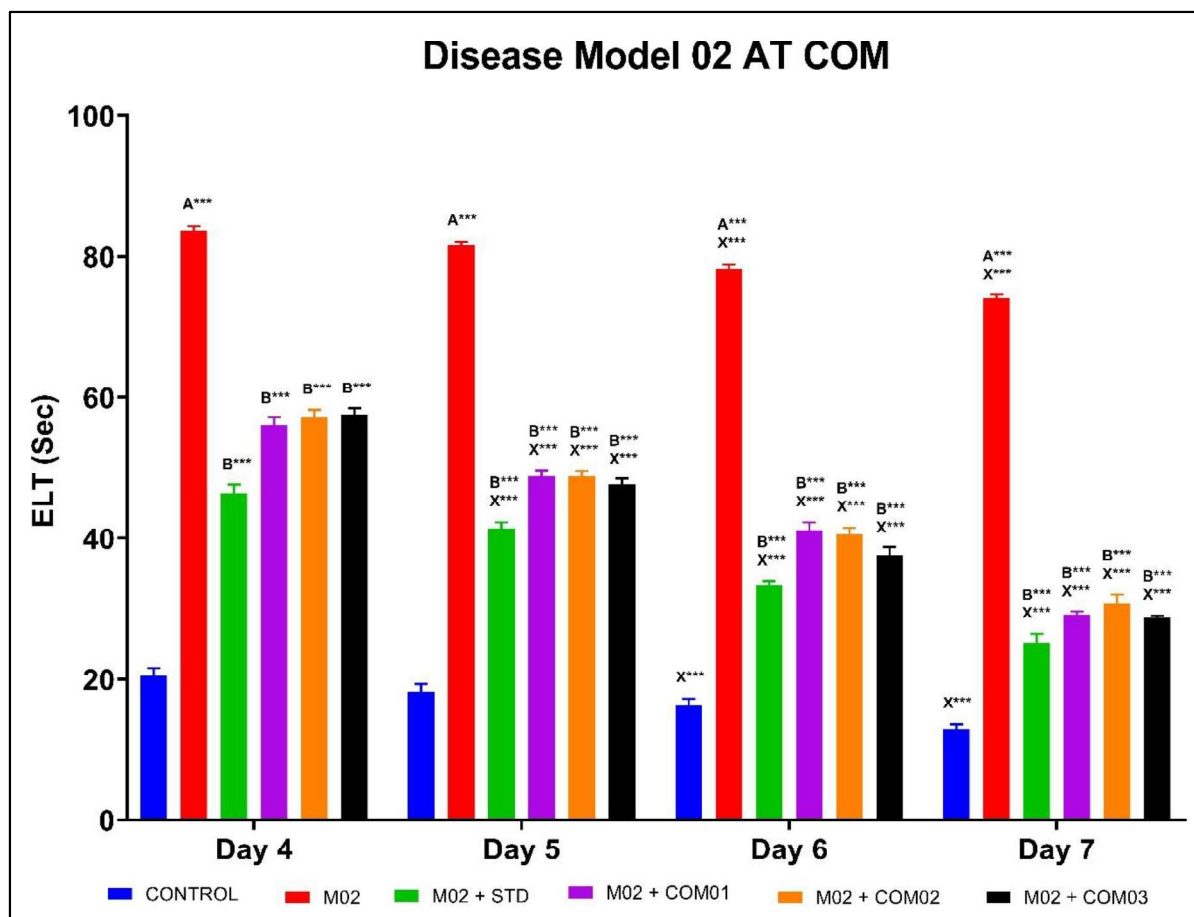


Figure No. 5.34: Effect on escape latency time (ELT) on days 04, 05, 06, and 07 during the acquisition trial.

Here A represent the comparison with the Control group, B represents the comparison with the Disease Model 01 group and X represent the comparison with the same group on day 04 and ***, **, and * have been used to represent $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

5.3.2.1.b RETRIEVAL TRIAL

Retrieval trials were conducted on days 04, 05, 06, and 07 in the evening.

From this study, it was observed that on day 04 and 05, the mean ELT of disease model 02 group was significantly increased with $p < 0.001$ as compared with the control group. The treatment groups, M02 + STD, M02 + COM01, M02 + COM02, and M02 + COM03, showed a significant decrease in the mean ELT when compared with the disease model 01 group with $p < 0.001$. On day 05 the mean ELT of treatment groups was found to be significantly ($p < 0.001$) better than the values of same groups on day 04.

On day 06 and 07, it was observed that the ELT of control groups was significantly lesser than the values of same group on day 04. On day 06 the mean ELT of control group was significantly lesser with $p < 0.01$ and on day 07 it was significantly lesser with $p < 0.001$. M02 + STD, M02 + COM01, M02 + COM02, and M02 + COM03 groups showed a significant reduction in the mean ELT as compared to Disease Model 02 group with $p < 0.001$. These groups also showed significant reduction in the mean ELT with a significance $p < 0.001$ as compared to the values obtained from these groups on day 04.

The results of the retrieval trial are depicted in figure no. 5.35, which is given below:

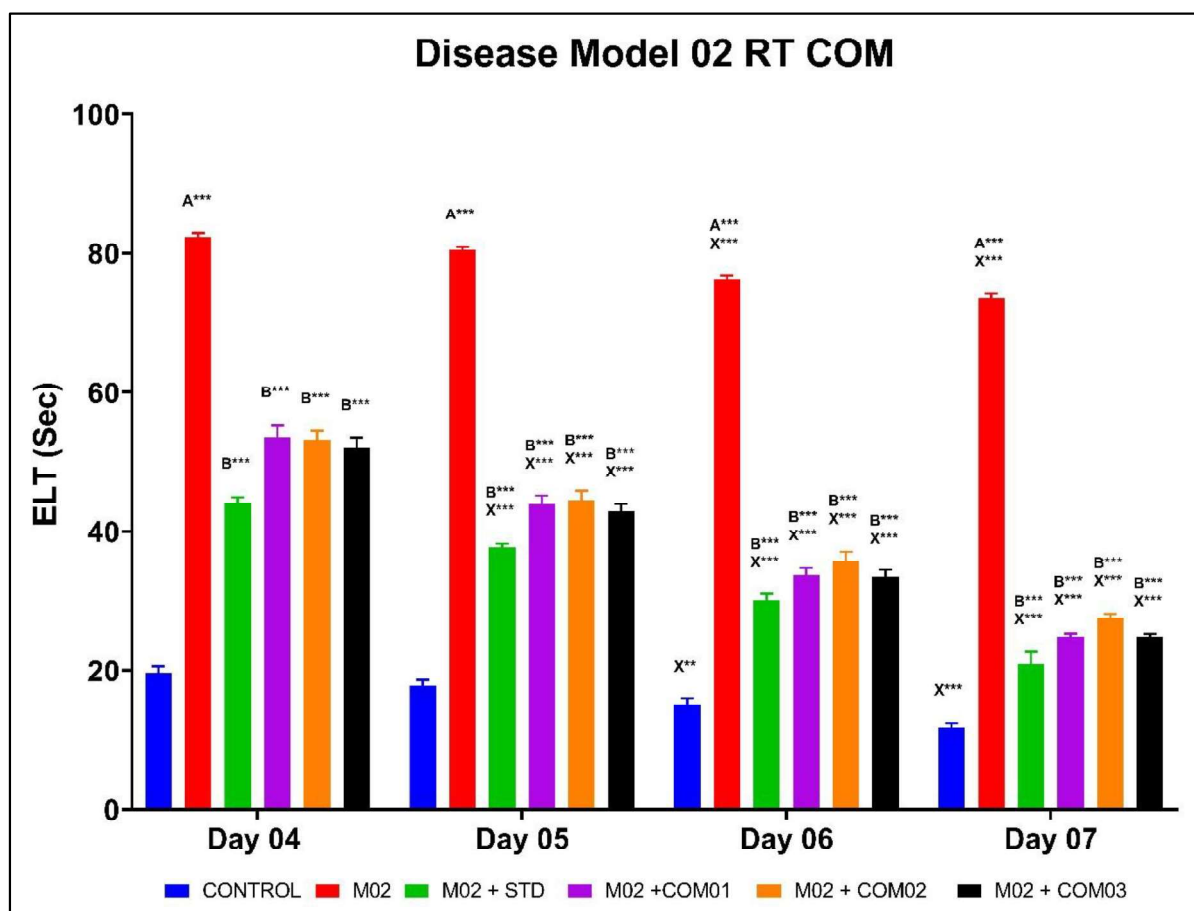


Figure No. 5.35: Effect on escape latency time (ELT) on days 04, 05, 06, and 07 during the retrieval trial.

Here A represent the comparison with the Control group, B represents the comparison with the Disease Model 01 group and X represent the comparison with the same group on day 04 and ***, **, and * have been used to represent $p < 0.001$, $p < 0.01$, and $p < 0.05$, respectively.

5.3.2.1.b EFFECT ON TSTQ

In this study the effect of the combination of test drugs was estimated on the TSTQ on day 08. It was estimated that the TSTQ value of the disease model 02 group was significantly less than the control group with a significance value of $p < 0.001$. All the treatment groups, i.e., M02 + STD, M02 + COM01, M02 + COM02, and M02 + COM03, had proven their potential by significantly increasing the TSTQ as compared to the disease model 02 group with a significance of $p < 0.001$. The results are depicted in figure no. 5.36, as follows:

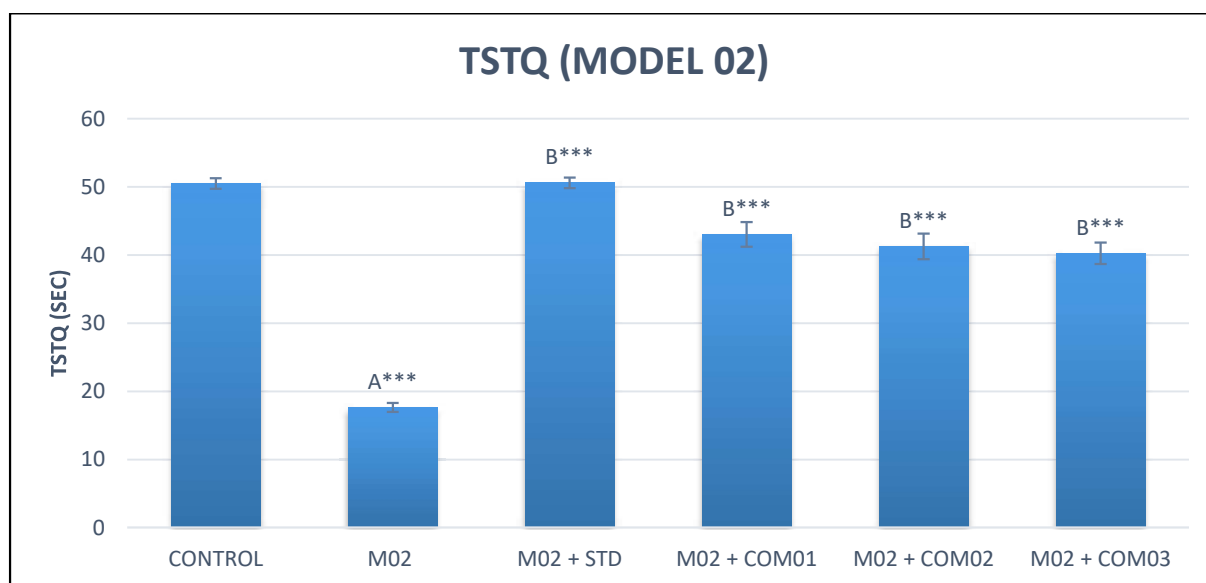


Figure 5.36: Effect of combination of test compounds and other interventions on TSTQ on day 08.

5.3.2.2 EFFECT ON TRANSFER LATENCY TIME USING ELEVATED PLUS MAZE

In this study, the effect of the combination of test drugs was observed on the transfer latency time using the elevated plus maze. The procedure was conducted on day 08 of the study. It was estimated that the mean TLT value was significantly high in the disease model 02 group when it was compared with the mean TLT value of the control group, with a significance value of $p < 0.001$. All the treatment groups, i.e., M02 + STD, M02 + COM01, M02 + COM02, and M02 + COM03, showed a significant reduction in the mean TLT value upon their respective comparison with the disease model 02 group with a significance level of $p < 0.001$. The results are depicted in figure no. 5.37, as follows:

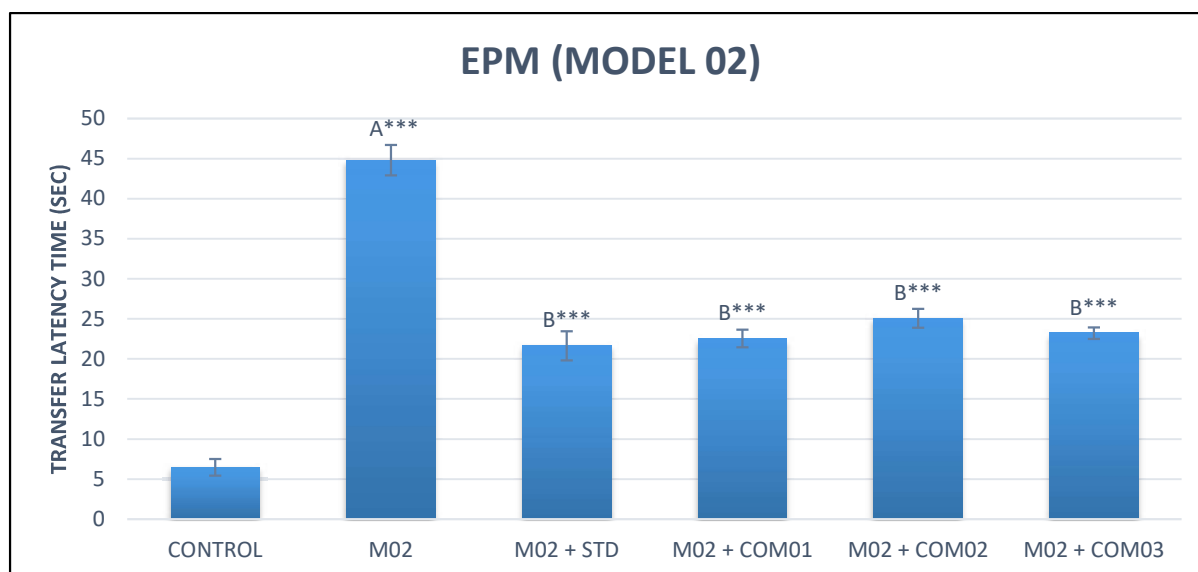


Figure 5.37: Effect of combination of test compounds and other interventions on transfer latency time using elevated plus maze.

5.3.2.3 EFFECT ON BRAIN AChE

It was estimated from the study that the brain levels of AChE significantly increased in the disease model 02 as it was compared with the value of the control group with a significance value of $p < 0.001$. Among the treatment groups, M02 + STD and M02 + COM02 groups showed significant decrease with a value of $p < 0.001$, M02 + COM01 showed significant decrease with a significance value of $p < 0.01$, and M02 + COM03 showed significant decrease with $p < 0.05$, upon their respective comparison with the disease model 02 group. The graphical presentation of the results is shown in figure no. 5.38, given below:

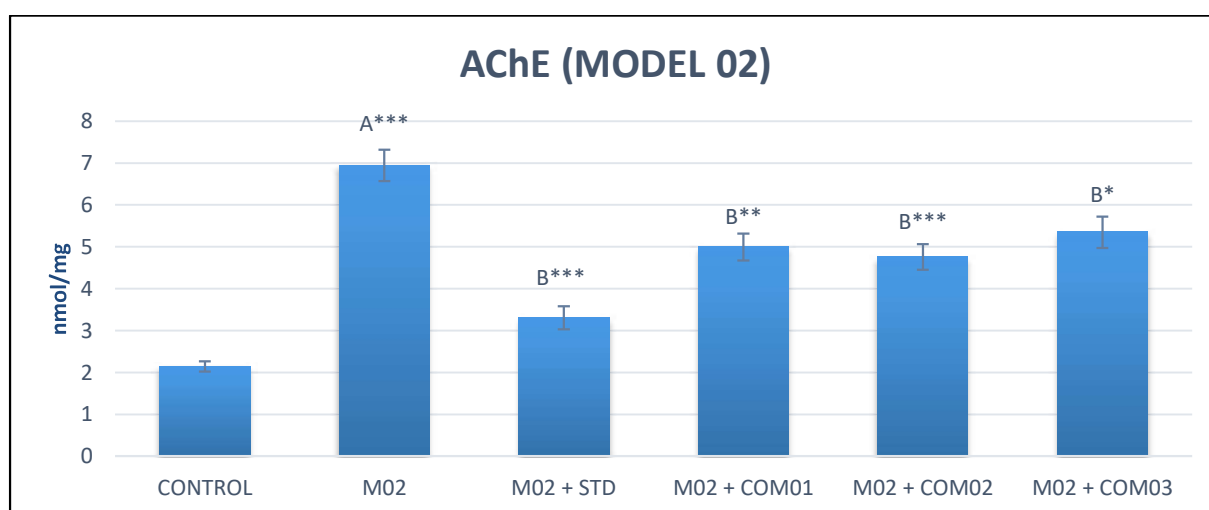


Figure 5.38: Effect of combination of test compounds and other interventions on brain AChE levels.

5.3.2.4 EFFECT ON BRAIN TNF- α

From this study it was evaluated that the brain levels of TNF- α of disease model 02 group were significantly high when it was compared with the control group with a significance value of $p < 0.001$. All the treatment groups, i.e., M02 + STD, M02 + COM01, M02 + COM02, and M02 + COM03, showed significant improvement in the brain TNF- α compared with the disease model 02 group with a significance value of $p < 0.001$. The results are depicted in figure no. 5.39, as follows:

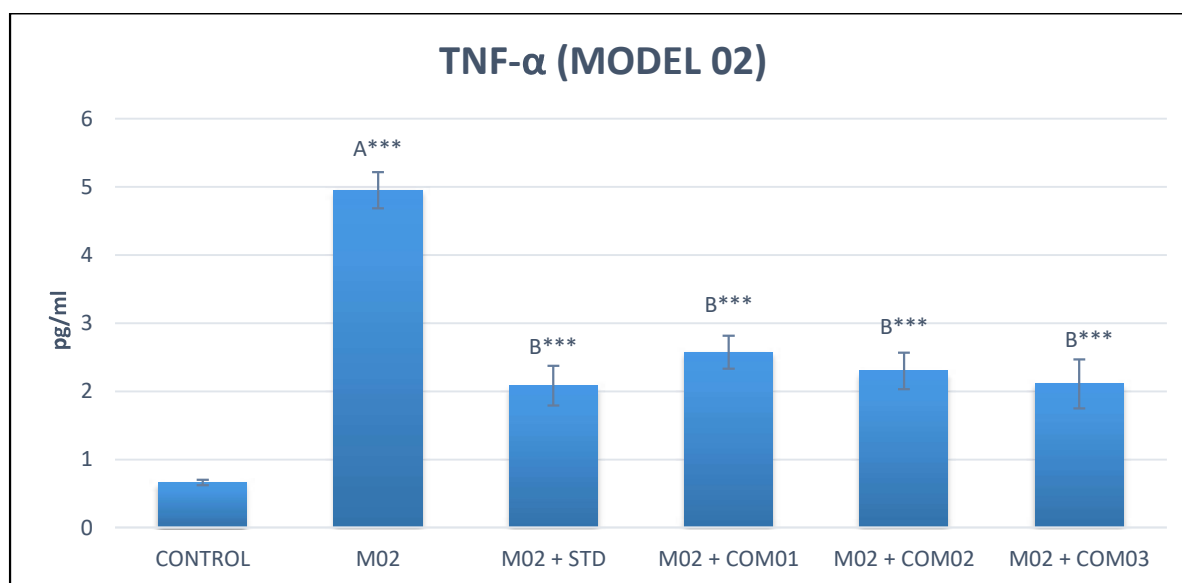


Figure 5.39: Effect of combination of test compounds and other interventions on brain TNF- α levels.

5.3.2.5 EFFECT ON BRAIN TBARS

From this study it was evaluated that the brain levels of TBARS of disease model 02 group were significantly high when it was compared with the control group with a significance value of $p < 0.001$. All the treatment groups, i.e., M02 + STD, M02 + COM01, M02 + COM02, and M02 + COM03, showed significant improvement in the brain TBARS compared with the disease model 02 group with a significance value of $p < 0.001$. The results are depicted in figure no. 5.40, as follows:

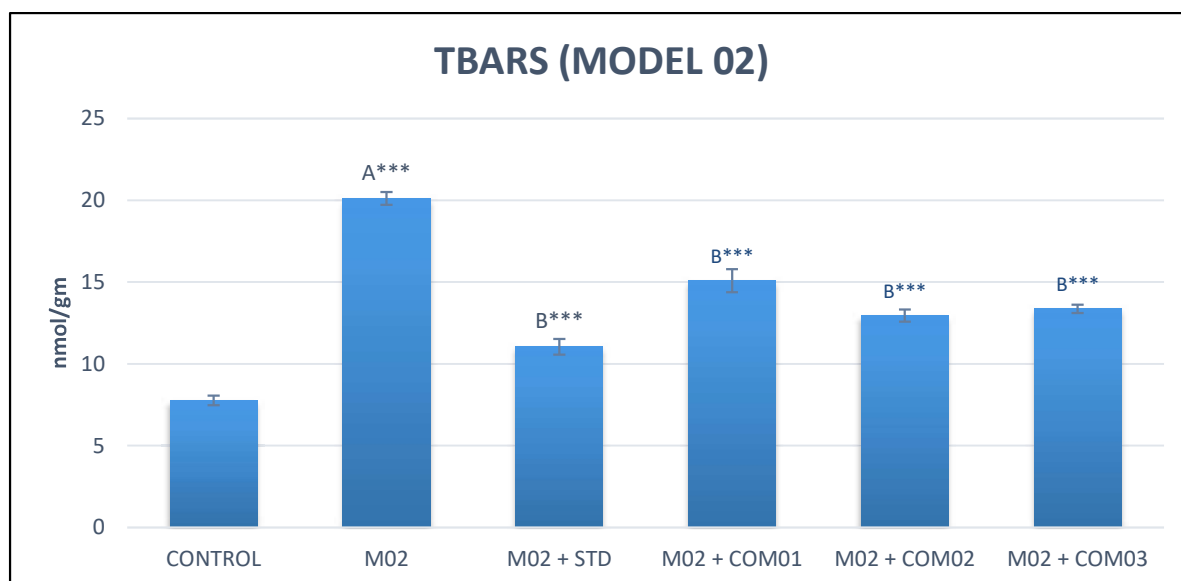


Figure 5.40: Effect of combination of test compounds and other interventions on brain TBARS levels.

5.3.2.6 EFFECT ON BRAIN GSH

From this study it was estimated that the brain levels of GSH were significantly lower in the disease model 02 group as compared to the control group with a significance value of $p < 0.001$. Among the treatment groups, all groups, i.e., M02 + STD, M02 + COM01, M02 + COM02, and M02 + COM03, showed a significant increase in the brain GSH levels upon their respective comparison with the disease model 02 group with a significance value of $p < 0.001$. The results are depicted in figure no. 5.41 as follows:

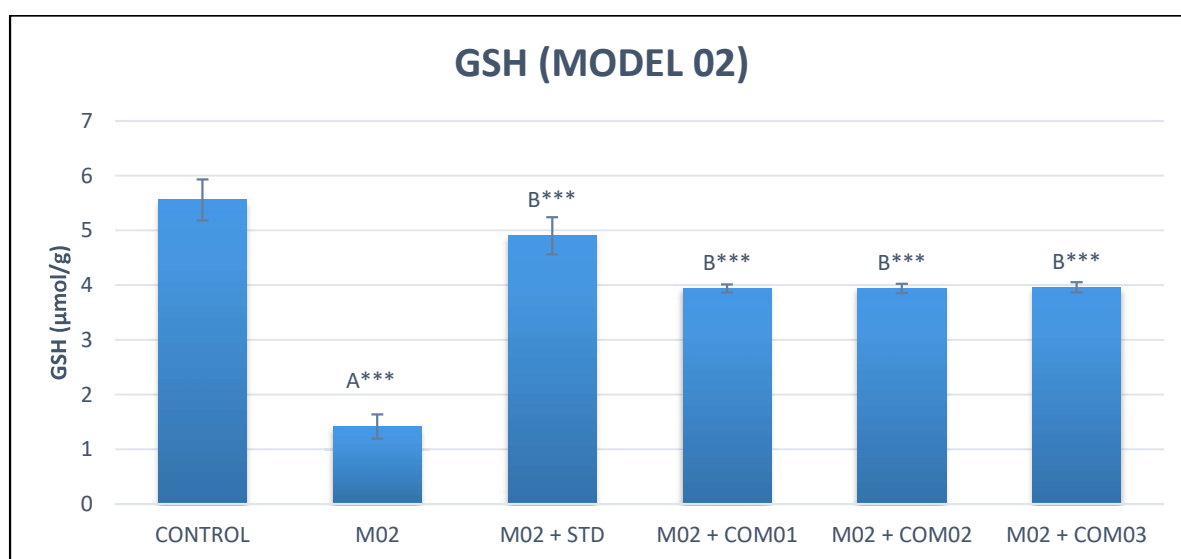


Figure 5.41: Effect of combination of test compounds and other interventions on brain GSH levels.

5.3.2.7 EFFECT ON BRAIN SUPEROXIDE DISMUTASE

From this study it was estimated that the brain levels of SOD were significantly lower in the disease model 02 group as compared to the control group with a significance value of $p < 0.001$. Among the treatment groups, all groups (i.e., M02 + STD, M02 + COM01, M02 + COM02, and M02 + COM03) showed a significant increase in the brain SOD levels upon their respective comparison with the disease model 02 group with a significance value of $p < 0.001$. The results are depicted in figure no. 5.42 as follows:

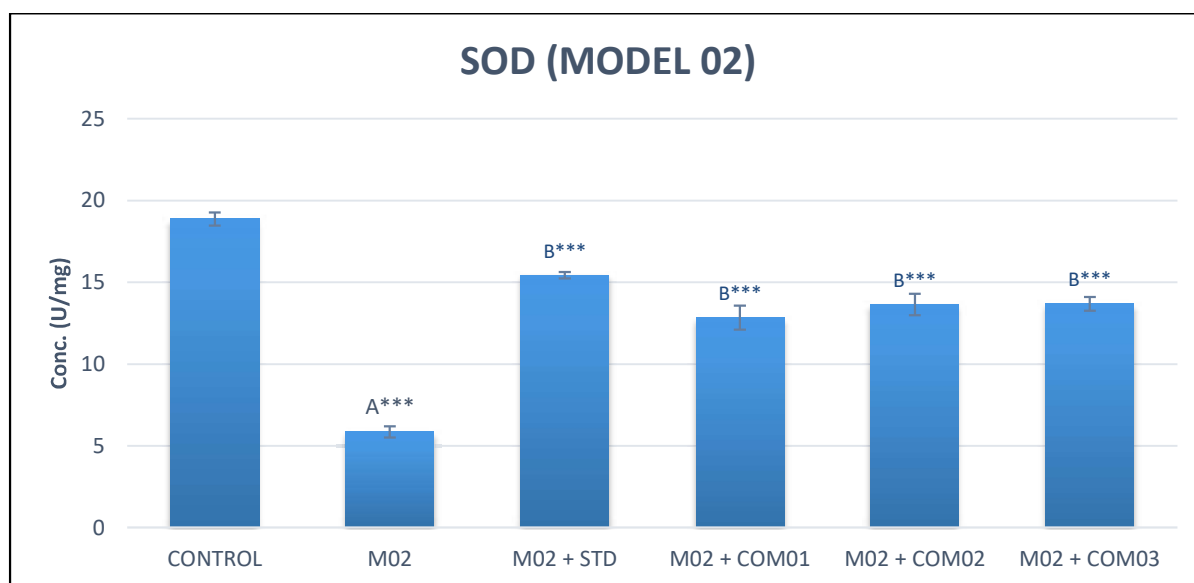


Figure 5.42: Effect of combination of test compounds and other interventions on brain SOD levels.

5.3.2.8 EFFECT ON BRAIN CATALASE

From this study it was evaluated that the levels of brain catalase significantly fall in the disease model 02 group as compared to the control group with a significance value of $p < 0.001$. Among the treatment groups, only two groups, i.e., the M02 + STD and M02 + COM03 groups, produced significant improvement in the brain catalase levels as compared to the disease model 02 group with a significance value of $p < 0.05$. All other treatment groups failed to produce any significant effect. The results are depicted in figure no. 5.43, as given below:

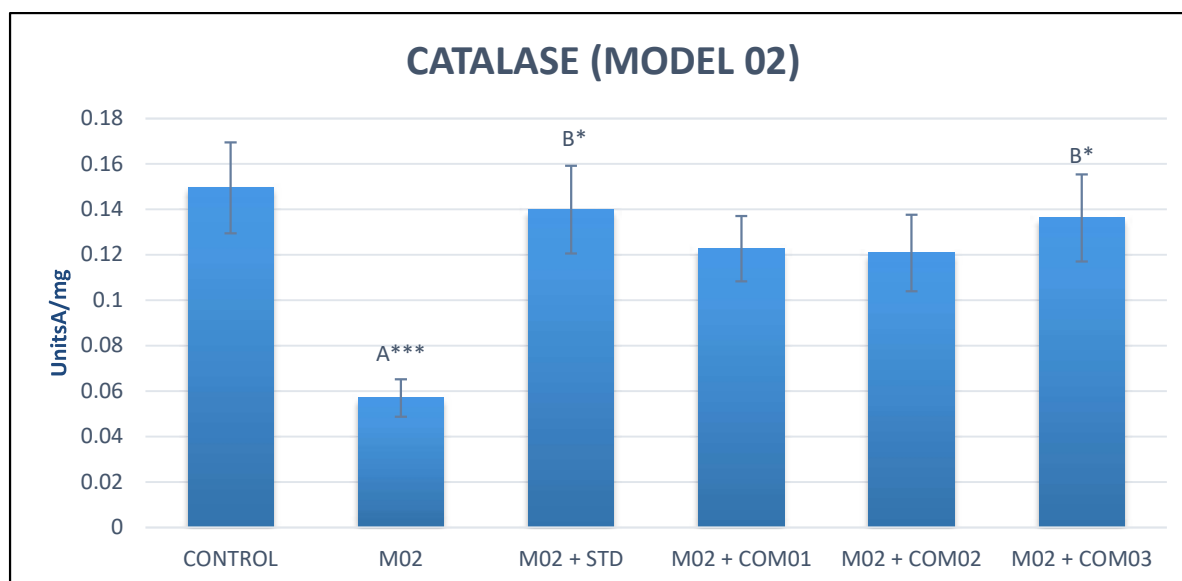


Figure 5.43: Effect of combination of test compounds and other interventions on brain CATALASE levels.

5.3.2.8 EFFECT ON BRAIN NITRITE

From this study it was evaluated that the brain levels of nitrite of disease model 02 group were significantly high when it was compared with the control group with a significance value of $p < 0.001$. All the treatment groups, i.e., M02 + STD, M02 + COM01, M02 + COM02, and M02 + COM03, showed significant improvement in the brain nitrite compared with the disease model 02 group with a significance value of $p < 0.001$. The results are depicted in figure no. 5.44, as follows:

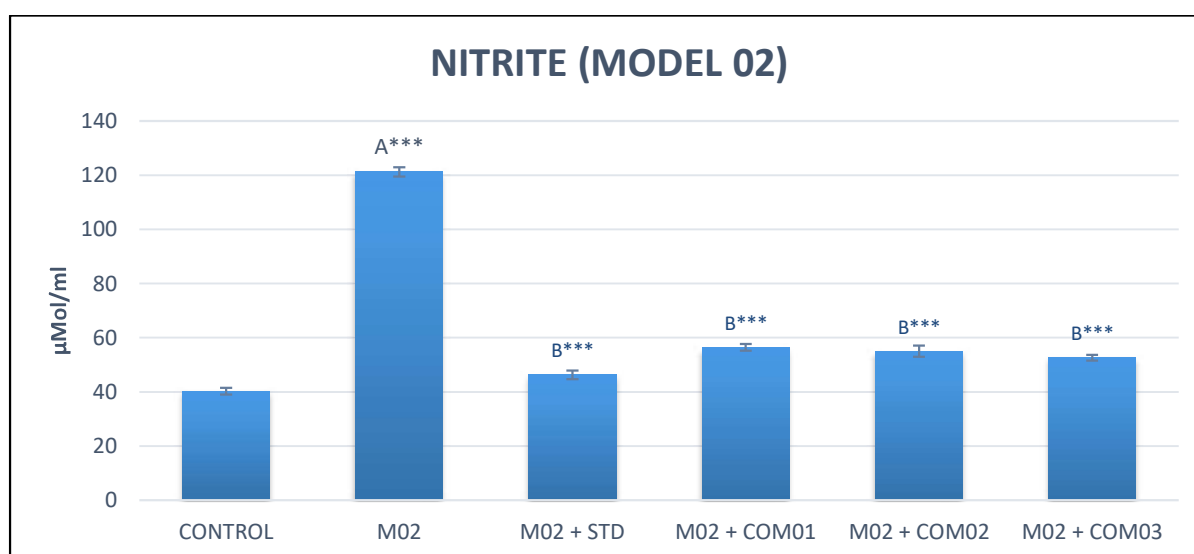


Figure 5.44: Effect of combination of test compounds and other interventions on brain NITRITE levels.

5.4 DISCUSSION:

This study investigated the effects of beta-caryophyllene, p-coumaric acid, and cinnamic acid and their combinations on oxidative stress markers (TBARS, GSH, CATALASE, SOD, and NITRITE), cholinergic markers (AChE), and inflammatory markers (TNF- α) in mice with dementia induced by AlCl₃ + D-galactose and L-methionine.

Aluminum is known to have neurotoxic effects that impede memory and cognitive function and contribute to neurodegeneration. According to research, aluminum chloride (AlCl₃) after crossing the blood brain barrier can reach to certain areas of brain like hippocampus and cerebral cortex, where it can increase the levels of inflammatory markers, can disturb the mitochondrial functionality, also can increase the formation of amyloid- β plaques by increasing the availability of NFT & Amyloid- β proteins and can also increase the levels of free radicals, by doing so it will promote the linked to neuronal degeneration (K. Kaur et al., 2022).

Another monosaccharide that has been connected to brain aging is D-galactose, which is produced when lactose is hydrolyzed in the intestine. Research has shown that D-galactose causes a decrease in brain-derived neurotrophic factors and a number of other processes that contribute to cognitive decline, such as mitochondrial dysfunction, elevated oxidative stress, inflammation, and apoptosis (Shwe et al., 2018).

From the previous studies it is understood that co-administration of AlCl₃ at a dose of 5 mg/kg p.o. and 60 mg/kg i.p. of D-galactose for a period of 90 days effectively induce the dementia in mice (Kumar et al., 2021).

The production of S-adenosyl methionine (SAM), a fundamental methyl donor for a range of biological methylation events involving RNA, proteins, carbohydrates, phospholipids, and neurotransmitters, depends on methionine, a crucial human amino acid. Furthermore, glutathione, a powerful antioxidant that guards against oxidative stress, and homocysteine, a neurotoxic intermediary in the methylation process, are both precursors of L-methionine (L-MET) (Loenen, 2010; Sharma et al., 2017; Trivedi & Deth, 2012).

Disturbances in methionine metabolism have been linked to mental and neurological disorders. Changes in L-MET and other metabolites in the one-carbon (C1) cycle have been connected to age-related neurodegenerative illnesses, including Alzheimer's disease (AD) and vascular dementia (Fayez et al., 2019; Y. Liu et al., 2022), as well as neurodevelopmental disorders like autism and schizophrenia (Indika et al., 2021).

According to research, giving L-methionine intraperitoneally twice a day for seven days at doses of 750 mg/kg at 9:00 am and 15 mg/kg at 3:00 pm can raise the brain's amyloid beta

levels, which can lead to cognitive impairments similar to those observed in Alzheimer's disease (W. Liu et al., 2019). Through glial cell activation and an increase in oxidative stress (Tapia-Rojas et al., 2015), this administration has also been connected to neuroinflammation (Grimble, 2006). These results demonstrate the fine balance needed for methionine metabolism and its consequences for brain function.

The dosage of beta-caryophyllene (100 mg/kg and 400 mg/kg; p.o.) was determined based on prior studies (da Costa Sobral et al., 2022). The oral LD₅₀ value for BCP is 2.85 g/kg, while the intraperitoneal LD₅₀ value is 5000 mg/kg of body weight (Schmitt et al., 2016). Prior research indicated adequate bioavailability in both central and peripheral systems (Mallmann et al., 2022). This study aims to evaluate BCP as a potential neuroprotective drug, specifically on its capacity to improve learning and memory deficits in chemical or genetic dementia models.

Doses of p-coumaric acid (100 mg/kg and 400 mg/kg; p.o.) were selected based on previous research (Sakamula & Thong-asa, 2018). For p-coumaric acid, the oral LD₅₀ value is 2.85 gm/kg, and the intraperitoneal LD₅₀ value is 1.16 gm/kg (Pei et al., 2016). Previous findings demonstrated satisfactory bioavailability, both centrally and peripherally. The present investigation looks to assess p-coumaric acid as a possible neuroprotective agent, particularly as it relates to its ability to ameliorate learning and memory deficits in either chemical or transgenic models of dementia.

Cinnamic acid dosage was selected on the basis of previous studies. In this study, two doses of the cinnamic acid were 100 mg/kg and 200 mg/kg p.o (Prorok et al., 2019). OECD and regulatory toxicology statistics show that the oral median lethal dosage (LD₅₀) of cinnamic acid in mice is higher than 5000 mg/kg body weight. Previous research on the pharmacology and toxicity of cinnamic acid had shown that giving it to mice by mouth at doses of 50 to 1000 mg/kg/day is safe and won't kill them (Lee et al., 2022). So, for this trial, doses were chosen that were in the sublethal range to make sure it was safe while also testing how well it worked as a treatment.

Among the behavioral estimations, Morris Water Maze was used to evaluate the escape latency time. The study was conducted for four days, in Model 01 it was conducted on 87, 88, 89, and 90th day and in Model 02 study it was conducted on day 04, 05, 06, and 07. Two trials were conducted every day, on trial was conducted in morning known as Acquisition trail and other was conducted in evening known as retrieval trial. The motive for acquisition trial was to provide the learning of the task and retrieval trial was conducted for the purpose of evaluating the memory of learned task. The test drugs successively improved the escape latency time on

all four days, also improve the values day to day. This improvement in the escape latency time denotes the improvement in the disease condition. On the 5th day of the study time spend in the target quadrant (TSTQ) was estimated, in Model 01 study TSTQ was estimated on the 91st day and in Model 02 study it was estimated on 08th day. Test drugs BCP, PCA, and CA showed the significant improvement in the TSTQ.

The second behavioral parameters used in the study was Elevated Plus maze test. The transfer latency time was estimated using the elevated plus maze. The test was conducted in two trial the first trial was considered as the learning trial and was not included in the results. In Model 01 the learning trial was conducted on the 90th day and in Model 02 it was conducted on 07th day. The second trail was conducted after 24 hrs from the leaning trial on day 91st in Model 01 study and 08th day on Model 02 study. The results of second trails were included in the study. The test compounds BCP, PCA and CA on both dose levels imparted significant improvements in the escape latency time. Hence, pointed toward the improvement in the memory and cognitive functions.

The AChE enzyme is an exemplary biochemical marker of cholinergic activities in the brain (Patocka et al., 2004). The principal metabolic role of AChE is to hydrolyze acetylcholine, a crucial neurotransmitter released into the synaptic cleft in several brain regions linked to memory and memory-related functions. Acetylcholine is systematically metabolized and decomposed to regulate memory and cognition (Roy et al., 2016). Excessive activity of AChE degrades acetylcholine, resulting in dysfunctional cognitive behavior (Walczak-Nowicka & Herbet, 2021). BCPHD, PCAHD, and CAHD not only markedly reduced brain AChE levels induced by L-methionine but also demonstrated a significant drop in AChE levels following treatment with AlCl₃ and D-galactose, corroborating the AChE induction noted in both disease-control groups. The mean levels of brain AChE in both interceptive models were dramatically reduced due to the potent AChE-inhibiting effect of donepezil (the standard medication) for each model. Consequently, our investigations indicate that beta-caryophyllene exerts an inhibitory impact on AChE, thereby restoring brain acetylcholine levels and enhancing memory and spatial learning capabilities in mice.

The significant significance of oxidative stress in the onset of dementia and Alzheimer's disease is recognized (Mao, 2013). In this diseased condition, it contributes by causing neurodegeneration, A β plaque formation, and NFT aggregation in the brain (Huang et al., 2016). Antioxidants serve as a crucial biochemical indicator for diagnosing many disease states, including SOD, catalase, GSH, TBARS, and nitrates. They each address distinct antecedents of free radical production, thereby mitigating oxidative stress. Animal groups

administered AlCl_3 + D-galactose and L-methionine exhibited reduced levels of SOD, GSH, and catalase, supporting the data related to SOD, GSH, catalase, TBARS, and nitrite in the brain (Lethem & Orrell, 1997; Rao & Balachandran, 2002). The concentrations of antioxidants and free radicals in interoceptive agent-treated mice have been markedly restored following beta-caryophyllene, cinnamic acid, and p-coumaric acid interventions against both the interoceptive models. Consequently, enhance the memory capacity in chemically induced mice.

Neuroinflammation, which refers to the excessive production of pro-inflammatory cytokines, including TNF-alpha (tumor necrosis factor-alpha) and IL-6 (interleukin-6), is implicated in the pathology of neurodegenerative diseases such as Alzheimer's disease (Pasqualetti et al., 2015). The initial sources of the production of pro-inflammatory cytokines in the CNS are microglial cells and astrocytes (D. Kaur et al., 2019). Microglia have resident and "activated" states; in the resident condition, they act as neuroinflammatory mediators; they transform into their activated state (Carson et al., 2006). Astrocytes, which provide support to the neurons and maintain the blood-brain barrier can also release cytokines to the injury or disease. In this study, interoceptive treatment was also shown to raise TNF-alpha in the brains of both disease control groups. High doses of beta-caryophyllene, cinnamic acid, and p-coumaric acid revealed a remarkable reduction in TNF-alpha levels in the brain. So, lowering inflammation in the brain and boosting cognitive troubles and memory problems.

In the combination study, three combinations were used. In the combination 01, high doses of beta-caryophyllene and p-coumaric acid were used. In the combination 02, high doses of beta-caryophyllene and cinnamic acid were put together. Lastly, in the combination 03, high doses of p-coumaric acid and cinnamic acid were used. The results of the combination groups were significantly better than the results that were obtained from the individual dose studies. All the combination groups i.e. COM01, COM02 and COM03 showed significant improvement in the escape latency time which was estimated by using the Morris water maze and transfer latency time using elevated plus maze. The effect of the combination groups on AChE was found to be significantly satisfactory; the combination groups produced a significant decrease in the brain levels of brain AChE against both the interoceptive models. The results on the antioxidant profile of all combination groups were significantly satisfactory. The combination groups also reduced the neuroinflammation; the reduction in the brain levels of TNF- α made it evident against both models of dementia.

Chapter 06
SUMMARY & CONCLUSION

6. SUMMARY & CONCLUSION

Dementia is a progressive condition characterized by a decay in cognitive functions, eventually impairing the ability to perform daily activities independently. While dementia is a broad term used for symptoms of memory and cognitive deterioration, it arises from various underlying diseases, each with distinct signs, symptoms, and neuropathological causes. The leading type of dementia is Alzheimer's disease (AD), followed by vascular dementia. Distinguishing between different types can be puzzling due to overlapping symptoms because the underlying mechanisms are unclear to distinguish. Still, finding out the exact underlying mechanism is elusive, but there are certain theories that can justify it. Among these theories is the amyloid beta theory, which suggests the accumulation of amyloid beta plaques, which disrupts the neuronal functions, triggers inflammation, and leads to cognitive deficits. There is another theory that explains the role of AChE in the development of the cognitive dysfunction. There is another theory that states that the mitochondrial dysfunction leads to deprived energy sources. Oxidative stress can also lead to cognitive dysfunction by causing an imbalance between the levels of free radicals and antioxidants, which can lead to neurodegeneration.

Dementia results from progressive brain cell death or neurodegenerative disorders. Research continues to explore potential treatments, but prevention strategies focus on lifestyle modifications, cognitive engagement, and overall brain health, more like symptomatic relief. Among the currently available drugs, donepezil is one of the most used drugs, which is an AChE inhibitor, and it tries to improve the cholinergic functions, which may lead to improvements in the disease conditions. Other drugs that inhibit AChE are rivastigmine and galantamine. Another class of drugs that is currently in use is NMDA receptor antagonists; memantine is the most used NMDA receptor antagonist.

From the previous findings, it is evident that disease model 01, which involves the administration of $AlCl_3$ and D-galactose, can induce oxidative stress, neuroinflammation, variations in the cholinesterase enzyme, and pathological mechanisms associated with Alzheimer's disease (AD). These factors contribute to increased cognitive dysfunction and memory impairment. In addition, disease model 02, characterized by high-dose administration of L-methionine, can lead to vascular dysfunction, neurodegeneration, neuroinflammation, oxidative stress, and AD-like pathological changes. In no previous study had both the interoceptive models been used in a single study. Consequently, both animal models offer a comprehensively broader perspective on the disease by targeting more pathological events and

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provide an opportunity to test the drugs beta-caryophyllene, p-coumaric acid, and cinnamic acid to their full extent.

In the odyssey to find the new therapeutic agents, we tried three phytoconstituents, i.e., beta-caryophyllene, p-coumaric acid, and cinnamic acid. Beta-caryophyllene has proven antioxidant properties. Along with its potential to activate the cannabinoid receptor type 2, which can actually help in cognitive dysfunctions. It is also reported to prevent the glutamate-induced excitotoxicity. P-coumaric acid and cinnamic acid are reported to have potent antioxidant properties as well as anticholinesterase and anti-inflammatory properties. So, it was hypothesized that these three phytoconstituents might help tackle the cognitive impairments caused by the interoceptive agents.

The findings of the study were propitious. All three phytoconstituents, either alone or in combination, showed significant results. They exhibited significant antioxidant activity by reducing the levels of TBARS and nitrites in the brain and elevating the reduced levels of GSH, SOD, and catalase. They successfully lowered the neuro-inflammation, which is evident by the significant reduction caused by these phytoconstituents in the brain TNF- α levels. Moreover, the phytoconstituents demonstrated noticeable AChE inhibitory activity. It was further supported by the results of behavioral parameters (Morris water maze test and elevated plus maze test), which were obtained during the course of the study. Furthermore, the results improved when the phytoconstituents were used in combinations. These improved results by employing the combinations might have been due to synergistic effects of these interventions because they shared some common effects, like antioxidant and anti-inflammatory effects, or might be because of broadening the course of action.

In conclusion, dementia is still not completely known and is still incurable and has a devastating impact globally. Various theories are available to explain the dementia, but still no definitive cure for dementia is available. This study gives us a future perspective for the treatment of dementia and cognitive disorders. The use of beta-caryophyllene, p-coumaric acid, and cinnamic acid not just proved its worth in treating the dementia but also uncovered the potential targets for treating the dementia. They target the imbalance between the free radical and antioxidants, which was the result of interoceptive models, and restore the balance, which resulted in improvement in the situation. They also targeted the neuro-inflammation and significantly reduced it by reducing various pro-inflammatory proteins, which was also the reason for inducing the memory and learning-associated deficits. They also significantly improved the cholinergic activities that are involved in memory and learning; they did so by reducing the effect of AChE in the brain. Furthermore, the study explored the synergistic aspect

SUMMARY & CONCLUSION

by using the combinations of these phytoconstituents. The results of the combinations of phytoconstituents were found to be significant and hence pointed towards the use of combinational drug therapies for the management of the dementia by using the interventions targeting different pathological factors, hence broadening the spectrum of action.

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APPENDICES

Appendix 01
(Letter of Candidacy)



Transforming Education Transforming India

**School of
Research Degree Programmes**

LPU/SRDP/EC/170913/069

Dated: 13th Sep 2017

Harsimran Singh
Registration Number: 41500120
Program Name: Ph.D. (Pharmacology) [Part Time]

Subject: Letter of Candidacy for Ph.D.

Dear Candidate,

We are very pleased to inform you that the Department Doctoral Board has approved your candidacy for the Ph.D. Programme on 9th March 2017 by accepting your research proposal entitled: "NEUROPROTECTIVE EFFECTS OF β -CARYOPHYLLENE, CINNAMIC ACID AND P-COUMARIC ACID AGAINST CHEMICAL-INDUCED DEMENTIA IN MICE" under the supervision of Dr. Navneet Khurana.

As a Ph.D. candidate you are required to abide by the conditions, rules and regulations laid down for Ph.D. Programme of the University, and amendments, if any, made from time to time.

We wish you the very best!!

In case you have any query related to your program, please contact School of Research Degree Programme.


HOS



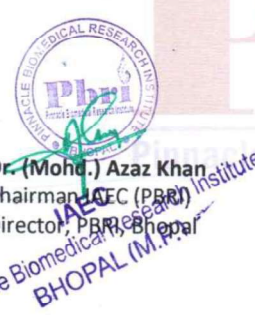
School of Research Degree Programme

Jalandhar-Delhi G.T.Road, Phagwara, Punjab (India) - 144411









Ph : +91-1824-444594 Fax : +91-1824-240830 E-mail : drp@lpu.co.in website : www.lpu.in

Appendix 02

IAEC Approval letter


<p>Ref. <u>PBRI/PHD/2024145</u> Date <u>31/05/24</u></p> <p style="text-align: center;">CERTIFICATE</p> <p>This is to certify that the project proposal no. PBRI/IAEC/PN-22113 entitled Neuroprotective Effects of β-caryophyllene, Cinnamic Acid, and <i>p</i>-Coumaric Acid Against Chemical-Induced Dementia in Mice, submitted by Mr. Harsimran Singh, has been approved/recommended by the IAEC Pinnacle Biomedical Research Institute, Bhopal (Organization), in its meeting held on 09 Dec. 2022 (Date), and 138 (Number and Species of animals) have been sanctioned under this proposal for a duration of the next 12 months.</p> <div style="text-align: center;">  </div> <div style="text-align: center;">  <p>Dr. (Mohd.) Azaz Khan Chairman, IAC (PBRI) Director, PBRI, Bhopal</p> <p style="color: blue; font-weight: bold;">Pinnacle Biomedical Research Institute BHOVAL (M.P.)</p> </div>
<p>PINNACLE BIOMEDICAL RESEARCH INSTITUTE (PBRI)</p> <p>Registered office :- Bharat Scout and Guide Campus, New Smart City Road, Shanti Marg, Shyamla Hills, Bhopal (M.P.) – 462003 Phone – 0755-4325540 +91 94258-90029 E-mail – info@pbri.in Web : www.pbri.in</p>

Appendix- 03
(Letter of Conduct of Research Work)

   	
<div style="display: flex; justify-content: space-around; background-color: #f0f0f0; padding: 5px;"> We Analyze We Innovate We Explore We Establish </div>	
Ref..P.B.R.I./PHD/2024/144	Date 31-05-24
<p><u>TO WHOMSOEVER IT MAY CONCERN</u></p> <p>It is verified that Mr. Harsimran Singh, Ph.D. research scholar with Lovely Professional University, Phagwara, Punjab, has conducted his work with Pinnacle Biomedical Research Institute, Bhopal. The IAEC protocol no. of his research work is PBRI/IAEC/PN-22113. He has physically conducted his research work (Animal Work) from our facility at Bhopal. He has conducted his study under the supervision of Dr. Megha Jha. The duration of his study was from October 2023 to May 2024.</p> <div style="text-align: center; margin-top: 20px;">  </div> <div style="display: flex; align-items: center; margin-top: 10px;">  <div> <p>Dr. M. A. Khan Director, PBRI, Bhopal</p>  </div> </div>	
<p>PINNACLE BIOMEDICAL RESEARCH INSTITUTE(PBRI)</p> <p>Registered office :- Bharat Scout and Guide Campus, New Smart City Road, Shanti Marg, Shyamla Hills, Bhopal (M.P.) – 462003 Phone– 0755-4325540 +91 94258-90029 E-mail – info@pbri.in Web. : www.pbri.in</p>	

Appendix- 04

(Publication)

- Singh, H., Khurana, N., Bafna, P. A., Mazumder, R., & Sharma, N. (2025). Neuroprotective effects of p-coumaric acid against chemical-induced dementia in mice. *Pharmacological Research - Modern Chinese Medicine*, 16(May), 100656. <https://doi.org/10.1016/j.prmcm.2025.100656>

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Neuroprotective effects of p-coumaric acid against chemical-induced dementia in mice

Harsimran Singh ^a, Navneet Khurana ^{a,*}, Pallavi Anand Bafna ^a, Rupa Mazumder ^b, Neha Sharma ^a

^a School of Pharmaceutical Sciences, Lovely Professional University, Phagwara, 144411, Punjab, India
^b Noida Institute of Engineering and Technology (Pharmacy Institute), Greater Noida, 201306, Uttar Pradesh, India

ARTICLE INFO

Keywords:
p-coumaric acid
Dementia
Neuroprotection
Oxidative stress
Cognitive impairment

ABSTRACT

Introduction: Dementia, a progressive and debilitating neurological disorder, affects millions of people worldwide and remains a significant global health challenge. Traditional plant-based medicines, especially those used in Chinese herbal practices, have shown promise in managing memory-related disorders. One such bioactive compound is p-coumaric acid, a naturally occurring phytoconstituent present in several traditional Chinese medicinal plants, including *Hedyotis diffusa*, *Fagopyrum cymosum*, and *Viola yedoensis*. Known for its antioxidant, anti-inflammatory, and neuroprotective properties, p-coumaric acid was investigated in this study for its potential to mitigate chemically induced dementia in mice.

Methods: A total of 54 Swiss albino mice weighing 25–35 g (either sex) were used in the present study, which were distributed into 9 groups. Two inter-receptive dementia models were utilized. In model 1, mice received aluminum chloride (5 mg/kg, orally) and D-galactose (60 mg/kg, intraperitoneally) for 90 days. In model 2, dementia was induced by administering L-methionine (750 mg/kg at 09:00 and 15 mg/kg, i.p. at 15:00) over seven days. Treatment groups received either p-coumaric acid at low (100 mg/kg, orally) or high (400 mg/kg, orally) doses, or the standard drug donepezil HCl (2 mg/kg, i.p.). Behavioral performance was assessed using the Morris water maze and elevated plus maze. At the end of the treatment period, brains were harvested for biochemical analyses including AChE, TBARS, SOD, catalase, GSH, nitrite, and TNF- α levels.

Results: p-Coumaric acid significantly improved spatial memory and cognitive function, as evidenced by reduced escape and transfer latency times and increased time spent in the target quadrant. It also restored antioxidant enzyme levels and reduced markers of oxidative stress and inflammation in both models.

Conclusion: Overall, the findings suggest that p-coumaric acid enhances cholinergic transmission, mitigates oxidative damage, and reduces neuroinflammation, indicating its potential as a therapeutic agent for dementia management.

1. Introduction

Worldwide, according to WHO, there were 57 million people who had dementia in 2021, and there are around 10 million new cases every year. As a result, the number is doubling every 20 years, with estimates exceeding 80 million by the year 2040 [1]. The condition is a result of progressive brain cell death or other neurodegenerative disorders. The main risk factor is age, with around 2 % of people aged 65–69 years having dementia, climbing to 20 % among people 85 years or older. The syndrome is so bad it primarily strikes the brain's most critical areas related to memory, linguistic and visual-spatial skills, attention, and

decision-making functioning. Dementia tends to have a gradual course, and by the time someone with dementia has impaired memory capacity, their brains have undergone substantial alterations over an extended period of time. It can cause a range of psychological and behavioral problems, including depression, agitation, tremors, speech and language problems, delusions, and restlessness. With dementia, depression is said to impact 20–30 % of people.

Experimental models: In the present study, AlCl₃ (5 mg per kg, oral) + D-galactose (60 mg per kg, intraperitoneal) were given for 90 days [2], and high doses of L-methionine (750 mg per kg and 15 mg per kg intraperitoneally) twice a day at different times were given [3], have

^{*} Corresponding author.
E-mail address: navneet.18252@lpu.co.in (N. Khurana).

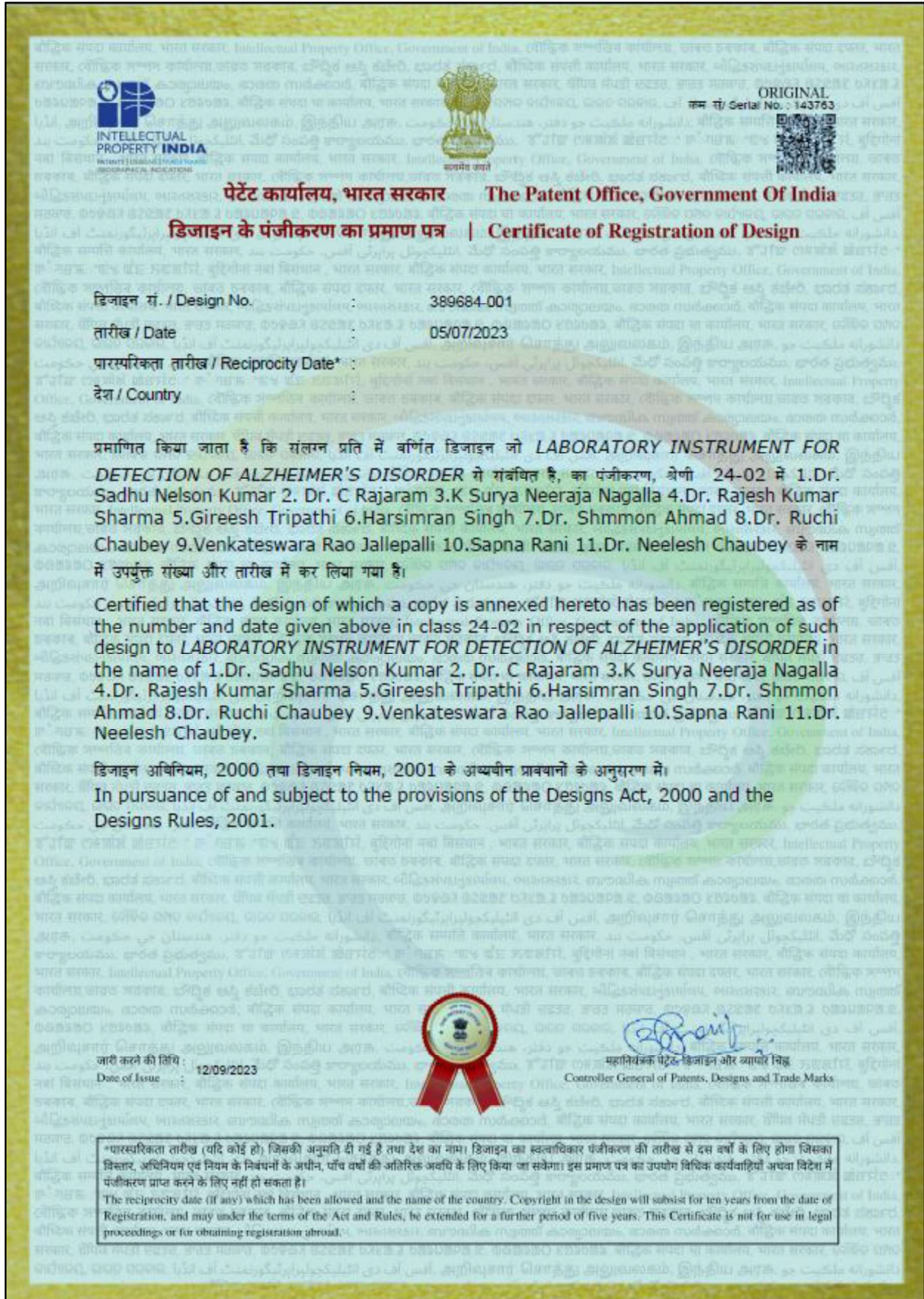
<https://doi.org/10.1016/j.prmcm.2025.100656>
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2667-1425/© 2025 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Publications in Communications:

1. Neuroprotective effect of beta caryophyllene against multiple chemical models of dementia in mice. (Research Paper)
2. Effect of beta-caryophyllene, p-coumaric acid, and cinnamic acid on memory deficits and brain oxidative stress. (Review Paper)

**Appendix 04
(Patent)**

1. Laboratory Instrument for detection of Alzheimer’s disorder.



Appendix 0 (Oral Presentations)

1. Certificate of oral presentation at national conference on the topic of “Current Trends on Pharmacoeconomics and Advanced Drug Delivery System”, held at Minerva College of Pharmacy, Indora (H.P.) on September 16, 2023.



2. Certificate of oral presentation at International Conference on the topic of “Harmonizing Informatics Tools with Pharmaceutical Research for Accelerated Translation in Healthcare: Academia and Industrial Perspective” held at Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, on February 14-16, 2025.



3. Certificate of oral presentation at National Conference on the topic of “Pharmaceutical Education and AI Coalescence Revolutionize a New Era” held at Guru Ram Dass College of Pharmacy, Malout, on April 19, 2025.

