Studies on nootropic effects of various extracts of *Gmelina arborea* and *Cayratia trifolia*

A Thesis Submitted to Gujarat Technological University

For the Award of

Doctor of Philosophy

in

Pharmacy

by

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Enrollment No. 119997290021

Under Supervision of

Dr. Sunil B. Bothara

GUJARAT TECHNOLOGICAL UNIVERSITY
AHMEDABAD

April 2019

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Abstract

Background and Objectives

Oxidative stress is intensely linked with neurodegenerative disorders, especially Alzheimer's disease (AD). Searching for medicinal plant with the nootropic activity for controling the development and progression of AD has received extensive consideration. The extensive literary survey was conducted and the plants *Gmelina arborea* and *Cayratia trifolia* were selected for the investigations. Previous studies have shown the antioxidant, analgesic, anti-inflammatory, etc. activities of these plants. Therefore, the objective of this study was to examine the nootropic effect of *Gmelina arborea* and *Cayratia trifolia* by using various animal models.

Materials and Methods

The in vivo models used for the study of nootropic activity of extract of *Gmelina arborea* and *Cayratia trifolia* were step down passive avoidance, conditioned avoidance response, sodium nitrite induced amnesia, and elevated plus maze while in vitro models includes antioxidant activity by DPPH & NO and estimation of brain reduced glutathione (GSH), estimation of acetyl cholinesterase enzyme activity in mice brain, estimation of brain total protein and estimation of catalase activity. In preliminary study different extracts like aqueous extract, methanolic extract, hydroalcoholic extract, petroleum ether extract and chloroform extracts were prepared. Based on effect on step down latency (SDL) using passive avoidance paradigm; we found that Aqueous Extract of *Cayratia trifolia* (AECT), Hydro-alcoholic Extract of *Cayratia trifolia* (HAECT), Chloroform Extract of *Gmelina arborea* (CEGA) and Hydro-alcoholic Extract of *Gmelina arborea* (HAEGA) has significant activity and further experiments are carried out by using these extracts. The AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) was suspended in distilled water and administered orally to mice for 15 days. Piracetam (140 mg/kg) was administered orally as a standard drug for 15 days. Control animals receive equivalent volume of distilled water.

Results

Effect on step down latency (SDL) using passive avoidance paradigm was measured during learning & memory trial to examine the memory formation based on negative reinforcement. SDL was defined as the time taken by the animal to step down from the

wooden platform to grid floor with all its paws on the grid floor. We found that AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) has significant activity. Animals injected with scopolamine and sodium nitrate were evaluated using step down passive avoidance test in mice for the development of amnesia. Scopolamine and sodium nitrate was administered prior the training session. Scopolamine and sodium nitrate control group significantly decreased the SDL in learning and memory trials. On treatment with AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) at all the doses significantly reversed scopolamine and sodium nitrate induced spatial memory impairment as compared to negative control group. Conditioned avoidance response behavior mainly affects cognitive behavior by mesocortical pathway of dopaminergic neurons. In our study, AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) significantly delayed time taken by the mice to climb the pole. In the Plus-maze test, TL might be shortened if the animal had previous experience of entering the open arm and the shortened TL could be related to memory. In our study we found that mice treated with AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), HAEGA (500 mg/kg) and standard drug piracetam (140 mg/kg) significantly reduced TL.

The brain AChE activity and total protein contents were estimated in this study. Pre-treatment with AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) at all doses significantly reduced Acetylcholinesterase (AChE) and reduction in the total protein levels as compared to control. In the current study, AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) treatment significantly significantly elevate the SOD, CAT activity and GSH levels. This effect of may be attributed to its antioxidant potential. Moreover, the antioxidant activity of AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) were comparable with standard vitamin C by using DPPH and nitric oxide methods.

Interpretation and Conclusion

Our study demonstrates the cognitive enhancing and/or anti-amnesic property of the plant extracts in the presence and/or the absence of amnestic agent suggests the nootropic activity.

Keywords

Nootopic; *Gmelina arborea* and *Cayratia trifolia*

Acknowledgement

Firstly, I would like to express my sincere gratitude to my guide ***Prof. Dr. Sunil B. Bothara*** *a brilliant academician for the continuous support of my Ph.D study and related research, for his punctuality, thought provoking discussions, patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D study. It's he who brought the best of my skills to practical use.*

Besides my supervisor, I would like to thank my Doctoral Progress committee members: ***Prof. Dr. Anita Mehta*** *and* ***Dr. Shitalkumar Zambad*** *for their insightful comments and encouragement, but also for the hard question which incented me to widen my research from various perspectives.*

Though the following dissertation is an individual work, I could never have reached the heights or explored the depths without the help, support, guidance and efforts of a lot of people. Thus my sincere gratitude goes to my parents, all my friends and my companions for their love, support, and patience over the last few years.

At this moment of completion of my work, the perpetual essence of life is seemingly at a turning point, I prefer my humble prostrations to the almighty who inculcated in me enough strength, courage and will power to discharge my duties immaculately and with whose showers of blessing this task was ventured.

It is impossible for me to complete this task without the firm support and inspiration from my strength my father, ***Mr. Natubhai Madia*** *he took all pains and always provided the greatest unsaid support to me. I owe all my success to him.*

I feel a deep sense of gratitude towards ***Dr. H. M. Tank*** *(Principal) of Atmiya Institute of Pharmacy, who always supported my work and gave me patient advice for the completion of my PhD and last but not least for providing all the necessary facilities required during the course of this work.*

Now I would like to mention my wife ***Monali****, without her support, none of my work could have been realized. Her undaunted faith, great encouragement and support help me to accomplish the task. I am really privileged to have wife with such an incredible patience, fortitude and service. I appreciate my son* ***Hitarth*** *for abiding my ignorance and the patience he showed during my thesis writing. Whose smiling face always made me happy and inspired me. Words would never say how grateful I am to both of you.*

I consider myself the luckiest in the world to have such a lovely and caring family, standing beside me with their love and unconditional support.

It will be unfair if, I don't thank my elder sister ***Arti,*** *her love and affection provide me the courage to fight with all situations.*

'Thanks' is a small word to ***Mr. Manharlal Madia (Mota Papa)****, Uncles* ***(Rameshbhai Madia, Gunvantrai Madia)*** *and Aunties* ***(Late. Chandrikaben, Susilaben and Jayshreeben),*** *cousins* ***(Bhaveshbhai, Manish, Nikunj, Janvi, Nidhi, Helly, Dhara)****, my sweet niece* ***(Maitri, Piya and Diya)****, and all my family members who not only supported me but also inspired me during the course of my study.*

Loving thanks to my dearest friends ***Dr. Ashok Agrawal, Pinakin Jadav, Kishan Madhani, Dr. Om Shukla, Dr. Dharmesh Golwala, Dr. Parag Rabara*** *and all my friends and well wishers for their moral support and encouragement during my study.*

It is very hard task to oblige to all the souls (Rats and Mice) who took pains and sacrifice themselves for my studies.

I am grateful to Lab assistant ***Rajivbhai, peon Kaushikbhai, Hiteshbhai & Jentibhai,*** *Librarian, Store Keeper and all Non-teaching staff for providing all facilities required for the successful completion of my research work.*

At last I also acknowledge to all those wonderful people without whose direct or indirect help, this work could not have been completed and oblige to them whom I hurt intentionally or unintentionally during my work.

I finish with a final silence of gratitude for my life.

My Sincere thanks to all.

April 2019 Madiya Darshan Natvarlal

Table of Content

List of Abbreviations

5-HT	Serotonin
AAMI	Age Associated Memory Impairment
ACh	Acetylcholine
AChE	Acetylcholinesterase
AD	Alzheimer's Disease
AECT	Aqueous Extract of Cayratia trifolia
AEGA	Aqueous Extract of Gmelina arborea
AMPA	(α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	One Way Analysis Of Variance
APP	Amyloid Precursor Protein
AT	Acquisition Trial
BDNF	Brain-derived neurotrophic factor
B.W.	Body Weight
CAR	Conditioned avoidance response
CECT	Chloroform Extract of Cayratia trifolia
CEGA	Chloroform Extract of Gmelina arborea
CNS	Central Nervous System
CPCSEA	Committee for the purpose of control and supervision on experiments on animals
DA	Dopamine
DNA	Deoxyribonucleic acid
DPPH	1,1-Diphenyl-2-Picryl Hydraryl
DTNB	5,5'-Dithiobis-2-nitrobenzoicacid
EEG	Electro encephalogram
FTD	Fornto-temporal dementia
FDA	Food And Drug Administration
FTD	Fornto-Temporal Dementia
GABA	Gamma-Aminobutyric acid
GSH	Glutathione
HAECT	Hydro-alcoholic Extract of Cayratia trifolia
HAEGA	Hydro-alcoholic Extract of Gmelina arborea

HD	Huntington's Disease
H_2O_2	Hydrogen peroxide
IAEC	Animal Ethical Committee
i.p.	Intra peritoneal
LBD	Lewy Body Disease
LTM	Long-Term Memory
LTP	Long Term Potentiation
MECT	Methanolic Extract of Cayratia trifolia
MEGA	Methanolic Extract of Gmelina arborea
MRI	Magnetic Resonance Imaging
$NaNO_2$	Sodium nitrite
NFT	Neurofibillary Tangles
NMDA	N-Methyl-D-Aspartate
NSAIDs	Nonsteroidal anti-inflammatory drugs
PEECT	Petroleum Ether Extract of of Cayratia trifolia
PEEGA	Petroleum Ether Extract of of Gmelina arborea
p.o.	Per oral
PS	Presenillin
RNA	Ribonucleic acid
RONS	Reactive Oxygen And Nitrogen Species
RT	Retention Trial
s.c.	Sub cutaneous
SDL	Step Down Latency
SFZ	Shock Free Zone
STM	Short-Term Memory
TNF	Tissue necrosis factor
TL	Transfer Latency
VaD	Vascular Dementia
WBC	White blood cells
βA	Beta Amyloid
DTNB	Dithiobisnitrobenzoic acid
SEM	Standard Error of Mean

List of Symbols

%	Percentage
µ	Micro
µl	Microliter
C	Celsius
g	Gram
h	Hour
IU	International Unit
Kg	Kilogram
mg	Milligram
min	Minute
ml	Milliliter
º	Degree
α	Alpha
β	Beta

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Chapter 1

INTRODUCTION

CHAPTER 1

Introduction

Cognitive dysfunction, amnesia, loss of memory are a serious global health problem. Dementia is a general term for a decline in mental ability severe enough to interfere with daily life (www.alz.org). Alzheimer's disease (AD) the most prevalent form of degenerative dementia (accounting for 50% - 80% of dementias) has been characterized by the progressive impairment of cognitive function and changes in behaviour and social adaptability (Katzman, 1986; Francis et al., 1999). Primary cause of AD and other cognitive dysfunctions remains a mystery which is not yet solved completely. Global investigation for understanding the fundamental pathological mechanisms of such disorders is still going on (Palit *et al.,* 2015). Ageing play an important role in development of cognitive dysfunction such as age associated memory impairment (AAMI) (Narwal *et al.,* 2012). The total percentage of aging population is increasing due to recent therapeutic advances that have prolonged the lifespan of human being.

Learning is the most characteristic attributes of the human and definite as the skill to alter behaviour on the basis of experience (Jain, 2007). Memory is special facility of brain which retains the events developed during the process of learning and both are mediated by nervous system (Chatterjee, 1997). Once memories have been stored in the brain, it becomes the part of brain process mechanism when it will recall in future (Guyton and Hall, 2006). Learning and memory are closely linked, all learning involves memory but all memory not involves to learning (Bijlani, 2004). Sensory information received through the eyes, ears, and other senses is registered very briefly in short-term or iconic memory (Greek; Iconic = Image). Some residue of information from the iconic memory persists for a few second in short term memory (Windhorst, 1996). Memory has been classified as i) Short term memory: includes memories that last for seconds or most minutes unless they are transformed into longer term memories. ii) Intermediate long term memories: which last for days to weeks but then fade away. iii) Long term memory: which once stored, can

be recalled up to years or even a life time later. iv) Working memory: includes mainly short term memory that is used during the course of intellectual reasoning but is terminated as each stage of the problem is resolved (Guyton and Hall, 2006).

Dementia is generally defined as the loss of logical abilities (medically called cognitive dysfunction) of sufficient capacity to interfere with social or occupational functioning (Shivakumar *et al.,* 2011). It can result from various diseases that cause damage to brain cells. There are many different types of dementia, each with its own cause and symptoms.

Alzheimer's disease is a most common type of dementia; accounts for an estimated 50 to 80 percent of cases. In affected persons progressive and permanent decline in memory and cognitive abilities beyond what might be expected from normal aging. Early symptoms showed difficulty in remembering names and recent events and apathy (episodic memory). During disease progression include impaired opinion, disorientation in time, place, and in person, executive functions, attention, problem solving capability, language and spatial orientation all begin to deteriorate (Newman *et al.,* 2007). Alzheimer's disease is a neurodegenerative disorder, which according to World Health Organization (WHO) affects 22 million people worldwide, out of which; over 3 millions are in India. Its prevalence rises stridently from about 5% at the age of 95 years. Though there are drugs available that aims at slow chain of disease, an affirmative cure to Alzheimer's disease still elude researchers (Gindi, 2011).

Alzheimer disease has a profound effect on both the patient and family, so appropriate treatment is needed. Nonpharmacological interventions are the current primary interventions for management of AD, and medications should be used in the context of multimodal interventions. (Dipiro *et al.,* 2017).

Current scenario of pharmacological treatment of dementia or AD is not satisfactory, as there is no cure for AD with existing medication (Francis *et al.,* 1999; Figueiro *et al.,* 2011). However Donepezil, Galantamine, Rivastigmine and Tacrine are drug of choice for treatment AD or dementia by U.S. Food and Drug Administration (FDA) to treat its symptoms. These drugs are commonly referred as "Cholinesterase Inhibitors". These drugs prevent the breakdown of neurotransmitter acetylcholine in the brain important for learning and memory. The fifth drug Memantine regulates the activity of a different

chemical messenger in the brain that is also important for learning and memory (Narwal *et al.,* 2004).

The theory and definition of a "nootropic drug" was first proposed in 1972 by C.E. Guirgea, the principal Piracetam researcher for UCB Pharmaceutical Company of Belgium that launched Piracetam. He coined the term "nootropic" from the italic words "noos" (mind) and "tropein" (to turn toward), to mean enhancement of learning and memory.

Widespread study of the modes of action of the nootropics has exposed various pharmacological effects. There may be no single major mode of action that is shared by the whole drug class. All these drugs however influence cholinergic function. By increasing high affinity choline uptake these drugs facilitate acetylcholine production and turnover with varying actions at both muscarinic and nicotinic receptors (Silverman, 2015).

Herbal medicines are currently in demand and their popularity is increasing day by day. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine (Verma and Singh, 2008).

Current pharmacological drugs are restricted towards the severe and later stage of dementia therapy. Therefore, the proper development of nootropic candidate with multiple drug targets for treatment of dementia, cognitive dysfunction and related neurodegeneration would be highly desirable (Palit *et al.,* 2015).

Gmelina arborea Roxb.

Gmelina arborea Roxb. belongs to the family Verbinaceae. Commonly known as Gamhar/shewan/Gambhari. It is found in part of India, Western Ghats, and from foot of North-West Himalaya to Chittagong and throughout Deccan Peninsula. (http://envis.frlht.org/indian-medicinal-plants-database.php).

It is one of the essential ingredients of the Dasamoola herbal formulation with a yearly demand of 1000 metric tons (Ved and Goraya, 2008).

The chemical constituents of plant consist of sesquiterpene, cerylalcohol, hentriacontanol-1, β-sitosterol, n-octacosanol, gmelinol, apiosylskimmin-a, apiofuranosyl-(1-6)-β-D-

glucopyranosyl, (1.0.7)-umbelliferone, stigmasterol, stigmastanol, campesterol, α-2-sitosterol, butulinol, luteolin, apigenin, quercetin, hentriacontanol, quercetogenin and other flavons (Tiwari *et al*., 2008).

The plant has various pharmacological activities like antioxidant activity, anthelmintc activity, anti microbial activity, diuretic activity, cardioprotective activity, anti-diabetic activity, immuno-modulatory activity, antipyretic and analgesic activity (Pathala *et al.,* 2015).

Thus, in the light of above, the goal of this investigation was to study the nootropic effect of plant extracts of *Gmelina arborea Roxb*. and *Cayratia trifolia Linn*.

Cayratia trifolia Linn.

Cayratia trifolia Linn. is a native of India, Asia and Australia. *Cayratia trifolia* is a perennial climber, woody at base, stem is more or less succulent, compressed and densely. Common name fox grape / bushgrape (in English), Amal-bel, Ramchana in Hindi and Amlavetash in Sanskrit belongs to family of Vitaceae (Jain *et al. 2005;* Singh *et al, 1998*). In India it is found in Jammu, Rajasthan, Assam, Tripura and West Bengal extending into peninusular India up to 600 m. (The Wealth of India, 1992).

The plant have trifoliated leaves with (2-3 cm) long petioles and ovate to oblong-ovate leaflets. Fruits are fleshy, juicy, spherical and about 1 cm in diameter of dark purple or black color. (Singh *et al.,* 2012).

The stem, leaves and roots of this plant contain hydrocyanic acid. Its leaves contain delphinidin, cyaniding and yellow waxy oil and sterols (The Wealth of India, 1992)

Leaves contain stilbenes such as piceid, resveratrol, viniferin and ampelopsin. Stem, leaves and roots are reported to possess hydrocyanic acid and delphinidin. (Kumar *et al.,* 2011).

The bark extract has been reported antibacterial, anticancer activity, anti-protozoal, antidiabetic activity and diuretic activities in animal models (Gupta *et al., 2010*).

Objective of Study

1. To prepare the extracts of *Gmelina arborea* and *Cayratia trifolia*.
2. To evaluate the nootropic activity of *Gmelina arborea* and *Cayratia trifolia* extracts by using various in-vivo and in-vitro animal models.
3. To public the results of investigation.

Chapter 2

REVIEW OF LITERATURE

CHAPTER

2

Review of Literature

2.1 Memory

Memory is the ability of an individual to record sensory stimuli, events, information, etc., retain them over short or long periods of time and recall the same at a later date when needed (Shete and Bodhankar, 2009). In psychology, memory is the ability of an organism to store, retain, and subsequently recall information. Although traditional studies of memory began in the realms of philosophy, the late nineteenth and early twentieth century put memory within the paradigms of cognitive psychology. In the recent decades, it has become one of the principal pillars of a new branch of science that represents a marriage between cognitive psychology and neuroscience, called cognitive neuroscience (Zola *et al.,* 1990).

2.1.1 Memory Formation and its Consolidation

Memory formation involves changes in synaptic transmission in specific neuronal pathways in CNS. These changes in synaptic transmission is brought about by various processes such as sprouting of axonal terminals, concentrations changes in the release of neurotransmitters, changes in the number of post synaptic receptors or by combination of all these mechanism (McCabe *et al.,* 2001).

This initial binding of information into a memory trace involves a short-term consolidation process which will complete within seconds. Memory consolidation process which will complete within seconds. Memory consolidation process can be divided into two phases: A protein and RNA synthesis-independent phase that lasts minutes to 1-3 h and a protein and RNA synthesis dependent phase that lasts several hours to days, weeks or even longer. During memory formation, protein synthesis thought to be required to transform newly learned information into stable synaptic modifications around the time of training or during the first

few hours post-training (J.R. Cooper, in International Encyclopedia of the Social & Behavioral Sciences, 2001).

At the cellular level consolidation involves different molecular events including the activation of several signaling cascades in specific brain regions, activation of these cascades in the hippocampus is initiated by receptor activation including AMPA, metabotropic and particularly NMDA glutamate receptor, and monoamines receptors, followed by the recruitment of second messenger systems and activation of different protein kinases and phosphatases. This leads to the activation of transcription and translation and ultimately the synthesis of proteins required for functional and structural changes (Lynn and Morris, 1997).

2.1.2 Classification of Memory (Guyton and Hall, 2006)

There are several ways of classifying memories, based on duration, nature and retrieval of information. From information processing perspective there are three main stages in the formation and retrieval of memory:

- Encoding (processing and combining of received information)
- Storage (creation of a permanent record of the encoded information)
- Retrieval/Recall (calling back the stored information in response to some cue for use in some process or activity).

2.1.2.1 Classification by duration

In cognitive psychology, memory is usually divided into three stores: the sensory, the short-term, and the long-term. The progress of information through these stores is often referred to as:

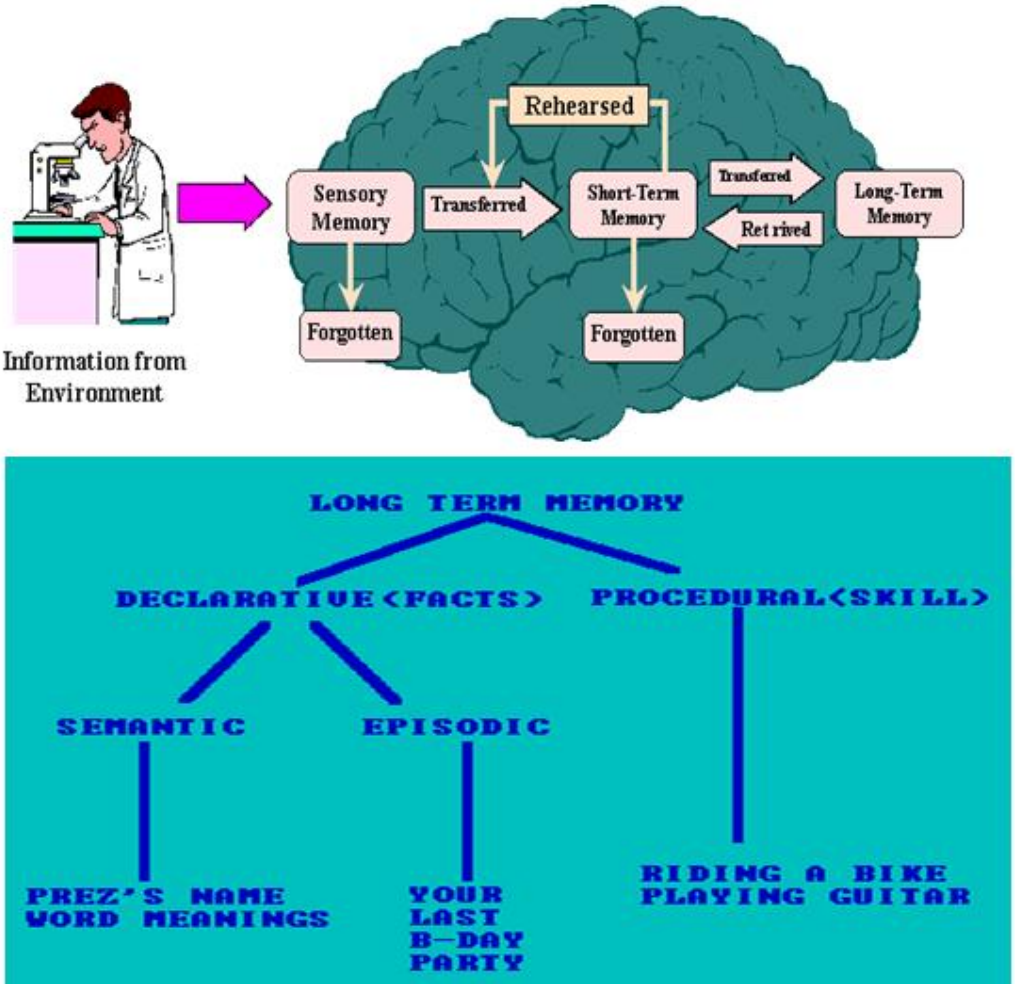


FIGURE 2.1.2.1: The information processing model

2.1.2.2 Sensory Memory

The sensory memory retains an exact copy of what is seen or heard (visual and auditory). Sensory memory is characterized by the duration of memory retention from milliseconds to seconds and short-term memory from seconds to minutes (Guyton and Hall, 2006).

Short-Term Memory (STM)

STM is characterized by:

- A limited capacity of up to seven pieces of independent information.
- The brief duration of these items last from 3 to 20 seconds.
- Decay appears to be the primary mechanism of memory loss.

- After entering sensory memory, a limited amount of information is transferred into short-term memory. Within STM, there are three basic operations:
- **Iconic memory** - The ability to hold visual images.
- **Acoustic memory** - The ability to hold sounds. Acoustic memory can be held longer than iconic memory.
- **Working memory** - An active process to keep it until it is put to use (think of a phone number you will repeat to yourself until you can dial it on the phone). Note that the goal is not really to move the information from STM to LTM, but merely put the information to immediate use.

The process of transferring information from STM to LTM involves the encoding or consolidation of information. This is not a function of time, that is, the longer a memory stayed in STM, the more likely it was to be placed into LTM; but on organizing complex information in STM before it can be encoded into LTM. In this process of organization, the meaningfulness or emotional content of an item may play a greater role in its retention into LTM. As instructional designers, we must find ways to make learning relevant and meaningful enough for the learner to make the important transfer of information to long-term memory (Guyton and Hall, 2006).

2.1.3 Long-term memory

Long-term memory is intended for storage of information over a long time (permanent storage). Information is stored on the basis of meaning and importance. Information from the working memory is transferred to it after a few seconds. Unlike in working memory, there is little decay (Guyton and Hall, 2006).

2.1.3.1 Classification by information type

Long-term memory are often divided into declarative (explicit) and procedural (implicit) reminiscences.

Declarative memory needs acutely aware recall, in this some acutely aware method should decision back the data. It’s typically referred to as express memory, since it consists of knowledge that's expressly keep and retrieved (Guyton and Hall, 2006).

Declarative memory are often additional sub-divided into long-term memory, that considerations facts taken freelance of context; and long-term memory, that considerations info specific to a specific context, like a time and place. Semantic memory permits the cryptography of abstract data regarding the planet, like "Paris is that the capital of France (Guyton and Hall, 2006).

Episodic memory, on the opposite hand, is employed for a lot of personal reminiscences, like the sensations, emotions, and private associations of a specific place or time. Autobiographical memory - memory for explicit events inside one's own life – is mostly viewed as either appreciate, or a set of, long-term memory.

Visual memory is a component of memory conserving some characteristics of our senses bearing on visual expertise. A tendency to square measure ready to place in memory info that resembles objects, places, animals or folks in style of a mental representation. Visual memory may result in priming and it's assumed some quite sensory activity figural system (PRS) underlies this development (Guyton and Hall, 2006).

In distinction, procedural memory (or implicit memory) isn't supported the acutely aware recall of knowledge, however on implicit learning. Procedural memory is primarily used in learning motor skills and will be thought-about a set of implicit memory. It’s disclosed once we do higher during a given task due solely to repetition - no new express reminiscences are shaped, however we have a tendency to square measure unconsciously accessing aspects of these previous experiences. Procedural memory concerned in motor learning depends on the neural structure and basal ganglia (Guyton and Hall, 2006).

2.1.3.2 Long-term memory processes

There are three main activities related to long-term memory: storage, deletion and retrieval. Information from short-term memory is stored in long-term memory by rehearsal. The repeated exposure to a stimulus or the rehearsal of a piece of information transfers it into long-term memory. Experiments also suggest that learning time is most effective if it is distributed over time. Deletion is mainly caused by *decay* and *interference*. Emotional factors also affect long-term memory (Ijsbrand M. Kramer, in Signal Transduction (Third Edition),

2016). However, it is debatable whether we actually ever forget anything or whether it becomes increasingly difficult to access certain items from memory. Having forgotten something may just be caused by not being able to retrieve it! Information may not be recalled sometimes but may be recognized, or may be recalled only with prompting. This leads us to the third prowess of memory: information retrieval (Ijsbrand M. Kramer, in Signal Transduction (Third Edition), 2016).There are two types of information retrieval: recall and recognition. In recall, the information is reproduced from memory. In recognition the presentation of the information provides the knowledge that the information has been seen before (Ijsbrand M. Kramer, in Signal Transduction (Third Edition), 2016).

2.1.4 Classification by temporal direction

Prospective Memory: Tying ribbon or string around a finger is the iconic mnemonic device for remembering a particular thought, which one consciously trains oneself to associate with the string.

A further major thanks to distinguish totally different memory functions is whether or not the content to be remembered is within the past, retrospective memory, or whether or not the content is to be remembered within the future, prospective memory. Thus, retrospective memory as a class includes long-term memory and episodic/autobiographical memory. In distinction, prospective memory is memory for future intentions, or memory to recollect. Prospective memory is any diminished into event- and time-based prospective memory (Ariel Y. Deutch, in Fundamental Neuroscience (Fourth Edition), 2013). Time-based prospective reminiscences are triggered by a time-cue, reminiscent of planning to the doctor (action) at 4pm (cue). Event-based prospective reminiscences are intentions triggered by cues, reminiscent of memory to post a letter (action) when seeing a mailbox (cue). Cues don't ought to be relating to the action (as the mailbox example is), and lists, sticky-notes, knotted handkerchiefs or string round the finger (see box) are all samples of cues that are created by folks as a technique to reinforce prospective memory (Ariel Y. Deutch, in Fundamental Neuroscience (Fourth Edition), 2013).

2.1.5 Potential neurotransmitters, neuromodulators and receptor systems involved in learning and memory:

Neurotransmitter/Neuromodulator	Receptor systems
➢ Glutamate	NMDA, AMPA receptors
➢ Acetylcholine	Muscarinic, nicotinic
➢ Dopamine	D1, D2 receptors
➢ Serotonin	5-HT_3, 5-HT_{1A} receptors
➢ Neuropeptides	G-protein-coupled peptidergic receptors
➢ GABA-β-carbolines	$GABA_A$/BZD receptor complex
➢ Neurosteroids	NMDA/$GABA_A$ receptors.

2.2 Neurotransmitter

Neurotransmitter are involve in concentration, mental focus, calculation ability, memory encoding, recall, creativity, and which further prevents most depressions. The three main neurotransmitters are acetylcholine, dopamine and serotonin (Dipiro *et al.,* 2008).

2.2.1 Role of Neurotransmitters

Thinking is a biologically demanding task. It involves the firing of neurons, which requires plenty of neurotransmitters, and even though these are reusable to some extent, they do get depleted. Depletion of neurotransmitters generally results in reduced mental performance, which may include difficulty in concentration, slowed reasoning, decreased learning efficiency, impaired recall, reduced coordination, lowered moods, inability to cope, increased response times, and mental fatigue (Claudia Gonzalez-Espinosa, Fabiola Guzman-Mejia, in Identification of Neural Markers Accompanying Memory, 2014). This also generally increases the likelihood of human error on tasks and activities performed. Stress causes neurotransmitters to be depleted even faster. Maintaining neurochemicals at optimal levels has a corresponding effect on brain performance, supporting improved mental ability and stamina, even beyond the individual's normal limits. The brain's neurotransmitters need to be replenished frequently, made by the body from substances ingested in the diet. As the brain ages, its ability to produce and maintain youthful levels of neurotransmitters declines. Thus, the theory is that by providing the brain with ample raw materials to make the

neurotransmitters to restore them to more youthful levels which maintain the cognitive function at vigorous youthful levels as well (Dipiro *et al.,* 2008).

2.2.1.1 Cholinergics

Cholinergics are substances which affect the neurotransmitter acetylcholine or the components of the nervous system which utilize acetylcholine. The cholinergic hypothesis claims that the decline in cognitive function in dementia is predominantly related to a decrease in cholinergic neurotransmission (Claudia González-Espinosa, Fabiola Guzmán-Mejía, in Identification of Neural Markers Accompanying Memory, 2014). The cholinergic muscarinic antagonist scopolamine is the drug most widely used to induce amnesia in experimental animals. Acetylcholinesterase inhibitors, which enhance the availability of acetylcholine in the synaptic cleft, were able to reverse the scopolamine induced deficit, indicating a neurotransmitter role for ACh in learning and memory. Muscarinic type I receptor antagonists, such as pirenzepine and the nicotinic antagonist mecamylamine, also have a negative effect on learning and memory performance (Dipiro *et al.,* 2008).

2.2.1.2 Dopaminergics

Dopaminergics are substances which affect the neurotransmitter dopamine or the components of the nervous system which utilize dopamine. Dopamine is produced in the synthesis of all catecholamine neurotransmitters, and is the rate limiting step for this synthesis. The mesocortical dopamine system also plays a crucial role in cognitive processes since the neurotransmitter dopamine predominantly controls the functions of prefrontal cortex (Claudia Gonzalez-Espinosa, Fabiola Guzman-Mejia, in Identification of Neural Markers Accompanying Memory, 2014). Dopamine D_1 receptors are implicated in the maintenance of reward-related learning, whilst D_2 receptors appear to be functionally related to the type of reinforcer involved. Quinpirole, a D_2 agonist, and D-amphetamine improved cognitive performance in various types of learning and memory tasks. It was argued that dopamine enhances learning processes but interferes with memory processes (Dipiro *et al.,* 2008).

2.2.1.3 Serotonergics

Serotonergics are substances which affect the neurotransmitter serotonin or the components of the nervous system which utilize serotonin. The role of serotonin in learning and memory has been receiving greater attention. Stimulation of serotonergic neurotransmission disrupts behavioural performance, whilst inhibition enhances performance in experimental animals (J.R. Cooper, in International Encyclopedia of the Social & Behavioral Sciences, 2001). 5-HT_3 (serotonin) receptor antagonists such as ondansetron and zacopride have shown promise in enhancing cognitive performance. It was hypothesized that 5-HT_3 receptors modulate cortical ACh release and may possibly act via an additional 5-HT_{1A} (serotonin) receptors are rich in the entorhinal cortex, a structure involved in learning and memory functions. Ipsapirone, a 5-HT_{1A} agonist, improved memory performance in rodents, whereas many other serotonergic agents failed to modulate learning and memory processes (J.R. Cooper, in International Encyclopedia of the Social & Behavioral Sciences, 2001).

2.3 Dementia

Dementia is an acquired syndrome of decline in memory and at least one other cognitive domine such as language, visio-spatial, or executive function that is sufficient to interfere with social or occupational function in an alert person. Dementia can also be defined as the progressive decline in cognitive function due to damage or disease in the brain beyond what might be expected from normal aging (Malaz *et al.,* 2003). Especially in the later stages of the condition, affected person may be disoriented in space in time and in person. Symptoms of dementia can be classified as either reversible or irreversible depending upon the etiology of the disease. Many diseases can cause the dementia syndrome. Alzheimer's disease and cerebrovascular ischemia (Vascular dementia) are the two most common causes, and some cases of dementia involve both of these disorders. Although some potentially reversible conditions, such as hypothyroidism or Vitamin B_{12} deficiency, are often thought to cause dementia, no more than 1.5% of cases of mild to moderate dementia is fully reversible (Malaz *et al.,* 2003).

2.3.1 Classification of Dementia

Dementia is classified into mainly into two categories:

Cortical Dementias: Alzheimer's disease, vascular dementia (also known as multifarct dementia), including Binswanger's disease, Dementia with Lewy bodies (DLB), Alcohol-Induced persisting Dementia (korsakoff's syndrome, wernicke's encephalopathy) Creutzfeldt-Jakob disease, Dementia pugilistic, Moyamoya disease (Shapira *et al.,* 1986).

Subcortical Dementias: Huntington's disease, Hypothyroidism, Parkinson's disease, Vitamin B_1 deficiency, Folate deficiency, Syphilis, Hypercalcaemia, Hypoglycemia, AIDS dementia complex (Shapira *et al.,* 1986).

2.3.2 Types of Dementia

2.3.2.1 Parkinson's Disease

Parkinson's disease (PD) was 1st time represented in the year of 1817 by the operating surgeon, as a medicine ill health consisting of resting tremor and an abnormal type of advanced motor incapacity (Samii *et al.,* 2004). Now a days, this enervating neurodegenerative disorder is the associate degreed is predicted to impose an increasing economic burden on societies. In the US, PD affects over fifty years older, and is associate degreed with economic burden of $25 billion annually (Scheife *et al.,* 2000). The cardinal symptom of PD includes resting tremor, bradykinesia, and rigidity. The resting tremor is taken into account to be the foremost common symptom and is shown because the 1st sign on seventieth of patients. Uneven initially, this tremor happens in hands, arms, legs, jaw, and face. Bradykinesia refers to slowed movement together with each nonvolitional and willing movement. It the foremost disabling symptom of early PD and at first manifests with difficulties with fine motor tasks appreciate doing up buttons or handwriting and reduced arm swing whereas walking (Samii *et al.,* 2004). The disguised feature is an example of slowed nonvolitional movement. Rigidity produces a resistance to passive movement that's uniform throughout the vary of motion of the limbs and joints. Varied non-motor options in PD have additionally been recognized within the past 10 years, together with involuntary disfunction, sensory symptoms, sleep disturbance, anxiety, depression, and insanity (Samii *et al.,* 2004).

The basic anatomy of the basal ganglia are reviewed, since it's thus vital for understanding PD pathophysiology. Motor, cognitive, and affectional activities are influenced by 2 major neural structure structures: the basal ganglia and neural structure (Hoshi *et al.,* 2005). The basal ganglia is most important anatomical telencephalic neural structure nuclei at the bottom of the prosencephalon and consists of corpus striatum, paleostriatum, nucleus niger, and cell nucleus. The basal ganglia and neural structure receive information from pallium comes and send their output right back to the cortex via the neural structure or on to the motor systems within the mesencephalon and neural structure. The output of the neural structure is excitative, whereas the basal ganglia output is repressive. The balance between these 2 systems permits for swish and coordinated movement and a disturbance in either system can cause movement disorders.

The pathological hallmark of PD is that the degeneration of dopaminergic neurons whose cell bodies are situated within the nucleus niger pars compacta (SNpc) and whose protrusive axons and nerve terminals are found within the corpus striatum (Przedborski 2005). The loss of nigral neurons within the nucleus niger leads to severe monoamine neurotransmitter depletion within the corpus striatum, touching the motor symptoms. The sickness is additionally thought of as a amino acid hydroxylase (TH) deficiency syndrome, since TH catalyzes the formation of L-3, 4-dihydroxyphenylalanine (levodopa), the rate-limiting step within the synthesis of monoamine neurotransmitter within the corpus striatum (Haavik & Toska 1998). In general, the looks of sickness symptoms is ascertained when loss of eightieth of striatal monoamine neurotransmitter and five hundredth of TH-immunoreactive dopaminergic neurons within the nucleus niger (Samii *et al.,* 2004).

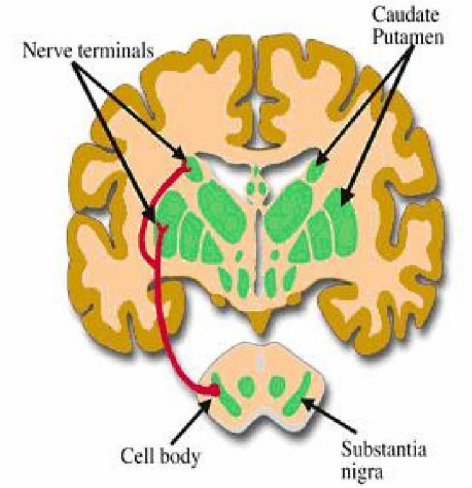


FIGURE 2.3.2.1a Schematic diagram of nigrostriatal dopaminergic pathway (Betarbet *et al.,* 2002).

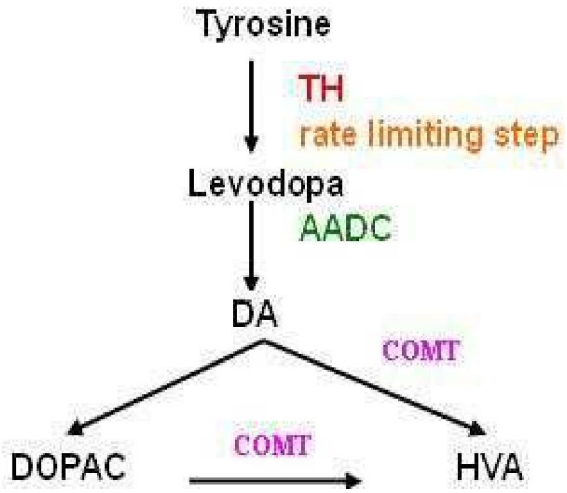


FIGURE 2.3.2.1b Main pathways of synthesis and metabolism of dopamine. The main enzymes involved are tyrosine hydroxylase (TH), aromatic amino acid decarboxylase (AADC), and catechol-O-methyltransferase (COMT). DA, dopamine; DOPAC, 3, 4-dihydroxyphenylacetic acid; HVA, homovanillic acid; levodopa, L-3, 4-dihydroxyphenylalanine.

The mechanism chargeable for death in metal continues to be unknown. However, growing proof advises that neuronic death within the SNpc could also be apoptotic (Lang & Lozano 1998). Apoptotic death could be a morphologically and biochemically outlined mode of death characterised by body substance condensation and aggregation to the nuclear margin, cytoplasmatic shrinkage, deoxyribonucleic acid fragmentation, and membrane blebbing. Blebbing of the membrane ends up in the cell budding faraway from itself into membrane-bound "apoptotic bodies", that square measure phagocytized by neighboring cells while not associate degree inflammatory response (Andersen 2001). In vitro studies with isolated

neurons and in vivo studies in animals treated with toxin have provided sturdy proof that neural structure death is preponderantly from cell death (Kaul *et al.,* 2003; Kostrzewa 2000). Additionally, autopsy studies have additionally rumored that dying neurons within the Parkinsonian brains displayed morphological characteristics of cell death together with cell shrinkage, body substance condensation, and deoxyribonucleic acid fragmentation (Andersen 2001; Anglade *et al.,* 1997; Mochizuki *et al.,* 1996).

Additional necessary distinguishing pathology of metal square measure lewy bodies, the intraneuronal inclusions of abnormal macromolecule aggregation (Emborg 2004). The lewy bodies square measure little spherical inclusions that comprise neurofilaments and ubiquitin (Blum *et al.,* 2001). Although the careful mechanisms square measure still beneath examination, the assembly of excessive folded proteins and also the compromised ubiquitin-proteasome system square measure supposed to play necessary roles within the formation of lewy bodies. The presence of lewy bodies isn't restricted to PD; the inclusions are discovered in Alzheimer's unwellness, suggesting the abnormal macromolecule aggregation in neurons is ubiquitously concerned within the pathological process of neurodegenerative diseases.

2.3.2.2 Neurotoxins induced PD

Subsequently the cause and mechanism were unknown and investigation the etiology and pathological process of the disease in humans is troublesome, neurotoxins are wont to induce experimental models of PD. The foremost common neurotoxins used are 6-hydroxydopamine (6-OHDA), rotenone, lactacystin, and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Beal 2001; Zhang *et al.,* 2005).

Neurotoxicity elicited by MPTP, a cyanogenetic byproduct within an underground blend of a pethidine analogue, is also most helpful for understanding palladium in developing methods. Various studies explored the mechanism of MPTP elicited Parkinson's. MPTP administration by selection damages dopaminergic neurons arising within the SNpc and causes the loss of dopaminergic nerve terminals within the basal ganglion that ends up in impaired dopaminergic neurotransmission (Burns *et al.,* 1983; Kalivendi *et al.,* 2003). MPTP elicited Parkinson's in primates replicates all the clinical signs of palladium, as well as tremor, rigidity, and bradykinesia. Additionally, primates treated with MPTP show a superb response

to the Dopastat precursor dihydroxyphenylalanine and to Dopastat receptor agonists treatment. Once administered to animals, MPTP crosses the blood brain barrier and is regenerate into its active matter, 1-methyl -4- phenylpyridinium (MPP+), by MAO group B. MPP+ is preoccupied by the plasma-membrane Dopastat transporter and accumulates within the Dopastatrgic neurons within the SNpc thanks to its similar structure to dopamine. Living thing MPP+ is preoccupied and accumulates within the mitochondria, wherever it inhibits the metabolism chain by inhibiting NADH dehydrogenase (Beal 2001). Since the transport of leptons down the electron transport chain (ETC) can unharness energy to form a nucleon and chemistry gradient for adenosine triphosphate formation, disruption of the ETC might cause the decrease in adenosine triphosphate that might have an effect on several processes within the neurons. MPP+-induced inhibition increases the superoxide radical formation, as NADH might now not transfer reduced equivalents to modify ubiquin-one, and also the high energy electrons can react with element to create superoxide (Fonck & Baudry 2003; Shults 2005). MPTP is so thought of a robust compound to induce neurodegeneration in animals, and MPP+ is employed in cellular models of palladium to exert aerophilous stress and induce programmed cell death.

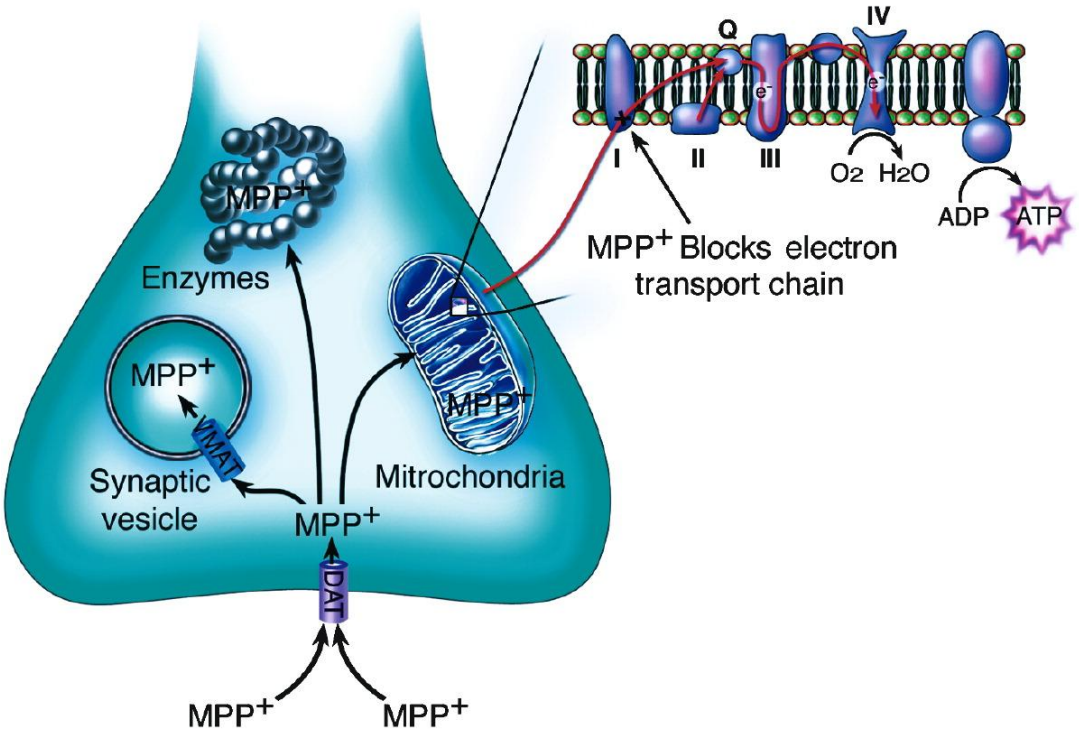


FIGURE 2.3.2.2 Schematic diagram of MPP+ intracellular pathway (Dauer & Przedborski 2003)

6-hydroxydopamine is a neurotoxins which induces experimental nigral degeneration and may be a hydroxylated analogue of the natural neurotransmitter, exhibiting a high affinity for many catecholaminergic cytomembrane transporters as well as the Dopastat transporters (DAT) and vasoconstrictive transporters. Consequently, 6-OHDA might enter each dopaminergic and noradrenergic neurons and injury each the peripheral and also the central nervous systems (Bove *et al.,* 2005). However, 6-OHDA couldn't cross the blood brain barrier (Schober 2004). Within the experimental models of PD, 6-OHDA is injected directly into the striate body, the neural structure, or the ascending medial neural structure bundle to wreck the nigrostriatal dopaminergic pathway. 6-hydroxydopamine by selection accumulates in Dopastat neurons, destroys nigral dopaminergic neurons, and depletes the Intropin} neurotransmitter within the striate body. 6-hydroxydopamine is recommended to induce nigrostriatal dopaminergic lesions via the generation of peroxide derived hydroxyl group radicals since it may be oxidatively deaminated by MAO to supply peroxide (Blum *et al.,* 2001). Moreover, 6-OHDA has been shown to cut back striatal glutathione (GSH) and SOD (SOD) catalyst activity and increase levels of macromolecule peroxidation merchandise (Schober 2004).

2.3.2.3 Risk factors of PD

Following are few risk factors of PD.

2.3.2.3.1 Age

Age is one of the possible risk issue PD as the most patients develop it around at fifty years aged. The prevalence of PD may increase with age, from 20/100,000 overall to 100/100,000 at age seventy (Dauer & Przedborski 2003; Tanner 1996).

Pathologically, aging is related to a decline of pigmented neurons within the SNpc. it's additionally urged that the aging nigrostriatal system could also be additional liable to injury caused by exogenous and endogenous toxins since the toxin MPTP causes additional severe pathological and organic compound injury in older mice (McCormack *et al.,* 2004).

2.3.2.3.2 Genetic susceptibility

Genetic part is usually recommended to be a very important risk issue. Medicine studies have known a positive case history of Parkinson illness together of the foremost vital risk factors for the illness (Allam *et al.,* 2005). In a study among 20000 male twins counsel that genetic factors seem to be a very important consider with onset before age fifty years (Tanner *et al.,* 1999). Discovery of a minimum of 9 factor loci in familial metal any demonstrates the genetic contribution to the present disorder (Samii *et al.,* 2004). The identification of those accountable genes for familial metal can offer vital clues for understanding the molecular pathological process of the illness (Kubo *et al.,* 2006).

2.3.2.3.3 Environment

The environmental factor of hypothesis is the invention for the Parkinsonian poisonous substance MPTP. MPTP was recognized as a neurolysin early in 1982, once many young drug addicts enigmatically developed a parkinsonian syndrome once the blood vessel use of dolophine hydrochloride analogue contaminated by its byproduct, MPTP (Przedborski *et al.,* 2001). This event reinforced the conception that exogenous chemicals will induce PD symptoms. Since then, many epidemiologic studies have prompt that exposure to totally different environmental agents as well as pesticides, pesticides, metals, and microorganism toxins might increase the chance of metal (Di four-card monte 2003; Kanthasamy *et al.,* 2005). Dieldrin, one among the foremost persistent bioaccumulative and deadly chemicals, is related to exaggerated incidence of metal (Kanthasamy *et al.,* 2005). Metals like metallic element, copper, iron, lead, and metal are coupled to metal in numerous etiological studies (Gorell *et al.,* 2004; Tanner 1996). Moreover, tobacco, caffeine, and tea appear to safeguard against development of PD due to their inhibitor properties (Checkoway *et al.,* 2002; Diamond State Lau & Breteler 2006; Gorell *et al.,* 2004; Hellenbrand *et al.,* 1997; Ross *et al.,* 2000).

2.3.2.3.4 Inflammation

Inflammation has conjointly been concerned within the pathologic process of PD. Studies have shown that several cases are in the midst of brain inflammation with reactive microglial cells (Arai *et al.,* 2006; McGeer *et al.,* 1988a; McGeer *et al.,* 1988b). Pro-inflammatory

cytokines like as IL-1β and TNF-α could induce neuroglia activation and injury to neurons. Cytokines may conjointly bind to the receptors on the dopaminergic neurons and trigger living thing death in sign pathways (Arai *et al.,* 2006). AN association between the low risk of metal use of the non-steroidal medicinal drug nonsteroidal anti-inflammatory has also been rumored (Chen *et al.,* 2005).

2.3.2.3.5 Oxidative stress

Oxidative stress elicited cell injury, as well as caspase-mediated cell death, could play a distinguished role in neurologic degeneration related to PD. Lipide peroxidation, and high levels of ATP consumption within the brain may source high aerobic metabolism and leads to free radicals throughout energy transduction (Valko *et al.,* 2007) and brain cells have low inhibitor competency (Crichton *et al.,* 2002). Moreover, SOD enzyme concentrations were 7 to 140-fold lower within the brain than the liver (Crichton *et al.,* 2002; Reddy & Clark 2004). Nigral monoamine neurotransmitterrgic neurons area significantly exposed to aerobic stress and dopamine is inactivated by MAO, H2O2 and ROS (Jenner 2003; Olanow & Tatton 1999; Yoritaka *et al.,* 1996, Koutsilieri *et al.,* 2002). On other hand, levodopa, the most important therapeutic agent for the treatment of PD, will increase aerobic stress and plays a major role in PD (Jenner 2003; mayonnaise *et al.,* 2005; Shulman 2000).

The prevalence of aerobic stress in PD is additionally supported by many studies. The deletion of reduced GSH and impairment of the inhibitor system were conjointly found in neural structure in PD cases that was related to a major increase in change GSH (Sofic *et al.,* 1992). Oxidative stress causes injury to proteins, DNA, and lipids and induces apoptotic death in dopaminergic neurons. Macromolecule carbonyls were found to be twofold higher within the SNpc compared to different regions in traditional postmortem brains, suggesting status to aerobic injury (Floor & Wetzel 1998). Moreover, macromolecule carbonyl concentration was higher in metallic element patients compared to age matched controls (Alam *et al.,* 1997a; Floor & Wetzel 1998). Similarly, augmented levels of the lipide peroxidation product malondialdehydes and lipide hydroperoxides were found within the neural structure within the metallic element patients (Olanow & Tatton 1999; Yoritaka *et al.,* 1996). Additionally, indicators of desoxyribonucleic acid injury, akin to 8-hydroxyguanine, were conjointly augmented within the neural structure also as another brain regions within the metallic

element patients (Alam *et al.,* 1997b; doctor 2003). Of these results recommend that aerobic injury is concerned in metallic element.

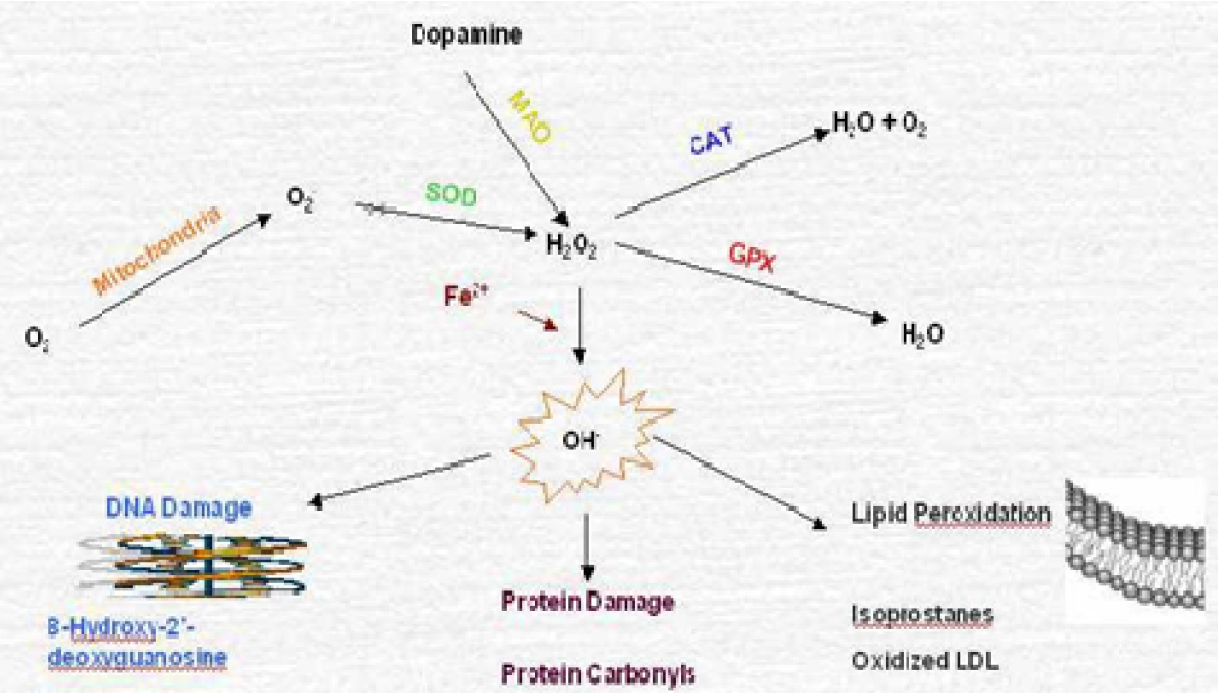


FIGURE 2.3.2.3.5 Pathway of ROS formation, the role of antioxidant and oxidative damage. CAT, catalase; GPX, glutathione peroxidase; SOD, superoxide dismutase; MAO, monoamine oxidase. (Alam *et al.,* 1997b).

2.3.2.4 The role of iron in PD

Metal ion might be related to the pathological process of PD by participation in macromolecule aggregation and stress (Gaeta & Hider 2005). Copper-mediated ROS generation might has enhanced stress burden in aging and neurodegeneration (Sayre *et al.,* 1999). Copper and iron stimulate, α-synuclein filament formation (Uversky *et al.,* 2001). The immersions of metals within the etiology of protein was additionally incontestible from epidemiologic study. Long-run activity exposure to bound metals resembling copper, manganese, lead, mercury, zinc, aluminum, and iron might increase the chance of protein (Gorell *et al.,* 1999; Gorell *et al.,* 2004; Uversky *et al.,* 2001). Exposure to high levels of Mn may cause neurotoxicity and induce a Parkinson's-like syndrome referred to as manganism (Latchoumycandane *et al.,* 2005; Olanow 2004).

Iron is an a vibrant nutrient for all living organisms since it plays several important roles in the body, as well as lepton transport, activation and transport of molecular O, albuminoid synthesis, tissue growth, neurochemical synthesis, and plenty of catabolic processes (Pollitt & Leibel 1976). Iron deficiency will cause changes in several metabolic processes, resembling aldohexose metabolism, neurochemical synthesis, and macromolecule synthesis (Beard 1990; Beard *et al.,* 1990; Brooks *et al.,* 1987).

Iron has ability to catalyse the assembly of extremely reactive hydroxyl radical radicals through the Haber-Weiss and Fenton reactions (Braughler *et al.,* 1986), that may cause aerophilous injury to membranes, nucleotides, and proteins.

Haber-Weiss reaction $H_2O_2 + O_2{-}\bullet \rightarrow O_2 + OH^- + OH\bullet$

Fenton reaction $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\bullet + OH^-$

Under biological conditions, iron cannot participate the higher than reactions. Iron is also concerned within the atom generation and might be venomous to cell survival. Iron is also free at hydrogen ion concentration values that may be seen in hardening of the arteries lesions (slightly under physiological pH) to induce supermolecule oxidization (Reddy & Clark 2004).

Iron is also a vital element of cytochromes a, b, and c, hemoprotein enzyme, and therefore the iron-sulfur complexes of the aerobic chain (Connor *et al.,* 2001). Iron is additionally concerned in ribonucleoside enzyme, the speed limiting catalyst of the primary metabolic reaction in DNA synthesis, and succinate dehydrogenase and aconitase of the TCA cycle (Domingo J. Pinero 2000).

Iron demand is high within the brain since the brain consumes high levels of ATP to take care of membrane ionic gradients, colligation transmission and nerve fibre transport. Iron is additionally a vital compound for several proteins that play essential roles within the traditional perform of neuronic tissues (Connor *et al.,* 1992; Zecca *et al.,* 2004). Iron could be a compound for the enzymes TH and tryptophane hydroxylase, that area unit concerned in neurochemical synthesis and destructive metabolism, severally (Beard & Connor 2003).

There are 2 classes of iron within the brain: pigment iron as a vicinity of hemoprotein and enzymes like peroxidases, and nonheme iron related to proteins (Haacke *et al.,* 2005). For the nonheme iron, siderophilin and protein area unit thought of key proteins in iron physiological state. Siderophilin could be a single chain, 80-KDa supermolecule that contains 2 iron-binding motifs and is chargeable for delivery of iron from/to peripheral tissues (Domingo J. Pinero 2000). Siderophilin (Tf) carries iron from the blood into brain tissue via siderophilin receptors (Tfr) placed within the brain's microvasculature (Haacke *et al.,* 2005). Tf-Tfr advanced may be preoccupied by cells via endocytosis and iron is free and exported into the living substance by powerfulness mental transporter one (DMT1) (Domingo J. Pinero 2000). Within the living substance, iron is accessible for necessary metabolic processes or is incorporated into the storage pool with protein for future would like. In most cells, the most regulator of iron physiological state is iron regulative supermolecule (IRP)/iron regulative part (IRE) system. Changes in iron standing like bronzed diabetes or iron depletion result in compensating changes during this system. Once iron is in excess, IRPs area unit in their inactive kind and don't bind the IREs on the mRNAs of protein and TfR. As a result, the protein is synthesized and therefore the TfR messenger RNA is degraded by nucleases. However, in conditions of iron depletion, IRPs binds to IREs on the mRNAs, preventing protein however permitting the synthesis of TfR by protective the messenger RNA from degradation (Zecca *et al.,* 2004).

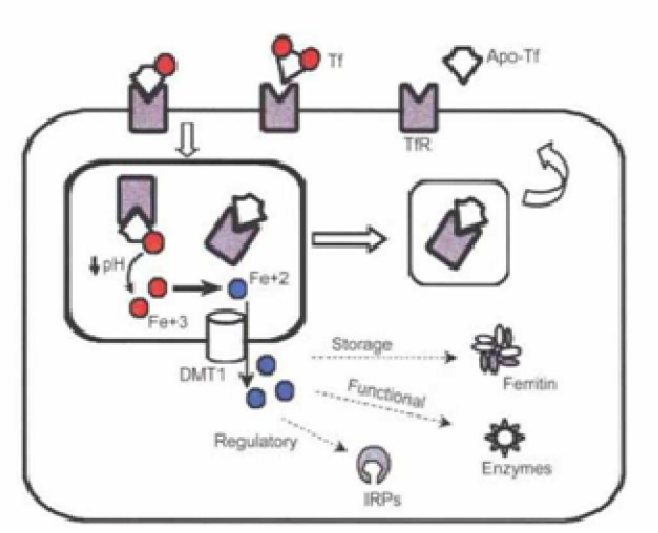


FIGURE 2.3.2.4a Cellular iron uptake (Domingo J. Pinero 2000)

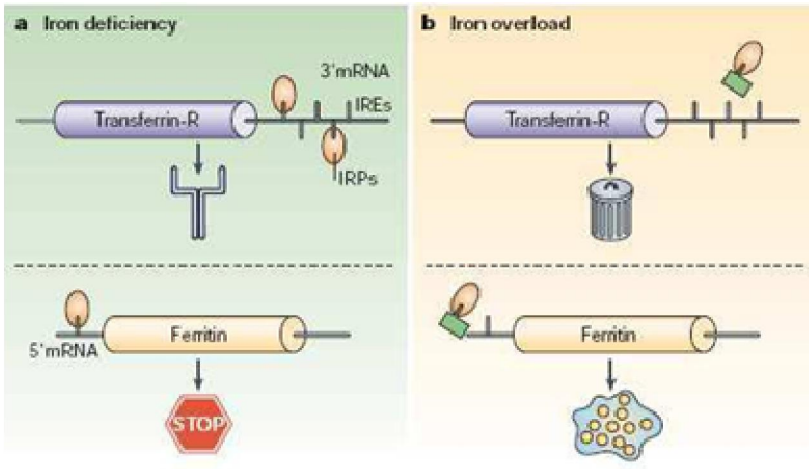


FIGURE 2.3.2.4b Translational regulation of the transferrin receptor and ferritin production (Zecca *et al.,* 2004).

The brain is extremely liable to excess iron since it's wealthy in unsaturated fatty acids (Schafer *et al.,* 2000). The neural structure has the best concentration of iron within the class brain. The neural structure has twenty ng/mg iron throughout the primary year of life, that will increase to two hundred ng/mg by the fourth decade (Zecca *et al.,* 2001). Excess iron may move with neuromelanin, a dark-colored pigment within the dopaminergic neurons, to induce aerobic stress and induce neurodegeneration (Double *et al.,* 2000).

Since excessive iron accumulation in neural structure was found in brains from paralysis agitans patients, the role of iron in palladium has recently gained attention (Berg *et al.,* 2002; Berg *et al.,* 2001). Recent studies additionally counsel that mis-regulation of iron metabolism, iron-induced aerobic stress, and radical formation square measure major unhealthful factors in palladium (Jellinger 1999). Protein is reportedly belittled within the neural structure of the palladium brain compared to older controls. H protein and L protein levels square measure reported to be seventy fifth and thirty seventh of traditional within the neural structure, severally (Domingo J. Pinero 2000). Exaggerated iron levels and low levels of protein permits for “free iron” that will be concerned in radical generation (Berg *et al.,* 2001; Bishop *et al.,* 2002). The neurotoxins MPTP and 6-OHDA unleash protein sure iron in each in vitro and in vivo condition (Grunblatt *et al.,* 2000). Though the mechanism isn't clear, MPTP disrupts iron metabolism through iron restrictive proteins by increasing globulin receptors, and therefore

inflicting exaggerated iron uptake by the brain (Blum *et al.,* 2001; LaVaute *et al.,* 2001).

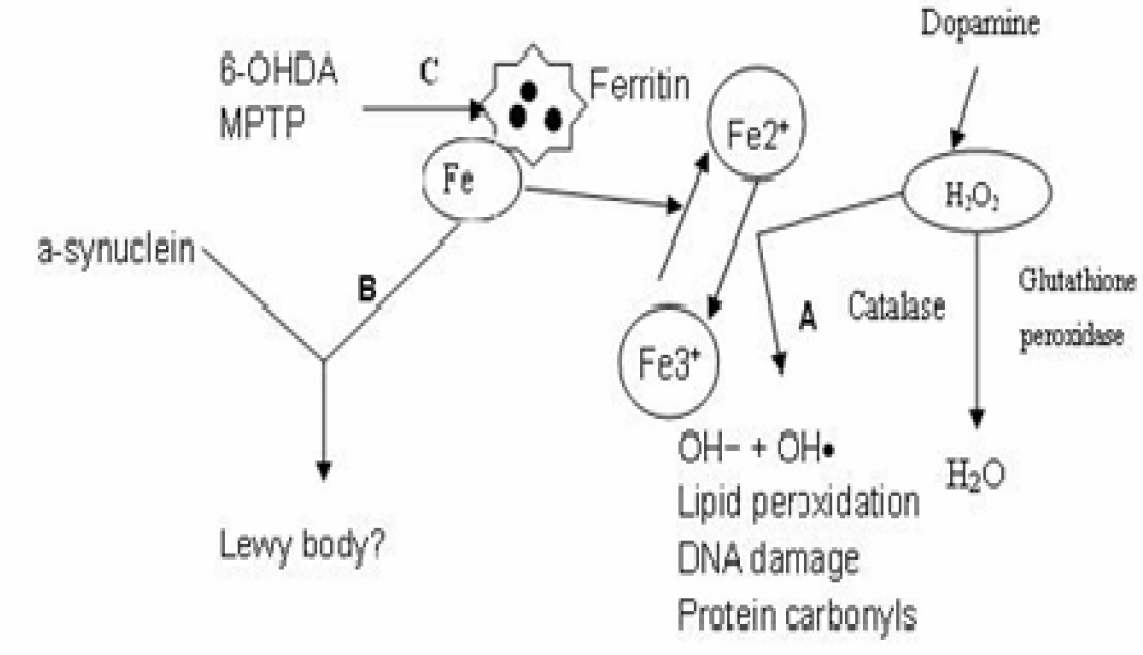


FIGURE 2.3.2.4c Hypothesized mechanism for the role of iron in PD (Blum *et al.,* 2001)

2.3.2.5 Treatment of PD

Larodopa was initial incontestible to be effective in reducing these motor deficits thirty years past and remains the foremost potent Antiparkinson drug (Hubble 1999; Samii *et al.,* 2004). This treatment will considerably attenuate the Parkinsonian symptoms and improve the standard of lifetime of Parkinsonian patients. Whereas Larodopa improves symptoms, however doesn't inhibit the progressive degeneration of dopaminergic neurons. Moreover, the bulk of patients receiving five years of Larodopa monotherapy can develop fluctuations and/or involuntary movements referred to as dyskinesias (Schelosky & Poewe 1993). Patients feel Larodopa effectiveness declines with time, and that they become slower and additional unsteady once future treatment with Larodopa (Samii *et al.,* 2004). Moreover, there's AN "on-off" phenomena within the patients, with sharp fluctuations between quality and immobility, with the "off" periods marked with severe palsy which will extend over many hours (Singh *et al.,* 2007). As a result, comparable to cabergoline, pramipexole, and ropinirole and are used as adjuvant therapies to Larodopa (Rinne 1981, Radad *et al.,* 2005). Most significant, of these treatments are restricted to symptomatic relief and don't attenuate the progress of the underlying pathology and also the natural course of the sickness (Bonuccelli & Pavese 2006).

Alternate therapeutic strategies

Alternate therapeutic methods should be thought-about to spot novel neuroprotective agents that effectively stop the progression of the neurodegenerative method. A variety of studies have examined whether or not antioxidants stop or cut back the progression of PD. Dietary supplementation with vitamin E and ascorbic acid were found to guard against 6-OHDA evoked striatal injury (Prasad *et al.,* 1999). Q10 may attenuate the MPTP evoked loss of striatal dopaminergic neurons and incorporates a protecting impact on metal (Ebadi *et al.,* 2001; Ernster & Dallner 1995). Additionally, estrogen, offers neuroprotection in associate degree MPTP mouse model (Tripanichkul *et al.,* 2006). Though there square measure several disputes regarding the effectivity of vitamin E within the bar or treatment of PD, each moderate and high intake of dietary vitamin E could have a neuroprotective impact and attenuate the danger of PD (de Rijk *et al.,* 1997; Etminan *et al.,* 2005). Long term, high dose vitamin E dietary supplementation (e.g. 1600-2000 IU d-α-tocopheryl succinate) starting within the third decade of life could function a roaring therapeutic strategy for the bar of metal (Fariss & Zhang 2003). Additionally, combination of α-tocopherol and ascorbate in patients with early metal would possibly delay the progression of the illness by a median of 5 years (Fahn 1992; Ricciarelli *et al.,* 2007).

Iron chelation is an efficient treatment of PD. Iron chelation via either transgenic expression of the iron binding macromolecule protein or oral administration of the metal chelator iodochlorhydroxyquin (CQ) reduced condition to MPTP for causation metal in animals (Kaur *et al.,* 2003). Pretreatment with the iron chelator, desferrioxamine (DFO), considerably protected (approximately 60%) against 6-OHDA (Ben-Shachar *et al.,* 1991; Zhang *et al.,* 2005). In another study, DFO reduced abnormal macromolecule aggregation and attenuated the lactacystin evoked monoamine neurotransmitter somatic cell loss (Zhang *et al.,* 2005). Desferrioxamine reduced the formation of reactive group radicals and lipoid peroxidation evoked by excess iron and MPTP, so leading to a major reversion of the reduction of striatal monoamine neurotransmitter levels (Lan & Jiang 1997).

However, the presently used iron chelators could cause some facet effects in patients. DFO is that the most potent iron chelator, however it should be administered at high dose to beat its

low ability to cross the blood brain barrier and causes some cyanogenetic effects (Zhang *et al.,* 2005). CQ has been incontestable to chelate each metallic element and ferrous iron and reduce total brain iron levels (Kaur 2002). Oral administration of CQ could shield against loss of striatal monoamine neurotransmitter evoked by MPTP (Kaur *et al.,* 2003) but causes vitamin B12 deficiency, which can cause pernicious anaemia (Kaur 2002; Yassin *et al.,* 2000).

Nutritional approaches to PD

Numerous food teams and specific nutrients are investigated for PD. High dose of vitamin E mainly 1600-2000 IU starting within the third decade of life, could forestall PD (Fariss & Zhang 2003). A mixture of vitamin E and water-soluble vitamin could also be helpful in patients with early PD (Prasad *et al.,* 1999). Tea is associate ancient potable and tea leaf polyphenols are currently thought to be therapeutic in PD since flavonoids possess iron chelating, antioxidant, and anti-inflammatory activities (Mandel *et al.,* 2006; Weinreb *et al.,* 2004). Tea leaf extract and its major polyphenol, (±)-epigallocatechin-3-gallate (EGCG), will shield against MPTP induced neurodegeneration in animal studies (Choi *et al.,* 2002; Levites *et al.,* 2001, Obata 2003).

Phytic acid is associate abounding variety of phosphorus in plant seeds and different plant tissues and is gift at two hundredth of the wet weight in whole grains, nuts, seeds, cereals, and legumes (Raboy 2003). Phytic acid was found to be a typical constituent of being cells and serves variety of functions reminiscent of signal transduction, cell proliferation and differentiation (Raboy 2003; Sasakawa *et al.,* 1995; Shamsuddin *et al.,* 1997). Phytic acid was thought of antinutrient by virtue of its ability to chelate powerfulness minerals and cut back their absorption (Reddy *et al.,* 1996). It will type chelating conjugates with essential metal cations reminiscent of copper, zinc, iron, and metal, and its affinity to bind these metals is as follows: $Cu^{2+} > Zn^{2+} > Co^{2+} > Mn^{2+} > Fe^{2+} > Ca^{2+}$ (Graf *et al.,* 1984). It inhibits •OH formation and reduces supermolecule peroxidation catalyzed by iron and ascorbate in human erythrocytes (Rao *et al.,* 1991). Phytic acid is one in every of the foremost effective agents for inhibiting chemical reaction in food materials. Giant amounts of phytate in plant seeds may supply protection from chemical reaction caused by high unsaturated fatty acids in soybeans

(Graf *et al.,* 1987). The inhibitor ability in vivo isn't clear, however in an exceedingly previous study it reduces supermolecule peroxidation and reduced liver iron stores by forty eighth in an exceedingly genetically iron overloaded-mouse model Hanson *et al.,* 2006). However, it could influence aerophilic stress freelance of iron-involved group formation and detoxifies the ROS (Shamsuddin *et al.,* 1997).

In distinction to different iron chelators, phytic acid is safe as a therapeutic and preventive agent and is non-toxic over long run administration (Vucenik *et al.,* 1992). Though intake could suppress the absorption of metal ions (Zhou & Erdman 1995), many studies indicate bodily function of enormous quantities of phytic acid with a diet poor in oligoelements could lead to poor bioavailability of minerals (Graf & Eaton 1993; Vucenik & Shamsuddin 2003). The lack of toxicity related to long run administration in animal models makes phytic acid appealing for interference of iron associated pathological process (Vucenik *et al.,* 1992).

Phytic acid is partly dephosphorylated to lower forms within the digestive tract by phytates from 3 totally different sources together with the diet, the viscus wall, and also the microorganism flora of the gut contents (Williams & Taylor 1985). The absorption of phytic acid in humans isn't well studied, however a really recent study has shown twenty eighth absorption of phytic acid (Agte *et al.,* 2005). However, in rodents it's well absorbed and is distributed to varied organs as early as 1-h when administration since rodents have phytase protein within the canal (GI) tract to hydrolyse phytic acid (Vucenik & Shamsuddin 2003). Though phytic acid could be extremely charged molecule, phytic acid may well be transported within the cells by bodily function (Shamsuddin *et al.,* 1997).

Phytic acid has several helpful effects relating to chelation of assorted metal ions and reducing aerophilic stress. Phytic acid attenuated heart muscle reperfusion injury and reduced the danger of concretion formation (Curhan *et al.,* 2004; Rao *et al.,* 1991). Phytic acid has anticancer activity in colonic cancer, duct gland cancer, murine transplanted and pathologic process fibrosarcoma, and chronic myeloid leukaemias (Deliliers *et al.,* 2002; Shamsuddin Vucenik 1999; Vucenik *et al.,* 1993; Vucenik & Shamsuddin 2003; Vucenik *et al.,* 1992). Though the precise mechanisms of anticancer activity don't seem to be renowned, it's going to exert its antitumor operate by decreasing phosphate pool and signal transduction pathways

(Graf & Eaton 1993; Shamsuddin & Vucenik 1999). To our data, suppression of MPP+ induced hydroxyl group radicals generation by chelating iron in rat corpus striatum with IP6 was rumored recently in one study (Obata, 2003).

2.3.2.6 Fornto-temporal Dementia (FTD)

The term "Fronto-temporal Dementia" or FTD refers to a bunch of diseases that ar normally misdiagnosed as Alzheimer's malady (AD). FTD a general term won't to refer disorders that also are named as: Pick's malady, Fronto-temporal body part. Degeneration, Progressive brain disease, linguistics insanity, FTD happens primarily between the ages of thirty five and seventy five. The malady affects each sex equally. Concerning four-hundredth of patients have a clear-cut case history. The foremost common symptoms are associate early modification in social and private conduct, characterised by issue to retort to a specific state of affairs. Typically this can be often related to an absence of inhibition, leading to impulsive or inappropriate behavior, comparable to swearing at in applicable times, outbursts of frustration, or lack of social tactfulness. Because the malady progresses, this could result in frank criminal behavior (e.g. shoplifting), poor money judgment or impulsive shopping for. At the acute, the impulsivity will be self, damaging, as once patients attempt to get out of a moving automobile. In some people, inappropriate sexual behavior happens. There may be repetitive or compulsive behaviors. this could embody a preoccupation with continuance specific acts (e.g. reading an equivalent book over and over) or continuance specific physical actions (e.g. walking to an equivalent location repeatedly) (Shapira *et al.,* 1986).

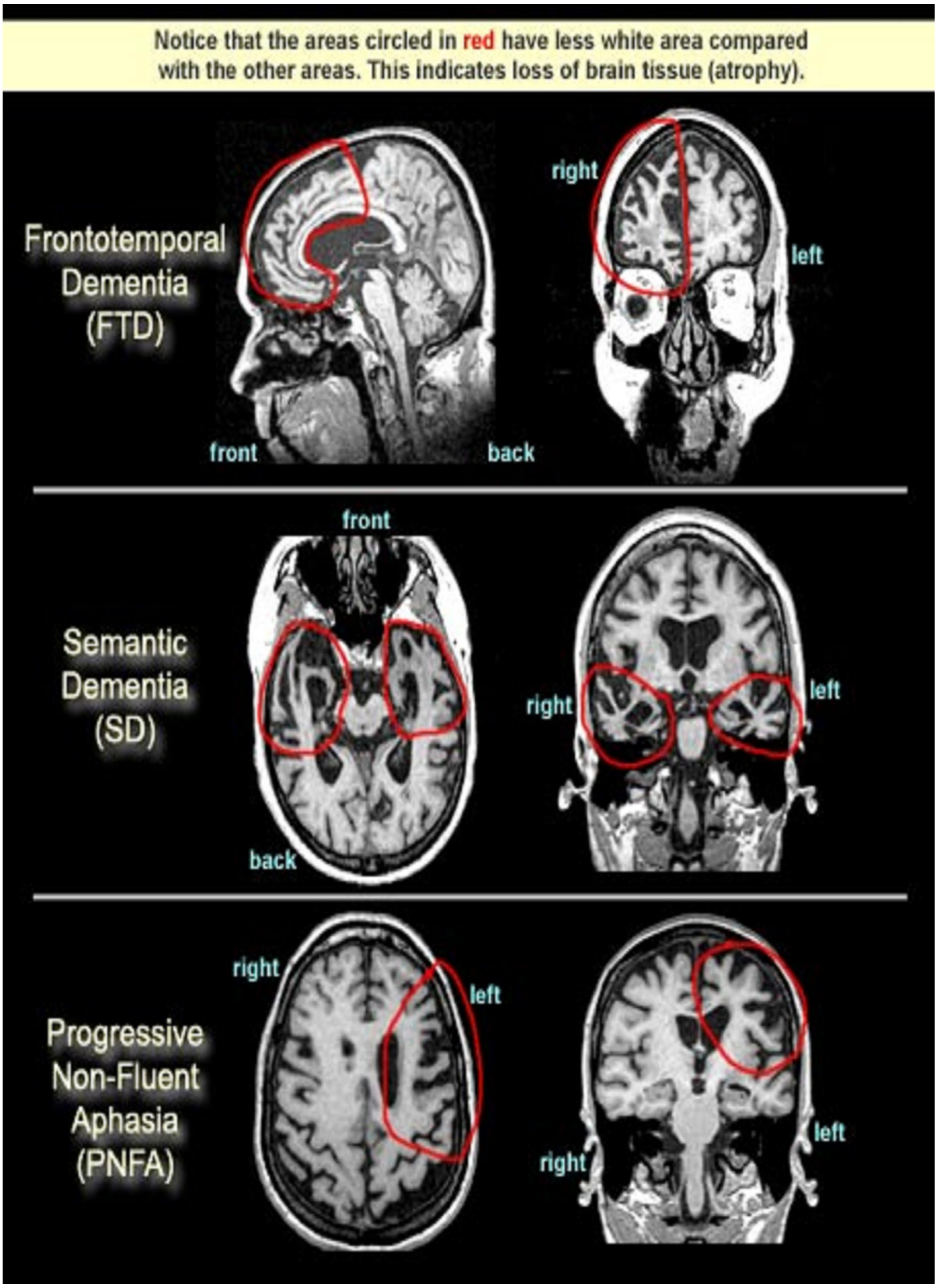


FIGURE 2.3.2.6 Atrophy associated with different types of Dementia (Shapira *et al.,* 1986).

2.3.2.7 Dementing Lewy Body Disease

Lewy Body malady (LBD) could also be the second most typical dementing disorder, accounting for between seven and twenty sixth of dementias. Lewy bodies are intraneuronal protoplasm inclusions composed of ubiquitin and neurofilment proteins, and may exist in each plant tissue and neural structure regions. They have an inclination to possess the best densities in parahippocampal cortex, cingulated, neocortex, temporal pole, nucleus niger, locus coeruleus, nucleus basalis of Meynert, and amygdale. Upon neuropathological examination, they're found in concerning two hundredth of brainsick brains, however might also occur within the absence of insanity. Their pathological process is unknown. as a result of the neuropathology of LBD includes each plant tissue and neural structure chages, patients with the malady tend to exhibit psychology options of each plant tissue and neural structure dementing syndromes (Elisabet, 2003).

2.3.2.8 Huntington's Disease

Huntington's unwellness (HD) is associate degree chromosome dominant neurodegenerative disorder related to basal ganglia and neural structure atrophy. HD is characterised by involuntary movements, psychological feature impairment, and activity abnormalities. It's caused by associate degree unstable dilated CAG repeat at intervals the secret writing region of the HD factor. Variability in age at onset, tendency of paternal transmission, and noncontinuous new mutations ar a number of HD's recognized clinical options (Aminoff and Katzung, 2003).

2.3.2.9 Alzheimer's Disease (AD)

2.3.2.9.1 History

Oskar Fischer, Francesco Bonfiglio and Graetano Perusini in 2006 explained the term Alzheimer's disease (AD) first time (Lage 2006). Kraepelin was one of the supervisor of Alzheimer (a psychiatrist and neuropathologist), and in the description of the symptoms and pathology of the disease, Kraepelin coined the name Alzheimer's disease, which has remained ever since. Alzheimer has delivered a lecture on Mrs. Auguste Deter in 1907 during the 37th Conference of South-West German Psychiatrists in Tubingen (Alzheimer 1907) and described the observation of the neuropathological lesions, neurofibrillary tangles in her brain. Her case

presented with memory aphasia, impairment, disorientation and psychosocial incompetence which has progressed gradually over the remaining years of her life, including experiencing worsening cognitive function and hallucinations.

This was not the first case of cognitive degeneration that Alzheimer encountered, however the case of Auguste Deter was also interesting due to her younger age. After her death, Alzheimer requested her brain be sent to him, from which he has examined tissue sections stained with a silver staining technique. From these microscopic analyses, he observed and described the presence of 'small miliary foci'and 'fibrillary bundles (Lage 2006, Alzheimer 1907). AD itself wasn't well thought-out a disease separate from dementia until the late 1960's, after studies (Blessed, Tomlinson & Roth 1968) showed that there was a connection between the characteristic hallmarks and cognitive decline as described by Lage (Lage 2006). Moreover, AD was different from normal aging (Kay, Beamish & Roth 1964) and identified mutations involved in hereditary forms of the disease (Tanzi *et al.,* 1996, Khachaturian 1985).

Unfortunately, because of the elusive nature of the disease, clinical diagnosis consists of terms like as 'possible' and 'probable' AD, and definite diagnosis as autopsy after substantiation of the presence of lesions – SP and NFT. Definition of AD consists of irreversible worsening of language, memory skills and judgement that progress over 10 to 15 years and are linked with the accumulation of neuropathological SP and NFT at postmortem evaluation (Braak, Braak 1991, Braak, Braak 1997, Khachaturian 1985, Mirra *et al.,* 1991).

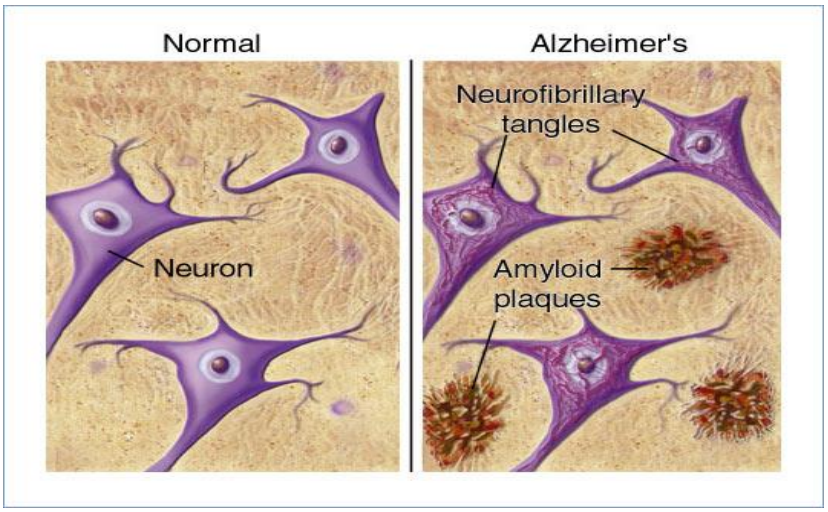


FIGURE 2.3.2.9.1 Neuron structural changes associated with Alzheimer's Disease (Mirra *et al.,* 1991).

2.3.2.9.2 Epidemiology

The aging of the population has been accompanied by a dramatic increase in the prevalence of dementia. 3% to 11% of persons older than 65 years of age and 25% to 47% of those older than 85 years of age have dementia (Malaz *et al.,* 2003).

2.3.2.9.3 Alzheimer's disease diagnosis

The diagnosis of AD can be divided into two parts. The first part deals with the symptoms that appear during a patient's life and involves measuring the worsening of cognition, behaviour, speech, understanding and memory. Various tests are available to make out the extent of the damage, and progression of the disease.

Mini-Mental State Examination (MMSE) (Folstein, Folstein & McHugh 1975) is a cognition test and are performed, at beginning and complemented by more sophisticated neuropsychological tests e.g. CERAD or WAIS to observe impairments in perceptual skills, memory, constructive abilities, language, orientation, problem solving, attention, and functional abilities. All this tests are carried out in combination with MRI and PET scans, to rule out other types of dementia (Khachaturian 1985).

The clinical diagnosis of AD is 90% accurate and confirmation of diagnosis carried out postmortem (Polvikoski *et al.,* 2001, Braak, Braak 1991, Khachaturian 1985, Mirra *et al.,* 1991, The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease, 1997) and includes important measurements of extracellular amyloid beta deposits as seen in figure 2.4.2.9.3.

The gold standard for assessing utilized the staging protocols as recommended by Braak and Braak (Braak, Braak 1991) in union with the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) (Mirra *et al.,* 1991) neuropathology scoring system, correlating well with AD prediction and diagnosis (Nagy *et al.,* 1998). Various studies had investigated postmortem brains from AD patients to measure a number of molecules thought to be involved in the pathogenesis of the disease. While silver impregnation techniques worn in the historical detection of SP and NFT (Aho *et al.,* 2010, Wirths *et al.,* 2001).

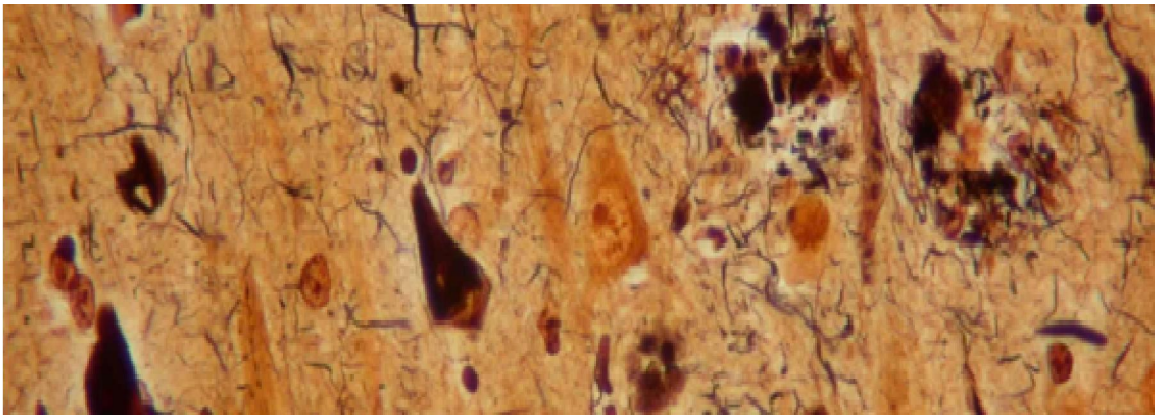


FIGURE 2.3.2.9.3 Senile plaque (SP; arrows) and neurofibrillary tangles (NFT; arrowheads) stained with Bielschowsky silver staining – the two primary hallmarks of Alzheimer's disease (Wirths *et al.,* 2001).

Moreover, immunohistochemical data also shows the presence of neurodegenerative structures mainly spongiform changes similar to those seen in Creutzfeldt-Jakob disease, intracellular Hirano bodies, synaptic loss and disturbances in many neurochemical systems (Duyckaerts, Delatour & Potier 2009). Atrophy of the amygdala, hippocampus, gyri and sulci is also observed (Kidd 2008, Duyckaerts, Delatour & Potier 2009).

A large amounts of dysfunctional proteins have also been identified within the affected regions of the AD brain (Haapasalo *et al.,* 2010, Cuervo, Wong & Martinez-Vicente 2010). The mRNA is only phenotypic variability leads to problems of AD (van Leeuwen *et al.,* 1998). Deletions of GAGAG motifs are the most common modifications that proteins undergo to develop variability. The PP gene has seven such sequences that causes mutated proteins, and disrupt subsequent pathways (van Leeuwen *et al.,* 1998). These mutant proteins can be detected in the brains of AD, as well as Down's syndrome (Wisniewski, Wisniewski & Wen 1985). These transcriptional changes affect postmitotic neurons (van Leeuwen *et al.,* 1998). Some individuals are more susceptible to these changes, either through lifestyle habits or genetic backgrounds (van Leeuwen *et al.,* 1998). In addition to immunohistochemical staining of brain tissue, many studies have investigated the levels of particular substances within patients' CSF and blood (Finch, Morgan 2007, Iqbal *et al.,* 2005, Fiala 2009). While a number of studies have published contradictory results and have questioned the ability of individual biomarkers (Fiala 2009) to interpret between memory problems and actual AD, results in collective measurements of tau protein, prostanes, A peptide species and inflammatory

molecules from CSF might give detailed information on disease progression and subtype (Fagan, Holtzman 2010).

2.3.2.9.4 Types of Alzheimer's disease

The less than 1% affected AD are dominant familial forms (van der Flier *et al.,* 2011) and may cause by mutations (van der Flier *et al.,* 2011, Miyoshi 2009).

2.3.2.9.5 Familial AD and Genetic mutations

Discoveries of families with early onset, dominant forms of the disease lead shows interlinked between amyloid precursor protein (Wisniewski, Wisniewski & Wen 1985) and two enzymes that cleave it (Presenilin 1 – gene of PSEN1, found on chromosome 14 (Levy-Lahad *et al.,* 1995) and Presenilin 2 – gene of PSEN2, found on chromosome 1 (St George-Hyslop *et al.,* 1992)), with the A peptide found in SP within the brains of AD. The mutations of the three genes (PP, PSEN1 and PSEN2) increases the levels of A peptide, which can lead to excess amounts of toxic forms of A peptide (Zhang *et al.,* 2001) which disrupt synapses (Gouras *et al.,* 2010, Takahashi *et al.,* 2004).

2.3.2.9.6 Sporadic AD

This form has no direct known causes and is considered to be a multifactorial disease. Many risk factors add together to initiate the dysfunction of brain as AD (Kukull, Ganguli 2000, Iqbal, Grundke-Iqbal 2010 and Iqbal *et al.,* 2005). The main hallmarks of AD are SP and NFT which have been observed occurring unreasonably in different cases (Jellinger, Attems 2007, Katzman *et al.,* 1988, Duyckaerts, Delatour & Potier 2009) as seen in figure 2.4.2.9.6**.** Many factors have been contributes to triggering AD including dietary, genetic, environmental, and/or combination of these might determine initiation, as well as disease progression.

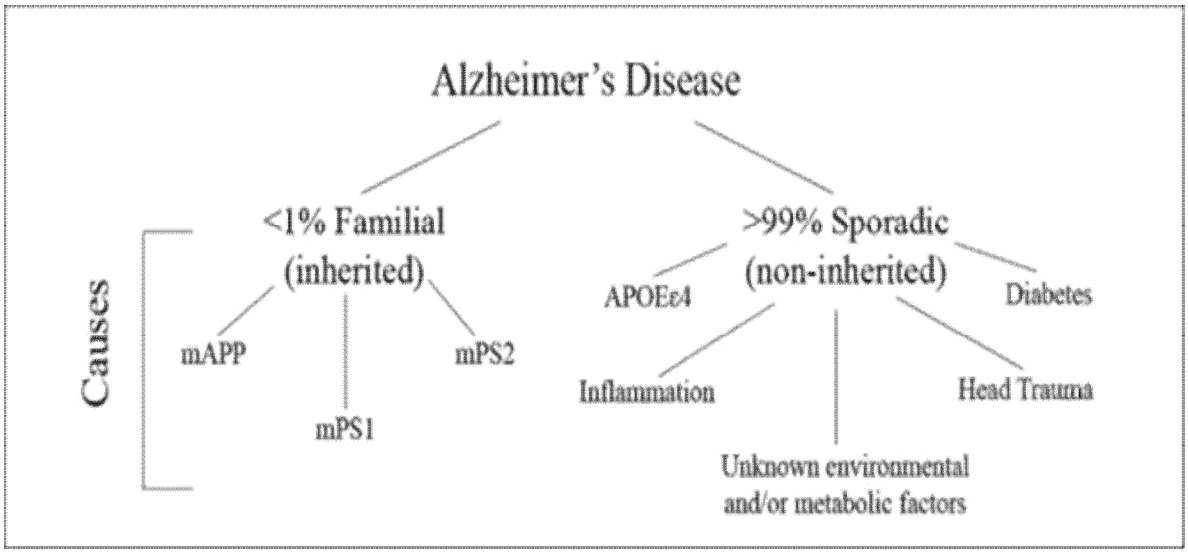


FIGURE 2.3.2.9.6 Proposed causes of Alzheimer's disease. Modified from the article (Iqbal, Grundke-Iqbal 2010). mAPP – mutations in the PP gene; mPS1 – mutations in the PSEN1 gene; mPS2 – mutations in the PSEN2 gene; APOE 4 – apolipoprotein epsilon 4 allele (Katzman *et al.,* 1988).

2.3.2.9.7 Clinical Features in the Three Stages of Alzheimer's Disease:

Stage I (1-3 years)

Memory – Impaired novel learning: mildly impaired remote memory

Visio-spatial Ability – Topographic disorientation; poor complex constructions

Language – impaired verbal fluency; mild anomia; empty, circumlocutory speech

Personality – Apathy; occasional irritability

Psychiatric Features – Sadness; delusions may be present

Motor system – Normal; EEG – Normal

CT/MRI – May be normal or may indicate generalized cortical atrophy, Hippocampal atrophy, or atrophy of entorhinal cortex atrophy, or atrophy of entorhinal cortex

Stage II (2-10 years)

Memory – Severe impairments in recent and remote recall

Visio-spatial Ability – Spatial disorientation; poor constructions

Language – Anomia; paraphasia; impaired comprehension

Calculation - Acalculia

Praxis – Ideomotor apraxia

Personality – Apathy; irritability

Psychiatric Features – Delusions and hallucinations may be present

Motor system – Restlessness; pacing or wandering

EEG – Slowing of background rhythm

CT/MRI – May be normal or may indicate generalized cortical atrophy. Hippocampal atrophy, or atrophy of entorhinal cortex.

Stage III (8-12 years)

Intellectual Function – Severely deteriorated

Language – Echolalia and reiterative speech disturbances; dysarthria; terminal mutism

Motor – Limb rigidity and flexion posture

Sphincter Control – Urinary and fecal incontinence

EEG – Diffuse slowing

CT/MRI – Generalized cortical atrophy, hippocampal atrophy, and atrophy of entorhinal cortex (Malaz *et al.,* 2003).

2.3.2.9.8 Neuropathology

Macroscopically brain is atrophic with reduced weight and secondary enlargement of ventricles. Alzheimer Disease is characterized by degeneration of neurons especially synapses and dendrites, amyloid (βA) plaques, NFT, granulovascular degeneration. Pathological changes are severe in temporal and parietal cortices and on later stages also on the frontal cortex. More than 50% of AD patients shown a white matter changes at the microscopic levels called selective incomplete white matter infarctions and is more common in senile dementia (late onset) rather presenile (young) type (Oliver F and Thomus 1999).

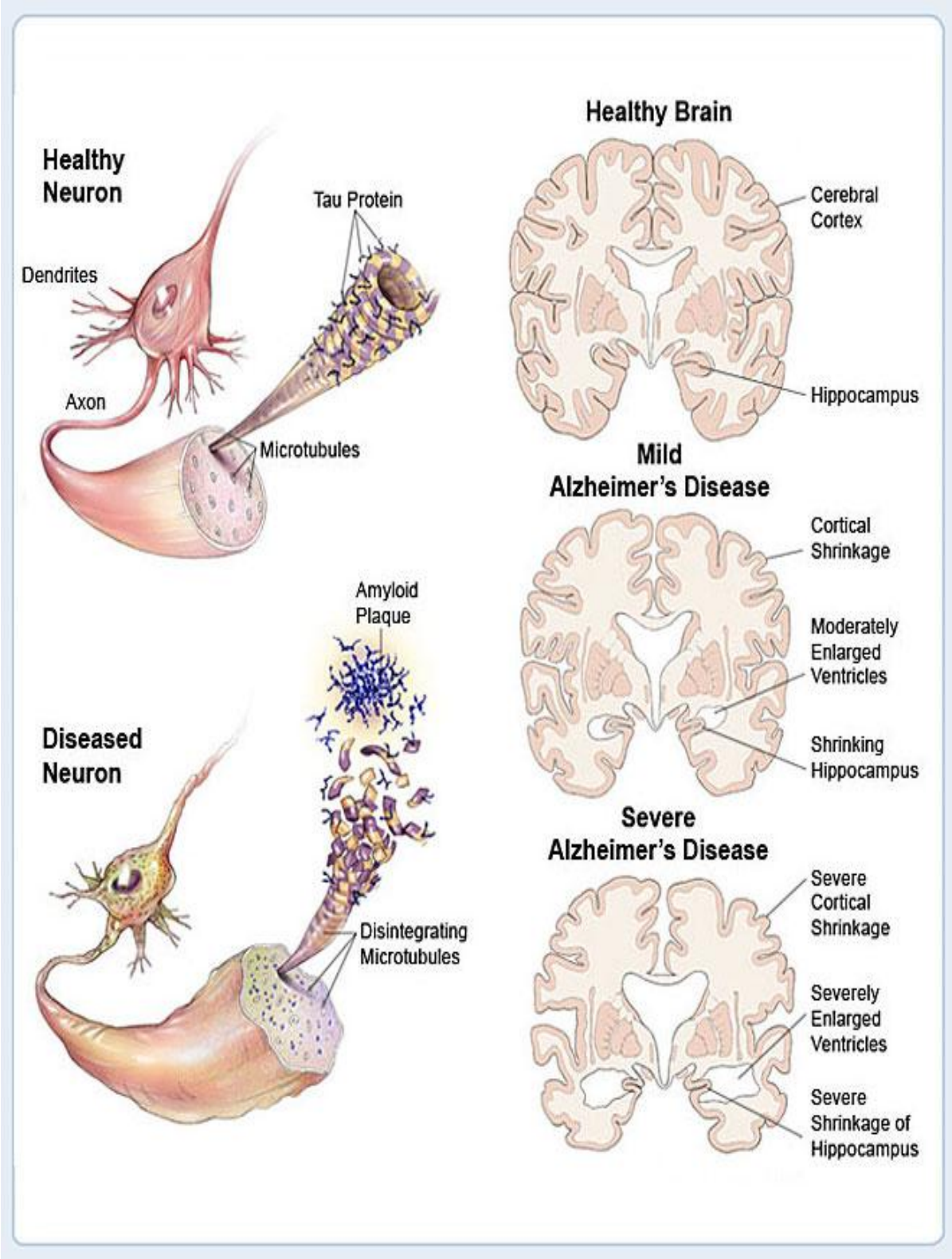


FIGURE 2.3.2.9.8 Neuropathological changes in AD (Oliver F and Thomus 1999).

Pathological changes are severe in temporal and parietal cortices and on later stages also on the frontal cortex. More than 50% of AD patients shown a white matter changes at the microscopic levels called selective incomplete white matter infractions and is more common in senile dementia (late onset) rather presenile (young) type.

2.3.2.9.9 Problems associated with AD and Dementia

Dementia causes a high burden of suffering for patients, their families, and society. For patients, it leads to increased dependency and complicates other co morbid condition. For families, it leads to anxiety, depression, and increased time spent caring for a loved one. The annual societal cost of dementia is approximately $100 billion (health care and related costs as well as lost wages for patients and family caregivers) (Malaz *et al.,* 2003).

2.3.2.9.10 Alzheimer's disease symptoms

Initial symptoms are often mistaken for stress-induced indicators and age-related problems. In early stages of the disease a noticeable changes in behavioural pattern, psychological pattern and inability to handle normal daily activities. Postmortem analyses of AD patients' brains demonstrate losses of neurons and synapses in the temporal/parietal lobes, cerebral cortex, and atrophy of the hippocampi (Khachaturian 1985, Alafuzoff *et al.,* 2008, Polvikoski *et al.,* 1995, Mirra *et al.,* 1991, Duyckaerts, Delatour & Potier 2009, The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease, 1997, Braak *et al.,* 2006).

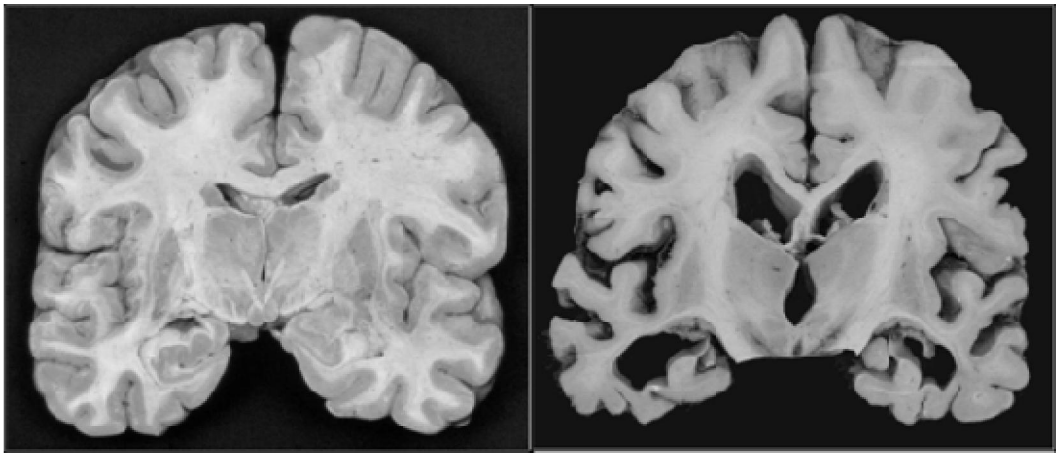


FIGURE 2.3.2.9.10 Diagram showing the shrinkage of an AD patient's brain (right) compared to a normal undemented individual's brain (left). The primary affected areas are the cortex and hippocampus. Image kindly supplied by Professor Hannu Kalimo (Polvikoski *et al.,* 1995).

2.3.2.9.11 Risk factors

Many factors can increase or decrease the risk of an individual's potential to develop the disease. Many years of research have tapering down on a number of mechanisms by which we can reduce the risk of getting AD, including diet (Grant *et al.,* 2002, Smith, Petot & Perry 1999), happiness (Berger *et al.,* 1999, Chen *et al.,* 1999) and exercise (Larson *et al.,* 2006). Some risk factors cannot be change, like gender (Behl 2002) and genetics (Bertram *et al.,* 2007). A few of the primary impacting factors are discussed below.

2.3.2.9.11.1 Environmental risks

A Longitudinal and retrospective studies (Kukull, Ganguli 2000, Grant *et al.,* 2002) have shaped many ideas for ways to maintain ones cognitive reserve and allow an individual to live healthily into old age with intact brain function. While clinical trials have failed to confirm some beneficial lifestyle habits, most likely due to the difficulty in designing such a long-lived study, and some argue against the presence of these risk factors (Daviglus *et al.,* 2010Grant *et al.,* 2002, Qiu, Kivipelto & Fratiglioni 2011).

Exercise is one of the way to maintain cognitive function (Larson *et al.,* 2006, Scarmeas *et al.,* 2009) which keeps the body operating effectively, help to keeps hormones and the immune balance, reduced body fat and keeping the cardiovascular system healthy (Kidd 2008). Knowing more than one language (Chertkow *et al.,* 2010) is also beneficial to brain maintenance. Cholesterol lowering drugs (Wolozin *et al.,* 2000) also implicit in AD. Many studies signify AD patients have significantly lower mean plasma concentration of HDL-cholesterol (Kuo *et al.,* 1998), high plasma concentrations of triglycerides, glucose and larger mean waist circumference compared to control (Razay, Vreugdenhil & Wilcock 2007, Altman, Rutledge 2010). The patients who took estrogen replacement therapy may also decrease the risk of developing AD (Behl 2002). A reduced level of oestrogen during and after menopause has been observed to increase the incidence of AD in post-menopausal women (Sunday *et al.,* 2007). Oestrogen is considered a neuroprotective hormone (Hua *et al.,* 2007) might be act as transcription regulator, antioxidant, enhance synaptic plasticity and connectivity (Candore *et al.,* 2010), and has been shown to protect neuronal cells against brain tissue (Gottfried-Blackmore, Croft & Bulloch 2008). A low incidence in pre-menopausal

women of ischemic stroke, suggestive of that oestrogen can also protect against neuro-trauma (Garcia-Segura, Azcoitia & DonCarlos 2001).

The food that contain antioxidants and vitamins (Smith, Petot & Perry 1999, Grant 1999), such as vitamin E (Morris *et al.,* 2005), are beneficial and help to retain cognition (Kidd 2008, de Rekeneire 2006, Kannappan *et al.,* 2011). Moreover moderate consumption of alcohol (Mukamal *et al.,* 2003, Criqui, Ringel 1994) especially red wine (Di Matteo *et al.,* 2007), that contains the antioxidant such as resveratrol (Kim *et al.,* 2006, Criqui, Ringel 1994). A cohorts study showed low incidences of AD who consumed curcumin (Ganguli *et al.,* 2000, Chandra *et al.,* 2001, Awasthi *et al.,* 2010). Other foods or diets, and beverages which are purportedly beneficial for preventing AD are coffee (Arendash, Cao 2010), liquorice (Kannappan *et al.,* 2011), and a Mediterranean diet (Scarmeas *et al.,* 2009), which lower cholesterol levels and prevent heart disease by providing dietary vitamins, antioxidants, anti-inflammatory molecules, omega-3 fatty acids and minerals (Grant *et al.,* 2002). The fat composition and fat quantity within a diet also warrant monitoring (Grant *et al.,* 2002, Altman, Rutledge 2010), as studies indicate fatty acids can initiate Presenilin-1 generation and saturated fatty acids can induce HP-tau formation (Altman, Rutledge 2010).

The deficiencies vitamin B12, can increase the risk of developing AD (Thomas, Fenech 2007, Aisen *et al.,* 2003). B12 is one of the most important nutrient that is necessary to maintain healthy nervous systems as it work as cofactor in the tricarboxylic acid cycle, and as a methyl carrier, involved in DNA metabolism (Thomas, Fenech 2007, Wagner *et al.,* 1995, Scarpa *et al.,* 2006). Adequate levels of this vitamin are needed to maintain these essential pathways, and improvements of cognitive impairment and lowered brain atrophy rates (Aisen *et al.,* 2003).

A gender (Gao *et al.,* 1998), a high fat diet (Solfrizzi *et al.,* 2008), family history (Breitner, Folstein & Murphy 1986, Breitner, Murphy & Folstein 1986, Breitner *et al.,* 1990), diabetes (Kroner 2009, Carlsson 2010), education (Addae, Youssef & Stone 2003), hypertension (Kalaria 2003), a history of head trauma (Guo *et al.,* 2000, Mayeux *et al.,* 1995), and susceptibility from particular genes (Bertram *et al.,* 2007) are risk factors for AD. The

preventative measures, such as lifestyle changes including avoiding toxins, overcoming depression (Berger *et al.,* 1999, Chen *et al.,* 1999) and being married (Helmer *et al.,* 1999, Daviglus *et al.,* 2010).

2.3.2.9.12 Concomitant diseases

Lifestyle diseases such as cardiovascular diseases (CVD) (Stampfer 2006, Kalaria 2003), diabetes (Kroner 2009) share many risk factors with AD and are also suggested to develop AD progression when present (Stampfer 2006, Luoto *et al.,* 2009, Stampfer 2006, Martins *et al.,* 2009, Iacono 2009, Stern 2009, Kivipelto *et al.,* 2005, Martinez *et al.,* 2002, Finch, Morgan 2007, Giunta 2008). The mechanisms by which CVD are causes AD are not clearly understood but dyslipidemia (Kalaria 2003), endothelial injury contribute to atherosclerotic CVD and this dysfunction within the brain is a key mediator of stroke and vascular dementia (Kalaria 2003), which are thought to contribute to disease development, progression or even cause AD (Kivipelto *et al.,* 2005, Luoto *et al.,* 2009). A cerebral amyloid angiopathy (CAA) is bring into being in up to 80% of AD patients (Altman, Rutledge 2010, Kalaria 2003), without atherosclerotic CVD. Type 2 diabetes diabetes also responsible for many risk factors for AD (Stampfer 2006, Figaro 2006, Lovestone 1999, Kroner 2009) and treatment of diabetes may reduce AD neuropathology (Beeri *et al.,* 2008, Tukiainen *et al.,* 2008 & Reiman *et al.,* 2005).

2.3.2.9.13 APOE and Lipidomics

AD patients having a minimum of one copy of the harmful allelomorph (Finch, Morgan 2007, Farrer *et al.,* 1997, Altman, Rutledge 2010). Sequence dose conjointly has a bearing on AD risk, relative risk of 3.2 for 3/ 4 carriers and 14.9 for 4/ 4 carriers to develop the disease (Farrer *et al.,* 1997), yet as moving the age of onset (Khachaturian *et al.,* 2004). The APOE sequence encodes a 34kDa conjugated protein, apolipoprotein E that has several roles in brain development, growth, function, maintenance, and anti-inflammatory properties, as well as repair (Horsburgh *et al.,* 2000). APOE may be a part of very-low-density lipoproteins (VLDLs) and is a receptor that participates in distribution of sterol and helps to manage lipide levels at intervals the brain and round the body (Finch, Morgan 2007, Altman, Rutledge 2010, Wolozin *et al.,* 2000, Jick *et al.,* 2000).

APOE is generated inside the brain by interstitial tissue cells (Altman, Rutledge 2010, Pitas *et al.,* 1987) and facilitate the pathophysiology of AD through promotion of NFT formation, amyloid deposition, neurotoxicity and aerophilous stress, moreover as increasing the porousness of the BBB (Altman, Rutledge 2010, Burns *et al.,* 2003, Jofre-Monseny *et al.,* 2007, Mas *et al.,* 2006). APOE might verify the incidence and severity of the many concomitant diseases by pathogens appreciate hepatitis C (Wozniak *et al.,* 2002) and herpes simplex (Itzhaki *et al.,* 1997).

The number of studies have investigated the mechanisms through that APOE 4 might add to AD compared to different alleles, and there's a wealth of data suggesting ways towards that treatments might be directed. APOE, is a part of VLDLs, is hydrolysed by compound protein enzyme (LpL), set at the brain microvascular epithelium, and will probably directly injury the BBB and facilitate production of pro-inflammatory mediators because of the high concentrations of lipolysis product it creates (Altman, Rutledge 2010). This lipoid accumulation that causes cell dysfunction and death, conjointly referred to as lipotoxicity, has been related to cell death, and dysfunction of mitochondria, similarly because the lysosomal and autophagy pathways (Altman, Rutledge 2010).

To more support the involvement of lipoid disfunction within the aetiology of AD, researchers investigated genes concerned in lipoid, and specifically steroid alcohol metabolism. Similarly as regulation lipoid and aldohexose pathways (Corre, Galibert 2005), USF1 has been shown genes concerned in reaction and cell cycle control similarly as PP transcription, colligation physical property, and neural survival and differentiation (Corre, Galibert 2005, Kovacs *et al.,* 1995, Yang *et al.,* 2002, Naukkarinen *et al.,* 2005). One study work polymorphisms among USF1 but, was negative for association with AD (Shibata *et al.,* 2006), though queries still stay on its involvement in pathological process, because of its vital role as a master transcriptional regulator.

Some studies have already steered enhancements in AD risk reduction through the employment of statins (Wolozin *et al.,* 2000, Jick *et al.,* 2000, Sparks 2005, Solomon 2009), though meta-analyses have usually been negative (Zhou 2007, McGuinness 2010). It can not

be prejudicious but, for a personal to scale back high steroid alcohol levels, considering the advantages obtained from a healthy heart. The helpful effects of statins could also be higher suited to bar of Alzheimer's sickness (Kivipelto 2005).

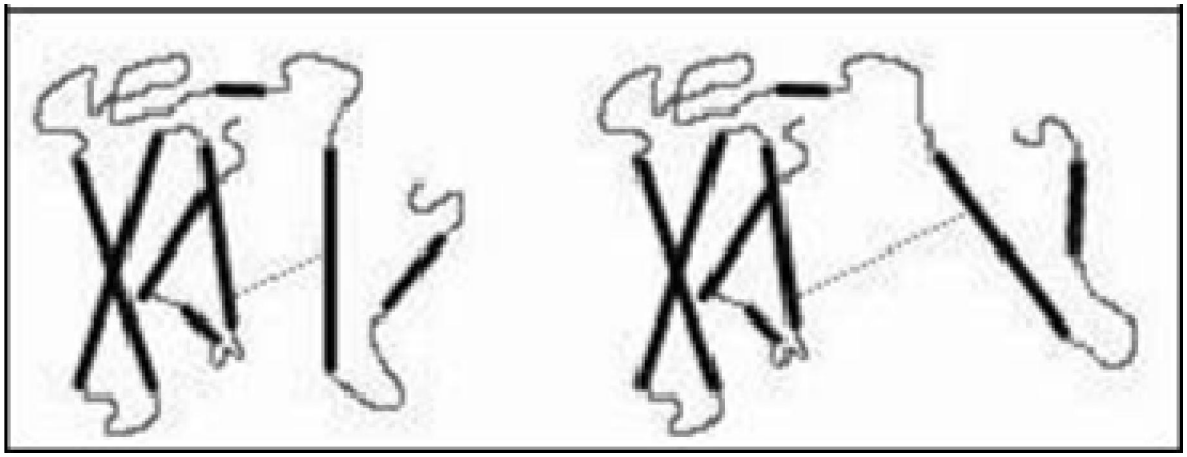


FIGURE 2.3.2.9.13 Schematic representation of APOE 4 confirmation before (left) and after (right) a high-fat meal. Dotted lines indicate the salt bridge between the amino acids R61 and E255 (Wolozin *et al.,* 2000).

2.3.2.9.14 Polymorphisms & Genes

Initial studies into the genetic risk of AD focused on pathways that were celebrated to be concerned within the unwellness, but thanks to the massive range of debatable studies, leading to an oversized range of potential unwellness factors, and therefore the little impact of the known risk genes, the tide is dynamical. Few newer studies are currently utilizing order wide association studies (GWAS), wherever up to five hundred 000 single ester polymorphisms (SNPs) will be detected at the same time (Lambert JC *et al.,* 2009, Beecham *et al.,* 2007 & 2009, Harold *et al.,* 2009).

CLU or clusterin, found on body is additionally called APOJ and as suspected, encodes a lipide transport molecule, ready to bind A amide like APOE (Jenne, Tschopp 1992, Jones, Jomary 2002). CLU possibly participates in a very amide transportation each out of and presumably into the brain (Jenne, Tschopp 1992, Jones, Jomary 2002, Calero *et al.,* 2000, DeMattos *et al.,* 2004). Consistent with the NCBI supermolecule information, CLU has been rumored as being concerned in death, tumor progression and neurodegenerative disorders. It's little effects on AD risk and possibly participates in unwellness pathologic process through organic phenomenon modulation or harm evoked Guerreiro *et al.,* 2010).

PICALM, found on body eleven, encodes the phosphatidylinositol binding clathrin assembly supermolecule and is assumed to be concerned in colligation neurochemical unharness and living thing trafficking (Dreyling *et al.,* 1996, Tebar, Bohlander & Sorkin 1999, Yao *et al.,* 2005). Consistent with NCBI, PICALM has several names and is concerned in endocytosis. Researchers recommend its involvement in AD relates to PICALM's location in epithelial tissue cells and is possibly related to transporting A amide through the BBB (Baig *et al.,* 2010).

EXOC3L2 encodes exocyst advanced part 3-like a pair of, is found on body nineteen, and its operate consistent with NCBI is unknown. Studies indicate it's transactivated by the viral hepatitis virus X substance (Seshadri *et al.,* 2010), that suggests it should participate within the inflammation method.

BIN1 encodes bridging measuring device one and is found on body a pair of. NCBI reports the supermolecule is concerned in colligation cyst endocytosis, as well as cyst formation, and studies indicate BIN1 facilitates cell death and incorporates a role in membrane organisation (Seshadri *et al.,* 2010).

CR1 produces complement part (3b/4b) receptor one, that is that the main receptor for the complement C3b supermolecule, a significant a part of the innate system and binds to amide (Rogers *et al.,* 2006, Wyss-Coray *et al.,* 2002). Through this mechanism it's thought to push clearance of amide and thus have an effect on AD risk. The sequence for it's found on body one, and therefore the membrane compound protein CR1 mediates cellular binding to immune complexes or particles that have activated the complement system (Rogers *et al.,* 2006, Wyss-Coray *et al.,* 2002, Kuo *et al.,* 2000, Zhou *et al.,* 2008).

SORL1 (also called LR11, SORLA or SORLA1), or sortilin-related receptor, is found on body 11q23 and produces a receptor for somatic cell APOE, denseness compound protein receptor category A. SORL1 binds A PP and regulates its sorting into endocytic-or recycling-pathways (Rogaeva *et al.,* 2007). High levels of this receptor are related to lower A amide production thanks to SORL1 promoting usage of PP, rather than transporting it to endosomes

or lysosomes wherever A amide is made (Rogaeva *et al.,* 2007). Several SORL1 SNPs are related to AD risk (Bertram *et al.,* 2007, Rogaeva *et al.,* 2007), but more factors, each genetic and non-, also are thought to have an effect on expression of this receptor, though the implications of those aren't absolutely understood.

GWA 14q32.13 (rs11622883) was known in GWAS and doesn't find to any celebrated sequence loci and thus it's operate is unknown (Grupe *et al.,* 2007). More analysis is going to be needed to work out the link SNP rs11622883 has with AD.

TNK1 encodes 'tyrosine enzyme, non-receptor, 1' and is found on body seventeen. It mediates living thing signalling behind receptor activation, consistent with (Azoitei *et al.,* 2007, Felschow, Civin & Hoehn 2000). Studies have determined that TNK1 could be a molecular switch that determines the properties of tumour necrosis factor signalling. By inhibiting NF B, TNK1 facilitates the tumour necrosis factor apoptotic pathway resulting in death (Azoitei *et al.,* 2007).

IL8 or lymphokine eight, settled on body four, produces associate degree inflammatory molecule celebrated for its pro- and anti- inflammatory actions as a chemokine (Li *et al.,* 2009). IL8 could be a major intermediator of inflammatory responses through its functions as a chemoattractant associate degreed properties as an angiogenic issue (Li *et al.,* 2009).

Many more genes are related to AD risk, but the chance effects are little. Several studies recommend it's possibly a mix of multiple genes, yet as environmental effects that impact on associate degree individual's AD risk, but studies revealing associations do reveal pathways which will be concerned within the aetiology of the unwellness.

2.3.2.9.15 Epigenetics

Epigenetics could be a comparatively new field of study (Vanyushin 2007) and involves the cell's means of turning on and off genes, which may be manipulated by food intake and different external pressures, additionally as moving the biological process stages of Associate in nursing individual's life cycle. Epigenetics additionally manipulate upregulation and down-

regulation of genes, additionally as regional changes in numerous organs because of native stimuli, as well as pathogens (Vanyushin 2007).

When factor promoters become alkyl group, they're physically blocked from binding to transcription factors and primarily inactivated, the factor suppressed and supermolecule production repressed (Lee, Ryu 2010). The alkyl group DNA is related to methyl-CpG-binding domain proteins (MBDs), that recruit different elements adore histones that inactivate the factor region or locus, forming what's referred to as chromatin granule (Lee, Ryu 2010, Suzuki, Bird 2008).

Studies have supported proof for this mechanism, as well as observations of inflammatory genes being hypomethylated in AD cortex (Akiyama *et al.,* 2000), and investigations into epigenetic modifications in monozygotic twins discordant for AD, indicating that DNA methylation was reduced within the AD-twin (Mastroeni *et al.,* 2009). The suggestions for causes behind these epigenetic changes vary, with some proposing age-related effects that gently lower the methylation content of genes, to others that counsel a decrease in surface-to-air missile levels – the first methyl group donor of cells – can be answerable (Scarpa *et al.,* 2006, Lee, Ryu 2010).

2.3.2.9.16 Causal theories

2.3.2.9.16.1 Brief history of causes

Since the outline of the sickness in 1907 by Alois Alzheimer (Alzheimer 1907), there are several theories on the reason for AD. Within the 1960's a correlation was found between the numbers of SP and NFT and psychological feature decline, gap up investigations into the causes behind these neuropathological lesions (Lage 2006). it had been not till the 1970's but, that AD was formally listed as a sickness and now not thought of a section of traditional aging, as mentioned within the review (Lage 2006).

Classified as a sickness, AD attracted intensive analysis into its causes. Proof of huge amounts of the metal metal found in NFT in AD patients' brains (Perl, Brody 1980, Terry, Pena 1965) cause theories that more than this metal caused AD. This theory is usually laid-off today, because of largely indirect evidence.

Most current theories close AD pathologic process involve the identification of abnormal or giant amounts of the molecules found in SP and NFT at intervals AD patients' brains, similar to A amide. Some researchers have advised imbalances within the brain's physiological condition atmosphere might cause AD (Crouch, White & Bush 2007), beside viruses and microorganism (Kamer *et al.,* 2008, Itzhaki, Wozniak 2004) that area unit able to cross the blood-brain-barrier, also because the risk that the system loses the flexibility to perform properly and effectively (Giunta 2008, Miklossy 2008).

2.3.2.9.16.2 Cholinergic hypothesis

The cholinergic hypothesis (Martorana, Esposito 2010, Contestabile 2010) was theorised as a result of investigations showed that AD brains had lower levels of neurotransmitter – a serious neurochemical within the brain – than non-demented older cases. Collectively of the oldest causative theories of AD, the disturbances within the cholinergic system have conjointly been the main target of most treatments accessible on the market, though they fail to produce abundant improvement in delaying clinical symptoms of the unwellness.

Treatments of AD supported the cholinergic hypothesis (donepezil, rivastigmine and tacrine etc.) are enzyme inhibitors that act just by deterring the actions of enzyme – associate degree catalyst that breaks down the neurochemical neurotransmitter. By reducing enzyme, the brain would be able to retain neurotransmitter and so operate properly.

Although newer compounds treating this avenue of disfunction are developed with fewer aspect effects (in order to produce higher drug doses) (Martorana, Esposito 2010), this is often not the sole factor that goes wrong in an advert brain and so researchers have looked to different causative theories and newer treatments which will have additional improvement on patients' symptoms.

2.3.2.9.16.3 Amyloid theory

The most ordinarily supported hypothesis for the reason behind AD relates to a macromolecule expressed in several cells, of unknown operate and concerned in familial AD

because of mutations within the cistron that code for it (Wisniewski, Wisniewski 1985). Athough its operate isn't fully understood, -amyloid precursor macromolecule (A PP) is usually recommended to be vital for nerve cell growth (Turner *et al.,* 2003, Vasto *et al.,* 2008, Priller *et al.,* 2006), signalling, and should additionally operate as Associate in Nursing inhibitor (Crouch 2007) and a metalloprotein, modulating copper transport and metabolism (Turner *et al.,* 2003, Priller *et al.,* 2006, Kong *et al.,* 2007).

The amyloidogenic version of the pathway involves cleavage by -secretase followed by the -secretase, emotional the 40-43 aminoalkanoic acid amyloid beta (A) amide, thought to cause the neurodegenerative illness (Selkoe 2001, Findeis 2007). The catalyst action of -secretase leaves a C-terminal fragment referred to as APP-CTF or C99, at intervals the membrane and releases APPs into the extracellular house. Once amide generation by -secretase from the C99 fragment, the A amide is extracellularly secreted (Rogaeva *et al.,* 2007).

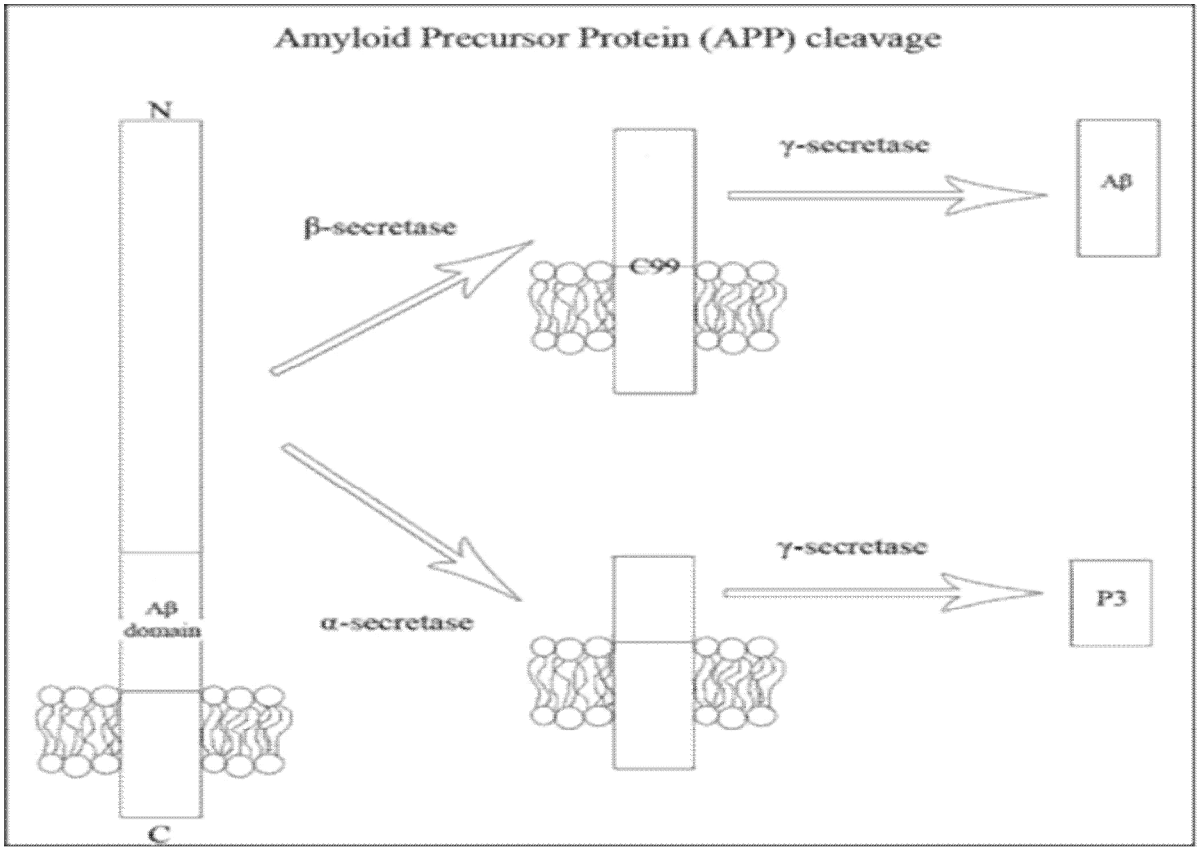


FIGURE 2.3.2.9.16.3 The amyloid beta component is formed by subsequent cleavage by the enzymes -secretase and -secretase of the amyloid precursor protein (APP), whilst initial cleavage with the -secretase results in the formation of the P3 peptide fragment and inhibits the formation of the neurotoxic amyloid beta protein (Findeis 2007).

2.3.2.9.16.4 Tau theory

Tau has six isoforms within the brain, that area unit all created from the one gene: MAPT, that is found on body seventeen (Lace, writer & Ince 2007, Andreadis, Brown & Kosik 1992). Variations between the isoforms relate to completely different numbers of binding domains and differential junction of the gene's sixteen exons, making six proteins starting from 352 – 441 amino acids long. though some studies have prompt there area unit completely different ratios of explicit isoforms in pathological states, all forms area unit gift in AD (Iqbal 2009, Lace, writer & Ince 2007, Andreadis, Brown & Kosik 1992).

Phosphorylation and de-phosphorylation of letter of the alphabet happens below traditional equilibrium conditions, with supposed hyperphosphorylation occurring in pathological brains (Lace, writer & Ince 2007). This phosphorylation situation causes the phosphorylated letter of the alphabet (HP-tau) to dissociate from microtubules and mixture into dense compact paired spiraling filaments (PHF) at intervals the cell – ultimately killing the somatic cell that it's speculated to be supporting and leading to the NFT ascertained in postmortem AD brains (Iqbal 2009). it's conjointly been ascertained that the letter of the alphabet gift in PHF has higher D-forms of aspartate and aminoalkanoic acid than L-forms, suggesting it's long lived and so might enhance aggregation (Shapira, Austin & Mirra 1988, Kenessey *et al.,* 1995). A ensuant stage within the life cycle of NFT happens after they survive once the death of the somatic cell, forgoing 'ghost' tangles that area unit simply seen with silver staining or immunohistochemical techniques (Duyckaerts, Delatour & Potier 2009).

NFT consist largely of HP-tau, however also are best-known to incorporate casein enzyme II, peptidase nexin I, polysaccharide salt proteoglycan, embryonic cell protein, tubule association protein-5, and ubiquitin, amongst alternative molecules (Duyckaerts, Delatour & Potier 2009, Hasegawa, Arai & Ihara 1990, Perry *et al.,* 1987, Rosenblatt, Geula & Mesulam 1989, Baum *et al.,* 1992). The participation of those molecules in NFT development is presently undetermined, but they might even be a secondary mechanism to the emergence of those brain lesions.

A staging system developed by Braak and Braak (Braak, Braak 1991) monitors the progression of AD into six supposed Braaks' stages, supported the placement and density of

NFT. Stages I and II describe neuropathology containing NFT found within the entorhinal, transentorhinal and CA1/subicular parts of the hippocampus. Increasing numbers of letter of the alphabet pathology within the limbic brain represent stages III and IV, wherever higher numbers within the hippocampus commonly correspond with the severity of dementedness. NFT found within the isocortical areas resolves stages V and VI and complete the progression of the sickness. Newer staging systems have used assay (Braak *et al.,* 2006) though some area unit nevertheless to be valid (Alafuzoff *et al.,* 2008).

HP-tau and NFT aren't solely seen within the brains of AD patients; they're conjointly characteristic lesions of alternative neurodegenerative diseases, together termed 'tauopathies' (Lace, writer & Ince 2007). Such diseases embody many entities within the major letter of the alphabet molecular category of frontotemporal body part degenerations (FTLD-tau), together with to Illustrate infrequent corticobasal degeneration, progressive supranuclear palsy, Pick's sickness and argyrophilic grain sickness (Mackenzie *et al.,* 2010). Some noted mutations within the cistron that code for letter of the alphabet, conjointly directly cause neurodegeneration within the style of hereditary frontotemporal dementedness and degenerative disorder joined to body seventeen (Lace, writer & Ince 2007), while not the presence of SP. The letter of the alphabet mutations directly disrupt the conventional functioning of the letter of the alphabet supermolecule and conjointly substantiate the neurotoxicity of HP-tau. Alternative mechanisms inflicting the event of NFT area unit projected to be through associate degree imbalance between the activities of kinases and phosphatases that management the phosphorylation of the letter of the alphabet supermolecule (Iqbal 2009). While toxin itself, HP-tau is believed to be secondary to a amide accumulation in AD (Iqbal 2009, Lace, writer & Ince 2007). This can be the commonly accepted theory, though there are a unit some researchers World Health Organization believe the letter of the alphabet supermolecule is answerable for the initiation of the neurodegeneration, thanks to its obvious neurotoxicity and its ability to be prophetical of dementedness severity (Thomas, Fenech 2007, Iqbal 2009, Lace, writer & Ince 2007). HP-tau pathology is commonly ascertained many decades before amide deposition and so advocates that letter of the alphabet may well be the cause behind SP and so AD (Lace, writer & Ince 2007, Braak, Del Tredici 2011), but studies have conjointly incontestable that A oligomers area unit capable of

instigating phosphorylation of the letter of the alphabet supermolecule (Lace, writer & Ince 2007), corroborating the A theory and feat this subject undecided.

2.3.2.9.17 Other potential causes

2.3.2.9.17.1 Inflammation

The plaques and tangles square measure additional specific to AD, there square measure alternative characteristics of the morbid brains that square measure broader in their disruption of brain perform and inflicting neurodegeneration. Inflammation, seen in several diseases of the older, is determined in AD brains and has long been thought to initiate the recognised pathology. New theories suggesting amide is associate acute part supermolecule (Soscia *et al.,* 2010, Kontush 2005) and concerned in immunity additionally support the participation of inflammation in AD aetiology. Additionally, a standard risk issue for the malady is brain injury (Guo *et al.,* 2000, Mayeux *et al.,* 1995), suggesting that chronic inflammation may initiate or a minimum of partake within the course of AD.

Multiple inflammatory markers square measure determined in postmortem AD brains, as well as pro-, anti- and post-acting molecules (Finch, Morgan 2007, McGeer, McGeer 2007), also because the activation of the resident immune (glial) cells within the central systema nervosum – neuroglia and astrocytes (Finch, Morgan 2007, McGeer, McGeer 2007), though this is often not continuously the case (Verkkoniemi *et al.,* 2001). These are found within the AD-affected regions of the brain and additionally localised with SP and NFT (Tuppo, Arias 2005). Alternative researchers have even instructed that SP and NFT square measure byproducts of the host response to basic infective processes (Castellani *et al.,* 2008). Though still alternative researchers have instructed that neuroglia lose practicality and this might participate in malady aetiology (Graeber, Streit 2010). Some samples of upregulated inflammatory elements found in AD brains square measure prostaglandins, complement element proteins, anaphylotoxins, adhesion molecules, cytokines, chemokines, proteases, enzyme inhibitors, free radicals, pentraxins cherish CRP and nuclear factor-kappa B (NF B), also as acute part proteins, amongst alternative inflammatory responses (Moreira 2008, Yasojima *et al.,* 2000, McGeer, McGeer 2007, Casadesus *et al.,* 2007, McGeer, Klegeris & McGeer 2005, Terai, Matsuo & McGeer 1996). These successively will initiate additional

inflammatory pathways and indicate that inflammation positively participates in malady progression, though to what extent remains undetermined.

Microglia reportedly turn out, or signal alternative cells to provide pro-inflammatory molecules interleukin-1 , interleukin-6 and tumor mortification factor-alpha (TNF), that recruits lymphocytes to inflamed areas by fixing tube cell adhesion (Ghoshal *et al.,* 2007). Also as being capable of directly killing cells (Ghoshal *et al.,* 2007), the excretion of neurotoxins and excitory neurotransmitters will increase and spiral out of management within the AD brain.

Astroglia cells square measure major players within the maintenance of the BBB, as well as regulation its porousness (Altman, Rutledge 2010). Studies have reported BBB integrity is essential to AD progression, though the explanation for this porousness has not been determined as exclusively the result of astroglial actions, or a combined effort of alternative additional elusive factors (Altman, Rutledge 2010). Whilst neuroglia and astrocytes square measure the resident immune cells at intervals the brain, the neurons themselves have additionally been determined responding to inflammatory signals that may participate in malady pathology (Jofre-Monseny *et al.,* 2007, Wozniak *et al.,* 2002, Itzhaki, Wozniak 2006, Itzhaki, Wozniak & Dobson 2002).

Further indications of inflammation's involvement in AD return from studies showing redoubled levels of inflammatory markers in morbid brains, also as in blood (Finch, Morgan 2007, Schmidt *et al.,* 2002). To boot, high levels of those markers can even indicate progression of the malady (Schram *et al.,* 2007), though what this result has on initiation of the malady remains debated. Such markers as CRP square measure capable of activating clearance of cellular rubble through the complement pathway and macrophages (Bottazzi 2006, Pepys, Hirschfield 2003). Studies have shown CRP created domestically at intervals the brain associated studies have also instructed an upregulation of CRP is gift close to SP and NFT (Yasojima *et al.,* 2000).

Additional restrictive molecules concerned in inflammatory responses cherish the COX enzymes (Ho *et al.,* 2006), that turn out prostaglandins by changing arachidonic acid, are instructed to tie inflammation in with redoubled production of A amide through redoubled autocoid E2 production (Ho *et al.,* 2006). The mechanism by that AD may develop with inflammatory responses taking part is not any doubt a fancy one and doubtless includes several alternative factors. Genes or polymorphisms that enhance associate individual's inflammatory response or condition to a specific malady (s), additionally to the decrease in effectiveness of maintenance systems in adulthood, may produce a system of chronic inflammatory activation and permit insults from extra diseases. This aging system with increased innate immune responses has been instructed by those backing the 'inflammaging' theory (Giunta 2008).

2.3.2.9.17.2 Oxidation and Mitochondrial dysfunction

The creation of reactive O species (ROS) with age results from several equilibrium mechanisms from manufacturing energy to replication of desoxyribonucleic acid for cellular growth. Throughout aging, the processes that maintain macromolecule integrity and cleanup systems scale back in potency and cause the build of varied errors (van Leeuwen *et al.,* 1998). These successively cause any mistakes to occur and also the system continues to suffer with the build-up of dysfunctional proteins and ROS. ROS themselves will directly oxidise and harm desoxyribonucleic acid, lipids and proteins and induce stress-responses, additionally as facilitate cell death through mitochondrial pathways (Altman, Rutledge 2010, Casadesus *et al.,* 2007).

A projected theory relating to oxidization and alternative purportedly are agents of AD suggest that some people have a better threshold for the harm (Stern 2009) caused throughout aging. This higher tolerance to aerobic (and alternative) harm prevents the individual from succumbing to the build-up of ROS and other affected molecules (proteins, lipids, A amide etc.) and so protects from the event of AD. This higher tolerance may well be relating to diet, environmental factors or genetic science (Dumurgier *et al.,* 2010), however yet remains AN unsure space that's tough to prove while not extremely elaborate longitudinal studies.

Mitochondria square measure the energy production factories of the cell (Cheng, Hou & Mattson 2010) and any defects inside these sometimes cause serious disorders (Gibson, Sheu & Blass 1998). Having to wear down giant amounts of energy generation for the cell, as well as ATP and NAD+ production, these industrial units have additionally developed ways in which of managing the Brobdingnagian amounts of waste that square measure created. throughout aging but, these systems dwindle economical and may break down and this can be one amongst the projected mechanisms that AD is believed to initiate – through mitochondrial pathology (Gibson, Sheu & Blass 1998, Onyango *et al.,* 2010, Swerdlow, Khan 2009, Twig, Hyde & Shirihai 2008).

The large quantity of ROS and alternative modify molecules, appreciate lipids and aldohexose, square measure burdensome to the mitochondrial machinery and become tougher to scrub up or take away with age, inflicting the mitochondria to perform less effectively, which might have forceful implications for each the individual cell and body as an entire (Swerdlow, Khan 2009). Additionally to the mechanism of aging generally, some studies have instructed haplotypes and alleles of mitochondrial genes square measure a lot of vulnerable to mitochondrial pathology (Swerdlow, Khan 2009, Maruszak *et al.,* 2009, van der Walt *et al.,* 2004). Studies have found associations between mitochondrial haplotypes H, U, K, J, T and faded mitochondrial perform, additionally as associations with AD itself (Maruszak *et al.,* 2009, van der Walt *et al.,* 2004).

Changes in mitochondrial dynamics, as delineate on top of, are related to the processes of learning and memory appreciate long run synergy (LTP) and recovery of synapses in phases of high colligation activity, that once not functioning properly are marked with modify desoxyribonucleic acid and ribonucleic acid and structural mitochondrial abnormalities (Cheng, Hou & Mattson 2010). In addition, mitochondrial desoxyribonucleic acid mutations accumulated over the cell's lifecycle for no matter reason might also contribute to aging and illness aetiology as instructed by animal studies (Trifunovic 2004), and proof of the presence of malformed mitochondria, as well as abnormal shapes and sizes (Trifunovic 2005). In addition, faded numbers of mitochondria were found in aged people (Trifunovic 2005). Whilst the proof of mitochondrial involvement in AD is substantial, it's unlikely that it's the

(only) method that initiates the illness and it's instructed that maybe it's a secondary event to the particular pathology that causes AD. While analysis still continues on this subject, it's arduous to once and for all agree on the mitochondrial role in AD pathological process.

2.3.2.9.17.3 Metal imbalance

The many previous studies recommended that excess amount of aluminum accumulated from food within the brain was one of the reason behind AD (Perl, Brody 1980, Crouch, White & Bush 2007, Adlard *et al.,* 2008). Another theories conjointly still counsel metals absorbed from the surroundings may well be answerable, comparable to animal studies indicating copper found in drinkable combined with a high sterol diet initiated amide accumulation (Marx 2003, Sparks, Schreurs 2003), and lead exposure throughout development that will increase A amide production within the brain later in life (Wu *et al.,* 2008).

Research has shown that metal chelators (Adlard *et al.,* 2008) may capable of disrupting SP and alternative amyloid deposits, though their toxicity and inability to cross the BBB has restricted their use as therapeutic treatments. Metals that are known in AD brains to be in dyshomeostasis square measure trace metals comparable to iron, zinc, and copper (Crouch 2007, Adlard *et al.,* 2008). The proof confirmative metal imbalance because the reason behind AD is proscribed, but any analysis ought to be pursued during this space because of some improvement in patients in results from treatments with metal chelators in clinical trials (Adlard *et al.,* 2008).

2.3.2.9.17.4 Viruses & bacteria

Because of an absence in definitive answers on the motive factors behind each the characteristic brain lesions, SP and NFT, and AD itself, researchers have begun to seem at morbific agents as potential causes and therefore therapeutic targets within the fight against the AD (Urosevic, Martins 2008). Correlations between brain levels of sure microorganism have instructed that maybe incursive pathogens are capable of facilitating general inflammatory responses and therefore setting off the suspected irreversible mechanisms behind AD (Kamer *et al.,* 2008). Samples of instructed microbes that correlate with either SP, insanity or AD prevalence embrace viruses reminiscent of herpes virus, herpes simplex virus

(HSV)-1 (Itzhaki, Wozniak 2006, Jamieson 1992), microorganism reminiscent of the Chlamydia (Gérard *et al.,* 2005), helicobacter, spirochete and spirochete species, and periodontic infections (Kamer *et al.,* 2008) with microorganism together with Aggregatibacter, Tannerella and Porphyromanos species, amongst others, as reviewed by Kamer (Kamer *et al.,* 2008).

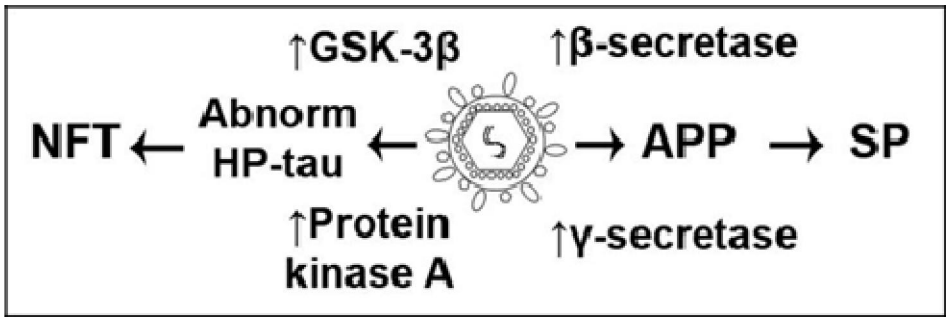


FIGURE 2.3.2.9.17.4. Proposed mechanisms for HSV-1 to cause Alzheimer's disease, as suggested by (Itzhaki, Wozniak 2008b).

2.4 Nootropic

Nootropics, popularly referred to as "smart drugs," are substances which boost human cognitive abilities (Lanni *et al.,* 2008). Typically, nootropics are alleged to work by increasing the brain's supply of neurochemicals (neurotransmitters, enzymes, and hormones), by improving the brain's oxygen supply, or by stimulating nerve growth. With a few notable exceptions, nootropics have very low or no toxicity, making overdose unlikely. Most have excellent tolerability and safety, and many nootropics, are alleged to potentiate each other. Most of the nootropic substances are herbs components and used by many people in personal cognitive enhancement regimens (Lanni *et al.,* 2008).

2.4.1 Treatment of Dementia

TABLE 2.4.1a List of drugs used in Treatment of Dementia

Drug	Mode of Action
Cholinergic Treatments precursors to Acetylcholine choline and Lecithin **Acetylcholinesterase Inhibitors** Tacrine, Galantamine, Velnacrine	Increases amount of ACh Prevent breakdown of ACh
Cholinergic agonists- Bethanicol	Muscarinic agonist
Other Therapeutic agents Acetyl-carnitine	Neuroprotective, promotes ACh synthesis
Anesthetics Procaine Hcl	Mild CNS stimulant with weak MAO inhibitor
Chelators EDTA, Desferoxamine	Proposed removal of toxins, calcium and aluminum
NSAIDs	Immune/ inflammatory effects may cause plaque and tangle formation could prevent degeneration
Nerve growth factor	May attenuate the degeneration of remaining cholinergic neurons
Neuropeptides ACTH, Somatostatin, Vasopressin **Calcium Channel Blocker** Nimodipine	May enhance the activity of endogenous Neurotransmitters Inhibit calcium influx associated with cellular Damage may slow progression of disease
Nootropic agents Piracetam	Enhances brain metabolism
Psychostimulants Methyl phenidate, Pemoline, Selegilline	CNS-stimulants Irreversible MAO inhibitor
Vasodilators Cyclandelate, isoxsuprine, Papaverine	Enhance blood flow to the brain

TABLE 2.4.1b List of currently available drugs with their side effects

Name of drugs	Adverse effects
Donepezil	Nausea, vomitting, diarrhea, muscle cramps, fatigue, anorexia, syncope.
Galantamine	Nausea, vomitting, diarrhea, anorexia, weight loss, abdominal pain, headache, dizziness, lethargy, confusion
Rivastigmine	Nausea, vomitting, diarrhea, dyspesia, anorexia, abdominal pain, insomnia, fatigue, dizziness, constipation, somnolence, tremor
Tacrine	Diarrhea, loss of appetite, clumsiness, vomitting, fainting, tachycardia, hyper- or hypotension, severe abdominal pain, hepatotoxicity

TABLE 2.4.1c List of Plant used as Nootropics

Name of Plant	Common Name	Nootropic / Memory enhancer effect
Bacopa monnieri	Brahmi	Memory Enhancer (Singh and Dhawan, 1997)
Acorus calamus	Bach	Improve memory functions (Achliya *et al., 2004*)
Withania somnifera	Ashwagandha	Improve memory functions (Kulkarni and Dhir, 2008)
Evolvulus alsinoides	Shankhpushpi	Improve memory functions (Nahata *et al., 2010*)

2.5 Animal Models for Nootropic Activity (Vogel *et al.*, 2002)

Based on the aversive stimuli, behavioural models for studying the neurobiology of learning and memory can be broadly classified into two types: *exteroceptive* (the aversive stimuli for learning and memory originating outside the body) or *interoceptive* (the aversive stimuli for learning and memory originating within the body). Accordingly, it is proposed that these two types of models may affect different neuronal systems involved in learning and memory. Once an animal has learned to escape from aversive events, the next favourable strategy is to try to avoid those aversive events totally. The aversive event is made predictable (conditioned stimulus) so that the animal can actively respond (active avoidance paradigms such as jumping on a pole or platform, shuttling to the safe part of the apparatus) or it suppresses/delays innate behaviour (passive avoidance paradigms such as reaching shock-free zone, voluntary delay in entering the dark comparment of the apparatus).

Various behavioural and pharmacological models currently available for the evaluation of learning and memory processes:

I. Exteroceptive aversive stimuli models

1. Behaviour on mazes
    - Elevated plus-maze
    - Radial arm maze
    - Radial water maze
    - Y-maze
    - Figure-8 maze
    - Morris water maze
    - Modular mazes
    - Stone T-maze
    - Furuya's three-panel runaway task
2. Avoidance behaviour on shuttle box
    - Two-way active avoidance task paradigm
    - Retention test performance of avoidance task
    - Step-down type passive avoidance test
    - Conditioned Avoidance Response
    - Modified passive avoidance test
    - Step-through type passive avoidance learning task

II. Interoceptive aversive stimuli models

1. Brain lesion-induced cognitive dysfunction
    - Electrolytic lesions
    - AF64A-induced NBM cholinergic lesions
    - Excitotoxin-induced lesions
2. Electroshock-induced amnesia
3. Hypoxic stress-induced learning deficits e.g. Sodium Nitrite induce amnesia

4. Pharmacological and discrimination assays
    - Scopolamine-induced amnesia
    - Dizocilpine-induced memory impairment
    - Clonidine-induced amnesia
    - Triazolam-induced cognitive dysfunction
    - Clozapine-induced cognitive dysfunction
    - Lignocaine-induced amnesia
    - Aluminum-induced learning deficits
    - Ethanol-induced state dependent learning
    - Carbon dioxide-induced amnesia

Characteristic features of various types of cognitive paradigms

Cognition	Paradigm
➢ Spatial long-term memory	Plus-maze
➢ Working memory	Radial arm maze
➢ Spatial working memory	Y-maze
➢ Spatial learning	Morris water maze, T-maze
➢ Short-term memory	Shuttle box
➢ Short-term memory	Rodent memory evaluator

2.5.1 Step-down Passive Avoidance

Fear-motivated avoidance tests are usually based on electric current as source of punishment. In many tests, the floor of the apparatus is made up by a grid that can be electrified. In consummatory conflict tests, the animal receives an electric shock when touching food or water. Avoidance tests are divided into two categories: passive avoidance and active avoidance. In passive avoidance, the animal has to refrain from executing a previously response, e.g., touch, food or water, step down from an elevated position (to a grid floor) or step into a narrow and apparently safer place (with a grid floor). Step-down or step-through tests are most frequently used to measure passive avoidance behaviour. The latency to refrain from performing the punished act expresses the ability to avoid. The most influential neurotransmitter on passive avoidance is dopamine according to the present findings. The

second most influential transmitter is glutamate. Drugs with effects of GABAnergic or cholinergic activity seem to have somewhat weaker impact. Half of the agents tested of serotonin-based neurotransmission were effective, whereas noradrenergic agents have weak influence on passive avoidance behaviour. Passive avoidance most likely involves both working memory and reference memory.

Different authors have followed different procedures to evaluate acquisition and memory using step-down apparatus. A few important studies have been reported here:
Kulkarni and Verma (1992) have followed a procedure in step-down passive avoidance, in which mice were put individually on the electric grid and allowed to explore for 1 min. The stimulus (20v) was then applied and latency to reach shock free zone (SFZ) recorded three consecutive times as basal readings. Animals that reached the SFZ in 2 min in the first trial were selected for the study. After 1 hr of the training, each animal was put on the electric grid again and the latency to reach SFZ and the number of mistakes (descents) the animal made in 15 min were recorded as parameters for acquisition and retention respectively.

Bhattacharya (1992) has followed a different method in which, the rat was placed on the elevated platform situated in the center of the passive avoidance box and the latency to step down was recorded. Immediately after stepping down, the rat received electric shock (0.5mA) of 3 sec duration through the grid floor, and was then returned to its home cage. On the following day (24 hr retention interval) the rat was once again placed on the platform and the step-down latency was recorded. Electric shock was not administered at this time. If the rat remained on the platform for the 5 min test duration, it was assigned a maximum score of 300 sec. Latency to step down was again assessed a week later on day 9 in order to assess the retention of passive avoidance learning.

Jaiswal *et al* (1991) has adopted the following procedure, in which step down latency (SDL) was measured on the first day, on the following day (24 hr retention interval) SDL was measured and given a 10 min inhibition period. Electric shock was not delivered on day two, when the animals remained for 10 min on the raised platform the animals were assigned max

score of 600 sec was given. At day 9 SDL was recorded to test the retention of the passive avoidance learning.

Joshi and Parle (2007) have followed a different method in which retention parameter SDL were noted for 90 min, 24 hr after the first test. Animals showing SDL in the range (2-15 sec) during the first test were used for the second session and the retention test.

2.5.2 Conditioned Avoidance Response

The nootropic activity is assessed by using the active avoidance paradigm. The Cook's Pole Climbing apparatus which uses the conditioned avoidance response as an index for evaluation of nootropic activity. The apparatus consist of a sound proof experimental chamber with the stainless-steel grid-floor, which could be electrified and with a provision for a buzzer tone. A wooden pole, screwed onto the inner surface of the lid of the chamber acts as the shock free zone (Turner, 1965).

Different authors have followed different procedures to evaluate acquisition and memory using Cook's Pole Climbing apparatus. A few important studies have been reported here,
Thakur and Mengi (2005) have followed a different procedure in which the evaluation of nootropic activity was carried out at the end of the first, 3rd and 7th day on the day of evaluation, 1 h post administration the acquisition trial (AT) was conducted and 24 h later the animals were subjected to the retention trial (RT). The acquisition trial consisted of 10 trial sessions interspersed with an interval of 30s. During each trial the rats were allowed to explore the apparatus for 10 s, followed by a buzzer tone of 50 Hz (conditioned stimulus) for 10 s. This was followed by the foot shock for 10 s. The animal learns to associate the buzzer tone with the impeding footshock and is capable of avoiding the foot shock on hearing the buzzer.

Sreemantula S *et al.,* (2005) adopted different procedure in which animal placing inside the perspex chamber of the apparatus. After an accustomed period of five minutes to the chamber, a buzzer was given followed by a shock through the grid floor. The rat had to jump on the pole to avoid foot shock. Jumping on the pole functionally terminates the shock and this was

classified as an escape while such jumping prior to the onset of the shock was considered as avoidance.

2.5.3 Sodium nitrite induced amnesia

Sodium nitrite induced amnesia is a type of interoceptive aversive stimuli model. Substantial impairment of cognitive functions such as mental skill, vigilance memory, logical reasoning and psychomotor performance has been observed at altitude above 3000m. Brain structures are most vulnerable to the oxidative stress especially hippocampus which is involved in memory. Reduced partial pressure of oxygen at high altitude causes oxidative stress by forming reactive oxygen and nitrogen species (RONS), which attack lipid, protein, DNA and activate the downstream pathway, leading to neuronal damage. The neuronal damage may finally lead to impairment in memory function (Gerhard, 2002).

Different authors have followed different procedures to induce chemical hypoxia and evaluate learning and memory using step-down apparatus. Bhattacharya (1994) has followed the procedure to induce chemical hypoxia by administration of sodium nitrite (35 mg/kg, sc), immediately after acquisition training.

2.6 Future perspectives

In recent years major development in nootropic drug discovery. Newer therapeutic development based on new drug targets which causing dementia or memory impairment. The new concept include possible use of stem cells to regenerate or grow new neurons to replace dead ones.

The future research mainly focous on targeting genome, neurons replacement and nerve growth factor (NGF). Erythropoietin (EPO) is also attractive approach for investigation in order to achieve neuronal protection and/or neuroregenration. EPO works as angiogenetic, antioxidant, antiapoptotic, neuronal plasticity, anti-inflammatory and stem cell modulation.

There are promising newer compounds are under various stages of proclinical and clinical trial with different approach or targets such as PDE4 inhibitors, L-type calcium channel modulator, 5 HT6 antagonists and nicotinic receptor agonist.

TABLE 2.6 List of Drugs in Development as Nootropics

Category	Drug	Indication	Status
Nicotinic alpha-7 Agonist	DMXBA (GTS-21), JN403, RO5313534	Alzheimer's Disease, Schizophrenia	Clinical Trial: Phase II Completed
PDE_4 Inhibitors	MEM 1414, MEM 1917	Alzheimer's Disease	Clinical Trial: Phase I Completed
L-Type Calcium Channel modulator	MEM 1003	Alzheimer's Disease	Clinical Trial: Phase IIa Completed
α4β2 nicotine AchR partial Agonist	AZD 3480 (TC 1734)	Age associated memory impairment mild cognitive impairment	Clinical Trial: Phase III
5HT6 Antagonists	MEM 68626, SB-742457, SAM-531, SGS-518, PRX-07034, SYN-114, SUVN-502		Pre-clinical Completed Clinical Trial: Phase II

2.7 Plant profile

Cayratia trifolia Linn is belongs to family Vitaceae and is a native of India, Asia and Australia **(**Kumar *et al.,* 2001).

2.7.1 Synonyms

Cayratia trifolia is also known by various synonyms such as:

1. *Vitis trifolia*
2. *Cissus carnosa*
3. *Vitis carnosa*
4. *Cissus trifolia*
5. *Cayratia carnosa*

2.7.2 Botanical distribution

Cayratia trifolia is a weak herbaceous climber, woody at base, stem is more or less succulent, compressed and densely. Leaves are trifoliolate with petioles 2-3-cm long. Leaflets are ovate to oblong-ovate, 2-8-cm long, 1.5-5-cm wide, pointed at the tip. Flowers are small greenish white 2.5mm, and brown on solitary cymes in leaf axils. Fruits are fleshy, juicy, dark purple or black, nearly spherical and about 1 cm in diameter. Seeds are triangular, apex rounded, ventral holes and ribs obtuse along margin, slightly raised **(**Kumar *et al.,* 2001).

FIGURE 2.7.2 *Cayratia trifolia Linn*. Plant **(**Kumar *et al.,* 2001).

2.7.3 Geographical distribution

It is found in India, southern China, Malaya, Caroline Islands Bangladesh, Burma, Ceylon, Combodia, Indonesia, Laos, Makaysia, Malacca, Pakistan, Thailand, Africa, Australia and Vietnam. In India it is found in Jammu, Rajasthan, Assam, Tripura and West Bengal extending into peninusular India up to 600 m **(**Kumar *et al.,* 2001).

2.7.4 Chemical constituents

Plant contains kaempferol, myricetin, quercetin, triterpenes and epifriedelanol. Whole plant of *Cayratia trifolia* has been reported to contain yellow waxy oil, steroids/terpenoids, flavonoids, tannins. Leaves contain stilbenes such as piceid, reveratrol, viniferin and ampelopsin. Stem, leaves and roots are reported to possess hydrocyanic acid and delphinidin. Several flavonoids such as cyanidin are reported in the leaves. Its seeds and fruits showed

presence of cyanogenic compounds. Fruits also contain calcium oxalate responsible for severe irritation in the mouth **(**Kumar *et al.,* 2001).

2.7.5 Pharmacological Activity

2.7.5.1 Antioxidant activity

The crude extract of ethyl acetate and methanol were tested for their biological activity including antioxidant activity by scavenging effect on DPPH (1,1-diphenyl-2-picryl hydraryl) radicals. The crude extract of *Cayratia trifolia* showed the ED_{50} values of 10.24 and 11.36 g/ml, respectively (Homhua *et al.,* 2007)

2.7.5.2 Anticancer activity

The methanolic extract is more potent than aqueous extract in exerting antineoplastic effect in both cell lines. The effect was analysed at different concentration level ranging from 50 to 500 g/ml. Delphidin and cyaniding which are anthocyanin and showed antiproliferative and proapoptotic properties in gastric adenocarcinoma and were also found to be protective against esophageal cancer in rodents (Rejitha and Das 2009).

2.7.5.3 Anti-inflammatory effect

In a rat model of carrageenan-induced Paw edema, inhibited both acute and chronic phases of the anti-inflammatory process (Gentilli *et al.,* 2001).

2.7.5.4 Cardioprotective effects

- It inhibits the vascular cell adhesion molecular expression (Ferrero *et al.,* 1988, Rotondo *et al.,* 1998)
- Inhibition of vascular smooth muscle cell proliferation (Haider *et al.,* 2005, Wang *et al.,* 2006, Poussier *et al.,* 2005)
- Stimulation of endolethelial nitric oxide synthase activity (Duffy and Vita, 2003, Wallerath *et al.,* 2002, Chen CK and Pace-Asciak, 1996)
- Inhibition of platelet aggregation (Olas *et al.,* 2005, Stef *et al.,* 2006)

2.7.5.5 Antidiabetic effect

The extract showed hypoglycemic and hypolipidemic effect in both Streptozotacin-induced diabetes rats and STZ-Nicotinamide-induced diabetes rats (Sharma *et al.,* 2006).

2.7.5.6 Antiviral effect

It inhibits herpes simplex virus types 1 and 2 replication by inhibition of an early step in virus replication cycle. *In vivo* studies in mice shows that resveratrol inhibits or reduce HSV replication in the vagina and limits extra-vaginal disease. Studies also show that resveratrol inhibits varicella-Zoster virus, certain influenza viruses, human cytomegalovirus. Furthermore, resveratrol synergistically enhances the anti-HIV-1 activity of several anti-HIV drugs (Docherty *et al.,* 2005).

2.8 *Gmelina arborea Roxb.*

2.8.1 Introduction

Gmelina arborea Roxb. belongs to the family Verbinaceae. It is found in part of India, Western Ghats, and from foot of North-West Himalaya to Chittagong and throughout Deccan Peninsula. It is a medium sized to rarely large deciduous tree attaining a height of 15-20m. *Gmelina arborea Roxb.* is one of the ingredients of most famous group Dashamoola and in particular Brihath panchamoola. It is popularly known as Coomb teak, Cashmeri tree, Candhar tree in english. Kashmarya, Kashmeeri, Gambhari in Sanskrit. Different parts of the plant can be used medicinally like root, fruit, leaf, flower, bark (Pathala *et al.,* 2015).

FIGURE 2.8.1 *Gmelina arborea Roxb.* (Pathala *et al.,* 2015)

2.8.2 Distribution

Found throughout India, from north-west Himalaya to Chittagong and throughout Deccan peninsula (Pathala *et al.,* 2015).

2.8.3 Family Features (Dutta, 1964)

Leaves: simple, opposite or whorled and sometimes pinnately or palmately compound.

Inflorescence: Raceme, panicle or spike (long or condensed), or a dichasial cyme.

Flowers: Bisexual, medianly zygomorphic, hypogynous and pentamerous. Bracts are sometimes in the form of involucres, as in Lantana.

Calyx: Sepals usually 5, rarely 4 or more and gamosepalous. The calyx is persistent.

Corolla: Petals usually 5, gamopetalous. They are initially 2- lipped and later 5 lobed. The tube may be long or short and the limb oblique is aestivation imbricate.

Androecium: Stamens are 4, didynamous and epipetalous (rarely 2 or 5). Often inserted or sometimes exserted and alternate with corolla lobes.

Gynoecium: Carpels are 2, rarely 4 and syncarpous. Superior ovary, entire or lobed, 2-locular with 1 or 2 ovules in each chamber or 4-locular with 1 ovule in each chamber. Terminal style.

Fruit: Drupe (2 or 4 pyrenes) and rarely capsule.

Seed: Exalbuminous seed.

2.8.4 Chemical Constituents (Pathala *et al.,* 2015)

Gmelo furan-α-furanosesquiterpenoid, sesquiterpene, cerylalcohol, hentriacontanol-1, β-sitosterol, n-octacosanol, gmelinol, and apiosylskimmin-α-apiofuranosyl- (1-6)-β-D-glucopyranosyl (1.0.7)-umbelliferone.

Root: Cluytylferulate, n-octacosanol, gmelinol, arboreol, 2-0- methyl arboreal, 2-0-ethylarboreol, isoarboreol,gmelanone, β-sitosterol, paulownin, 6”-bromoisoarboreol, 4-hydroxysesamin, 4,8-dihydroxysesamin, 1,4- dihydroxysesamin (gummadiol), 2-piperonyl-3-(hydroxymethyl)-4 (α-hydroxy-3-,4-methylenrdioxybenzyl)-4- hydroxy tetrahydro furan (1), 4-epigummadiol-4-0-glucoside, 1,4-dihydroxy-2,6-dipiperonyl-3,7-dioxabicyclo [3,3,0]-octane, gmelanone, palmitic, oleic and linoleic acids, stigmasterol, stigmastanol, campesterol, α-2-sitosterol, and butulinol.

Leaf: Luteolin, apigenin, quercetin, hentriacontanol, β- sitosterol, quercetogenin and other flavons.

Fruits: Butyric and tartaric acids, saccharine substances and little tannin, β- sitosterol, ceryl alcohol, gmelinol, arborone, arboreal, luteolin, apigenin, quercetin, hentriacontanol, and quercetogenin.

2.8.5 Pharmacological Activities

2.8.5.1 Antioxidant Activity

The various in vitro assays method which showed free radical scavenging activity. The activity could be at the same concentration to that of standard ascorbic acid which was due to proton donating ability and could serve as free radical inhibitors or scavengers (Kaswala *et al.,* 2012).

2.8.5.2 Anthelmintc Activity

Alcoholic and aqueous leaves extracts of *Gmelina arborea Roxb.* Showed anthelmintic activity in dose dependent manner giving shortest time of paralysis and death compared to piperazine citrate (Kaswala *et al.,* 2012).

2.8.5.3 Anti-Microbial Activity

The crude leaf and stem bark extracts of *Gmelina arborea Roxb.* showed significant anti-microbial activities against gram positive and gram negative organism and the activity may be due to the presence alkaloids, saponins, carbohydrates, phenolics, tannins and anthraquinone (Kaswala *et al.,* 2012).

2.8.5.4 Diuretic Activity

Methanolic extract of *Gmelina arborea Roxb.* has shown significant diuretic activity on albino rats. The diuretic activity due to synergistic action of (HCO3-/Cl-), (HCO3+/H+) exchangers and the (N+/H+) antiporter by inhibiting tubular re-absorption of water and accompanying anions to cause dieresis (Kaswala *et al.,* 2012).

2.8.5.5 Cardioprotective

Ethanolic extract of *Gmelina arborea Roxb.* has shown potential protective effect against doxorubicin induced cardiactoxicity by increasing cardiac markers activities like SGOT (Serum glutamic oxaloacetic transaminase), SGPT (Serum glutamic pyruvic transaminase) and ALP (Alkaline phosphate test) in plasma (Kaswala *et al.,* 2012).

2.8.5.6 Anti Diabetic Activity

Ethanolic extract of *Gmelina arborea Roxb*. bark was found to reduce the increase of blood sugar in streptozotacin induced diabetes (Kaswala *et al.,* 2012).

2.8.5.7 Immuno Modulatory Activity

Methanolic extract of *Gmelina arborea Roxb.* and ethyl acetate fraction of methanolic extract have been found to increase the total WBC count, which was lowered by cyclophosphamide, a cytotoxic drug. The drug is also capable of normalizing the levels of neutrophils and lymphocytes. The results indicates that the *Gmelina arborea Roxb.* can stimulate the bone marrow activity. As the drug is capable of reducing the cyclophosphamide induced toxicity, it can be useful in cancer therapy also (Kaswala *et al.,* 2012).

2.8.5.8 Antipyretic and Analgesic Activity

Gmelina arborea Roxb. bark extract reduced the hyperthermia 1 h after the administration and its effect is comparable to that of the standard antipyretic drug paracetamol. Chloroform and benzene extract reduced the temperature 3 h after their administration but have mild effects. However the analgesic activity of ethanolic and aqueous extract (test compounds) was found to be more significant on acetic acid induced test than tail flick test as compared to standard diclofenac sodium (Kaswala *et al.,* 2012).

2.8.5.9 Anti-inflammatory Activity

Gmelina arborea Roxb. root extract significantly showed the anti-inflammatory activity when compared to control group. *Gmelina arborea Roxb.* extract showed comparable results in parameters like reducing inflammation, percentage inhibition of paw edema and dry

granuloma weight in acute carrageenan paw edema and sub-acute inflammation cotton pellet granuloma models with standard aspirin treated group (Gandigawad *et al.,* 2019).

Chapter 3

MATERIALS AND METHODS

CHAPTER 3

Materials And Methods

3.1 Animals

Experiments were carried out on Wistar rats (200-250g) and Swiss albino mice (20-25g). They were obtained from Zydus Cadila, Ahmedabad. The animals were housed in polypropylene cages of dimension 16"x11"x 6" for rats and 10"x 8.5"x 6" for mice. Paddy husk was provided as bedding material, which was changed twice per week. The cages were maintained clean. They were fed with standard pellet diet and water *ad libitum.* They were kept in a well-aerated room and a 12-hour light and dark cycle was maintained. The room temperature was maintained at 22 ± 2ºC. The institutional animal ethical committee (IAEC) approved the protocol of this study. In the span of experiments, the animals were properly examined for any disease / disorder, infections and protected from any injury or stress.

3.2 Plant Material Collection

The heartwood and leaves respectively of *Gmelina arborea Roxb.* and *Cayratia trifolia Linn.* were obtained from botanical garden of Atmiya Institute of Pharmacy, Rajkot. The heartwood of *Gmelina arborea* and leaves of *Cayratia trifolia* were identified by Prof. Muliya, Taxonomist Department of Botany, Christ College, Rajkot. The parts of both plants washed under running tap water and dried in shade for 3 weeks. Dried leaves of *Cayratia trifolia* and heartwood of *Gmelina arborea* were coarsely powdered, and passed through sieve of mesh size no.22. 300 g of coarse powder was extracted with different solvents like hydroalcohol (30:70), chloroform, methanol, pet-ether and distilled water by Soxhlet extraction method (Vasudevan and Parle, 2007). The extracts were labeled and stored in airtight glass container at 4ºC throughout the study.

TABLE No. 3.2 Summary of Selected Plants and Extracts Prepared

Plant Name →	***Gmelina arborea Roxb.***	***Cayratia trifolia Linn.***
Part Selected →	Heartwood	Leaf
Extraction	Methanolic (MEGA)	Methanolic (MECT)
	Petroleum Ether (PEEGA)	Petroleum Ether (PEECT)
	Chloroform (CHGA)	Chloroform (CHCT)
	Aqueous (AEGA)	Aqueous (AECT)
	Hydro-alcoholic (HAEGA)	Hydro-alcoholic (HAECT)

3.3 Drugs, Chemicals and Solvents

Piracetam (Torrent Pharmaceuticals, India), Scopolamine (Cadila Healthcare, Indai), Sodium nitare (Merck Specialties, India), Methanol and Chloroform (Modern scientific, Nashik), Fehling's A, Fehling's B, Benedict's reagent, Barfoed's reagent, Million's reagent, Ninhydrin solution, Dragendorff's reagent, Hager's reagent, Mayer's reagent (Qualigens Fine Chemicals, Mumbai).

3.4 Method of administration

The herbal extracts were suspended in distilled water administered orally to mice and rats (for conditioned avoidance response model) for 15 days and 21 days (for conditioned avoidance response model). Piracetam (140 mg/kg, p.o.) was administered orally as a standard drug for 15 days and and 21 days (for conditioned avoidance response model) (Pulok *et al.,* 2007). Control group received same volume of distilled water via the oral route for equal time period. In the sodium nitrite induced amnesia group, to induced hypoxia sodium nitrite (35 mg/kg b.w. s.c) has been used immediately after acquisition training (Elisabet, 2007).

3.5 Phytochemical Investigation

Phytochemical investigations of the both *Gmelina arborea Roxb.* and *Cayratia trifolia Linn.* extracts were carried out as described by Kokate *et al.,* 2006.

Preliminary phytochemical investigation of extracts

The preliminary phytochemical investigations were carried out for extracts of *Gmelina arborea* and *Cayratia trifolia.*

A) Detection of Carbohydrates

Extracts were dissolved individually in distilled water. The filtrate was used to test the presence of carbohydrates using fallowing tests.

a) Molisch's test

Filtrates were treated with few drops of alcoholic α-naphthol (1%) solution in a test tube and 2 ml concentrated H_2SO_4 was added cautiously along the sides of the test tubes. Presence of violet ring at the junction of two solution indicates the presence of carbohydrates.

b) Benedict's test

Taken 2 ml of filtrate in test tube and add Benedict's qualitative reagent and heated on water bath. Formation of orange red precipitate confirm the presence of reducing sugars.

c) Fehling's test

Filtrates were hydrolyzed with 10% Hcl, neutralized with alkali and heated on water bath with Fehling's A and B reagents. Formation of red precipitate confirm the presence of reducing sugars.

B) Detection of Alkaloids

Extracts were dissolved individually in 10% hydrochloric acid and filtered. The filtrates were tested carefully with alkaloid reagents.

a) Mayer's test

Filtrates were treated with Mayer's reagent (potassium mercuric iodide), formation of a yellow cream precipitate indicate the presence of alkaloids.

b) Wagner's test

Filtrates were treated with Wagner's reagent (iodine in potassium iodide) and observed.

Formation of brown/reddish brown precipitate indicates the presence of alkaloids.

c) Dragendroff's test

Filtrates were treated with Dragendroff's reagent. Formation of red precipitate indicates the presence of alkaloids.

d) Hager's test

Filtrates were treated with Hager's reagent. Formation of yellow colored precipitate indicates the presence of alkaloids.

C) Detection of Glycosides

Extracts were hydrolyzed with dilute Hcl, and hydrolysate were subjected to glycosides tests.

a) Modified Borntrager's test

Extracts were treated with ferric chloride solution and heated for 5 min on boiling water bath. The mixtures were cooled and added benzene with proper shake and mix well. The seprated benzene layer was treated with half of its volume of ammomia solution. Development of cherry red pink color in the ammonia layer indicates the presence of anthranol glycoside.

b) Legal's test

Extracts were treated with sodium nitroprusside in methanolic and pyridine alkali. The development of pink to red color indicate the presence of cardiac glycoside.

c) Killer killani test

Extracts were dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solutions. This was then under laid with 1 ml of concentrated H_2SO_4. A brown ring obtained at the presence of a cadenolides.

D) Detection of Saponins

a) Froth's test

The extracts were diluted with distilled water to 20 ml shaken in a measuring cylinder for 15 min. The development of 1 cm layer of foam suggest the presence of saponins.

b) Liberman buchard's test

Extract were treated with chloroform and filtered. Filtrates were treated with few drops of acetic anhydride then boiled and cooled under running tap water. Concentrated H_2SO_4 was added through the sides of the test tube. The development of brown ring at the junction indicated the presence of steroidal saponins.

E) Detection of Phytosterols

a) Salkowski's test

Extract were taken and mix with chloroform and filtered them. Added few drops of concentrated sulphuric acid in filtrates, shaken well and allowed to stand for few min. If lower layer turns red, sterols are present. If lower layer turns golden yellow triterpenes are present.

F) Detection of Fixed Oils and Fats

a) Stain test

Slight quantity of extracts were pressed in between two filter paper. Filter paper shows oily stain indicates the presence of fixed oil in extracts.

b) Soap test

Extracts were boiled on water bath with 0.5 N alcoholic KOH solutions. Development of soap shows the presence of fats and fixed oils in extracts.

G) Detection of Phenolic Compounds and Tannins

a) Ferric chloride test

The extracts were treated with few drops of neutral ferric chloride solution. The formation of bluish black color indicates the presence of phenolic nucleus.

b) Gelatin test

To the extracts, 1% gelatin solution containing sodium chloride was added. The formation of white precipitate indicates the presence of tannins.

c) Lead acetate test

The extracts were treated with few drops of lead acetate solution; formation of yellow precipitate indicates the presence of flavonoids.

d) Alkaline reagent test

The extracts were treated with few drops of sodium hydroxide separately. Formation of intense yellow color, which becomes colorless on addition of few drops of dilute acid, indicates the presence of flavonoids.

e) Shinoda test

The extracts were treated with few fragments of magnesium metal separately, followed by drop wise addition of concentrated hydrochloric acid. The formation of magenta color indicates the presence of flavonoid.

H) Detection of Amino acids and Proteins

a) Millons test

Extracts were taken and few ml of Millons reagent added. Formation of white precipitate, on heating which turns to red, indicates the presence of proteins.

b) Biuret test

Extracts were taken and add 1ml of 10% sodium hydroxide solution and heated over water bath. A drop of 0.7% copper sulphate solution added to mixtures. The formation of purple violet color indicates the presence of proteins.

c) Ninhydrin test

Extract were treated with 0.25% ninhydrin reagent was and boiled for few minutes. Formation of deep blue color indicates presence of amino acid.

3.6 Acute Toxicity

Toxicity as per OECD guideline 423

Acute oral toxicity of extracts was carry out by using female, mice weighing 18-22 g. All the animals were fasted for 3 hrs with water ad libitium prior to the experiment. The extarcts were

administered in dose of 300, 2000 and 5000 mg/kg p.o. to groups of mice (n=3) and percentage mortality was noted after 24 h and daily thereafter for total 14 days.

3.7 Screening Models for Memory Activity

3.7.1 Step down passive avoidance (Dhingra and Kulkarni, 2004)

Passive avoidance response is extensively used for the screening of drugs effecting learning and memory. The test involves training of mice to avoid punishment (normally an electric shock) by curbing a normal behavior (such as an exploratory behavior). At specified interval after training, the animals will be tested again for retention of such learning.

Apparatus

The apparatus consisted of a box (30×30×40 cm in height) having three walls of wood and one wall of plexiglas, featuring a grid floor (made up of 3 mm stainless steel rods set 8-mm apart), with a wooden platform (4×4×4 cm) in the center of the grid floor. The box was illuminated with a 15-W bulb during the experimental period. Electric shock (20 V, AC) was delivered to the grid floor.

Procedure

A typical paradigm consists of three phases.

Phase 1 Familiarization

The animal was placed on the elevated platform situated in the center of the passive avoidance box and the latency to step down was recorded. After 15 sec of exploration, it is returned to the home cage. Animals showing step down latency (SDL) of more than 15sec in the first training session were excluded from the experiment.

Phase 2 Learning

Immediately after stepping down, the animal received unavoidable foot shock (20 V, AC) and was then returned to its home cage.

Phase 3 Retention test

On the next day (24 hr retention interval) the mice were once again placed as above mentioned and the step down latency were recorded. Electric shock or stimuli was not given at this time. If the mouse remained on the shock free zone for the 300 sec time, it was

considered a maximum score of 300s. Latency to step down was again assessed a fortnight later, on day 15 learning of passive avoidance to assess. Before 30 min of retention trial administered scopolamine butyl bromide. The SDL was recorded on the days of examine i.e. 1st day and 15th day after 60 mins of drug administration.

TABLE No. 3.7.1 Treatments Groups of Step down passive avoidance test:

Group No.	Treatment Groups (Dose)
I	Control (Distilled water, p.o.)
II	Scopolamine (0.4 mg/kg, p.o.)
III	Piracetam (140 mg/kg, p.o.)+Scopolamine (0.4 mg/kg, p.o.)
IV	MECT (500 mg/kg, p.o.) + Scopolamine (0.4 mg/kg, p.o.)
V	PEECT (500 mg/kg, p.o.)+ Scopolamine (0.4 mg/kg, p.o.)
VI	CECT (500 mg/kg, p.o.)+ Scopolamine (0.4 mg/kg, p.o.)
VII	AECT (500 mg/kg, p.o.)+ Scopolamine (0.4 mg/kg, p.o.)
VIII	HAECT (500 mg/kg, p.o.)+ Scopolamine (0.4 mg/kg, p.o.)
IX	MEGA (500 mg/kg, p.o.)+ Scopolamine (0.4 mg/kg, p.o.)
X	PEEGA (500 mg/kg, p.o.)+ Scopolamine (0.4 mg/kg, p.o.)
XI	CEGA (500 mg/kg, p.o.)+ Scopolamine (0.4 mg/kg, p.o.)
XII	AEGA (500 mg/kg, p.o.)+ Scopolamine (0.4 mg/kg, p.o.)
XIII	HAEGA (500 mg/kg, p.o.)+ Scopolamine (0.4 mg/kg, p.o.)

Note:

In preliminary study, we used different extracts *Gmelina arborea* and *Cayratia trifolia* as mentioned in above table. Based on effect on step down latency (SDL) using passive avoidance paradigm; we found that Aqueous Extract of *Cayratia trifolia* (AECT), Hydro-alcoholic Extract of *Cayratia trifolia* (HAECT), Chloroform Extract of *Gmelina arborea* (CEGA) and Hydro-alcoholic Extract of *Gmelina arborea* (HAEGA) has significant activity and further experiments were carried out by using these extracts only.

3.7.2 Sodium nitrite induced amnesia

Sodium nitrite induced amnesia is a type of interoceptive aversive stimuli model. Sodium nitrite (35 mg/kg, s.c.) was administered immediately after the learning trial on day 1 to induce amnesia in mice as described by Bhattacharya, 1994. Apparatus and procedure is of same as step down passive avoidance test.

TABLE No. 3.7.2 Treatments Groups of Sodium nitrite induced amnesia:

Group No.	Treatment Groups (Dose)
I	Control (Distilled water, p.o.)
II	Sodium nitrite (35 mg/kg, s.c.)
III	Piracetam (140 mg/kg, p.o.) + Sodium nitrite (35 mg/kg, s.c.)
IV	AECT (500 mg/kg, p.o.) + Sodium nitrite (35 mg/kg, s.c.)
V	HAECT (500 mg/kg, p.o.) + Sodium nitrite (35 mg/kg, s.c.)
VI	CEGA (500 mg/kg, p.o.) + Sodium nitrite (35 mg/kg, s.c.)
VII	HAEGA (500 mg/kg, p.o.) + Sodium nitrite (35 mg/kg, s.c.)

3.7.3 Pole Climbing Test

Cook‘s Pole Climbing Apparatus use to study cognitive function, mainly a response to conditioned stimuli during learning and its retention. The apparatus has an experimental chamber (25 × 25 × 25 cm) with the floor grid in a soundproof enclosure. Scrambled shock (6mA) is delivered to the grid floor of the chamber composed of stainless steel rods. A pole, 2.5 cm in diameter, hangs inside the chamber through a hole in the upper center of the chamber. The study rat was placed in the chamber and allowed to explore the chamber for 45 seconds. Conditioned stimulus (CS) i.e buzzer signal was turned on and unconditioned stimulus (US) i.e electric shock delivered through grid floor for 45 Sec. Animal learned to associate the buzzer with the impending foot shock and was capable of avoiding the foot shock by climbing the pole after buzzer signal. Avoidance response was defined as climbing reaction time 10 sec. Every rat was subjected to maximum 05 trials on 1st day, and 24 hrs later, rat was subjected to Relearning trials (2nd day 3 trials and on 3rd day one trial) and transfer latency was noted to check the retention of Conditioned Avoidance Response (CAR)

and escape response. Animals were screened by using this model and those who demonstrated at least one escape response either on day one or two were included in the study. Latency to climb the pole was recorded on 21th day after extracts and piracetam administration of 60 min. (Cook and Weidley, 1957).

TABLE No. 3.7.3 Treatments Groups of Pole Climbing Test:

Group No.	Treatment Groups (Dose)
I	Control (Distilled water, p.o.)
II	Piracetam (140mg/kg, p.o.)
III	AECT (500 mg/kg, p.o.)
IV	HAECT (500 mg/kg, p.o.)
V	CEGA (500 mg/kg, p.o.)
VI	HAEGA (500 mg/kg, p.o.)

3.7.4 Elevated plus maze

Elevated plus maze (EPM) was used as exteroceptive stimuli behavioral method to evaluate the spatial long-term learning and memory in mice. The model was performed for testing learning and memory, followed as per the neuropsychopharmacological principle of remembering of learned tasks. Apparatus consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm) with arms extended from a central platform (5 cm × 5 cm). Apparatus height from floor was 25cm. Transfer latency (TL) was count as the time taken by a mouse to enter in covered arm with all four its legs. Each mouse was placed at the end of the one open arm facing away from the central platform and the TL was recorded when the mouse entered one of the covered arm. Each mouse was then permitted to explore the apparatus for 10 secs. The cut off time for TL was recorded as 90 secs, if the mouse did not enter the covered arm within 90 secs. TL was examine and recorded 24 hrs after the 1st exposure to the apparatus. The TL was recorded on the days of examine i.e. 1st day and 15th day after 60 mins of drug administration (Kulkarni and Verma, 1993).

TABLE No. 3.7.4 Treatments Groups Elevated plus maze:

Group No.	Treatment Groups (Dose)
I	Control (Distilled water, p.o.)
II	Piracetam (140mg/kg, p.o.)
III	AECT (500 mg/kg, p.o.)
IV	HAECT (500 mg/kg, p.o.)
V	CEGA (500 mg/kg, p.o.)
VI	HAEGA (500 mg/kg, p.o.)

3.7.5 Estimation of Acetyl cholinesterase enzyme activity in rat brain

Acetylcholinesterase activity was measured by the method of Ellman *et al.* (1961). A photometric method for determining acetylcholinesterase activity of tissue extracts has been described. The substrate used in the assay system is acetylthiocholine, the ester of thiocholine and acetic acid. The mercaptan formed as a result of the hydrolysis of the ester then reacts with an oxidizing agent 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) which is split into two products, one of which (5-thio-2-nitrobenzoate) absorbs at 412nm. The enzyme activity is measured by following the increase of yellow color produced from thiocholine when it reacts with DTNB. It is based on coupling of these reactions:

(Enzyme)

Acetylcholine ----------→ Thiocholine + Acetate

Thiocholine + DTNB ----------→ Yellow color + Colorless

Reagents

Buffer: Phosphate, 0.1 M, pH 8.0.

Substrate: Acetylthiocholine iodide, 0.075 M (21.67 mg/ml). This solution was used successfully for 10-15 days if kept refrigerated.

Ellman's reagent: Dithiobisnitrobenzoic acid (DTNB) 0.01 M (39.6 mg) was dissolved in 10 ml pH 7.0 phosphate buffer (0.1 M) and 15 mg of sodium bicarbonate were added. The reagent was made up in buffer of pH 7 in which it was more stable than in that of pH 8.

Procedure

After the behavioral study, the rats were decapitated and brains were excised and kept on a cold petridish, which is kept on crushed ice. Brains were washed superficially with isotonic saline to remove blood.

The tissue was homogenized (approximately 20 mg of tissue per ml of phosphate buffer (pH 8.0, 0.1 M) in a homogenizer.

A 0.4 ml aliquot of this homogenate was added to a cuvette containing 2.6 ml of phosphate buffer (pH 8.0, 0.1 M).

Of the DTNB reagent, 100 µl were added to the photocell. The absorbance was measured at 412 nm (Elico SL 159 UV-VIS Spectrophotometer); when this had stopped increasing, the photometer slit was opened so that the absorbance was set to zero.

Of the substrate, 20 µl were added. Changes in absorbance were recorded and the change in absorbance per min was calculated.

Calculation

The rate in moles of the substrate hydrolyzed per minute per gram of tissue was calculated by:

$$R = \frac{\Delta A}{1.36\,(10^{4})} \times \frac{1}{(400/3120)C_o} = 5.74\,(10^{-4})\,\frac{\Delta A}{C_o}$$

Where,

R = rate, in moles substrate hydrolyzed per min per g of tissue;

ΔA = change in absorbance per min;

C_o = original concentration of tissue (mg/ml)

3.7.6 Estimation of protein

The protein content of brain tissue was estimated using bovine serum albumin as standard Lowery et al., 1951.

Reagents

- 1 N NaOH: 20 g NaOH dissolved in 450 ml distilled water and volume made upto 500 ml with distilled water.
- Copper-tartarate-carbonate reagent:
- 1 % copper sulphate (CuSO4 5H2O); 2% Sodium potassium tartarate (NaKC4H4O6 4H2O); 25 % sodium carbonate (Na2CO3) solutions.
- These solutions were mixed in following sequence;
- 1ml of copper sulphate (1 %) and 1 ml of sodium potassium tartarate (2 %) were mixed in a beaker. To that, 100 ml of sodium carbonate solution was added (1:1:100) and mixed thoroughly.

3.7.7 Estimation of superoxide dismutase (SOD)

SOD was estimated as per the method described by Misra and Fridovich (1972). Supernatant (0.1ml) of sample was mixed with 0.1 ml EDTA ($1x10^{-4}$ M), 0.5 ml of carbonate buffer (pH 9.7) and 1 ml of epinephrine ($3x10^{-4}$ M). The optical density of formed adrenochrome was read at 480 nm for 3 min at an interval of 30 sec.

3.7.8 Estimation of catalase (CAT)

Decomposition of H_2O_2 in presence of catalase was estimated by the method of Aeibi et al. (1974). A 50 ul of supernatant was added to buffered substrate (50 mM phosphate buffer, pH 7 containing 30 mM H_2O_2) to make total volume 3 ml. The decrease in the absorbance was read at 240 nm for 2.5 min at an interval of 15 sec. Results were expressed as mean absorbance of catalase activity.

3.7.9 Estimation of level of reduced glutathione (GSH)

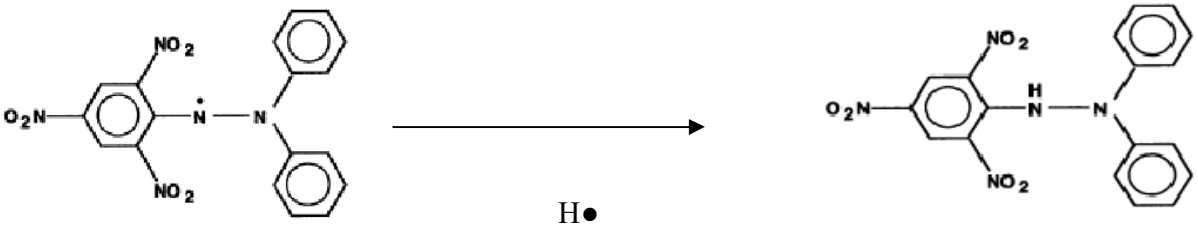
Reduced GSH levels in. tissue homogenates were estimated as per the method described by Beutler et al. (1963). The supernatant (2 ml) was mixed with 10% chilled trichloroacetic acid. The mixture was kept in ice bath for 30 min and centrifuged at 1000 x g for 10 min at 4 °C. Supernatant (0.5 ml) was mixed with 2.0 ml 0.3 M disodium hydrogen phosphate and 0.25 ml 5, 5'-dithiobis-2-nitrobenzoic acid (40mg/100ml in 1% sodium citrate) was added just before measuring the absorbance at 412 nm. Standard curve for GSH was prepared using standard

glutathione enzyme. Results were expressed as pmole of GSH/g tissue. All the parameters were estimated spectrophotometrically (UV 1601, Shimadzu (Asia Pacific) Pvt. Ltd., Sydney, Australia) at their respective specified wavelengths.

3.8 *In- vitro* Antioxidant Property

3.8.1 DPPH (1, 1-diphenyl-2-picryl hydrazyl) Method

DPPH radical scavenging reaction:



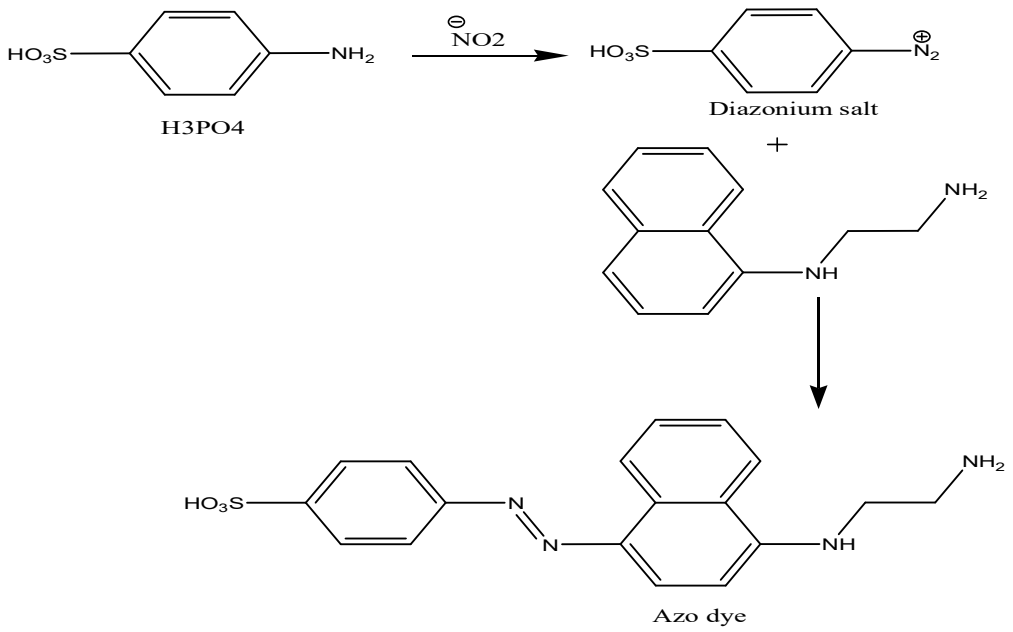
Diphenylpicrylhydrazyl (free radical) Diphenylpicrylhydrazine (non- radical)

Procedure: DPPH (2.365 mg) was dissolved in 10 ml of 95% ethanol and the *Ceratonia siliqua* was dissolved in 95% ethanol to make the stock solutions, which was diluted to give concentrations ranging from 10-200 µg/ml. The *Ceratonia siliqua* of varying concentration (1.5 ml) were added in the test. DPPH (1.5 ml) was added to both blank as well as in test and finally 95% ethanol (1.5 ml) was added in both blank and test. The tubes were kept aside for 20 min allowing the reaction to take place. After 20 min the absorbance (A) of the solutions was recorded against the respective blanks at 517 nm by using colorimeter (Keto *et al.,* 1998).

$$\% \text{ scavenging activity} = \frac{\text{Absorbance of blank - Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

3.8.2 Nitric Oxide Scavenging Activity

Nitric Oxide scavenging reaction:



Procedure

The reaction mixture containing sodium nitropruside (15 mM, 1ml) in phosphate buffer saline (pH 7.3), with or without the plant extracts at different concentrations (1ml), was incubated for 150 min (Marcocci *et al.,* 1994). The NO· radical thus generated would interact with oxygen to produce nitrite ion (NO_2^-), which was assayed by mixing with an equal amount (1ml) of Griess reagent and absorbance read at 546 nm by using colorimeter.

3.9 Statistical Analysis

The values are expressed as Mean ± Standard error mean (SEM) and analyzed by one way analysis of variance (ANOVA) followed by Dunnett's test using Graph pad Software version 5.0. $p<0.05$ was considered to be statically significant.

Chapter 4

RESULTS

CHAPTER 4

Results

4.1 Preliminary phytochemical investigation of *Cayratia trifolia Linn.* Leaf extracts

The plant extract showed the presence of following chemical constituents as mentioned in Table 4.1.

TABLE 4.1: Phytochemical investigation of *Cayratia trifolia Linn.* Leaf extracts

Chemical Constituents	MECT	PEECT	CECT	AECT	HAECT
Carbohydrates	-	-	-	+	+
Alkaloids	-	-	-	-	-
Glycosides	-	-	-	-	-
Saponin Glycoside	-	-	-	-	-
Flavonoids	-	-	-	-	+
Phytosterol	+	+	+	+	+
Fixed oil & Fats	-	-	-	+	-
Phenolic compounds & Tannis	-	-	+	+	+
Protein & Amino acids	-	-	-	-	-

+ = Present, - = Absent

Methanolic extract of *Cayratia trifolia* (MECT)

Petroleum Ether extract of *Cayratia trifolia* (PEECT)

Chloroform extract of *Cayratia trifolia* (CECT)

Aqueous extract of *Cayratia trifolia* (AECT)

Hydro-alcoholic extract of *Cayratia trifolia* (HAECT)

4.2 Preliminary phytochemical investigation of *Gemelina arborea Roxb.* Leaf extracts

The plant extract showed the presence of following chemical constituents as mentioned in Table 4.2.

TABLE 4.2 Phytochemical investigation of *Gemelina arborea Roxb.* Leaf extracts

Chemical Constituents	MEGA	PEEGA	CEGA	AEGA	HAEGA
Carbohydrates	-	-	-	+	+
Alkaloids	+	-	+	-	+
Glycosides	+	-	+	+	+
Saponin Glycoside	-	-	-	-	-
Flavonoids	-	-	-	-	-
Phytosterol	-	-	-	-	-
Fixed oil & Fats	-	+	+	-	-
Phenolic compounds & Tannis	-	-	-	-	-
Protein & Amino acids	+	-	-	+	+

+ = Present, - = Absent

Methanolic extract of *Gmelina arborea* (MEGA)

Petroleum Ether extract of *Gmelina arborea* (PEEGA)

Chloroform extract of *Gmelina arborea* (CEGA)

Aqueous extract of *Gmelina arborea* (AEGA)

Hydro-alcoholic extract of *Gmelina arborea* (HAEGA)

4.3 Effects of *Cayratia trifolia Linn.* on behavioural parameters, clinical signs of abnormality and mortality

TABLE 4.3 Effects of various extracts of *Cayratia trifolia Linn.* on behavioural parameters, clinical signs of abnormality and mortality as mentioned in Table 4.3.

Evaluating parameters	MECT (5000 mg/kg)	PEEGA (5000 mg/kg)	CECT (5000 mg/kg)	AECT (5000 mg/kg)	HAECT (5000 mg/kg)
1) Loss of reflex					
a) Righting reflex	-	-	-	-	-
b) Pinna reflex	-	-	-	-	-
c) Corneal reflex	-	-	-	-	-
2) Changes in the body weight	No change compared to control	No change compared to control	No change compared to control	No change compared to control	No change compared to control
* Control group showed slight weight gain					
3) Clinical abnormality	-	-	-	-	-
4) Mortality					
a) within 24 hr	Nil	Nil	Nil	Nil	Nil
b) within 72 hr	Nil	Nil	Nil	Nil	Nil
c) within 14 days	Nil	Nil	Nil	Nil	Nil
5) Behavioral Parameters	Tremors, convulsions, salivation, diarrhoea and lethargy were not observed	Tremors, convulsions, salivation, diarrhoea and lethargy were not observed	Tremors, convulsions, salivation, diarrhoea and lethargy were not observed	Tremors, convulsions, salivation, diarrhoea and lethargy were not observed	Tremors, convulsions, salivation, diarrhoea and lethargy were not observed

4.4 Effects of *Gemelina arborea Roxb.* on behavioural parameters, clinical signs of abnormality and mortality

TABLE 4.4 Effects of various extracts of *Gemelina arborea Roxb*. on behavioural parameters, clinical signs of abnormality and mortality as mentioned in Table 4.4.

Evaluating parameters	**MEGA (5000 mg/kg)**	**PEEGA (5000 mg/kg)**	**CEGA (5000 mg/kg)**	**AEGA (5000 mg/kg)**	**HAEGA (5000 mg/kg)**
1) Loss of reflex					
a) Righting reflex	-	-	-	-	-
b) Pinna reflex	-	-	-	-	-
c) Corneal reflex	-	-	-	-	-
2) Changes in the body weight	No change compared to control	No change compared to control	No change compared to control	No change compared to control	No change compared to control
* Control group showed slight weight gain					
3) Clinical abnormality	-	-	-	-	-
4) Mortality					
a) within 24 hr	Nil	Nil	Nil	Nil	Nil
b) within 72 hr	Nil	Nil	Nil	Nil	Nil
c) within 14 days	Nil	Nil	Nil	Nil	Nil
5) Behavioral Parameters	Tremors, convulsions, salivation, diarrhoea and lethargy were not observed	Tremors, convulsions, salivation, diarrhoea and lethargy were not observed	Tremors, convulsions, salivation, diarrhoea and lethargy were not observed	Tremors, convulsions, salivation, diarrhoea and lethargy were not observed	Tremors, convulsions, salivation, diarrhoea and lethargy were not observed

4.5 Step down passive avoidance

a) Effects after 24 h

The mice showed significant increase in SDL in piracetam ($p<0.001$), AECT ($p<0.001$), HAECT ($p<0.001$), CEGA ($p<0.001$) and HAEGA ($p<0.001$) as compared to scopolamine groups. While there is no significant increase in MECT, PEECT, CECT, HEGA, PEEGA and AEGA treated groups after 24 h respectively. The results are shown in figure 4.5a.

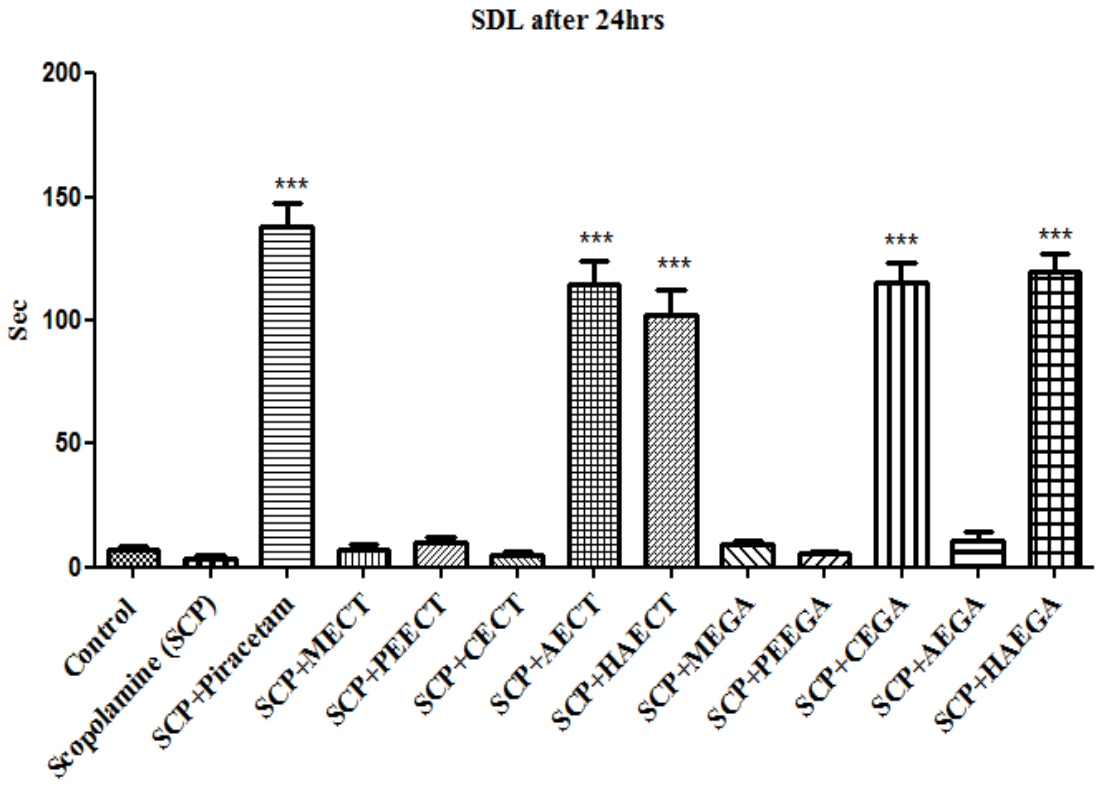


FIGURE 4.5a Effect of treatments on step down latency (SDL) of scopolamine induced cognitive deficit mice by using step down apparatus. Data expressed as mean ± SEM values, n=10. ***$p<0.001$ compared to scopolamine using one-way ANOVA followed by Dunnett's test.

b) Effects on 15 days

The mice showed significant increase in SDL in piracetam ($p<0.001$), AECT ($p<0.001$), HAECT ($p<0.001$), CEGA ($p<0.001$) and HAEGA ($p<0.001$) as compared to scopolamine groups. While there is no significant increase in MECT, PEECT, CECT, HEGA, PEEGA and AEGA treated groups after 15 days respectively. The results are shown in figure 4.5b.

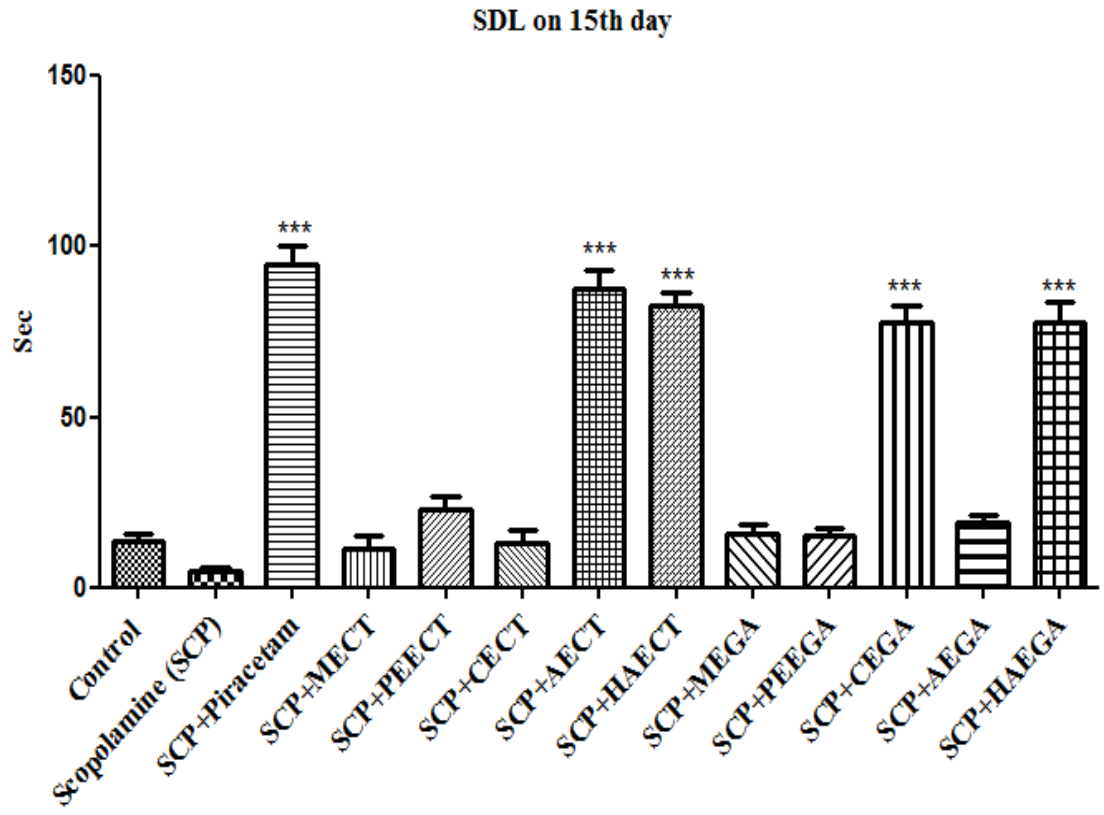


FIGURE 4.5b. Effect of treatments on step down latency (SDL) of scopolamine induced cognitive deficit mice by using step down apparatus. Data expressed as mean ± SEM values, n=10. ***$p<0.001$ compared to scopolamine using one-way ANOVA followed by Dunnett's test.

4.6 Sodium nitrite induced amnesia

a) Effects after 24 h

The mice showed significant increase in SDL in piracetam ($p<0.01$), AECT ($p<0.01$), HAECT ($p<0.01$), CEGA ($p<0.01$) and HAEGA ($p<0.01$) as compared to sodium nitrite treated groups on after 24 h respectively. The results are shown in figure 4.6a.

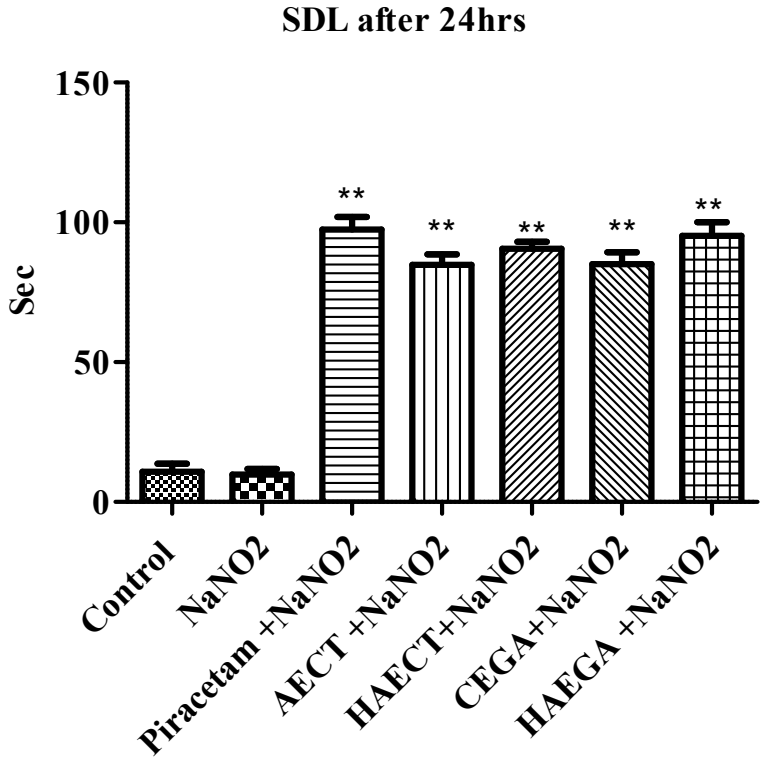


FIGURE 4.6a. Effect of treatments on step down latency (SDL) of $NaNO_2$ induced cognitive deficit mice by using step down apparatus. Values are shown in mean ± SEM values, n=10. ***p<0.01* compared to sodium nitrite using one-way ANOVA followed by Dunnett's test.

b) Effects on 15 days

The mice showed significant increase in SDL in piracetam ($p<0.01$), AECT ($p<0.01$), HAECT ($p<0.01$), CEGA ($p<0.01$) and HAEGA ($p<0.01$) as compared to sodium nitrite treated groups on after 15 days respectively. The results are shown in figure 4.6b.

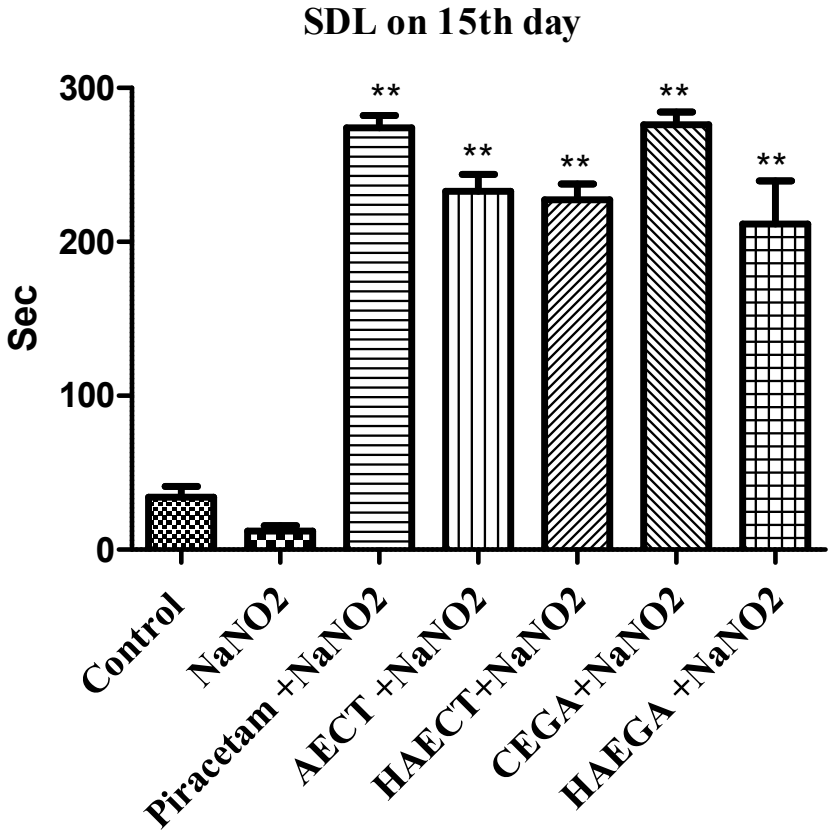


FIGURE 4.6b. Effect of treatments on step down latency (SDL) of $NaNO_2$ induced cognitive deficit mice by using step down apparatus. Values are shown in mean ± SEM values, n=10. ***p<0.001* compared to sodium nitrite using one-way ANOVA followed by Dunnett's test.

4.7 Conditioned avoidance response

Inhibition of conditioned response was assessed on day 21st to assess the inhibition of conditioned avoidance learning as an index for evaluation of nootropic activity. The results are shown in figure to indicate that there is significant effect of piracetam ($p<0.001$), AECT ($p<0.05$), HAECT ($p<0.05$), CEGA ($p<0.05$) and HAEGA ($p<0.01$) on the retention of Inhibition of conditioned response in rats on 21st day when compared to control.

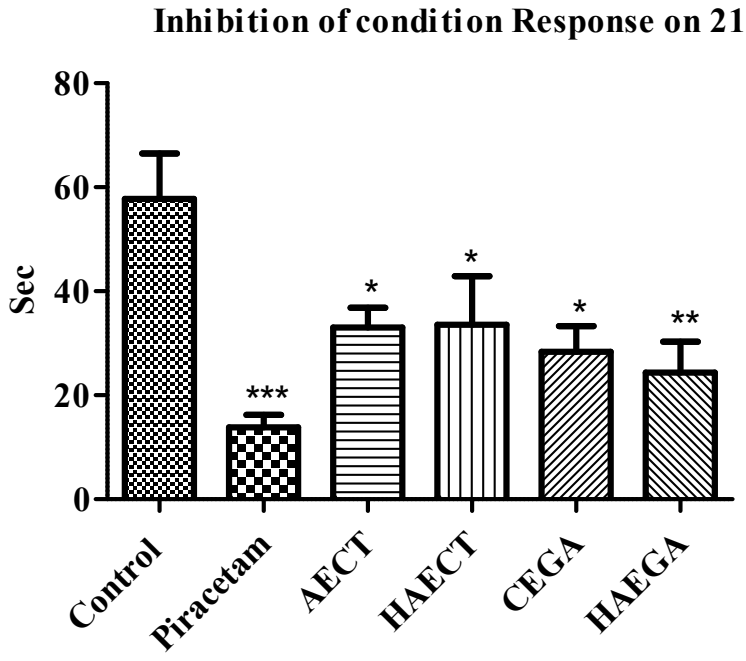


FIGURE 4.7 Effect of treatments on inhibition of conditioned response of rat by using Cook's pole climbing apparatus. Values are shown in mean ± SEM values, n=10. ***$p<0.001$, **$p<0.01$, *$p<0.05$ compared to control using one-way ANOVA followed by Dunnett's test.

4.8 Elevated Plus Maze

a) Effects after 24 h

The mice showed significant decrease in TL in piracetam ($p<0.001$), AECT ($p<0.001$), HAECT ($p<0.001$), CEGA ($p<0.001$) and HAEGA ($p<0.001$) as compared to control groups after 24 h respectively. The results are shown in figure 4.8a.

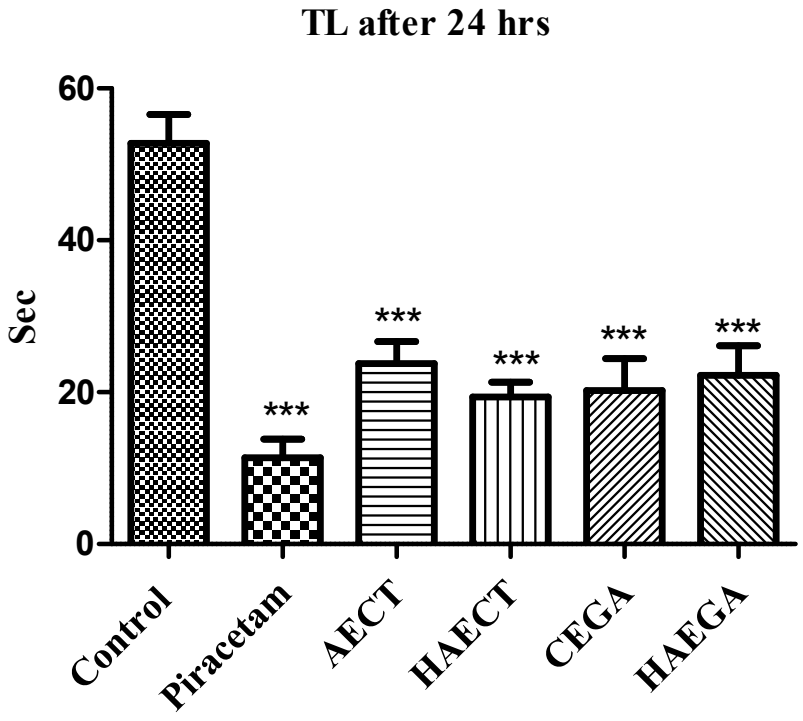


FIGURE 4.8a Effect of treatments on TL of mice by using Elevated Plus Maze apparatus. Values are shown in mean ± SEM values, n=10. ****p<0.001* compared to control using one-way ANOVA followed by Dunnett's test.

b) Effects on 15 days

The mice showed significant decrease in TL in piracetam ($p<0.001$), AECT ($p<0.001$), HAECT ($p<0.001$), CEGA ($p<0.001$) and HAEGA ($p<0.001$) as compared to control groups on 15 days respectively. The results are shown in figure 4.8b.

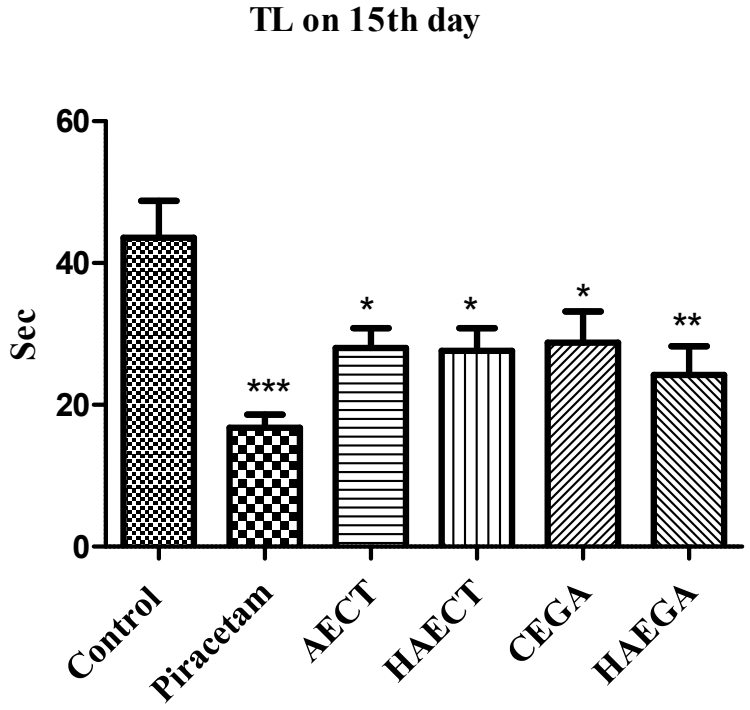


FIGURE 4.8b Effect of treatments on TL of mice by using Elevated Plus Maze apparatus. Values are shown in mean ± SEM values, n=10. ****p<0.001* compared to control using one-way ANOVA followed by Dunnett's test.

4.9 Estimation of acetyl cholinesterase enzyme activity

The mice showed significant decrease in AchE activity in piracetam ($p<0.05$), AECT ($p<0.05$), HAECT ($p<0.05$), CEGA ($p<0.05$) and HAEGA ($p<0.05$) as compared to control groups. The results are shown in figure 4.9.

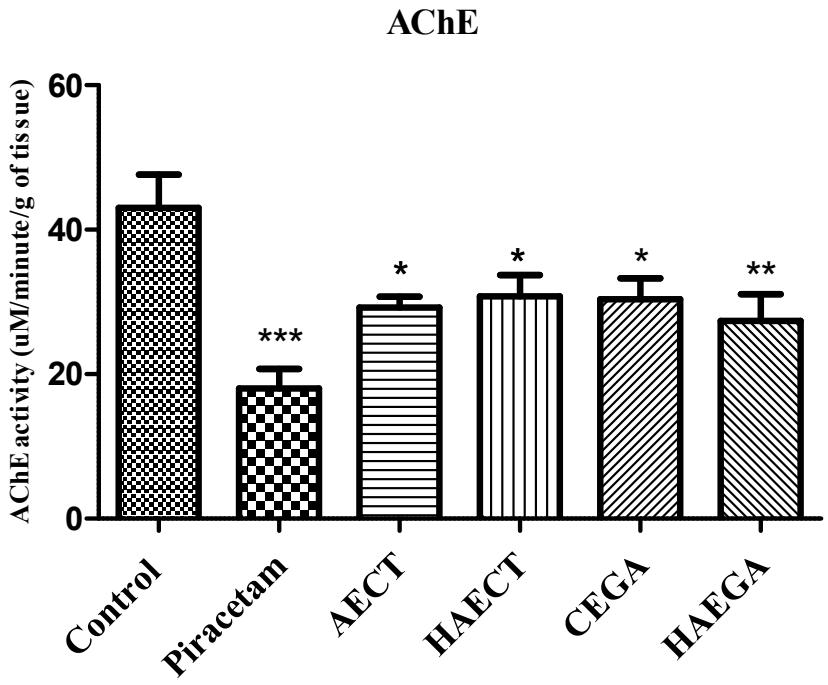


FIGURE 4.9 Effect of treatments on AChE enzyme activity. Values are shown in mean ± SEM values, n=6. ***$p<0.001$, **$p<0.01$, *$p<0.05$ compared to control using one-way ANOVA followed by Dunnett's test.

4.10 Estimation of total protein

The mice showed significant decrease in total protein in piracetam *p<0.001*), AECT (*p<0.001*), HAECT (*p<0.001*), CEGA (*p<0.001*) and HAEGA (*p<0.01*) as compared to control groups. The results are shown in figure 4.10.

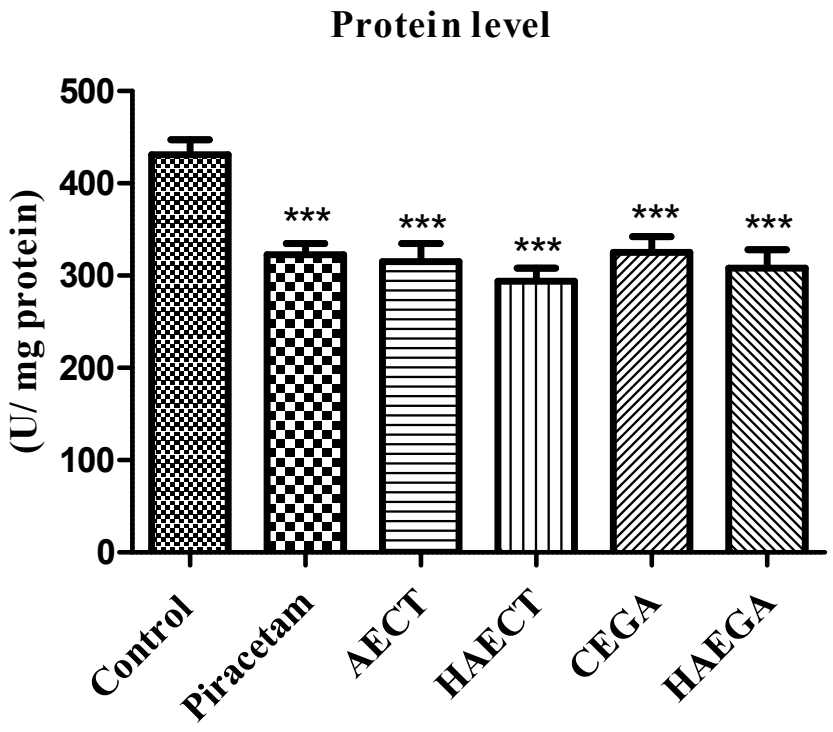


FIGURE 4.10 Effect of treatments on total protein. Values are shown in mean ± SEM values, n=6. ****p<0.001* compared to control using one-way ANOVA followed by Dunnett's test.

4.11 Estimation of Catalyse activity

The mice showed significant increase in catalyse activity in piracetam ($p<0.001$), AECT ($p<0.01$), HAECT ($p<0.001$), CEGA ($p<0.001$) and HAEGA ($p<0.001$) as compared to control groups. The results are shown in figure 4.11.

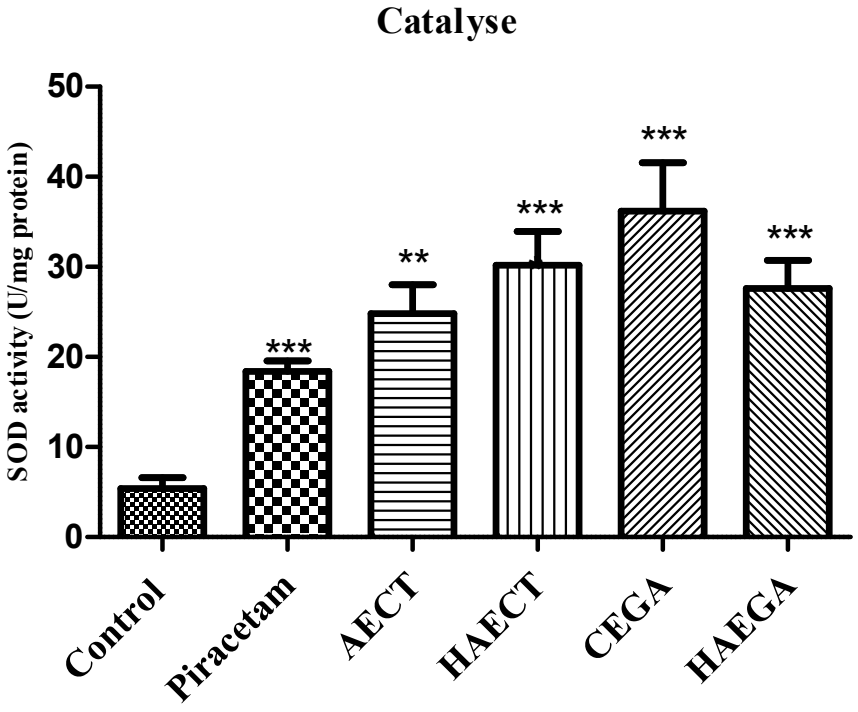


FIGURE 4.11 Effect of treatments on catalyse. Values are shown in mean ± SEM values, n=6. ***$p<0.001$, **$p<0.01$ compared to control using one-way ANOVA followed by Dunnett's test.

4.12 Estimation of SOD activity

The mice showed significant increase in SOD activity in piracetam (*p<0.001*), AECT (*p<0.001*), HAECT (*p<0.001*), CEGA (*p<0.001*) and HAEGA (*p<0.01*) as compared to control groups. The results are shown in figure 4.12.

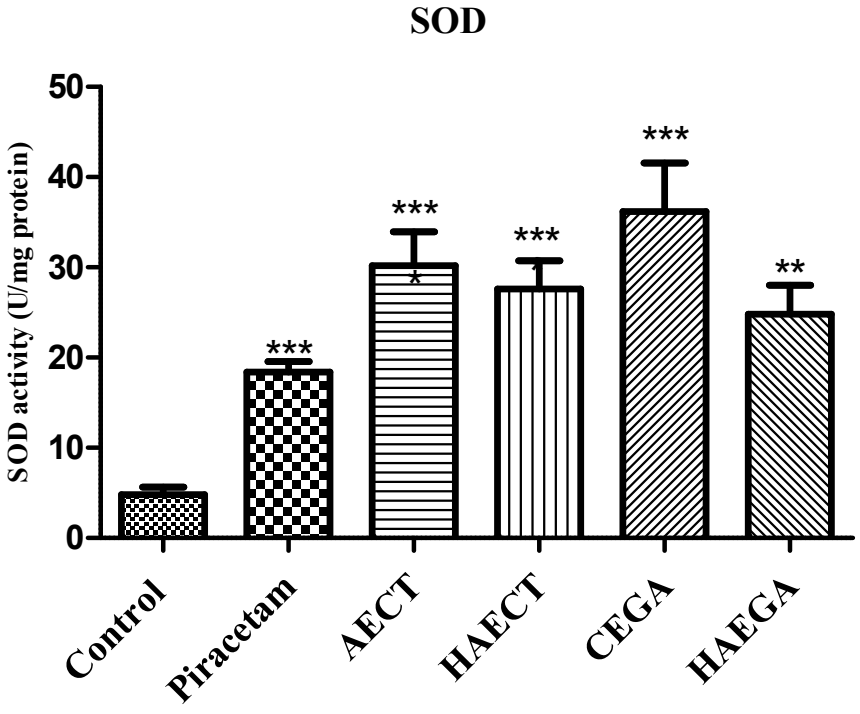


FIGURE 4.12 Effect of treatments on SOD. Values are shown in mean ± SEM values, n=6. ****p<0.001*, ***p<0.01* compared to control using one-way ANOVA followed by Dunnett's test.

4.13 Estimation of GHS activity

The mice showed significant increase in GSH activity in piracetam ($p<0.05$), AECT ($p<0.01$), HAECT ($p<0.05$), CEGA ($p<0.05$) and HAEGA ($p<0.05$) as compared to control groups. The results are shown in figure 4.13.

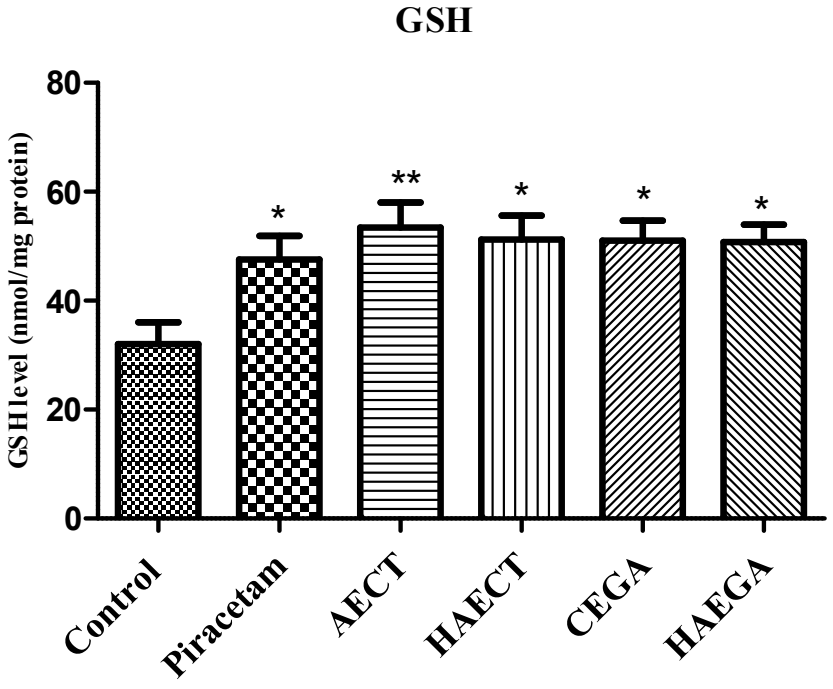


FIGURE 4.13 Effect of treatments on GSH. Values are shown in mean ± SEM values, n=6. $^{**}p<0.01$, $^{**}p<0.05$ compared to control using one-way ANOVA followed by Dunnett's test.

4.14 In- vitro Antioxidant Property

4.14.1 DPPH (1, 1-diphenyl-2-picryl hydrazyl) Method

TABLE 4.14.1: DPPH scavenging potential of the selected plant extracts

AECT	70.2 ± 3.52
HAECT	76.4 ± 5.09
CEGA	76.6 ± 4.56
HAEGA	62.4 ± 4.26
Vitamin C	81.8 ± 2.95

Values are shown in mean ± SEM values, n=5.

4.14.2 Nitric oxide (NO) Method

TABLE 4.14.2: NO scavenging potential of the selected plant extracts

AECT	42.8±4.70
HAECT	44.8±2.97
CEGA	46.8±2.83
HAEGA	47.6±3.06
Vitamin C	53.2±4.65

Values are shown in mean ± SEM values, n=5.

Chapter 5

DISCUSSION

CHAPTER 5 Discussion

Human nervous system is one of the most vital system for proper functioning and regulation of the entire body and also responsible for intelligence, emotion, wanting, perceiving, learning and memory. Memory is the ability of an individual to record sensory stimuli, events, information, etc., retain them over short or long periods of time and recall the same at a later date when needed (Shete and Bodhankar, 2009). Cognition, broadly defined, includes perception, learning, memory, and decision making, in other words, all ways in which animals take information about the world through the senses, process, retain, and decide to act on it can be called as cognition (Sheenaja *et al*., 2011). Besides age and gender, stressful and sedentary lifestyle, dietary excess, emotional disturbances, education level, social activities and burdens are among the factors that may lead to amnesia, anxiety, high blood pressure and dementia. Cognitive deficits have long been recognized as severe and consistent neurological disorders associated with numerous psychiatric and neurodegenerative states such as Alzheimer's disease. The Alzheimer disease is a most ordinary neurodegenerative disorder without an effective treatment. Dementia is the paramount feature of Alzheimer's disease (Grossberg, 2003). Nootropic agents are known to facilitate learning and memory, and prevent impairment of cognitive functions induced by diseases and brain insults. The several acetylcholinesterase inhibitors such as donepezil, rivastigmine and galantamine for mild to moderate cases, and the N-methyl D-aspartate antagonist memantine for the treatment of moderate to severe dementia are the only available treatments of cognitive dysfunction and memory loss associated with dementia. All these drugs seem to produce modest symptomatic improvements, but none appears to be able to cure the dementia or to stop its progression. There is a need for development of novel therapeutic strategies that target or even better prevent the molecular mechanisms leading to AD dementia (Florent-Bechard, 2007).

A variety of therapeutic targets have been identified as relevant in the treatment of cognitive disorders, including modulation of the cholinergic system, which may be achieved by the inhibition of acetylcholinesterase (AChE), and neuroprotection against

glutamate-induced overstimulation of N-methyl-D-aspartate (NMDA) receptors, by the use of NMDA receptor modulators. Other activities considered to be relevant in the alleviation of cognitive impairment include anti-inflammatory, antioxidant, antistress, antidepressant, and estrogenic activities. Accordingly in late Nineties, nootropic agents have enjoyed reputation as widely used class of agents that improve cognitive functions like memory and learning, provide neuroprotective effects from various insults and have excellent tolerability and safety (McDaniel, 2002). Development of cognition enhancers is still a difficult task because of complexity of the brain functions, poor predictability of animal tests and lengthy and expensive clinical trials as well as the lack of a common, generally accepted, mechanism of action (Gualtieri, 2002). Thus various nootropic agents like piracetam have not been accepted globally. After the early serendipitous discovery of first generation cognition enhancers, current research is based on a variety of working hypotheses, derived from the progress of knowledge in the neurobiopathology of cognitive processes (Gualtieri, 2002). All these problems in the development of the new leads for the pharmacotherapy and desperate need of the treatment prompted many researchers to search the option from the traditional system of medicines for this relentless progressive and devastating illness to transform it into a manageable chronic disease.

Herbs have been highly valued and used regularly for thousands of years by the peoples of the world as the medicine of the masses. Man has always searched for that herb that heals the body and soothes the mind. The nature provides a new opportunity to regain one's full mental capacity. A number of herbs traditionally employed in the Indian system of medicine "Ayurveda", to alleviate memory impairment both in healthy individuals and those with disease states which are now recognised as specific cognitive disorders such as Alzheimer's disease (Parle and Vasudevan, 2007). Two of the currently licensed drugs used to treat cognitive symptoms in AD, galantamine and rivastigmine were derived from plant sources and have been characterised as inhibitors of AChE. However, some plant preparations which occur as a complex mixture of components, such as Ginkgo biloba extract, have demonstrated relevant biological activities in relation to cognitive function, but the compounds responsible for the observed effects or the mechanisms of action have not been well characterised. Amongst the many plants reputed to enhance cognitive function in a variety of traditional medicines including Ayurvedic, Chinese, European, African and South American medicines, relatively few have been extensively studied to determine any pharmacological basis for their historical uses (Howes and Houghton,

2009). All these aspects of the lacunae in the available pharmacotherapy prompted us to undertake the search for the promising leads from various plants which were already mentioned in the classical texts of Ayurveda for cognitive enhancement. Plants, especially used in Ayurveda can provide biologically active moieties and lead structures for the development of modified derivatives with enhanced activity and /or reduced toxicity.

The extensive literary survey was conducted and the plants *Gmelina arborea* and *Cayratia trifolia* were selected for the investigations. The objective of the present work was confined to investigate the in vivo pharmacological activity of the *Gmelina arborea* and *Cayratia trifolia* stepwise manner in utmost possible pharmacologically valid model systems. The present work started in a stepwise manner by collecting the plant material from geographical area, Rajkot and authentication was done. The qualitative phytochemical investigations of the plants *Gmelina arborea* and *Cayratia trifolia* were conducted. *Gmelina arborea* revealed the presence of Carbohydrates, Alkaloids, Glycosides, Fixed oil & Fats, Protein and Amino acids. *Cayratia trifolia* revealed the presence of Carbohydrates, Flavonoids, Phytosterol, Phenolic compounds, Tannins and Phytosterol.

Amnesia inducing agents are the potential tool in the study of behavioural and neurobiological basis of learning and memory which may provide critical data for understanding and treating disorders of cognitive functions (Kulkarni, 2008). Furthermore pharmacologically induced impairment of cognitive tasks with scopolamine has become routine tools for the elucidation of specific neurotransmitters and receptor systems in the manipulation of learning and memory process. Scopolamine a nonselective muscarinic antagonist blocks cholinergic signalling and produces memory deficits that are similar to those found in age related senile CNS dysfunction (Vaisman, 2009; Vasudevan and Parle, 2006). Animals injected with scopolamine were evaluated using step down passive avoidance test in mice for the development of amnesia. Chemical hypoxia induced by administration of sodium nitrite, resulting in the reduction of oxygen carrying capacity of blood with conversion of haemoglobin to methemoglobin. Moreover, Hypoxia induced with sodium nitrite reduces incorporation of choline into acetylcholine, thus decreases the synthesis of acetylcholine. Scopolamine and sodium nitrate were administered prior the training session. Effect on step down latency (SDL) using passive avoidance paradigm was measured during learning and memory trial to examine the memory formation based

on negative reinforcement. SDL was defined as the time taken by the animal to step down from the wooden platform to grid floor with all its paws on the grid floor. This is a passive avoidance task as the normal exploratory behaviour of the animal is inhibited in this task.

In passive avoidance task, the animal learns that a specific place should be avoided when it is associated with a disturbing event (electrical shock). This is based on fear-conditionng behaviour, which originates from hippocampus and the amygdale. (Izadpanah *et al.,* 2016). Scopolamine, a muscarinic receptor antagonist, crosses the blood brain barrier and induces cognitive impairement and dementia by blocking postsynaptic muscarinic receptors and there by perturbing central cholinergic neuronal function leading to disruption of memory in experimental animals (Drachman & Leavitt, 1974), resulting in disruptions in the learning, acquisition, short-term retention (working memory) and retrieval from reference (long term memory), attention by blocking cholinergic neuro-transmission and reducing hipocampal volume during dementia (Yamada *et al.,* 2008). Hippocampal acetylcholine plays important role in learning and memory (Braida *et al.,* 2014; Hasselmo, 2006; Laursen *et al.,* 2014). Hippocampus contains various cholinergic receptors including muscarinic acetylcholine receptors (mAChR) (Freund *et al*., 2016; van der Zee and Luiten, 1999). mAChR antagonist, such as scopolamine, is used for screening candidates for dementia because it impairs learning and memory (Ebert and Kirch, 1998; Jung *et al*., 2016; Sitaram *et al*., 1978). In preliminary study, we studied the effects of all extracts MECT (500 mg/kg), PEECT (500 mg/kg), CECT (500 mg/kg), AECT (500 mg/kg), HAECT (500 mg/kg), MEGA (500 mg/kg), PEEGA (500 mg/kg), CEGA (500 mg/kg), AEGA (500 mg/kg) and HAEGA (500 mg/kg). We found that AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) has significant activity. A significant rise in SDL by AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) as compared to control suggesting beneficial effect either on learning or memory or on both process respectively. The significant effects demonstrated by plant preparations was found to be statistically comparable with established nootropic agent piracetam, thus suggesting memory facilitating effect of plant preparations. However, as shown by our passive avoidance task results, the shorter latency time induced by scopolamine was significantly reversed by AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg). So for further investigation were carried out by using this extracts only.

Selected plant preprations significantly restored the scopolamine-induced cognitive dysfunction induced by a cholinergic blockade in Passive avoidance test an exteroceptive behavioral model. It may due to increase of acetylcholine in the hippocampus through its acetylcholinesterase inhibitory effect. Hence selected plant preparations could be used successfully for the therapeutic management of the AChE induced intervention of cholinergic transmission related to dementia.

Oxygen free radicals are implicated in the process of aging and may be responsible for development of AD. Using sodium nitrite ($NaNO_2$) hypoxia, a model of aging brain, the core mechanism of amnesia development due to $NaNO_2$ is by induction of mild anaemic hypoxia (Gibson and Duffy, 1981). Sodium nitrite causes hypoxia due to the impairment in oxidative metabolism and cholinergic functions (Vogel and Vogel, 1997). This leads to due to increased generation of oxidative free radical which impairs ACh synthesis in the cholinergic neurons and exposure to hypoxia also elevates NO level in hippocampal region of brain (Maiti et al., 2007). Therefore neurodegeneration and consequent loss of memory functions may be linked with excessive accumulation of NO in neuronal cells.
Further, increased free radical generation also disrupts the activity of antioxidant enzymes such as SOD, CAT, glutathione peroxidase and glutathione reductase which protect the cholinergic neurons (Naik *et al*., 2006). These events lead to severe cholinergic neuronal dysfunction with $NaNO_2$. In sodium nitrate induced amnesia; control group significantly decreased the SDL in learning and memory trials. On treatment with AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) at all the doses significantly reversed sodium nitrate induced spatial memory impairment as compared to negative control group. The nootropic effect revealed by AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) was found to be similar to that of standard piracetam.

Fear conditioning is often conducted as a type of avoidance task in which the cue associated with the aversive stimulus is the environmental context. The fear conditioning task used extensively by (Fanselow *et al*., 2005) Conditioned avoidance response behavior mainly affects cognitive behavior by mesocortical pathway of dopaminergic neurons (Cook and Catania, 1964). It is an avoidance escape procedure used to separate nootropics from antipsychotics. Whereas, sedative compounds suppress both avoidance and escape responding at approximately the same doses, neuroleptic drugs reduce avoidance

responding at lower doses than those affecting escape responding. In our study, AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) significantly delayed time taken by the rats to climb the pole. This revealed that memory retrieval potential of AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg).

Memory for locations in space is primarily the province of the hippocampus and related structures. Damage to the hippocampus in rodents is nearly always associated with deficits in acquiring spatial memory, as well as deficits encoding spatial position (Nadel and Keefe, 1978). Elevated plus maze test used to measure the spatial long-term memory in animals. In the elevated plus-maze test, TL might be shortened if the animal had previous experience of entering the open arm and the shortened TL could be related to memory. In our investigation results of elevated plus maze confirm the amelioration of amnesia, improvement in learning skill and cognitive impairment in mice treated with AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg) and HAEGA (500 mg/kg), whereas similar type of cognitive enhancing activity was obtained from standard drug piracetam. The shortening of TL by Piracetam as well as AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) indicates improvement in memory.

The acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions. It is a critical component for dementia related memory deficits and underlying process of cognitive disorders (Das *et al*., 2005). Acetylcholine facilitates NMDA receptor function and this can induce large long-term potentiation (LTP) (Blitzer et al., 1990; Markram and Segal, 1990). The most successful approach for AD treatment currently is to inactivate AChE, an enzyme present in the synapse that rapidly cleaves ACh and prevents neuronal signaling (Cutler & Sramek, 2001). AChE inhibitors such as physostigmine, tacrine and velnacrine can enhance cognitive process in animals and humans (Dawson et al., 1991). Therefore, inhibition of AChE is an attractive strategy for the treatment of dementia and associated cognitive disorders (Terry and Buccafusco, 2003; Trinh *et al*., 2003). Our investigation validated the earlier concept regarding management of dementia by promising ex vivo inhibition of AChE inhibition exists. It may also enhance the cholinergic activity, brain acetylcholine level in synapses (Lane *et al*., 2006), and there by ameliorate the cognitive and memory dysfunction in the dementia related neurodegenerative diseases. A specific marker for cholinergic neurons (hydrolysed

content of acetylcholine) was measured to provide a putative rational basis for the beneficial memory effects of plant preparations.

The brain AChE activity and total protein contents were estimated in this study along with the passive avoidance. Pre-treatment with AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) at all doses significantly reduced AChE and reduction in the total protein levels as compared to control. The results are comparable to standard piracetam. Alterations in the levels of various neurochemicals play a crucial role in the pathophysiology of memory deficits of laboratory animals and patients with Alzheimer's disease. The impairment of central acetylcholine neurotransmission in the brain is believed to be a principle neuropathological feature of Alzheimer's disease (Zhang, 2004). All the preparations seem to have addressed the cholinergic deficiency by augmenting Acetylcholine levels in brain through cholinesterase inhibition. Thus AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) improved basal performance and comparable with reference standard piracetam claiming their nootropic potential.

The oxidative stress is the state of imbalance between the level of antioxidant defense mechanism and production of the free radicals that favors potential brain damage. Brain tissue is particularly susceptible to oxidative damage due to its high oxygen content, low level of antioxidant protection, and high level of polyunsaturated fatty acids (Moreira *et al.*, 2005). Therefore, it is believed that pharmacological modification of oxidative damage is one of the most promising strategies in the treatment of neurodegenerative diseases. Superoxide anion, hydrogen peroxide, and the highly toxic hydroxyl radical, collectively known as reactive oxygen species (ROS), are the most abundant free radicals in living cells. Accumulation of these free radicals in specific brain areas, which can lead to neurodegeneration, is believed to be a major factor in Alzheimer's disease (Glover and Sandler, 1993). Reactive oxygen species (ROS)-induced damage to biomolecules (lipids, proteins, DNA and RNA) plays an important role in the advancement of aging and age-related neurodegenerative disorders such as AD (Liu et al., 2003). Mitochondrial dysfunction resulting from increased reactive oxygen species generation has also been involved in aging and neurodegenerative disorders. The interaction between oxidative stress and mitochondrial dysfunction probably forms a vicious downward spiral that

amplifies the deficits and thus is believed to play an important role in the neurodegenerative disorders like AD (Swerdlow, 2011).

Basal forebrain and amygdala are the areas which are involved in learning and memory formation and are the most vulnerable areas susceptible to oxidative stress (Mattson *et al.,*1999). superoxide dismutase (SOD) and catalase (CAT) are the key enzymes, that remove these ROS by antioxidant defence mechanisms. An effective antioxidant should have the capability of increasing the intracellular concentrations of the above mentioned key enzymes. The selected plant extracts were established to increase the SOD and CAT in the rat brains after 21 days of administration. To exert this effect in the brain, the active constituents of selected plant extracts should cross the blood–brain barrier (BBB). Brain membrane contains a relatively high degree of polyunsaturated fatty acids. Due to the lipophilic nature of the membrane, it exhibits selective permeability to lipophilic molecules across BBB.

Reduced GSH is the most abundant non-protein thiol, which buffers free radicals in brain tissue (Dringen *et al*., 2000). Glutathione is an endogenous antioxidant found in all animal cells. The redox system of glutathione consists of GSH and an array of functionally related enzymes, of which Glutathione Reductase (GR) is responsible for the regeneration of GSH, whereas Glutathione peroxidase (GPx) works together with GSH in the decomposition of H_2O_2 and other organic hydroperoxides (Ding *et al*., 2013). A reduction in the levels of GSH may impair H_2O_2 clearance and promote formation of OH, the most toxic moiety to the brain, leading to more oxidant load and consequently oxidative damage. In this study, the treatments of animals with selected plant extracts prevented this process which may be the mechanism responsible for facilitating effects of inhibitory avoidance memory. Damage to myelin sheath by stress may lead to cognitive dysfunction (Sims *et al*., 2013).

The enhanced antioxidant effect may be due to augmentation of antioxidant enzyme protective system involving SOD and CAT activities both in hippocampus and striatum (Singh et al., 2009). In the current study, AECT (500 mg/kg), HAECT (500 mg/kg), CEGA (500 mg/kg), and HAEGA (500 mg/kg) treatment significantly increase the SOD, CAT activity and GSH levels. This effect of may be attributed to its antioxidant potential.

There are numerous methods available for evaluating in vitro antioxidant activity. Due to the oxidative process, however, the total antioxidant activity of an antioxidant cannot be evaluated by using a single method. Therefore at least two methods should be employed in order to evaluate the total antioxidant activity. The stable radical 1,1-diphenyl-2-picryl-hydrazyl is a useful reagent for investigating the radical scavenging activity. DPPH• accepts an electron from a hydrogen radical to become a stable diamagnetic molecule. The decrease in radical absorbance by DPPH caused by antioxidants results in the scavenging of the radical by hydrogen donation (Soares *et al*., 1997).
We evaluated ex vivo antioxidant property by DPPH method. The antioxidant activity of AECT, HAECT, CEGA and HAEGA were comparable with standard vitamin C by using DPPH method.

Cognition is a multi-factorial process with delicate balance among the factors interacting with each other at physiological and molecular level. NO, being a diffusible neurotransmitter, modulates cognitive process by regulating cell viability and death through the formation of toxic peroxynitrite and inhibition of cytochrome oxidase depending on its concentration (Beckman et al., 1990; Sachdeva et al., 2011). Chronic exposure to hypoxia elevates NO level in hippocampal region of brain (Maiti et al., 2007). Therefore neurodegeneration and consequent loss of memory functions may be linked with excessive accumulation of NO in neuronal cells. However, the component(s) responsible for scavenging of NO synthesis in selected plant extracts is not clear and more studies are necessary to unfold the underlying mechanisms.

Thus our investigation marvellously demonstrates the cognitive enhancing and/or anti-amnesic property of the plant preparations in the presence and/or the absence of amnestic agent suggests the nootropic activity of the plant preparations. The nootropic effect may be attributed to inhibition of AChE activity and thus improving the spatial memory formation. The neuroprotection could be ascribed to its strong antioxidant potential, inhibitory role on AChE activity and NO scavenging property. Thus, the extracts can also be utilized in form of nutraceuticals after safety assessment. From the finding of the present investigations the study prospect selected plant preparation as the promising therapeutic candidate to be screened further in neurodegenerative disorders like AD.

Chapter 6

CONCLUSION

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The prominent outcome of the present investigations is that *Gmelina arborea* and *Cayratia trifolia* would appear to be active and have a profound memory enhancing effects comparable to piracetam. The observed nootropic activity may be due to enhancement of cholinergic and antioxidant activity might boost the nootropic activity. In conclusion, plant preparations share number of physiological actions that are potentially beneficial in slowing cognitive decline and it seems reasonable to suggest that they could be considered as a strategy to prevent or slow down the development of neurodegenerative diseases such as dementia and alzheimer's disease at an early stage.

Chapter 7

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Chapter 8

PUBLICATION

List of Publications

1. Evaluation of nootropic activty of Ca*yratia trifolia* in experimental animal models published in World Journal of Pharmacy and Pharmaceuticals Sciences. 2018:7(6);1558-68.
2. Evaluation of nootropic activty of *Gmelina arborea* in experimental animal models published in Inventi Impact Ethanopharmacology 2018(4):187-191.

Appendix

A

GTU Code : 263

AICTE LETTER F.No.: 06/01/GUJ/ PHARMA/2006/004
PCI LETTER F. NO : 32 - 995 / 2006 PCI

SMT. R. D. GARDI B. PHARMACY COLLEGE

Managed by : Shri Saraswati Education & Charitable Trust-Rajkot
(Courses : B. Pharm. & M. Pharm.)

- Approved by : AICTE, PCI, New Delhi & Govt. of Gujarat
- Affiliated to Gujarat Technological University (GTU), Ahmedabad.

Ref. No. : 191/b/2012-13

Date : 08/09/2012

APPROVAL CERTIFICATE

This is certify that the project title **"Studies on nootropic effect of various extract of *Gmelina arborea, Vitex negundo* & *Cayratia trifolia*"** has been approved **(01/2012/IAEC/RDGPC)** by the IAEC, Smt. R.D.Gardi B.Pharmacy College, Nyara, Rajkot – 360 110.

Dr. Shital D. Faldu,
Chairperson,
IAEC,
Smt. R. D. Gardi B. Pharmacy College,
Rajkot.

Dr. Sudhir A. Mehta,
CPCSEA Main Nominee,
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Smt. R. D. Gardi B. Pharmacy College,
Rajkot.

Nyara, Jamnagar Road, Opp. Garden Dinner Club, Nr. Cricket Stadium, Rajkot. Pin : 360 110. (Gujarat)
Phone : (0281) 2926408 / 7 Fax : (0281) 2585501, Mo. 090990 63163
E-mail : rdgardipharmacy@yahoo.com Website : www.pharmacy.saraswatiedutrust.org.

Appendix

B

Gmail Darshan Madiya <darsh.pharma@gmail.com>

Requesting permission to use copyrighted material - IMAGE of Schematic Diagram of Nigrostriatal Dopaminergic Pathway

Betarbet, Ranjita <rbetarb@emory.edu> 18 March 2019 at 19:08
To: Darshan Madiya <darsh.pharma@gmail.com>
Cc: Bothara Sunil <botharasb1@yahoo.co.in>

Sure, please use the schematic and cite the paper as required.

Best

Ranjita

Ranjita Betarbet, PhD

Assistant Professor and

Administrative Director,

Center for Neurodegenerative Disease,

Emory University,

505G, Whitehead Research Building,

619 Michael Street

Atlanta 30322

Tel – 404 727 9104

Fax – 404 727 3728

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Gmail Darshan Madiya <darsh.pharma@gmail.com>

Requesting permission to use copyrighted material - IMAGE - Neuron structural changes associated with Alzheimer's Disease

Ed Berger <eberger@brightfocus.org> 19 March 2019 at 21:31
To: Darshan Madiya <darsh.pharma@gmail.com>
Cc: Bothara Sunil <botharasb1@yahoo.co.in>

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- I can give you permission to use the first image, if you provide this citation:

The medical illustration is provided courtesy of Alzheimer's Disease Research, a BrightFocus Foundation program.
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- https://www.linkedin.com/in/bobmorreale

Hope that helps!

Ed

Ed Berger

Manager of Online Operations

BrightFocus® Foundation
Cure in Mind. Cure in Sight.

22512 Gateway Center Drive

Clarksburg, Maryland 20871

301.556.9348

eberger@brightfocus.org

www.brightfocus.org

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Gmail Darshan Madiya <darsh.pharma@gmail.com>

Requesting permission to use copyrighted material - IMAGE - Cellular iron uptake

permissions (US) <permissions@sagepub.com> 20 March 2019 at 21:36
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Cc: Bothara Sunil <botharasb1@yahoo.co.in>
Subject: Re: Requesting permission to use copyrighted material - IMAGE - Cellular iron uptake

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From: Darshan Madiya <darsh.pharma@gmail.com>
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Subject: Requesting permission to use copyrighted material - IMAGE - Cellular iron uptake

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I request you to kindly grant me permission to quote / use **" Cellular Iron uptake(image)" [**Volume 6, Number 6, 2000 ; ISSN 1073-8584; *Neuroscientist, 6(6), 435–453.* doi:10.1177/107385840000600607**]** in the thesis. There is **no commercial use** of the thesis **purely academic purpose**.

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Yours sincerely,

Darshan Madiya