To Study The Ameliorative Effect Of Hydroalcholic Extract Of *Caryota urens* (Arecaceae) On Streptozotocin Induced Alzheimer's Model In Mice

Dissertation submitted to

THE TAMIL NADU DR.M.G.R.MEDICAL UNIVERSITY, CHENNAI

In partial fulfilment for the award of the degree of

MASTER OF PHARMACY

IN

PHARMACOLOGY

By

Register No: 261425013

UNDER THE GUIDANCE OF
DR.P.MURALIDHARAN, M.Pharm., Phd.

DEPARTMENT OF PHARMACOLOGY

C.L.BAID METHA COLLEGE OF PHARMACY

(AN ISO 9001-2008 CERTIFIED INSTITUTION)

CHENNAI – 600097

OCTOBER – 2016

Phone : 24960151, 24962592, 24960425
e-mail : clbaidmethacollege@gmail.com
Website : www.clbaidmethacollege.com

C.L. Baid Metha College of Pharmacy

An ISO 9001:2008 approved institution
Jyothi Nagar, Old Mahabalipuram Road,
Thorapakkam, Chennai - 600 097.

ISO 9001 : 2008
Reg. No.: BQ91C032

Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai.
Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education, New Delhi

Dr.P.Muralidharan, M.Pharm., Phd

Prof & Head

Department of Pharmacology

CERTIFICATE

This is to certify that Project entitled **To Study The Ameliorative Effect Of Hydroalcholic Extract Of *Caryota urens* (Arecaceae) On Streptozotocin Induced Alzheimer's Model In Mice** submitted by Regn No: **261425013** in partial fulfilment of the course for the award of the degree of **Master of Pharmacy in Pharmacology.** It was carried out at the Department of Pharmacology in C.L. Baid Metha College of Pharmacy, Chennai-97 under my guidance during the academic year 2015-2016.

Place: Chennai

Date: (**Dr.P.MURALIDHARAN**)

Prof. Dr. GRACE RATHNAM, M.Pharm., Ph.D.,

Principal

CERTIFICATE

This is to certify that Project entitled **To Study The Ameliorative Effect Of Hydroalcholic Extract Of *Caryota urens* (Arecaceae) On Streptozotocin Induced Alzheimer's Model In Mice** submitted by Regn No: **261425013** in partial fulfilment of the course for the award of the degree of **Master of Pharmacy in Pharmacology.** It was carried out at the Department of Pharmacology in C.L. Baid Metha College of Pharmacy, Chennai-97. Under the supervision of *Professor Dr.P.Muralidharan* during the academic year 2015-2016.

Place: Chennai-97

Date: (**Prof. Dr. GRACE RATHNAM**)

.

DECLARATION

Register No: **261425013** hereby declare that this dissertation entitled, **To Study The Ameliorative Effect Of Hydroalcholic Extract Of *Caryota urens* (Arecaceae) On Streptozotocin Induced Alzheimer's Model In Mice** has been originally carried out by me under the guidance and supervision of **Prof. Dr.P.Muralidharan, M.Pharm,. PhD,** Head of the department of pharmacology C.L. Baid Metha College of Pharmacy, Chennai-97 for the academic year 2015-2016. This work has not been submitted in any other degree at any other university.

Place: Chennai-97

Date: Register No: **261425013**

ACKNOWLEDGEMENTS

<div align="right">

M.PRASANTH

</div>

LIST OF TABLES:

LIST OF GRAPHS

LIST OF FIGURES

CONTENTS:

ABBREVATIONS

AchE	: Acetylcholinesterase
AChEIs	:Acetylcholinesterase Inhibitors
ACSF	: Artificial Cerebrospinal Fluid
AD	: Alzheimer's disease
AlCl3	: Aluminimum Trichloride
ALS	: Amyotrophic Lateral Sclerosis
ANOVA	: Analysis Of Variance
ApoE	: Apolipoprotein
APP	: Amyloid Precursor Protein
ATC	: Acetylthiocholine
ATP	: Adenosine Triphosphate
Aβ	: Beta Amyloid
BBB	: Bloob Brain Barrier
CAT	: Catalase
CLF	: Chloroform Fraction
CMC	: Carboxy Methyl Cellulose
CNS	: Central Nervous System
CON	: Control
CPCSEA Experiments	: Committee For The Purpose Of Control And Supervision Of on Animals
CSF	: Cerebrospinal Fluid
CT	: Computed Topography
DA	: Dopamine
DDW	: Double Distilled Water
DM	: Diabetes Melitis
DNA	: Deoxyribonucleic Acid
DPPH	: 1,1-Diphenyl-2-Picrylhydrazyl
DTNB	: 5,5'-Dithiobis-(2-Nitrobenzoic Acid)
EDTA	: Ethylene Diamine Tetra Acetic Acid Disodium Salt
EL	: Escape Latency

FALS	: Familial Amyotrophic Lateral Sclerosis
FTIR	: Fourier Transport Infrared Spectroscopy
GDP	:Gross Domestic Product
GFAP	: Glial Fibrillary Acidic Protein
GHS	: Globally Harmonized System
GLUT4	: Glucose Transporter
GPx	: Glutathione Peroxidase
GSH	: Reduced Glutathione
GSK-3	: Glycogen Synthase Kinase-3
H2O2	: Hydrogen Peroxide
HAECU	: Hydroalcoholic Extract *CARYOTA URENS*
HD	: High dose
HM	: Henry Molaison
HNF	: 4- Hydroxy-2-Nonenal
HPTLC	: High-Performance Thin-Layer Chromatography
i.c.v.	: Intracerebroventricular
IAEC	: Institutional Animal Ethical Committee
IGF	: Insulin-Like Growth Factor
IR	: Insulin Receptor
IRS Substrate	: Nicotinamide Adenine Dinucleotide Phosphate Insulin Receptor
LD	: Low dose
LPO	: Lipid Peroxidase
MRI	: Magnetic Resonance Imganing
mRNA	: Messenger RNA
MWM	: Morris Water Maze
NADP	: Nicotinamide Adenine Dinucleotide Phosphate
NADPH	: Nicotinamide Adenine Dinucleotide Phosphate Reduced Tetra Sodium Salt
NC	: Negative control
ND	: Neurodegeneration

NFT	: Neurofibrillary Tangles
NMDA	: N-methyl-D-aspartate
NMR	: Nuclear Magnetic Resonance
NO	: Nitric Oxide
NPs	: Neuritic Plaques
NTs	: Neuropil Threads
OECD	: Organisation for Economic Co-operation and Development
OPT	: O-phthalaldialdehyde
ORT	: Object Recognition Test
PET	: Positron Emission Tomography
p.o.	: per oral
PG	: Prostaglandin
PI3K	: Phosphatidylinositol-3 kinase
PNS	: Peripheral Nervous System
RA	: Retrograde Amnesia
SAD	: Sporadic Alzheimer Disease
SDL	: Step Down Latency
SDS	:Sodium Dodecyl Sulphate
SEM	: Standard Error Mean
SOD	: Superoxide Dismutase
SP	: Senile Plaques
STZ	: Streptozotocin
STD	: Standard
TBA	: Thiobarbituric Acid
TPC	: Total Phenolic Content
TFC	: Total Flavonoid Content

1. INTRODUCTION

Nature is the abundant source of medicinal plants. The use of medicinal plants is from ancient times onward. Till today majority of people rely on such traditional remedies. Many of compounds used for the production of modern medicines were also derived from the herbs in the surroundings.

Because of their ability to synthesize a wide variety of chemical compounds that can be used to perform important biological functions, their phytochemicals have been processed for beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. At least 12,000 such compounds have been isolated so far, a number estimated to be less than 10% of the total.[1]

The term "herbs" refers to plants or parts of them, including grasses, flowers, berries, seeds, leaves, nuts, stems, stalks and roots, which are used for their therapeutic and health-enhancing properties. Generations of skilled herbal practitioners, researchers and scholars have refined and tested the vast science of herbology, producing thousands of plant-based remedies that are safe and effective.

An estimated eighty percent (80%) of the world's population employs herbs as primary medicines

Ayurveda, Siddha and Unani systems of medicine provide good base for scientific exploration of medicinally important molecules from nature.

India is rightly called the botanical garden of the world and perhaps the largest producer of medicinal herbs. India recognizes over 3000 plants for their medicinal value. It is estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine i.e. 75% of the medicinal needs of the world countries. Medicinal herbs are used in one form or another, under indigenous systems of medicine like Ayurveda, Siddha & Unani.

India holds its history in herbal remedy and found to be so popular that the government of India has created a separate department 'AYUSH' under the Ministry of Health & Family Welfare. In 2000, the National Medicinal plants Board was established by Indian government in order to deal with the herbal medicinal system.

Traditional therapy is supported by evidence on safety and effectiveness. Usually, these evidences were found on sources such as pharmacopoeias, traditional scriptures, and/ or clinical experience registered over hundreds of years. At present, an increasing number of scientific studies support the use of herbal therapy. The advantages of traditional medicine include its diversity and flexibility, availability and affordability. It is of comparatively low cost and merely requires relatively low level of technological input. However, there is a need for an increase in research to improve the evidence base as regards to efficacy.[2]

PLANT MEDICINES, SAFER AND TIME-TESTED

Plant medicines are far and away safer, gentler and better for human health than synthetic drugs. This is so because human beings have co-evolved with plants over the past few million years.[3]

2. LITERATURE REVIEW

2.1 MEMORY [4,5]

Memory is the processes by which information is encoded, stored, and retrieved.

There are three main stages in the formation and retrieval of memory:

Encoding or registration (receiving, processing and combining of received information)

- **Storage** (creation of a permanent record of the encoded information)
- **Retrieval**, *recall* or *recollection* (calling back the stored information in response to some clue for use in a process or activity)
- Encoding allows information that is from the outside world to reach our senses in the forms of chemical and physical stimuli. In this first stage we must change the information so that we may put the memory into the encoding process. Storage is the second memory stage or process. This entails that we maintain information over periods of time. Finally the third process is retrieval. This is the retrieval of information that we have stored. We must locate it and return it to our consciousness. Some retrieval attempts may be effortless due to the type of information.

SENSORY MEMORY

Sensory memory corresponds approximately to the initial 200–500 milliseconds after an item is perceived. The ability to look at an item, and remember what it looked like with just a second of observation, or memorisation, is an example of sensory memory.

Short term memory

Short-term memory allows recall for a period of several seconds to a minute without rehearsal. Its capacity is also very limited. However, memory capacity can be increased through a process called chunking
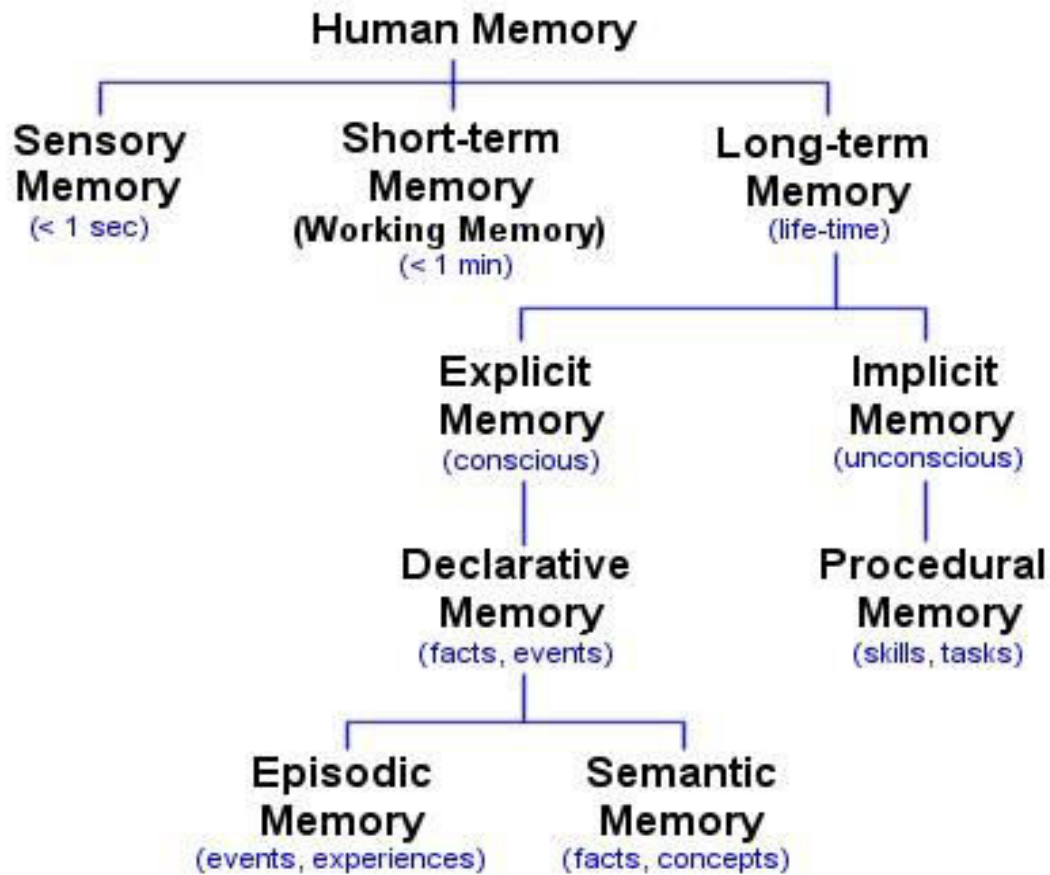
Long-term memory

Long-term memory can store much larger quantities of information for potentially unlimited duration (sometimes a whole life span). Its capacity is immeasurably large. Long-term memories, on the other hand, are maintained by more stable and permanent changes in neural connections widely spread throughout the brain. The hippocampus is essential (for learning new information) to the consolidation of information from short-term to long-term memory, although it does not seem to store information itself. Without the hippocampus, new memories are unable to be stored into long-term memory, as learned from HM after removal of his hippocampus, and there will be a very short attention span. Furthermore, it may be involved in changing neural connections for a period of three months or more after the initial learning. Several studies have demonstrated that memory depends on getting sufficient sleep between training and test. Additionally, data obtained from neuroimaging studies have shown activation patterns in the sleeping brain which mirror those recorded during the learning of tasks from the previous day, suggesting that new memories may be solidified through such rehearsal

2.2 AMNESIA[6]

Amnesia is a condition in which one's memory is lost. The causes of amnesia have traditionally been divided into certain categories. Memory appears to be stored in several parts of the limbic system of the brain, and any condition that interferes with the function of this system can cause amnesia. Functional causes are psychological factors, such as post-traumatic stress or, in psycho analytic terms, defense mechanism.

- **Anterograde amnesia** is the loss of long-term memory, the loss or impairment of the ability to form new memories through memorization. People may find themselves constantly forgetting a piece of information, people or events after a few seconds or minutes, because the data does not transfer successfully from their conscious short-term memory into permanent long-term memory. Primarily in older men, transient global amnesia causes severe loss of memory for minutes or hours.

- **Retrograde amnesia**, the loss of pre-existing memories to conscious recollection, beyond an ordinary degree of forgetfulness. This type of amnesia first targets the patient's most recent memories. The amount of memories lost depends on the severity of the case. The person may be able to memorize new things that occur after the onset of amnesia (unlike in antero grade amnesia), but is unable to recall some or all of their life or identity prior to the onset. The effect of retrograde amnesia (RA) occurs on fact memory to a lower degree than it occurs on autobiographical memory, which can be affected over the whole lifespan of the patient by RA. There have also been some cases where retrograde amnesia is a result of hypoglycemia in insulin-dependent diabetic patients.

However, there are different types of memory, for example procedural memory (i.e. automated skills) and declarative memory (personal episodes or abstract facts), and often only one type is impaired. For example, a person may forget the details of personal identity, but still retain a learned skill such as the ability to play the piano.

In addition, the terms are used to categorize patterns of symptoms rather than to indicate a particular cause (etiology). Both categories of amnesia can occur together in the same patient, and commonly result from drug effects or damage to the brain regions most closely associated with episodic memory: the medial temporal lobes and especially the hippocampus.

An example of mixed retrograde and antero grade amnesia, may be a motorcyclist unable to recall driving his motorbike prior to his head injury (retrograde amnesia), nor can

he recall the hospital ward where he is told he had conversations with family over the next two days (antero grade amnesia).

The most influential case on anter ograde amnesia was H.M. This patient had to undergo bi-lateral removal of the hippocampus and amygdala in order to treat his severe epilepsy. In fact, H.M. was reported having up to 10 seizures per day. Although the epilepsy was fixed through surgery H.M. ended up with antero grade amnesia.

The effects of amnesia can last a long time even after the condition has passed. Some sufferers claim that their amnesia changes from a neurological condition to also being a psychological condition, whereby they lose confidence and faith in their own memory and accounts of past events.

Another effect of some forms of amnesia may be impaired ability to imagine future events. A 2006 study showed that future experiences imagined by amnesics with bilaterally damaged hippocampus lacked spatial coherence, and the authors speculated that the hippocampus may bind different elements of experience together in the process of re-experiencing the past or imagining the future.

2.3 NEURODEGENERATION

Neurodegeneration is the umbrella term for the progressive loss of structure or function of neurons, including death of neurons. Many neurodegenerative diseases including ALS, Parkinson's, Alzheimer's, and Huntington's occur as result of neurodegenerative processes. As research progresses, many similarities appear that relate these diseases to one another on a sub-cellular level. Discovering these similarities offers hope for therapeutic advances that could ameliorate many diseases simultaneously. There are many parallels between different neurodegenerative disorders including atypical protein assemblies as well as induced cell death. Neurodegeneration can be found in many different levels of neuronal ranging from molecular to systemic.[7]

The term neurodegeneration is a combination of two words - "neuro," referring to nerve cells and "degeneration," referring to progressive damage. The term "neurodegeneration" can be applied to several conditions that result in the loss of nerve structure and function.

This deterioration gradually causes a loss of cognitive abilities such as memory and decision making. Neurodegeneration is a key aspect of a large number of diseases that come under the umbrella of neurodegenerative diseases. Of these hundreds of different disorders, so far attention has been mainly focused on only a handful, with the most notable being Parkinson's disease, Huntington disease and Alzheimer's disease. A large proportion of the less publicized diseases have essentially been ignored.

All of these conditions lead to progressive brain damage and neurodegeneration. Although all three of the diseases manifest with different clinical features, the disease processes at the cellular level appear to be similar. For example, Parkinson's disease affects the basal ganglia of the brain, depleting it of dopamine. This leads to stiffness, rigidity and tremors in the major muscles of the body, typical features of the disease. In Alzheimer's disease, there are deposits of tiny protein plaques that damage different parts of the brain and lead to progressive loss of memory. Huntington's disease is a progressive genetic disorder that affects major muscles of the body leading to severe motor restriction and eventually death.[8]

2.4 NEURODEGENERATIVE DISEASES

ALZHEIMER'S DISEASE

Alzheimer's disease is characterized by loss of neurons and synapses in the cerebral cortex and certain subcortical regions. This loss results in gross atrophy of the affected regions, including degeneration in the temporal lobe and parietal lobe, and parts of the frontal cortex and cingulate gyrus.

Alzheimer's disease has been hypothesized to be a protein misfolding disease (proteopathy), caused by accumulation of abnormally folded beta amyloid and tau proteins in the brain. Plaques are made up of small peptides, 39–43 amino acids in length, called beta-amyloid Beta-amyloid is a fragment from a larger protein called amyloid precursor protein (APP), a transmembrane protein that penetrates through the neuron's membrane. APP is critical to neuron growth, survival and post-injury repair. In Alzheimer's disease, an unknown process causes APP to be divided into smaller fragments by enzymes through proteolysis. One of these fragments gives rise to fibrils of beta-amyloid, which form clumps that deposit outside neurons in dense formations known as senile plaques.[9]

Parkinson's disease

Parkinson's disease is the second most common neurodegenerative disorder and manifests as bradykinesia, rigidity, resting tremor and posture instability. Parkinson's disease is a degenerative disorder of the central nervous system. It results from the death of dopamine- generating cells in the substantia nigra, a region of the midbrain; the cause of cell-death is unknown.

The mechanism by which the brain cells in Parkinson's are lost may consist of an abnormal accumulation of the protein alpha-synuclein bound to ubiquitin in the damaged cells. The alpha- synuclein-ubiquitin complex cannot be directed to the proteosome. This protein accumulation forms proteinaceous cytoplasmic inclusions called Lewy bodies.[10]

Huntington's disease

Huntington's disease causes astrogliosis and loss of medium spiny neurons. Areas of the brain are affected according to their structure and the types of neurons they contain, reducing in size as they cumulatively lose cells. The areas affected are mainly in the striatum, but also the frontal and temporal cortices. The striatum's subthalamic nuclei send

control signals to the globus pallidus, which initiates and modulates motion. The weaker signals from subthalamic nuclei thus cause reduced initiation and modulation of movement, resulting in the characteristic movements of the disorder.

Mutant Huntington is an aggregate-prone protein. During the cells' natural clearance process, these proteins are retro grade transported to the cell body for destruction by lysosomes. It is a possibility that these mutant protein aggregates damage the retrograde transport of important cargoes such as BDNF by damaging molecular motors as well as microtubules.[11]

Amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS/Lou Gehrig's Disease) is a disease in which motor neurons are selectively targeted for degeneration. In 1993, missense mutations in the gene encoding the antioxidant enzyme Cu/Zn superoxide dismutase 1 (SOD1) were discovered in subsets of patients with familial ALS. This discovery led researchers to focus on unlocking the mechanisms for SOD1-mediated diseases. However, the pathogenic mechanism underlying SOD1 mutant toxicity has yet to be resolved. More recently, TDP-43 and FUS protein aggregates have been implicated in some cases of the disease, and a mutation in chromosome 9 (C9orf72) is thought to be the most common known cause of sporadic ALS.[12]

2.5 ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is a progressive brain disorder that gradually impairs memory and ability to learn, reasoning, judgment, communication and daily activities. AD is characterized clinically by cognitive impairment and pathologically by the deposition of β amyloid plaques and neurofibrillary tangles, and the degeneration of the cholinergic basal forebrain[13]. In the advance stage, motor weakness increases that leads to muscular contractures which produces immobility such as pneumonia, pulmonary embolism and death. At present no direct ante mortem confirmatory test exists[14]. Although the risk of developing Alzheimer's disease increases with age in most people, symptoms first appear after age 60[15]. According to World Health Organization projections, about three-quarters of the estimated 1.2 billion elders will be living in low- and middle-income countries by the year 2025. Epidemiological studies conducted in India between 1996 indicated that dementia affects 2.7% of the population, AD being the most common cause (1.3%).[16]

HISTORY

Auguste D. was first alzheimer patient who admitted to Frankfurt's *Hospital for the Mentally Ill and Epileptics* in 1901. Dr. Alois Alzheimer, had been a doctor at the hospital for some 13 years but was still baffled by her case and so took an unusual interest in it. Even after a career move to the Royalc Psychiatric Clinic in Munich in 1903, Alzheimer continued to follow the progression of Auguste D.'s illness from a distance. As a consequence, when she died in April 1906, the director of the Frankfurt hospital sent her brain to Alzheimer, who used it to produce the recently rediscovered 250 sample slides. Hence the disease was named after him.[17]

2.6. CAUSES [18]

Increased risk

Although it's still unknown what triggers Alzheimer's disease, several factors are known to increase your risk of developing the condition.

Age

Age is the single most significant factor in the development of Alzheimer's disease. The likelihood of developing the condition doubles every five years after you reach 65 years of age. However, it's not just older people who are at risk of developing Alzheimer's disease. Around 1 in 20 people with the condition are under 65. This is called early onset Alzheimer's disease and it can affect people from around the age of 40.

Family history

The genes you inherit from your parents can contribute to your risk of developing Alzheimer's disease, although the actual increase in risk is small if you have a close family member with the condition. However, in a few families, Alzheimer's disease is caused by the inheritance of a single gene, and the risks of the condition being passed on are much higher. If several of your family members have developed dementia over the generations, it may be appropriate to seek genetic counselling for information and advice about your chances of developing Alzheimer's disease when you are older.

Down's syndrome

People with Down's syndrome are at a higher risk of developing Alzheimer's disease. This is because the genetic fault that causes Down's syndrome can also cause amyloid plaques to build up in the brain over time, which can lead to Alzheimer's disease in some people.

Head injuries

People who have had a severe head injury have been found to be at higher risk of developing Alzheimer's disease.

Cardiovascular disease

Research shows that several lifestyle factors and conditions associated with cardiovascular disease can increase the risk of Alzheimer's disease. these include

- smoking

- obesity

- diabetes

- high blood pressure

- high cholesterol

2.7 THEORIES OF ALZHEIMER'S DISEASE [19]

Five theories are warrant to determine the actual cause of this disease:

1. Chemical Theory: Biochemical Deficiencies: Brain cells communicate with each other through biochemical substances called as neurotransmitters. Studies of Alzheimer's disease brains have uncovered diminished levels of various neurotransmitters that are thought to influence intellectual functioning and behavior. Toxic Chemical Excesses: Increased deposits of metal ions such as aluminum, Lead, etc have been found in Alzheimer's disease brains.

2. Genetic Theory: This theory explains the inheritance of a gene which directs the production of apolipoprotein (ApoE). In early-onset Alzheimer's, mutation on chromosome 14 which accounts for 10% of Alzheimer's cases. Additionally, a mutation was also found out on chromosomes 1 and 21. In 1997, another mutation on chromosome 12 effectively linked to late onset Alzheimer's.

3. Autoimmune Theory: The body's immune system, which protects against potentially harmful invaders, may erroneously begin to attack its own tissues, producing antibodies to its own essential cells.

4. Slow Virus Theory: A slow-acting virus has been identified as a causative agent for Alzheimer's disease.

5. Blood Vessel Theory: Defects in blood vessels supplying blood to the brain are being studied as a possible cause of Alzheimer's.

2.8 ALZHEIMER STATISTICS

- Worldwide, at least 47.5 million people are living with dementia, making the disease a global health crisis that must be addressed. Alzheimer's disease is the most common cause of dementia and may contribute to 60–70% of cases.

- The global number will increase to an estimated 75.6 million in 2030, and will almost triple by 2050 to 135.5 million.[20]

- Total health-care costs for people with dementia amount to more than 1 per cent of the global gross domestic product (GDP), or US$604 billion in 2010.[21]

- The number of Americans living with Alzheimer's disease is growing fast. An estimated 5.4 million Americans of all ages have Alzheimer's disease in 2016.Of the 5.4 million Americans with Alzheimer's, an estimated 5.2 million people are age 65 and older, and approximately 200,000 individuals are under age 65.These numbers will escalate rapidly in coming years. By 2050, the number of people age 65 and older with Alzheimer's disease may nearly triple, from 5.2 million to a projected 13.8 million.[22]

- An estimated 564,000 Canadians are living with dementia

- In china about 9.2 million people suffering from dementia (2010)

2.9 ALZHEIMERS IN INDIA

- In India, more than 4 million people have some form of dementia**.**

- being second largest country in world, India has less number of alzheimers patient.

- curcumin, commonly known as *haldi* in India, helps reduce the risk of Alzheimer's disease, a brain disorder that results in memory loss, personality changes and a decline in the thinking ability. These adverse impacts, scientists believe, are related to the death of brain cells and a breakdown of connections. [23]

- As per a study conducted by scientists from the US -based University of California, curcumin inhibits the accumulation of destructive beta amyloids inert substances responsible for Alzheimer's in the brain. The study, involving genetically altered mice, suggests curcumin is far more effective in inhibiting formation of the amyloids than drugs currently being tested to treat the disease

- They assert the extensive use of curcumin is possibly why India has the lowest rate of the disease in the world about 4.4 times less among adults aged 70-79 than the rate in the us.[24]

2.10 STAGES FOR ALZHEIMER [25]

Evaluating Prescription Drugs Used to Treat: Alzheimer's Disease • Consumer Reports Best Buy Drugs

Not Alzheimer's

- Forgetting things occasionally.
- Misplacing items, like keys, eye glasses, bills, paper work.
- Forgetting the names or titles of some things, like movies, books, people's names.
- Some reduction in ability to recall words when speaking.
- Being "absent-minded" or sometimes hazy on details.
- "Spacing out on things," such as appointments.

Early stage

- Short-term memory loss, usually minor.
- Being unaware of the memory lapses.
- Some loss, usually minor, in ability to retain recently learned information .
- Forgetting things and unable to dredge them up, such as the name of a good friend or, even, family member.
- Function at home normally with minimal mental confusion, but may have problems at work or in social situations.
- Symptoms may not be noticeable to all but spouse or close relatives/friends.

Middle-stage

- Short-term memory loss deepens, may begin to forget conversations completely, or names of loved ones
- Mental confusion deepens, trouble thinking logically
- Some loss of self-awareness
- Friends and family notice memory lapses
- May become disoriented, not know where you are
- Impaired ability to perform even simple arithmetic
- May become more aggressive or passive
- Difficulty sleeping

Late-stage

- Severe cognitive impairment and short-term memory loss

- Speech impairment

- May repeat conversations over and over

- May not know names of spouse, children, or care givers, or what day or month it is

- Very poor reasoning ability and judgment

- Neglect of personal hygiene

- Personality changes; may become abusive, highly anxious, agitated, delusional, or even paranoid

- Needs extensive assistance with activities of daily living
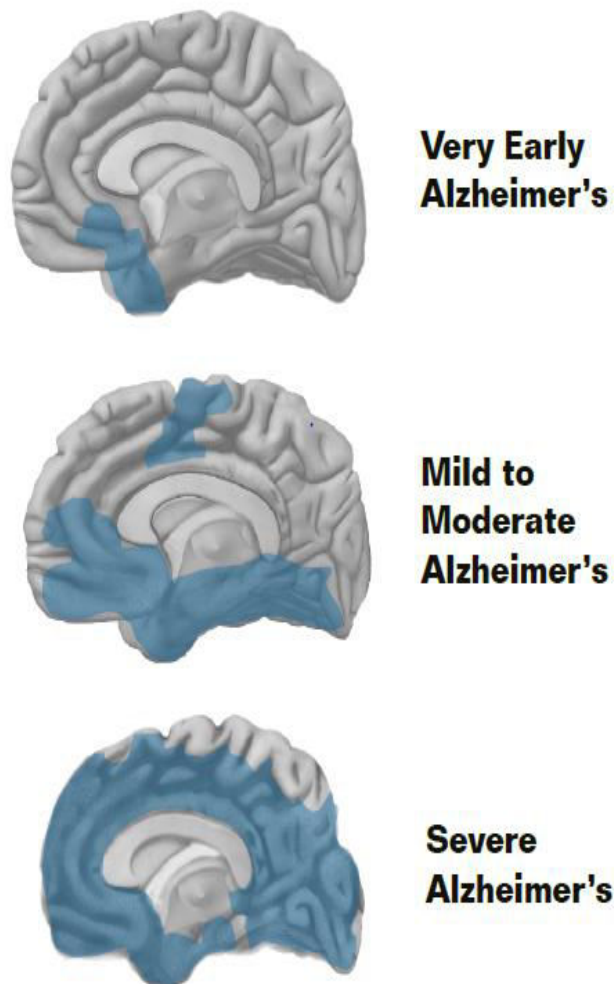
FIG 1: Stages of Alzheimer's disease

As Alzheimer's disease progresses, neuro fibrillary tangles spread throughout the brain shown in blue

2.11 SIGNS [26]

Memory loss that affects day-to-day abilities

It's normal to occasionally forget appointments, colleagues' names or a friend's phone number and remember them later. A person with Alzheimer's disease may forget things more often or have difficulty recalling information that has recently been learned.

Difficulty performing familiar tasks

Busy people can be so distracted from time to time that they may forget to serve part of a meal and only remember later. A person with Alzheimer's disease may have trouble completing tasks that have been familiar to them all their lives, such as preparing a meal or playing a game.

Problems with language

Everyone has trouble finding the right word sometimes, but a person with Alzheimer's disease may forget simple words or substitute words, making sentences difficult to understand.

Disorientation in time and space

It's normal to forget the day of the week or your destination - for a moment. But people with Alzheimer's disease can become lost on their own street, not knowing how they got there or how to get home.

Impaired judgment

From time to time, people can make questionable decisions such as putting off seeing a doctor when they have an infection. A person with Alzheimer's disease may experience changes in judgment or decision-making, for example not recognizing a medical problem that needs attention or wearing heavy clothing on a hot day.

Problems with abstract thinking

From time to time, people may have difficulty with tasks that require abstract thinking, such as balancing a cheque book. Someone with Alzheimer's disease may have significant difficulties with such tasks, for example not understanding what numbers are and how they are used.

Misplacing things

Anyone can temporarily misplace a wallet or keys. A person with Alzheimer's disease may put things in inappropriate places: an iron in the freezer or a wristwatch in the sugar bowl.

Changes in mood and behaviour

Everyone becomes sad or moody from time to time. Someone with Alzheimer's disease can exhibit varied mood swings - from calm to tears to anger - for no apparent reason. Alzheimer Society of Canada

2.12 DIAGNOSIS [27]

Diagnostic Criteria

The only method of definitively diagnosing AD is a brain autopsy. However, mental and behavioral tests and physical examinations allow physicians to make an accurate diagnosis of AD in 90 percent of cases.

The first step in finding a diagnosis is obtaining the patient history. During this time, the physician will determine what symptoms are present, when they began, and how they have progressed over time. The family history of illness is also pertinent. The physician will perform a physical examination, including blood tests and urinalysis. This is done to rule out other potential causes of dementia, such as hormone imbalance, vitamin deficiency, and urinary tract infections. Brain scans may also be performed to exclude tumors, cerebro vascular accidents, traumatic brain injury, and infections. These scans are also helpful in identifying the characteristic tangles and plaques seen in AD. Structural imaging scans, including magnetic resonance imaging (MRI) and computed tomography (CT), provide information about the shape and volume of the brain. Functional imaging allows the physician to determine how effectively the brain cells are working. A functional MRI or positron emission tomography (PET) scan can be used

2.13 DETECTION TECHNIQUES [27]

CT SCAN

CT scans can be accurate in diagnosing AD and ruling out other possible causes of the symptoms. However, this type of scan is more effective during the later stages of the disease. This technique is most often used to identify the neuro fibrillary tangles and beta-amyloid plaques seen during advanced stages of AD. In early diagnosis, research has shown that both the MRI and PET scans are more effective.

MRI

Magnetic resonance imaging (MRI) techniques, first , create two or three-dimensional images of the body that can be used to diagnose injury and illness. The MRI was used to detect the hippocampal volume and determine its significance as an indicator of AD neuropathology

PET

Positron emission tomography (PET) uses radiation signals to create a three dimensional color image of the human body. A PET scan has the capacity to detect changes in metabolism, blood flow, and cellular communication processes in the brain

2.14 PATHOLOGY

PLAQUES AND TANGLES

There is two abnormal structures in their Alzheimer brains, both amyloid plaques and neuro fibrillary tangles. These are particularly common in regions of the brain that are very important in the creation of memory. Plaques consist mainly of dense, insoluble deposits of protein and cellular material that build up around and outside neurons. Tangles are twisted, insoluble fibres that develop inside nerve cells. While such structures can be found in limited numbers in the brains of many healthy elderly people, they are present to a much greater degree in those who have shown the symptoms of Alzheimer's disease. [28]

Healthy Brain   **Severe Alzheimer's**
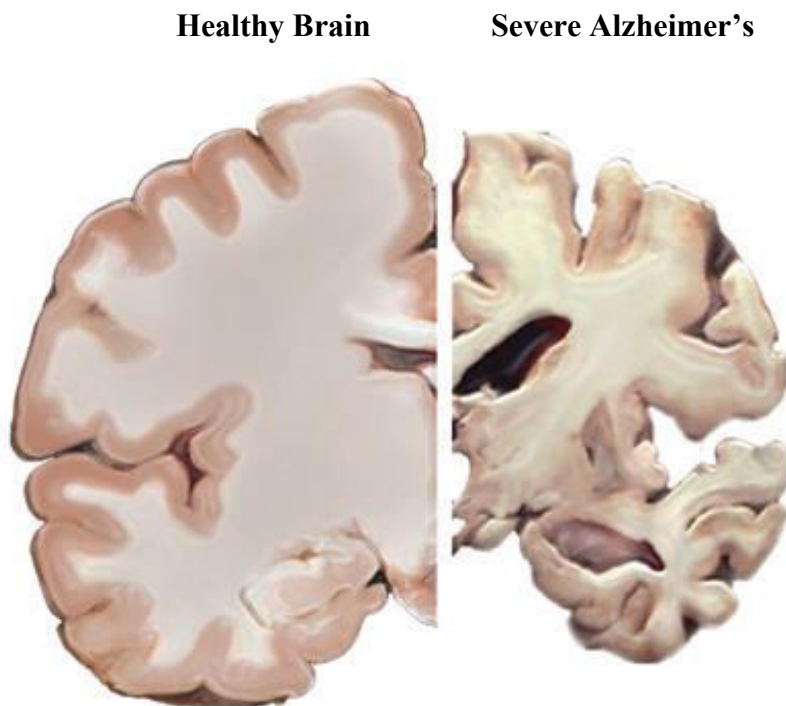
Fig 2: Cross sections of the brain show atrophy, or shrinking, of brain tissue caused by Alzheimer's disease.

Beta amyloid

In the normal brain, a larger protein the amyloid precursor protein is broken down into three smaller protein fragments known as alpha-amyloid, beta-amyloid, and gamma-amyloid. In those individuals with Alzheimer's disease, when amyloid precursor protein breaks down it creates a disproportionate amount of beta-amyloid but less alpha-amyloid and gamma-amyloid protein than usual. This excess of beta-amyloid protein overwhelms the brain's capacity to remove it and so it accumulates as insoluble gum like plaques, which may also contain other molecules, neurons, and non-nerve cells.

Such plaques "gum up the works" by damaging the connection points that is the synapses, between neurons and, as a consequence, interfere with such cells' ability to communicate. In Alzheimer's disease, early plaques develop in the hippocampus, a brain structure involved in encoding memories, and also in other parts of the cerebral cortex that are necessary for thought and decision making. As the disease progresses, additional plaques form in the frontal lobes of the brain. The more severe the symptoms of Alzheimer's disease, the more plaques will typically be found in the patient's brain during autopsy. Such beta-amyloid plaques also trigger an inflammatory response. Part of this process involves the creation of oxygen free radicals highly reactive molecules which can damage or kill other cells by creating holes in their membranes or binding to their DNA and interfering with survival. This plaque-related inflammatory process appears to destroy large numbers of brain cells in Alzheimer's patients and its effects are obvious in stained brain sections. This could be why taking anti-inflammatory drugs, for other health problems, may accidentally reduce the probability of developing Alzheimer's disease. [29]
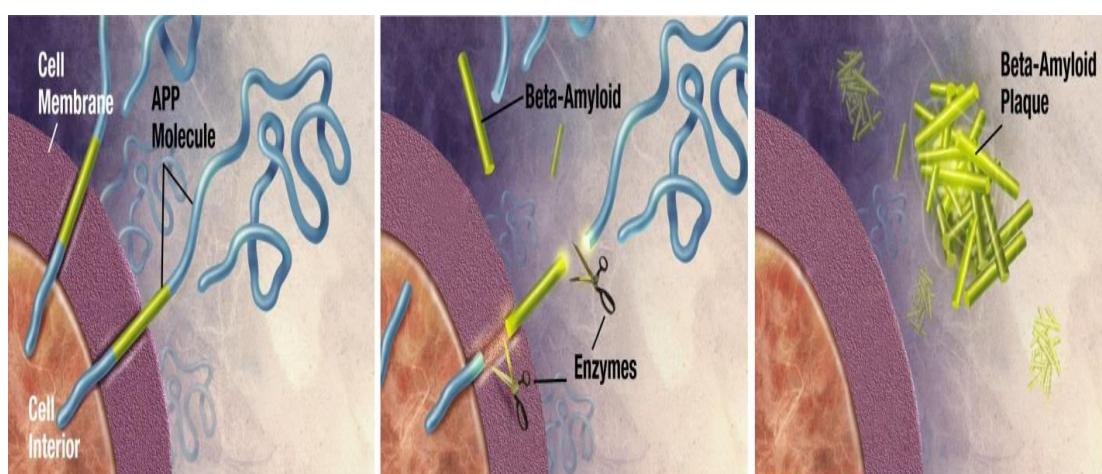
Fig 3 : Formation Of Amyliod Plaques

Tangles

Tau is important protein,that normally binds with tubulin, which is used to form structures known as microtubules. These microtubules are of great biological significance because they act like the pillars and girders of a building, giving shape and structure to cells. In patients with Alzheimer's disease, tau becomes chemically abnormal and begins to pair with other threads of tau and become tangled. As this happens, neuron microtubules disintegrate. These "tangles" prevent the movement of nutrients and other molecules to the nerve endings of the neurons, and as a result, communication malfunctions can occur, often followed by cell death. Tangles initially interfere with the functions of the brain's temporal lobe, causing memory loss and difficulties in reading and writing. As plaques and tangles begin to appear in the frontal lobes, personality disorders and other symptoms appear. While tangles can be seen also in the brains of healthy older people, they are relatively rare. In Alzheimer's patients, the worse the symptoms, the more common tangles are usually found to be on brain autopsy.

Normally tau protein undergoes phosphorylation, the addition of a phosphate (PO4) group to a protein or to a small molecule. Phosphorylation provides a very fast way of regulating proteins. If a protein, is regulated by phosphorylation it is always present in "standby" mode. When an activating signal arrives, the protein is phosphorylated and then performs in the way intended .the tau in the brains of Alzheimer's patients is abnormal in that it is hyperphosphorlylated, that is phosphorlylated to excess. It has been corrupted by several extra molecules of phosphorus. As a result, the tau malfunctions and becomes unable to support tubulin's role in the production of microtubules, which, therefore, lack integrity and begin to twist. Communication and cell nourishment is compromised and eventually declines to zero. The neuron cannot be sustained and begins to wither. The cell membranes collapse and every part of the neuron disintegrates and with it synapses, each representing a memory fragment. [30]
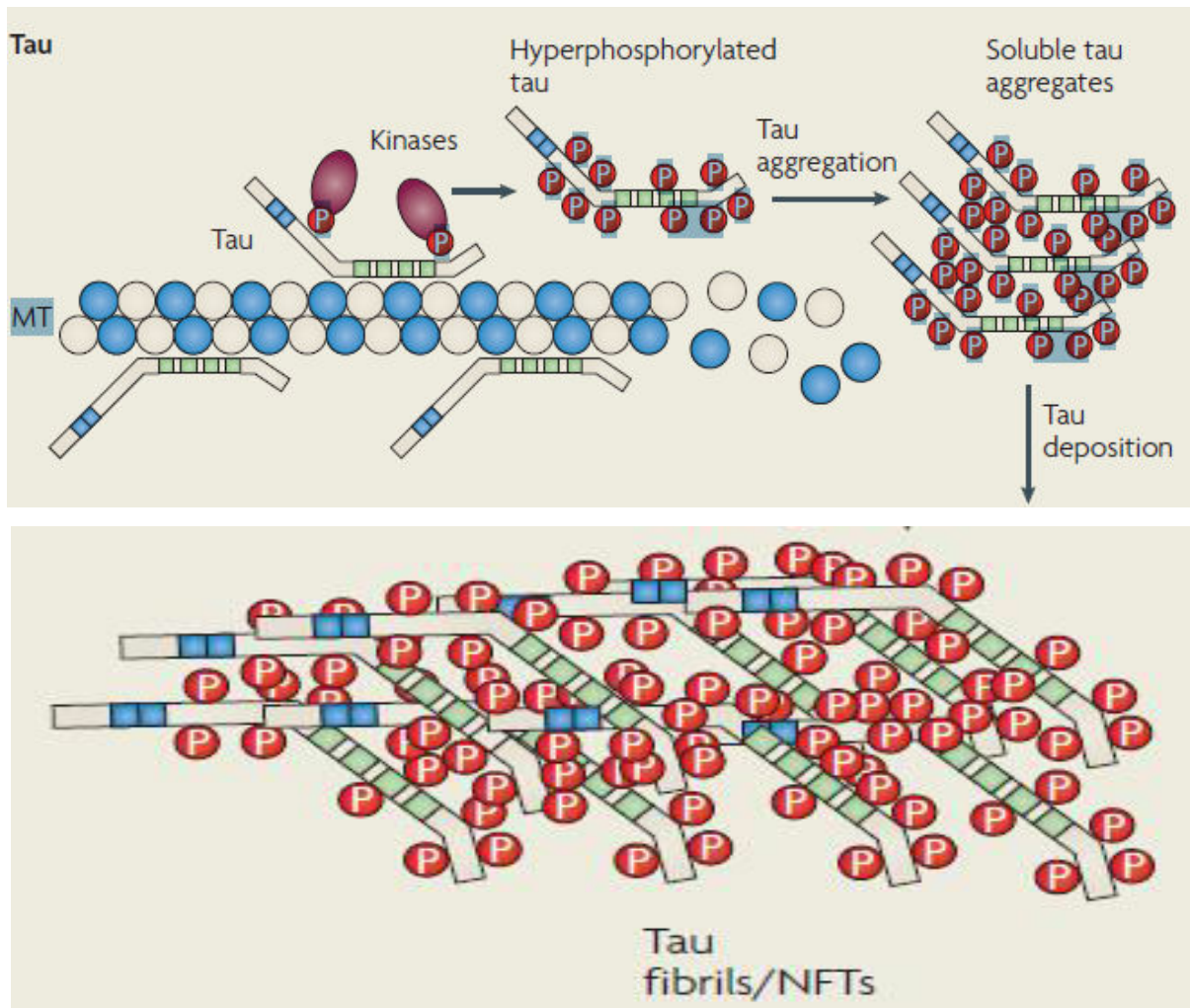
Fig 4 : Formation Of TAU tangles

Nucleus Basalis Of Meynert

Plaques and tangles do not seem to be the only cause of Alzheimer's related neuron death. Certain neurons found at the base of the brain known as the Nucleus Basalis of Meynert, appear to die in Alzheimer's disease without any evidence of the interference of plaques or tangles. Such neurons produce acetylcholine, a chemical messenger used in communication between neurons. Normally acetylcholine-manufacturing neurons have long branches that reach into the hippocampus and cortex and are thought to play an important role in learning and memory. Their death in Alzheimer's disease means that less acetylcholine is available to the brain, probably interfering with both its memory and learning capacity. [31]

Genetics

Alzheimer's disease seems linked, to some degree, to a gene in chromosome called the APO E gene, which codes for apolipoprotein E. This protein is involved in the cellular movement of cholesterol throughout the body. There are three slightly different types (known as alleles) of the APO E gene, namely APO E2, APO E3, and APO E4. Everybody has inherited two copies of this gene, one from each parent. The E3 variant is the most common and occurs in between 40 and 90 percent of the populations of particular regions; E2 and E4 are less common, being present in 2 percent and 6 to 37 percent of people respectively.It has been demonstrated that the probability of developing sporadic late-onset Alzheimer's disease is much higher in those possessing the EPO E4 allele. Indeed, anyone who has inherited copies of the APO E4 alelle from both parents has a 15 times greater risk of developing sporadic Alzheimer's disease than someone without this form of the APO E gene. Consequently, in Alzheimer's patients, carriers of the APO E4 are common, with this allele being present in approximately 40 percent. [32]

The Cholinergic System

Many of the cognitive deficits seen in Alzheimer's disease seem linked to problems in the cholinergic system, Choline acetyltransferase is reduced, especially in the temporal cortex and the hippocampus. In addition, Alzheimer's affected brains, again in their cortical and hippocampal areas, show a marked decrease in forebrain cholinergic neurons. Concentrations of cerebrospinal fluid acetylcholine are also low in Alzheimer's disease patients and are positively correlated with dementia scale test scores. All this evidence, combined with studies show that anticholinergic drugs cause a decline in memory, which support the view that many of the cognitive deficits that occur in Alzheimer's disease patients are probably caused by cholinergic abnormalities.[33]

It has been the prevailing view that the symptomatic efficacy of AChEIs is attained through their augmentation of acetylcholine-mediated neuron-to-neuron transmission. However, there is evidence that AChEIs may slow disease progression and hippocampal atrophy and may have disease modifying effects. In addition, symptomatic improvement in AD patients is not restricted to agents that enhance acetylcholine function in the brain, as is the case for memantine which acts on another neurotransmitter. Interestingly, memantine,

whose benefits also appear to be best for moderate-to-severe AD, has been recently linked to modulation of inflammation also.[34]

THE CATECHOLAMINERGIC

The neurotransmitters of the nervous system dopamine and serotonin can be derived from the amino acid tyrosine. The enzyme tetrahydrobiopterin, required for the synthesis of these two neurotransmitters, is significantly depressed in the cerebrospinal fluid of patients suffering from Alzheimer's disease.

It is not surprising, therefore, that the brains of patients with this type of dementia contain less dopamine and serotonin than usual. Several studies have demonstrated that the subnormal production of these neurotransmitters appears to be linked to the death of dopamine receptors and noradrenergic and serotonergic neurons, in the cortex and elsewhere in the Alzheimer's brain. [35]

The loss of the D2 receptor-enriched modules in the brains of Alzheimer's patients contributed to disturbances in information processing that may be responsible for cognitive and noncognitive impairments. Among the others, dopaminergic system may play a relevant role in the mechanisms involved in learning and memory processes, showing strong synaptic interaction with acetylcholine in different brain areas.[36]

Most of the publications dealing with the role of serotonin receptors in AD focus on the possible interplay between the serotonergic system and the amyloid-mediated part of pathophysiology. This is mostly based on the experimental finding demonstrating that administration of selective serotonin reuptake inhibitors (SSRI) in mouse models of AD reduces the production of toxic amyloid proteins and amyloid plaques. The fact that SSRIs seem to inhibit the production of toxic amyloid species makes them a promising tool to slow-down the progression of AD. Whereas serotonin receptors can influence processing of Aβ via modulation of activity of corresponding secretases , the role of serotonin receptors in NFT formation have not yet been analysed. However, there is indirect evidence suggesting the involvement of the serotonergic system in tau hyper-phosphorylation.

An additional and even more intrigue connection between tau pathology and 5-HT receptors involves the receptor-mediated modulation of the Brain-derived neurotrophic factor (BDNF) concentration. It is known that BDNF attenuates tau and Aβ pathologies by activating the phosphatidylinositol 3 kinase/GSK-3β pathway. Activation of this pathway, which leads to the inhibition of GSK-3β activity, attenuates phosphorylation of tau and, consequently, reduces pathology [37]

2.15 TREATMENTS

Table 1: Pharmacological [38]

Generic	Brand	Approved for	Side effects
Donepezil	Aricept	All stages	Nausea, vomiting, loss of appetite and increased frequency of bowel movements.
galantamine	Razadyne	Mild to moderate	Nausea, vomiting, loss of appetite and increased frequency of bowel movements.
memantine	Namenda	Moderate to severe	Headache, constipation, confusion and dizziness.
rivastigmine	Exelon	Mild to moderate	Nausea, vomiting, loss of appetite and increased frequency of bowel movements.
memantine + donepezil	Namzaric	Moderate to severe	Headache, diarrhea, dizziness, loss of appetite, vomiting, nausea, and bruising.

Table 2: Effect of Alzheimer's drug [39]

Drug	Slowing of cognitive decline	Activities of daily living	Overall risk of adverse events	Stopped taking medication because of adverse effects
Donepezil	Very small effect	Very small effect	Upto 88%	On average 19%
Galantamine	Very small effect	Very small effect	Upto 90%	On average 17%
Memantine	Very small effect	Very small effect	Upto 84%	Upto 10%
Rivastigmine	Very small effect	Very small effect	Upto 96%	On average 24%
Tacrine	Data not reported	Data not reported	Upto 51%	On average 43%

Table 3: New Medicines in Development for Alzheimer disease [39]

Drug name	Indication	Company	Development Status
ABT-126 acetylcholinesterase inhibitors	Alzheimer disease	Abbott	Phase 2
ABT-126	Alzheimer disease	Abbott	Phase 2
LY2886721	Alzheimer disease	Eli Lilly and Company	Phase 1
AZD3480	Alzheimer disease	Targacept Inc.	Phase 2
AVP-923 (dextromethorphan/quinidine)	Alzheimer disease, mild cognitive impairment	Avanir Pharmaceuticals	Phase 2
MABT5102A	Alzheimer disease	Genentech	Phase 2
AZD5213	Alzheimer disease	AstraZeneca	Phase 2
Gantenerumab	Alzheimer disease	Hoffmann-La Roche	Phase 3
AAB-003 (PF-05236812)	Alzheimer disease	Pfizer	Phase 1
BMS-241027	Alzheimer disease	Bristol-Myers Squibb	Phase 1
MABT5102A	Alzheimer disease	Genentech	Phase 2
BIIB037	Alzheimer disease prodromal or mild AD	Biogen Idec	Phase 1
GSK2647544	Alzheimer disease,	GlaxoSmithKline	Phase 1

MEDICINAL HERBS TO TREAT ALZHEIMER[40]

Centellaasiatica L. (Umbelliferae)

Extract from the leaves of *Gotu Kola* (*Centella asiatica*) has been used as an alternative medicine for memory improvement in the Indian Ayurvedic system of medicine for a long time.

Ginkgo biloba L. (Ginkgoaceae)

Ginkgo Biloba is the best known herb for Alzheimer's disease and its associated symptoms. In controlled clinical trials, using a placebo and control group, *ginkgo biloba*extracts showed therapeutic benefits in Alzheimer's, similar to prescription drugs such as Donepezil or Tactrin, with minimal undesirable side effects.

Salvia officinalis (Lamiaceae)

Sage as it is more commonly referred for Alzheimer's disease treatment. It has been reported to assist the brain in the fight against AD. Sage contains the antioxidants carnosic acid and rosmarinic acid. These compounds are thought to protect the brain from oxidative damage.

Curcuma longa L. (Zingiberaceae)

Curcuma longa (Turmeric) has been used as a source of Curcumin (diferuloylmethane), an orange-yellow component of turmeric or curry powder. Studies have proved that Curcumin has anti-inflammatory and antioxidant activities, and it helps in combating Alzheimer's Disease (AD). Regular consumption of this herb helps in keeping the mind balanced. The dose of curcumin can be reduced by making it to colon targeting.

Matricariarecutita (Asteraceae)

Matricariarecutita is said to stimulate the brain, dispel weariness, calm the nerves, counteract insomnia, aid in digestion, break up mucus in the throat and lungs, and aid the immune system. Chamomile can relieve anxiety, and in higher doses, leads to drowsiness, according to the University of Maryland Medical Center

Lipidium meyenii Walp (Brassicaceae)

Lipidium meyenii, is known as Maca. Maca shows beneficial improvement in memory and learning. Black maca improves experimental memory impairment, induced by ovariectomy, due in part, to its antioxidant and AChE inhibitory activities. Results demonstrated that black maca can enhance learning and memory in OVX (ovariectomized) mice

Commiphora whighitti (Burseraceae)

Commiphora whighitti , a plant resin, contains the major constituent of guggulipid, which is guggulsterone. The guggulipid has been seen to be a potential cognitive enhancer for improvement of memory in scopolamine-induced memory deficits

Withania somnifera (Solanaceae)

Active glycowithanolides of *Withania somnifera* (Ashawgandha) have a significant antioxidant function, which is accomplished by increasing the activities of superoxide dismutase, catalase, and glutathione peroxidase. *Ashwagandha*is also reported as a Nervine tonic that rejuvenates the cells and boosts energy.

NONPHARMACOLOGY TREATMENT [41]

- Cognitive enhancement
- Individual and group therapy
- Regular appointments
- Communication with family, caregivers
- Environmental modification
- Attention to safety

2.16. LITERATURE REVIEW OF ALZHEIMER'S DISEASE

1.Brain insulin system dysfunction in streptozotocin intracerebroventricularly treated rats generates hyperphosphorylated tau protein.

   Grünblatt E et. al .investigated that the intracerebroventricular (icv) application of streptozotocin (STZ) in low dosage was used in 3-month-old rats to explore brain insulin system dysfunction. Three months following STZ icv treatment, the expression of insulin-1 and -2 mRNA was significantly reduced to 11% in hippocampus and to 28% in frontoparietal cerebral cortex, respectively. . Insulin receptor (IR) mRNA expression decreased significantly in frontoparietal cerebral cortex and hippocampus (16% and 33% of control). These abnormalities may point to a complex brain insulin system dysfunction after STZ icv application, which may lead to an increase in hyperphosphorylated tau-protein concentration. Brain insulin system dysfunction is discussed as possible pathological core in the generation of hyperphosphorylated tau protein as a morphological marker of sporadic Alzheimer's disease. [42]

2. Changes in hippocampal synapses and learning-memory abilities in a streptozotocin-treated rat model and intervention by using fasudil hydrochloride.

 Hou Y et.al. elucidate the anti-dementia role of FH(Fasudil hydrochloride, which is a Rho kinase inhibitor used to treat neurological diseases) in Alzheimer's disease. Twenty-four Sprague-Dawley rats were randomly divided into four groups: (1) sham-operated group (control), (2) sham-operated followed by FH administration group (sham+FH), (3) streptozotocin (STZ)-treated group (STZ), and (4) STZ treatment followed by FH administration group (STZ+FH). The results indicate that STZ induced deficit in learning/memory, decrease in SYP expression, degeneration in synaptic structures, and increase in the expressions of p-LIMK2 and p-cofilin. These changes were reversed by the administration of FH, suggesting that FH has anti-dementia properties that protect synaptic structure and function. FH induced dephosphorylation (inactivation) of LIMK2 and subsequent dephosphorylation (activation) of cofilin, which may be responsible for the amelioration of neuronal synaptic structure and function.[43]

3.Long-term abnormalities in brain glucose/energy metabolism after inhibition of the neuronal insulin receptor: implication of tau-protein.

 Hoyer S et.al. stated that the triplicate intracerebroventricular (icv) application of the diabetogenic compound streptozotocin (STZ) in low dosage was used in 1-year-old male Wistar rats to induce a damage of the neuronal insulin signal transduction (IST) system and to investigate the activities of hexokinase (HK), phosphofructokinase (PFK), glyceraldehyde-3-phosphate dehydrogenase (GDH), pyruvate kinase (PK), lactate dehydrogenase (LDH) and alpha-ketoglutarate dehydrogenase (alpha-KGDH) in frontoparietotemporal brain cortex (ct) and hippocampus (h) 9 weeks after damage. In parallel, the concentrations of adenosine triphosphate (ATP), adenosine diphosphate (ADP), guanosine triphosphate (GTP) and creatine phosphate (CrP) were determined. The fall of the activities of the glycolytic enzymes HK, PFK, GDH and PK was found to be more marked after 9 weeks of damage when compared with 3- and 6-week damage whereas the diminution in the concentration of energy rich compound was stably reduced by between 20 and 10% relative to control. The abnormalities in glucose/energy metabolism were discussed in relation to tau-protein mismetabolism of experimental animals, and of sporadic AD.[44]

4.Spatial discrimination learning and choline acetyltransferase activity in streptozotocin-treated rats: effects of chronic treatment with acetyl-L-carnitin

Prickaerts J et.al. studied the effects of chronic treatment with ALCAR on spatial discrimination learning in the Morris task and choline acetyltransferase (ChAT) activity of middle-aged STREP-treated rats. Chronic treatment with ALCAR attenuated both the STREP-induced impairment in spatial bias and the decrease in hippocampal ChAT activity. These findings indicate that ALCAR treatment has a neuroprotective effect, although further studies are needed to characterize the mechanism of action of ALCAR in this model.[45]

5. Cognitive performance and biochemical markers in septum, hippocampus and striatum of rats after an i.c.v. injection of streptozotocin: a correlation analysis

Fahrig T et.al evaluated the effects of an intracerebroventricular injection of streptozotocin on cognitive behavior and biochemical markers in the brain of middle-aged Wistar rats. Intracerebroventricular injected streptozotocin has previously been reported to decrease the central metabolism of glucose. They found that streptozotocin treated rats showed an impaired cognitive performance in the delayed non-matching to position task and the Morris water escape task. Glial fibrillary acidic protein, an indicator of reactive astroglial changes,

was measured in three different (soluble, Triton X-100 soluble and crude cytoskeletal) protein fractions and its content in the fractions of the septum, hippocampus and striatum of streptozotocin-treated rats was increased.[46]

6. Tau Is Hyperphosphorylated at Multiple Sites in Mouse Brain In Vivo After Streptozotocin-Induced Insulin Deficiency

Buffie J Clodfelder-Miller et.al tested the hypothesis that insulin depletion caused by administration of streptozotocin may cause tau hyperphosphorylation in mouse brain by using site-specific phosphorylation-dependent tau antibodies to obtain precise identification of the phosphorylation of tau on individual residues. A massive (fivefold average increase) and widespread at multiple residues (detected with eight different phosphorylation-dependent tau antibodies) increase in the phosphorylation of tau was found in mouse cerebral cortex and hippocampus within 3 days of insulin depletion by streptozotocin treatment. This hyperphosphorylation of tau at some sites was rapidly reversible by peripheral insulin administration.[47]

2.17 GENERAL METHODS OF ALZHEIMER'S INDUCTION

Aβ1-40, aluminium and growth factor β1 induced alzheimer's

A new animal model of AD was establish by stereotaxically injecting Aβ1-40, aluminium and growth factor β1 into lateral cerebral ventricle, and transforming growth factor β1 into anterodorsal nucleus of thalamus at the same time. This was ideal animal model of AD that more mimic the complicated etiology, the praxiological features, pathological features and biochemistry changes of AD. The results showed that the cognitive ability of the model rats was damaged, neurons became derangement and karyopyknosis, moreover, senile plaques (SP) and neurofibrillary tangles (NFTs) were found in the cortex and hippocampus over 3 months, choline acetyl transferase was decreased, and Aβ was increased.[48]

AluminiumTrichloride Induced Alzheimer's

Different doses of AlCl3.6H2O (50, 70 and 100 mg/kg I.P) were used. The progression of AD was also determined for all used dose levels of AlCl3 after different periods (4, 5 and 6 weeks), as well as after one week of stopping ALCL3 injection (70 and 50 mg/kg I.P for 6 weeks) .t he present results showed that rats injected with AlCl3.6H2O at dose 70 mg/ kg I.P for 6 weeks represent the most exact model that mimics AD where the hippocampus neuronal degeneration and pyknosis were more pronounced he present results showed that rats injected with AlCl3.6H2O at dose 70 mg/ kg I.P for 6 weeks represent the most exact model that mimics AD where the hippocampus neuronal degeneration and pyknosis were more pronounced.[49]

Beta Amyloid Induced Alzheimer's

Administration of beta amyloid (25-35) 10µl by intra cerebral ventricle results in development alzheimer's. it causes accumulation of beta amyloid in brain leads to neuronal degeneration. Histopathology examination shows formation tangles and plaques in hippocampus.[50]

Colchicine Induced Alzheimer's

Colchicine is a microtubule-disrupting agent that produces marked destruction of hippocampal granule cells, mossy fibers and septo-hippocampal pathways. It induces neurofibrillary degeneration by binding to tubulin, the structural protein of the microtubule,

which is associated with loss of cholinergic neurons and decrease in acetylcholine transferase, thereby, resulting in impairment of learning and memory. Colchicine 7.5 µg in 5µl in ACSF is used to induce Alzheimer's. [51]

Streptozotocin induced alzheimer's

Streptozotocin (STZ) is a glucosamine-nitrosourea compound which was originally identified as an antibiotic. It is toxic to beta cells of pancreas and usually transported through glucose transporter 2 and commonly used to induce experimental diabetes in animals. STZ administration through route such as intra cebroventricular or intra peritoneal injection produces reduced cognition and increased cerebral aggregated Aβ fragments, total tau protein, and Aβ deposits. These changes were accompanied with decreased glycogen synthase kinase (GSK-3) alpha/beta ratio phosphorylated total in the brain. Administration of STZ in a rodent's brain has been shown to produce neuro inflammation, oxidative stress and biochemical alterations, which is considered to be a valid experimental model of the early pathophysiological changes in neurodegenerative disease. [52]

Transgenic mouse model for alzheimer's

Alzheimer's can be induced by altering the genes in mouse. Genes that are responsible for amyloid, tau ,presenilin, cholinergic pathways were identified and altered to induce alzheimer's. [53]

Amyloid – TGF (transgene)

Presenilin – Ps (transgene)

2.18 STREPTOZOTOCIN

Streptozotocin(2-deoxy-2-(3-(methyl-3-nitrosoureido)-D-glucopyranose)) is a betacytotoxic drug which, following peripheral (parenteral) administration at high doses, selectively destroys insulin producing/secreting β cells in the pancreas, and causes type I diabetes mellitus in adult animals.[54]

STZ mechanism of action

Central mechanism of action of streptozotocin

Central STZ administration caused neither systemic metabolic changes nor diabetes mellitus. STZ has been administrated mostly in doses ranging from 1–3 mg/kg body weight, injected 1–3 times, either uni-or bi-laterally into the lateral cerebral ventricles. The mechanism of central STZ action and its target cells/molecules have not yet been clarified but a similar mechanism of action to that in the periphery has been recently suggested. GLUT2 may also be responsible for the STZ induced effects in the brain. So the changes were accompanied with decreased glycogen synthase kinase (GSK-3) alpha/beta ratio phosphorylated/total) in the brain. Administration of STZ in a rodent's brain has been shown to produce neuro inflammation, oxidative stress and biochemical alterations, which is considered to be a valid experimental model of the early pathophysiological changes in neurodegenerative disease. [52]

Peripheral mechanism of streptozotocin

In the periphery, STZ causes selective pancreatic β cell toxicity results from the drug's chemical structure which allows it to enter the cell via the GLUT2 glucose transporter. The predominant site of GLUT2 localization is the pancreatic beta cell membrane. Following peripheral administration, STZ causes alkylation of β -cell DNA which triggers activation of poly ADP-ribosylation, leading to depletion of cellular NADH and ATP). When applied intra peritoneally in high doses (45-75 mg/kg) STZ is toxic for insulin producing/secreting cells, which induces experimental DM type 1. Low doses (20-60 mg/kg) of STZ given intra peritoneally in neonatal rats damages IR and alters IR signaling and causes diabetes mellitus type. [55]
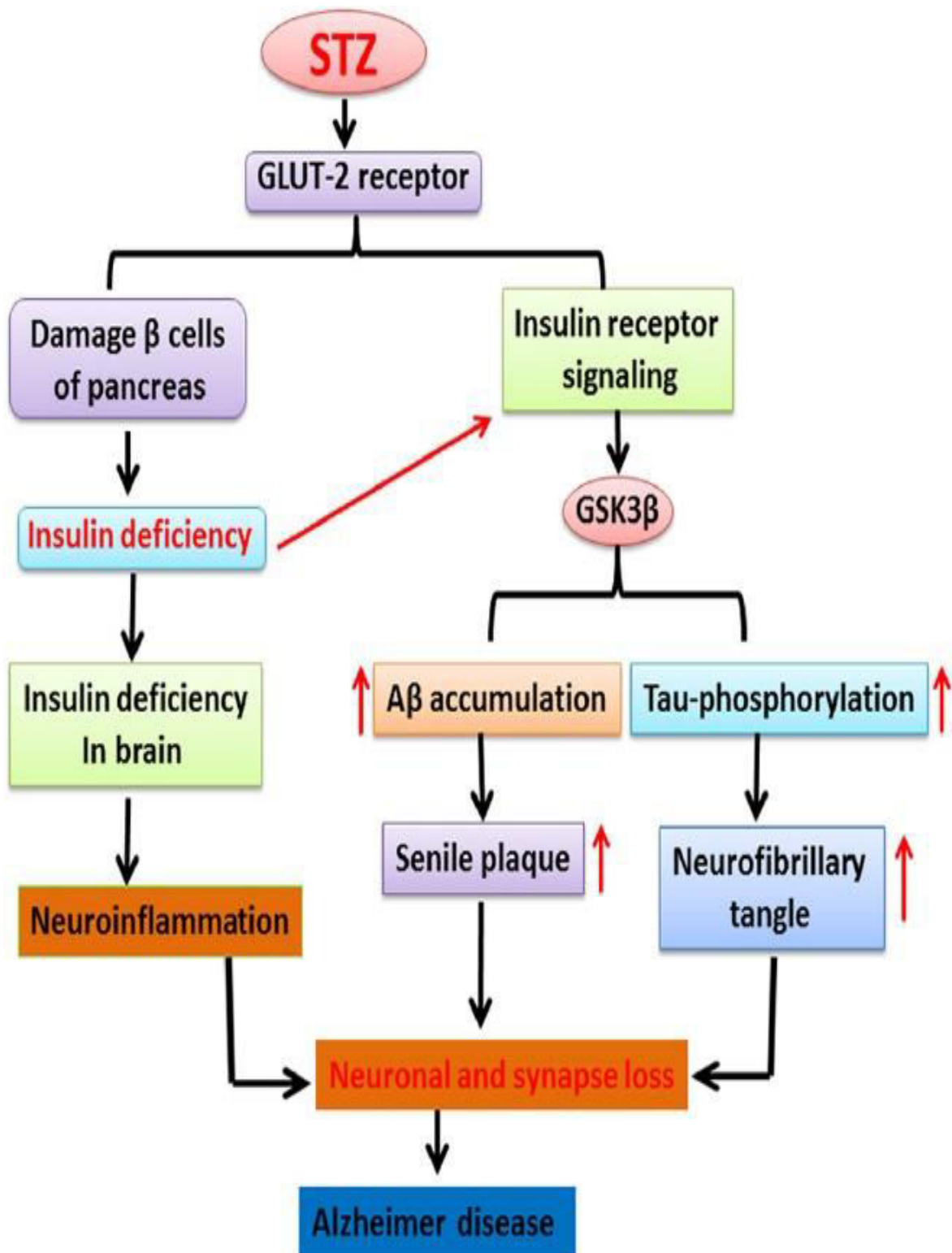
Fig 5 : Mechanism of STZ in induction of Alzheimer's
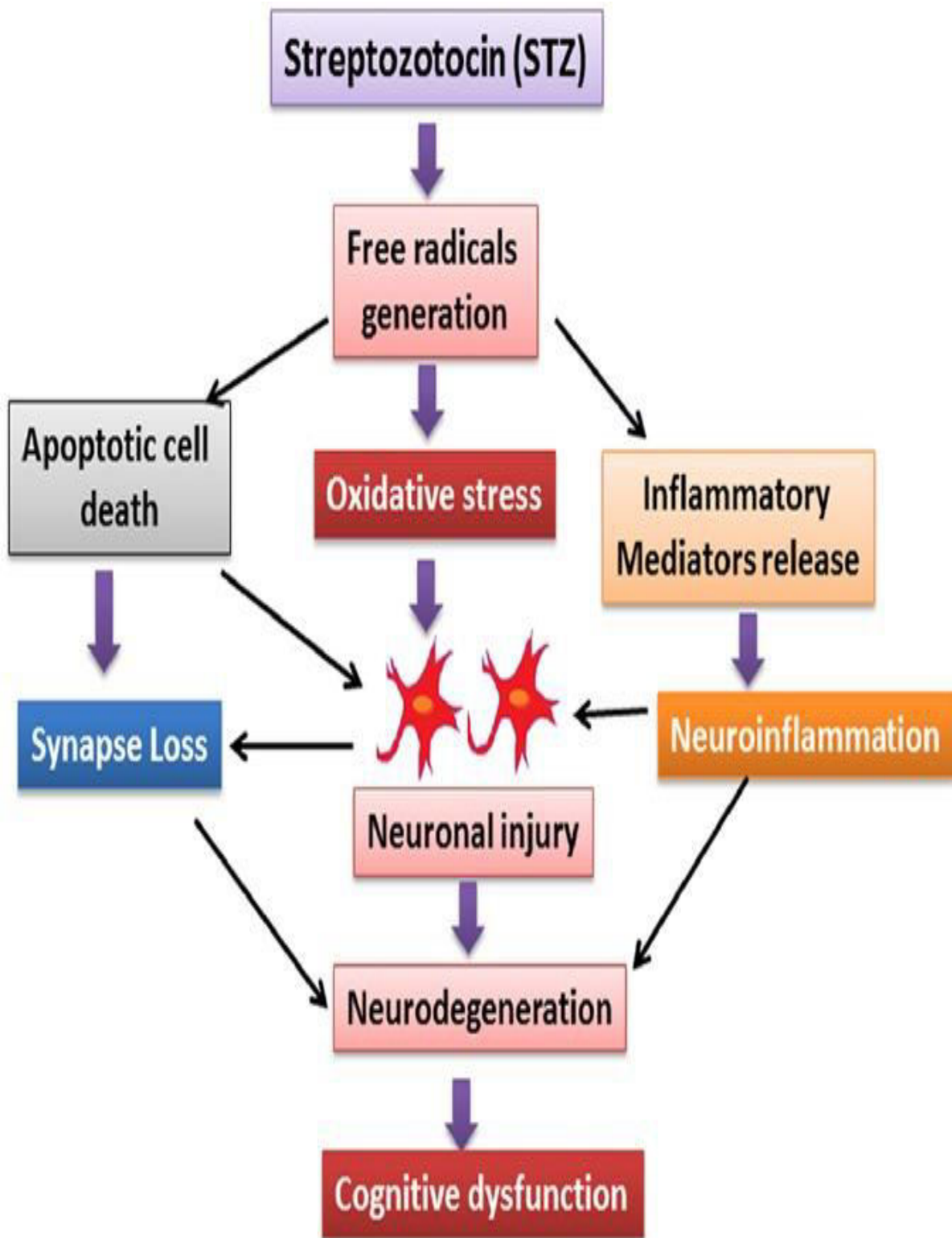
FIG 6 : Mechanism of STZ in Cognitive dysfunction

STZ mediated neuroinflammation:

Neuroinflammation is a important pathology of AD and precedes plaque and tangle formation during AD progression.. There is a close association of neuro inflammation with the pathogenesis of AD and involves the activation of glial cells in neurodegenerative diseases such as AD . STZ treatment caused enhanced neuro inflammatory mediators and altered redox stress that contribute to the neurodegenerative processes. These free radicals also further trigger the neuronal damage *via* formation of pro-inflammatory mediators and associated cytotoxic products during neuro inflammation that can be detrimental to neuronal function. Reposts suggested that neuronal insulin protects from neuro inflammation and redox stress thus any impaired insulin function in brain hamper neuronal function. [52]

Neuroprotective role of insulin in brain:

Interestingly, insulin can directly modulate synaptic plasticity and learning and memory, and disturbances in insulin signaling pathways in the periphery and in the brain have recently been implicated in AD and aging brain. Insulin also negatively regulates the metabolism of Aβ and tau proteins which are key building blocks of amyloid plaques and neurofibrillary tangles, and are well documented neuropathological hallmarks of AD. These negative regulations by insulin lead to more devastating for neurological function. Conversely, insulin injected into the brain intracerebroventricularly can improve performance of memory tasks in animals .Also, insulin delivered intranasally increased the performance of attention-related tasks in humans . A recent study reported that insulin receptor levels are down-regulated in the brains of AD patients . Insulin receptors were found to be internalized in neurons, and both insulin receptors (IRs)-1 and IRS-2 were reduced which taken together leads to reduced insulin signaling activity. It is becoming increasingly apparent that alteration of signaling molecules that are known to be involved in insulin signal transduction may play a role in the pathogenesis of AD .Although the actions of brain insulin are not fully understood

, binding of insulin to its receptor initiates autophosphorylation of the receptor's β-subunit, leading to binding and tyrosine phosphorylation of multiple insulin receptor substrates including IRS-1 and IRS-2, which play a role in synaptic plasticity and memory formation.[52]

3.PLANT PROFILE

Botanical classification [56]

Kingdom	-	Plantae
Phylum	-	Tracheophyta
Class	-	Liliopsida
Order	-	Arecales
Family	-	Arecaceae
Genus	-	Caryota
Species	-	*C.urens*

FIG 7 :   Whole Tree   **Flowering Bud**

3.1 BOTANIC DESCRIPTION

Caryota urens is an unarmed, hapaxanthic, solitary or clustered, medium sized palm up to 20 m tall; straight, unbranched, obscured at first by persistent fibrous leaf bases and sheaths, conspicuously ringed with narrow leaf scars, internodes elongated. Leaves bipinnate (pinnate in juveniles), induplicate with a terminal leaflet; sheath triangular, disintegrating into strong black fibres, densely hairy;petiolechannelled above; leaflets numerous, obliquely wedge shaped, upper margin irregularly toothed. Inflorescence axillary, solitary, pendulous, branched to 1 order or rarely unbranched, bisexual; prophyll tubular; peduncular bracts up to 8, large; distal portion of rachis bearing spirally arranged, protandrous triads of 2 male flowers and 1 female flower. Flowers with 3 sepals and 3 petals.Male flower with free petals; stamens 6, filaments short, sometimes connate at base. Female flower globose; petals connate up to half way; ovary superior, 3-locular with a single ovule per cell, stigma 3-lobed.The smooth epicarp of the drupaceous and globose fruit turns dark scarletred at maturity. Mesocarp is fleshy, filled with abundant, irritant, needlelike crystals. The endocarp is not differentiated. Each fruit has 2 large hemispherical seeds with ruminate endosperm.[57]

Distribution

Native	- India, Malaysia, Myanmar, Nepal, Sri Lanka
Exotic	- Papua New Guinea, Thailand, Vietnam

Other names:

Bengali	-	sopari
English	-	fishtail palm, Indian sago palm, wine palm, jaggery palm, kitul palm
Hindi	-	mari
Sanskrit	-	mada, dirgha
Sinhala	-	kitul
Tamil	-	kundalpanai, koondalpanai, thippali, tippili, kondapanna

Uses

It is used to treat gastric ulcers, migraine headaches, snake-bite poisoning and rheumatic swellings. The root is used for tooth ailments, the bark and seed to treat boils, and the tender flowers for promoting hair growth. [57]

3.2 PLANT LITERATURE REVIEW

Evaluation Of Central Nervous System Activity Of *Caryota urens* Fruit Extract On Various Animal Models

Murshid .V, studied the influence of *Caryota urens* on various CNS disorders like epilepsy, depression, anxiety, neuralgia, motor in coordination, motility using experimental models. This study demonstrared the extract of *Caryota urens* may be helpful in protecting effects in CNS disorders.[58]

Antioxidant Activity of *Caryota urens*L. (*Kithul*) Sap

P. Ranasingheet. alstudied the antioxidant properties of *C.urens* sap, using 1,1-diphenyl-2-picryl-hydrazyl (DPPH), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS+), Ferric Reducing Antioxidant Power (FRAP) and metal ion chelating assays. Radical scavenging activity and FRAP value were calculated. This study shows, for the first time, antioxidant activity of C. urens sap, as determined by free radical scavenging activity, electron donating reducing power and metal ion chelating capacity.[59]

Antioxidant and anti-diabetic properties of *Caryota urens* (*Kithul*) flour

G. E. M. Wimalasiri et.al studied the antioxidant properties of *C. urens* flour using different in vitro assays namely, 2,2-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS+), ferric reducing antioxidant power, oxygen radical absorbance capacity and ferrous ion chelating assays. Total phenolic Content (TPC) and Total flavonoid Content (TFC) were also evaluated. Anti-diabetic properties were estimated using alpha amylase and alpha glucosidase enzyme inhibition assays. He concluded from results that *Caryota urens* flour has moderate antioxidant property but did not contain any marked anti-diabetic properties.[60]

Qualitative phytochemical screening, anti-oxidant and anti-microbial activity studies on ethanolic flowers extract of caryota urenes linn.

A.Charles et.al studied the anti-oxidant activity of *C.urens* flowers on DPPH, Hydrogen per-oxide, and reducing power scavenging activity methods. This study also includes antimicrobial activity using 20 bacterial species and 7 fungal species. The results

describes promising antioxidant property of *C.urens* and the zone of inhibition concentration of bacteria and fungus was found to be 25µg/25µl.[61]

Phytochemical analysis on *Caryota urens* (fishtail palm) fruit from VIT university campus for pharmaceutical use

Vaishnavi R et.al studied the phytochemical analysis on *C.urens*. The results shows the presence of carbohydrate, alkaloids, flavanoids and phenols, organic and inorganic nature.[62]

Chemical Constituents from the Base Leaves of *Caryota urens*

Hasan M.H. Muhaisen isolated the chemical constitutens from base leaves of the plant. The results shows the presence of following compounds Triacontane, Lupeol, Myricadiol ,Sitosterol, Tetracosonid, Ursolic acid. [63]

Identification of Flavonoids in Methanolic Extract of *Caryota urens* (Fish Tail Palm): A Phytochemical Screening Involving Structure Analysis by FTIR Spectroscopy

V. Devi Rajeswari et.al. This study involves isolation of flavonoids from *Caryota urens* using thin layer and high performance liquid chromatography. Isolated compounds were subjected to NMR and FTIR spectroscopy, The different frequencies of the peaks in FTIR spectrum is found to fall in the same range of frequencies of functional groups which is present in rutin.[64]

***In Vitro* Estimation Of Antioxidant Activity Of *Caryota urens* Fruits**

Md. SahabUddin studied the antioxidant activity of chloroform fraction (CLF), carbon tetra chloride fraction (CTF) and n-Hexane fraction (NHF) of methanolic extracts of *Caryota urens* (CU) fruits, using DPPH (2,2-diphenyl-2-picrylhydrazyl) radical scavenging assay from the results he concluded among three different fractions CLF showed the highest antioxidant activity (61.58 % scavenging) at 400 µg/ml concentration followed by CTF and NHF. The IC50 values for the DPPH radical scavenging test were in the order of CLF (93.45 ± 3.09 µg/ml) > CTF (473.01 ± 12.95) > NHF (613.13 ± 7.64). This study suggested that CLF of CU fruits had strong antioxidant effect compared to CTF and NHF. [65]

4. SCOPE OF THE WORK

Alzheimer's disease (AD) is a neurodenerative disorder that gradually impairs memory and ability to learn, reasoning, judgment, communication and daily activities. AD is characterized clinically by cognitive and memory impairment. An estimated 5.4 million Americans of all ages have Alzheimer's disease in 2016.Of the 5.4 million Americans with Alzheimer's, an estimated 5.2 million people are age 65 and older. By 2050, the number of people age 65 and older with Alzheimer's disease may nearly triple, from 5.2 million to a projected 13.8 million.

There is currently no cure for Alzheimer's disease. But currently some category of drugs like AChE inhibitors and NMDA antagonists were used along with some antioxidants and some other supportive therapy. Therefore there is a lot of promising scope in the development of drug therapy for this serious and debilitating disorder.

Since ancient time the herbal medicines are effective in the treatment of various ailment, many plants have been used in the treatment of several dreadful disease but they are not scientifically exploited or improperly used. Therefore, these plant drugs deserve detailed studies in the light of modern science.

The present study is to prove the memory enhancement and cognitive effect of *caryota urens* on Alzheimer's induced mice using various memory retention experiments such as Y maze , Morris water maze, Passive avoidance etc.

5. PLAN OF WORK

```
                        ┌──────────────────────────┐
                        │    Literature  review    │
                        └──────────────────────────┘
                    ↓                              ↓
┌──────────────────────────┐      ┌──────────────────────────┐
│      Plant selection     │      │  Selection of experimental│
│                          │      │    methods and animals    │
└──────────────────────────┘      └──────────────────────────┘
              ↓                                  ↓
┌──────────────────────────┐                     │
│   Plant collection and   │                     │
│      authentification    │                     │
└──────────────────────────┘                     ↓
              ↓                    ┌──────────────────────────┐
┌──────────────────────────┐      │      Acute toxicity      │
│   Preparation of exract  │      └──────────────────────────┘
└──────────────────────────┘                     ↓
              ↓                    ┌──────────────────────────┐
┌──────────────────────────┐      │      Selection dose      │
│  Phytochemical screening │      └──────────────────────────┘
└──────────────────────────┘
```

Animal pre treatment for 21 days

Induction of alheimer's by STZ in I.C.V

Invivo study   Neurotransmitters estimation   Histopathological study

Statistical analysis

Invivo

- **Assesment of Habituation**
  - 1.Open field activity
  - 2.Closed field activity

- **Assesment of Memory retention**
  - 1.Step down passive avoidance test
  - 2.Y maze
  - 3. Morris water maze

Invitro

- **Neurotransmitter estimation**
  - 1.Dopamine
  - 2.Serotonin
  - 3.Acetylcholinesterase

- **Antioxidant estimation**
  - 1.Superoxide dismutase
  - 2.Catalse
  - 3.Lipid peroxidation
  - 4.Glutathione reductase
  - 5.Glutathione oxidatase

6. MATERIALS AND METHODS

6.1 Collection and authentication:

The fresh flowering bud of plant of *crayota urens* (Arecaceae) was collected from kelambakkam, chennai district of Tamilnadu in the month of December. The plant material was identified and authenticated by Dr.D.Aravind, Assistant Professor, specialization in medicinal plants, Department of Botany, National Institute Of Siddha, Ministry of AYUSH (Govt.Of India), and Chennai-47.A voucher specimen was submitted at C.L.BaidMetha college of pharmacy, Chennai-97.

6.2 Preparation of hydroalcohlic extract of *Caryota urens (*HAECU*)*

The fresh flowering bud of plant of *crayota urens* was collected and washed with running water. It was shade dried at room temperature and that dried plant material was made into coarse powder. The powder was extracted with ethanol (70%) and water 30%) in soxhlet extractor.

6.3 Method of extraction

The extraction was carried out in Soxhlet apparatus. The coarse powder weighing 200g was extracted with ethanol in water bath at room temperature for 72 hours. The solvent was then removed by filtration and fresh solvent was added to the plant material. The extract process was twice repeated. The combined filtrates were then evaporated under reduced pressure to give dark green viscous mass. The extract was stored at 0-4°C.

6.4. Percentage yield:

The hydroalcoholic extract yielded dark greenish brown semisolid residues, and percentage yield was found to be (15 %w/v) and the extract was preserved by refrigeration for further use.

Table 4: CHEMICALS USED

S.no	Chemicals Name	Company Name
1.	5,5-Dithio-bis-2-nitro benzic acid (DTNB) Ellman's reagent	Sisco Research Laboratories pvt. Ltd, Mumbai
2.	Acetic acid	S.D. Fine-Chem Ltd, Mumbai
3.	Acetylthiocholine iodide	S.D. Fine-Chem Ltd, Mumbai
4.	Acetylthiocholine esterase	SRL chemicals, sisco lab, Maharastra.
5.	Carboxy methl cellulose	LobaChemie Pvt. Ltd, Mumbai
6.	Chloroform	LobaChemie Pvt. Ltd, Mumbai
7.	Distilled water	Andavar Distilled Water Company, Chennai
8.	Disodium Dihydrogen Phosphate	LobaChemie Pvt. Ltd, Mumbai
9.	Donepezil	Eisai Pharmaceuticals, Mumbai
10.	Ethyl acetate	Ranbaxy Fine Chemical Ltd, NewDelhi
11.	Ethylene diamine tetra acetic acid (EDTA) Disodium Salt	Chemspure, Chennai
12.	Ethanol	LobaChemie Pvt. Ltd, Mumbai
13.	Formalin	Paxy speciality chemicals, Chennai
14.	Glutathione oxidased	SRL chemicals, sisco lab, Maharastra
15.	Glutathione reduced (GSH)	SRL chemicals, sisco lab, Maharastra
16.	Hydrogen peroxide	Chemspure, Chennai
17.	Hydrochloric acid	LobaChemie Pvt. Ltd, Mumbai
18.	Magnesium chloride	LobaChemie Pvt. Ltd, Mumbai
19.	Perchloric acid	LobaChemicals Pvt Lyd Mumbai
20.	Potassium chloride	Chemspure, Chennai
21.	Pyridine	Merck Specialties Pvt Ltd
22.	Sodium Azide	SRL Chemicals, Maharastra

23.	Sodium dodoecyl sulphate	S.D fine Chemicals ,Mumbai
24.	Sodium hydroxide	Chem pure , Chennai
25.	Sodium bicarbonate	S.D fine Chemicals, Mumbai

7. PRELIMINARY PHYTOCHEMICAL ANALYSIS [66]

The Hydro-alcoholic extract of *Caryota urens* was subjected to preliminary phytochemical screening for the presence or absence of phytoconstituents by the following methods.

1)   Test for alkaloids:

The extract was treated with dilute hydrochloric acid and filtered. The filtrate is used in the following tests.

a) Mayer's reagent (Potassium Mercuric Iodine Solution)

0.5ml of the extract was treated with Mayer's reagent and the appearance of cream color indicates the presence of alkaloid

b) Dragendroff's test (Potassium Bismuth Iodide)

0.5ml of the extract was treated with Dragendroff's reagent and the appearance of reddish brown color precipitate indicates the presence of alkaloid.

c) Hager's test (Saturated solution of Picric acid)

0.5ml of the extract was treated with Hager's test and the appearance of yellow color precipitate indicates the presence of alkaloid.

d) Wagner's test (Iodine-Potassium Iodide Solution)

0.5ml of the extract was treated with Wagner's test and the appearance of brown color precipitate indicates the presence of alkaloid.

2) Test for Carbohydrates

a) Molisch's test:

The extract was treated with 3ml of alpha-napthol in alcohol and concentrated sulphuric acid was added along the sides of the test tube carefully. Formation of violet color ring at the junction of two liquids indicates the presence of carbohydrates.

b) Fehling's test (CuSO4.7H2O+KOH+Potassium Tartarate):

The extract was treated with Fehling's solution A and B heated in boiling water for few minutes. The appearance of reddish brown color precipitate indicates the presence of reducing sugars.

c) Benedict's test (Sodium citrate + sodium carbonate + CuSO4.7H2O)

The extract was treated with Benedict's test and heated in boiling water for few minutes. The appearance of reddish orange color precipitate indicates the presence of reducing sugars.

d) Barfoed's test (Copper Acetate+ Glacial acetic acid)

The extract was treated with Barfoed's test and heated in boiling water for few minutes. The appearance of reddish orange color precipitate indicates the presence of non- reducing sugars.

3)Test for steroids

a) LibermannBurchard test:

The extract was treated with small quantity of concentrated sulphuric acid, glacial acetic acid and acetic anhydride. The appearance of green color indicates the presence of steroids

4) Test for proteins

a) Biuret's test:

The extract was treated with copper sulphate and sodium hydroxide solution. The appearance of violet color indicates the presence of proteins.

b) Millon's test:

The extract was treated with Millon's reagent. The appearance of pink color indicates the presence of proteins.

5) Test for Tannin's

a) The extract was treated with 10% lead acetate solution. The appearance of white precipitate indicates the presence of tannins.

b) The extract was treated with aqueous bromine solution. The appearance of white precipitate indicates the presence of tannins.

6) Test for Phenols

a) The extract was treated with neutral ferric chloride solution. The appearance of violet indicates the presence of phenols.

b) The extract was treated with 10% sodium chloride solution. The appearance of cream color indicates the presence of phenols.

7) Test for Flavonoid's

a) 5ml of extract solution was hydrolysed with 10%v/v sulphuric acid and cooled. Then, it is extracted with diethyl ether and divided into three portions in three separate test tubes. 1ml of diluted sodium carbonate, 1ml of 0.1N sodium hydroxide, and 1ml of strong ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow color demonstrated the presence of flavonoids.

b) Shinoda's test: The extracts were dissolved in alcohol, to that one piece of magnesium is added followed by concentrated hydrochloric acid along the sides of the test tube drop wise. It is heated in a boiling water bath for few minutes. The appearance of magenta colour indicates the presence of flavonoids.

8) Test for Gums and Mucilage

The extract was treated with 25ml of absolute alcohol and then solution was filtered. The filtrate was examined for its swelling properties.

9) Test for Glycosides

The extract was dissolved in the glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated sulphuric acid, formation of red ring at the junction of two liquids indicates the presence of glycosides.

10) Test for Saponins

1ml of the extract was diluted to 20ml with distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicates the presence of saponins.

11) Test for Terpenes

The extract was treated with tin and thionyl chloride, appearance of pink color indicates the presence of terpenes.

12) Test for sterols

The extract was treated with 5% potassium hydroxide solution; appearance of pink color indicates the presence of sterols.

8. ACUTE TOXICITY STUDIES [67]

The acute toxicity was done by using OECD guidelines 423.The acute toxic class method (423) was step wise procedure with 3 animals of single sex per step. Depending on the mortality and/or morbidity status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the substances. This procedure results in the use of a specified number of animals while allowing for acceptable data- based scientific conclusion.

This method uses defined doses of drug (2000mg/kg body weight) and results allow a substance to be ranked and classified according to the Globally Harmonized System (GHS) for classification of chemical which cause acute toxicity.

Procedure:

Adult female Swiss albino mice weighing 20- 30gms were used for the study. The starting dose level of 2000mg/kg body weight p.o of hydro-alcoholic extract *Caryota urens* was given. Since most of the crude extracts possess LD50 value more than 2000 mg/kg, p.o. so starting dose 2000mg/g p.o. was used. Dose volume administered was 1ml/100 gm body weight to mice which were fasted overnight with water *ad libitum*. Food was withheld for further 3-4hrs after oral administration of drugs and observed for the signs of toxicity.

Body weight of mice before and after determination were noted and any changes in skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic & central nervous system, motor activity and behavior pattern were observed and also sign of tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity also noted.

9. EXPERIMENTAL ANIMALS:

The Swiss albino mice weighing 22-30gm were used for this study. The inbreed animals were procured from the animal house of C.L.BaidMetha college of pharmacy, Thoraipakkam, Chennai- 97.They were housed six per kg under standard laboratory conditions at a temperature 22±2°C with 12:12 hrs light and dark circle. The animals were provided with standard animal feed, water and ad libitum. The animals were adapted to laboratory conditions one week prior to initiation of experiments. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study was approved by Institutional Animal Ethical Committee (IAEC)

9.1 EXPERIMENTAL DESIGN:

On the first day of experiment the animals were divided randomly into five groups of six animals each. Amnesia is induced by i.c.v. of streptozotocin for the II, III, IV, V groups were performed on the 21st day of the pretreated animals and treatment was continued for 5 days. Control animals were given 1%w/v of CMC orally by using intragastric catheter, were the last dose was given 60 min prior to behavioral testing and on 30th day scarification of animal were done for invitro studies. [68]

Experimental dementia of AD in mice was induced by i.c.v. STZ. Mice were anesthetised with anesthetic ether and i.c.v. injections were made with a hypodermic needle of 0.4 mm external diameter attached to a 10 μl Hamilton microlitre syringe. STZ was dissolved in freshly made ACSF (25 mg/ml) solution. (ACSF (147 mMNaCl; 2.9 mMKCl; 1.6 mM MgCl2, 1.7 mM dextrose)). [69]

IAEC REFERENCE NO: IAEC/XLVII02/CLBMCP/2015

GROUPING

S.no	Groups	Treatment
1	Group I	Carboxy methyl cellulose(1%w/v) p.o
2	Group II	Negative control Streptozotocin(2mg/kg, i.c.v)
3	Group III	Standard Donepezil (5mg/kg, i.p.)
4	Group IV	Pretreatment with HAECU(200mg/kg,p.o)
5	Group V	Pretreatment with HAECU(400mg/kg,p.o)

10.METHODS AND ASSESSMENTS

10.1 ASSESSMENT OF HABITUATION BEHAVIOUR

10.1.1 Open field activity[70]

Exploratory behavior was evaluated in an open field paradigm. The open field was made up of plywood and comprises of 40 x 50 x 60 cm dimension. The entire apparatus was painted black and divided into 16 squares with white lines on the floor. Each animal was placed at the corner of the apparatus and for next five minutes they were observed for their ambulation such as line crossings and head dipping.

10.1.2 Closed field activity [71]

The locomotor activity was measured by using an Actophotometer. The actophotometer consisted of a square arena (30 x 30 x 25 cm) with wire mesh bottom, in which the animal moves. Six lights and six photocells were placed in the outer periphery of the bottom in such a way that a single mouse can block only one beam. The movement of the animal interrupts a beam of light falling on a photocell, at which a count was recorded and displayed digitally. The locomotor activity was measured for a period of 10minutes. Technically its principle is that, a photocell is activated when the rays of light falling on the photocell are cut off by animals crossing the beam of light. As the photocell activated, a count is recorded. The photocells are connected to an electronic automated counting device which counts the number of ―cut offs‖.

10.2 ASSESSMENT OF MEMORY AND RETENTION

10.2.1 Morris water maze test [72- 73]

The Morris water maze test is performed to evaluate spatial working and reference memory. In this model the animals are placed into a large circular pool of water and they can escape on to a hidden platform. The platform is hidden by its placement just below the water surface and by opaque water. Therefore the platform offers no local cues to guide the escape behavior. The animal can escape from swimming by climbing on to the platform and with time the animal apparently learns the spatial location of the platform from any starting position at the circumference of the pool. Morris water maze consists of a large circular tank made of black opaque polyvinyl chloride or hardboard coated with fiber glass and resin and

then surface painted white (1.8-2m in diameter and 0.4-0.6m high). The pool is filled upto a height of 30cm with water maintained at around 25°C and rendered opaque by addition of small quantity of milk or non-toxic white color. The pool is provided with filling and draining facilities and is mounted at waist level. The tank is hypothetically divided into 4 equal quadrants the platform (11cm2) of 29 cm height is located in the centre of one of these 4 quadrants. The platform remains fixed in the position during the training session. Each animal is subjected to four consecutive trials for four days (21-24) during which they are allowed to escape on the hidden platform and allowed to remain for 20seconds. Escape latency time to locate the hidden platform in water maze is noted as an index of acquisition or learning. In case the animal is unable to locate the hidden platform within 120seconds, it is gently guided by hand to the platform and allowed to remain there for 20seconds. On the 29th day, 60minutes after last dose, platform is removed and time spent by each animal in target quadrant searching for the hidden platform is noted as an index of retrieval and measured.

10.2.2 Passive Shock Avoidance Test: [74]

Passive avoidance behavior based on negative reinforcement was used to examine the long term memory. The apparatus consisted of a box (27x27x27 cm) having three walls of wood and one wall of Plexiglass,featuring a grid floor (3mm stainless steel rods set 8mm apart) with a wooden platform (10x7x1.7cm) in the centre of the grid floor. Electric shock (20V,A/C) was delivered to the grid floor. During training session ,each mouse was gently placed on the wooden platform set in the centre of the grid floor, when the mouse stepped down and placed all its paw on the grid floor, shocks were delivered for 15seconds and the Step Down Latency(SDL) was recorded. SDL was defined as time taken by the mouse to step down from the wooden platform to grid floor with its entire paw on the grid floor. Animals showing SDL in the range of 2 to 15 seconds during the first were used for the second session and the retention test. The second session was carried out 90minutes after the first test. During second session, if the animals stepped down before 60seconds, electric shocks were delivered once again for 15seconds. During the second test, animals were removed from shock free zone ,if they did nt step down for a period of 60seconds and subjected to retention test. On the 29th day, after the treatment of last dose training was given and memory retention was examined after 24 hours (i.e., on 30th day) in a similar manner, except that the electric shocks were not applied to the grid floor observing an upper cut off time of 300 seconds.

10.2.3. Y Maze Test [75]

  Immediate working memory performance was assessed by recording spontaneous alternationbehavior in a single session in a Y maze made up of black painted wood. Each arm was 40cmlong, 12cm high, 3cm wide at the bottom and 10cm wide at the top and converged in an equilateral triangular central area. Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8 minute session. The series of arm entries was recorded visually. Entry was considered to be complicated when the hind paws of the mouse had completely entered the arm. Alternation was defined as successive entries into the three different arms (A, B and C) on overlapping triplet sets. The percentage of traids in which all three arms were represented i.e, ABC, CAB or BCA but not BAB was not recorded as an ―alternation‖ to estimate short term memory. Percentage alternation was calculated as the ratio of actual to possible alternation (defined as the total number of arm entries minus two), multiplied by hundred as shown %alternation= {(No. of alternations) **/** (Total arm entries -2)} x 100. On the 29th day, 90 minutes after the treatment of last dose arm entries was recorded visually and percentage alternation was calculated.

11. NEUROTRANSMITTERS ESTIMATION

11.1. Acetylcholinesterase(AChE) enzyme determination [76]

Acetylcholinesterase enzyme activity was estimated by Elman method.

Reagents

1. 0.1M phosphate buffer

2. DTNB Reagent

3. Acetylthiocholine (ATC)

Procedure

The mice were decapitated,brains are removed quickly and placed in ice cold saline. Frontal cortex, hippocampus and septum are quickly dissected out on a petri dish chilled on crushed ice.the tissues were weighed and homogenized in 0.1M phosphate buffer(pH 8).0.4ml aliquot of the homogenate is added to a cuvette containing 2.6ml of phosphate buffer(0.1M,pH 8) and 100μl of DTNB. The contents of the cuvette are mixed thoroughly by bubbling air and absorbance is measured at 412nm in a spectrophotometer. When absorbance reaches a stable value,it is recorded as basal reading.20μl of substrate i.e., acetylthiocholine is added and change in absorbance is recorded. Change in absorbance/min is thus determined.

Reagents	Sample	Blank
Phosphate buffer solution	2.6ml	2.7ml
Supernatant	0.4ml	0.4ml
DTNB	0.1ml	……

Calculations

The enzyme activity is also calculated by using the following formula

$$\text{Acetylcholinesterase } \textit{activity (M/ml)} = \frac{\textit{A/min X Vt}}{\varepsilon \text{ X b X VS}}$$

where,

A/min= Change in absorbance per min

ε= 1.361x 104 M/cm

b= path length (1cm)

Vt= total volume (3.1ml)

VS= sample volume (0.4ml)

The final reading of enzyme activity is expressed as μ moles/min/mg tissue

$$\textit{μ moles/min/mg protein} = \frac{\textit{μ moles/ml sample}}{\textit{mg protein/ml sample dilution}}$$

11.2. Estimation of dopamine [77]

Preparation of tissue extracts

Reagents

1. HCl-butanol

2. Heptanes

3. 0.1M HCl: (0.85ml conc. HClupto 100ml of water)

Procedure

On the day of experiment mice was sacrificed, whole brain was dissected out and the subcortical region (including the striatum) was separated. Weigh the tissue and homogenized in 5ml HCl-butanol for about 1 min. the sample was then centrifuged for about 10 min at 2000rpm. An aliquot supernatant phase (1ml) was removed and added to centrifuge tube containing 2.5ml heptane and 0.31ml of 0.1M HCl. After 10 min of vigorous shaking , the tube was centrifuged under the same conditions as above in order to separate two phases, and the overlaying organic phase was discarded. The aqueous phase (0.2ml) was then either taken for the dopamine assay. All the steps were carried out at 0°C. it was taken in between 50-75 mg of the tissue for homogenate with 5ml of HCl-butanol in correlation of same tissue concentration of 0.1ml of HCl-butanol in Schlumpf method.

Reagents

1. 0.4M HCl: 3.4ml conc. HClupto 100ml water

2. Sodium acetate buffer pH(6.9)

3. 5M NaOH:

4. 0.1M Iodine solution(in ethanol)

5. Sodiumthiosulphate

6. 10M acetic acid: 57ml of glacial acetic acid dissolved in distilled water upto 100ml.

Procedure

To the 0.2ml of aqueous phase**,** 0.5ml 0.4M HCl and 0.1ml of EDTA/sodium acetate buffer (pH6.9) were added, followed by 0.1ml iodine solution (0.1M in ethanol) in oxidation. The reaction was stopped after 2 min by addition of 0.1ml Sodium thiosulphate solution. 0.1ml acetic acid is added after 1.5 min. the solution was then heated to 100°C for 6 min when the sample again reached room temperature, excitation and emission spectra were read from the spectrofluorimeter. The readings were taken at 330-370nm.

Unknown conc $\quad = \dfrac{\text{intensity of unknown} * \text{conc of std}}{\text{intensity of std}}$

11.3. Estimation of Serotonin [77]

The serotonin content was estimated by the method of Schlumpf.

Reagents

1. O-phthalaldialdehyde (OPT) reagent

Procedure

To 0.2ml of aqueous extract 0.25ml of OPT reagent was added. The flurophore was developed by heating to 100°C for 10 min. after the samples reached equilibrium with the ambient temperature, readings were taken at 360-470nm in the spectrofluroimeter. For serotonin tissue blank, 0.25ml conc. HCl without OPT was added. Internal standard: 500µg /ml each of noradrenaline, dopamine and serotonin are prepared in distilled water: HCl-butanol in 1:2 ratios.

Unknown conc $= \dfrac{\text{intensity of unknown} * \text{conc of std}}{\text{intensity of std}}$

12. Estimation of Antioxidant Enzyme

12.1. Estimation of Superoxide dismutase (SOD) [78]

Reagents

1. Carbonate buffer (100mM, pH 10.2)
2. Epinephrine (3mM)

Procedure

The SOD activity in supernatant was measured by the method of Misra and Fridovich. The supernatant (500µl) was added to 0.800ml of carbonate buffer (100mM, pH 10.2) and 100µl of epinephrine (3mM). The change in absorbance of each sample was then recorded at 480nm in spectrophotometer for 2min at an interval of 15sec. Parallel blank and standard were run for determination of SOD activity.

One unit of SOD is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto oxidation.

Reagents	Uninhibited (Standard)	Inhibited (Sample)	Blank
Carbonate buffer	0.900ml	0.800ml	1.0ml
Supernatant	-	0.1ml	-
Epinephrine	0.1ml	0.1ml	-

The reaction mixtures are diluted 1/10 just before taking the readings in spectrophotometer

Calculation

$$\%\textbf{Inhibition} = \frac{\Delta A_{480nm}\ /min\ Uninhibited - \Delta A_{480nm}/min\ inhibited}{\Delta A_{480nm}\ /min\ Uninhibited - \Delta A_{480nm}/min\ Blank} \times 100$$

$$\textbf{Units}\ /\ \textbf{ml enzyme} = \frac{\%Inhibition\ \times\ V_t}{(50\%)\ \times\ V_s}$$

$$\textbf{Units}\ /\ \textbf{mg protein} = \frac{Units\ /\ ml\ enzyme}{mg\ protein\ /\ ml\ enzyme}$$

Where,

V_t= Total volume (1.0ml)

V_s= Sample volume (0.1ml)

12.2. Estimation of Catalase (CAT) [79]

Reagents

a. Phosphate buffer solution (50mM)
b. Hydrogen peroxide (H_2O_2) 30mM

Procedure

Catalase activity was measured by the method of Aebi. 0.1ml of supernatant was added to cuvette containing 1.9ml of 50mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0ml of freshly prepared 30mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from changes in absorbance at 240nm. Activity of catalase was expressed as units/mg protein. A unit is defined as the velocity constant per second.

Reagents	Sample	Blank
Phosphate buffer	1.9ml	2.9ml
Supernatant	0.1ml	0.1ml
H_2O_2	1ml	-

The reaction occurs immediately after the addition of H_2O_2.

Solutions are mixed well and the first absorbance (A_1) is read after 15sec (t_1) and the second absorbance (A_1) after 30sec (t_1). The absorbance is read at wavelength 240nm.

Calculation

$$K = \frac{V_t}{V_s} \times \frac{2.3}{\Delta t} \times \log \frac{A_1}{A_2} \times 60$$

Where,

K = Rate constant of the reaction

$\Delta t = (t_2 - t_1) = 15\text{sec}$

A_1 = Absorbance after 15sec

A_2 = Absorbance after 30sec

V_t = Total volume (3ml)

V_s = Volume of the sample (0.1ml)

12.3. Estimation of Lipid peroxidase (LPO) [80]

The level of Lipid peroxidase was estimated by Thiobarbituric acid reaction method described by Ohkawa*et al.*

Reagents

1. Sodium dodecyl sulphate (SDS) (8.1% w/v)
2. Acetic acid (20%; pH 3.5)
3. Thiobarbituric acid (TBA) (0.8%)
4. n-butanol/pyridine mixture (15:1)

Procedure

To 0.2ml of test sample, 0.2ml of SDS, 1.5ml of acetic acid and 1.5ml of TBA were added. The mixture was made up to 4ml with water and then heated in a water bath at 95°C for 60min. After cooling, 1ml of water and 5ml of n-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4000rpm for 10min, the organic layer was taken and its absorbance was read at 532nm. The level of lipid peroxides was expressed as nmoles of MDA released/g wet tissue.

Reagents	Sample	Blank
SDS	0.2ml	0.2ml
Supernatant	0.2ml	-
DDW	1.6ml	1.8ml
Acetic acid	1.5ml	1.5ml
TBA	1.5ml	1.5ml
n-butanol/pyridine	5ml	5ml

Calculation

$$\textbf{Concentration of MDA} = \frac{\text{Absorbance at 532nm}}{\text{L} \times \text{\Large\epsilon}} \times \text{D}$$

Where,

L = Light path (1cm)

ϵ = Extinction co-efficient 1.56×10^5 $M^{-1}cm^{-1}$

$$\text{D} = \frac{\text{Total volume (10ml)}}{\text{Volume of the sample (0.2ml)}}$$

12.4. Estimation of Glutathione peroxidase [81]

Reagents

1. Phosphate buffer, pH 7.0 (75mM)
2. Glutathione reductase (60mM)
3. Sodium azide (0.12M)
4. Disodium EDTA (0.15mM)
5. NADPH (3mM)
6. H_2O_2 (7.5mM)

Procedure

3ml cuvette containing 2.0ml of phosphate buffer (75mmol/L, pH 7.0), 50µl of glutathione reductase (60mmol/L), 50µl of NaN_3 (0.12mol/L), 0.1ml of Na_2EDTA (0.15mM/L), 100µl of NADPH (3.0mmol/L) and tissue supernatant were added. Water was added to make a total volume of 2.9ml. The reaction was started by the addition of 100µl of (7.5mmol/L) H_2O_2, and the conversion of NADPH to NADP was monitored by a continuous recording of the change of absorbance at 340nm at 1min interval for 5min. Enzyme activity of GSHPx was expressed in terms of mg of protein.

Reagents	Sample	Blank
Phosphate buffer	2.0ml	2.9ml
Glutathione reductase	0.05ml	0.05ml
Sodium azide	0.05ml	0.05ml
Disodium EDTA	0.1ml	0.1ml
NADPH	0.1ml	0.1ml
Supernatant	0.1ml	0.1ml
H_2O_2	0.1ml	-
DDW	0.5ml	0.6ml

Calculation

$$\textbf{Enzyme activity }(\textbf{M/min/ ml}) = \frac{A_{340} / \min \times V_t}{\varepsilon \times d \times V_s}$$

Where,

$\varepsilon = 6.22 \times 10^6 \ M^{-1}cm^{-1}$

$d = 1cm$

V_t = Total volume (3.0ml)

V_s = Sample volume (0.1ml)

12.5. Estimation of Glutathione reductase (GRD) [81]

Glutathione reductase was assayed by the method of Stahl *et al*.

Reagents

1. Phosphate buffer (0.3M; pH 6.5)
2. EDTA (0.25M)
3. Glutathione oxidized, GSSG (0.012M)
4. NADPH (0.03M); Nicotinamide Adenine Dinucleotide Phosphate reduced tetra sodium salt, NADPH.Na_4 (Mw.833.35)

Procedure

The reaction mixture containing 1ml phosphate buffer, 0.5ml EDTA, 0.5ml GSSG and 0.2ml of NADPH was made up to 3ml with distilled water. After the addition of 0.1ml of tissue homogenate, the change in optical density at 340nm was monitored for 2min at 30sec interval.

One unit of the enzyme activity was expressed as nmoles of NADPH oxidized/min/mg protein

Reagents	Sample	Blank
Phosphate buffer	1.0ml	1.5ml
EDTA	0.5ml	0.5ml
GSSG	0.5ml	-
NADPH	0.2ml	0.2ml
Supernatant	0.1ml	0.1ml
DDW	0.8ml	0.8ml

Calculation

$$\textbf{Enzyme activity (M/min/ ml)} = \frac{A_{340} / \min \times V_t}{\varepsilon \times d \times V_s}$$

Where,

$\varepsilon = 6.22 \times 10^6 \ M^{-1}cm^{-1}$

$d = 1cm$

V_t= Total volume (3.1ml)

V_s= Sample volume (0.1ml)

13. METHODS FOR HISTOPATHOLOGICAL STUDY

The mice from each group were anaesthetized using intraperitoneal injection of thiopentone sodium. The brain was carefully removed without any injury after opening the skull. The collected brain was washed with ice cold normal saline and fixed in 10% formalin saline. Paraffin embedded sections were taken 100μm thickness and processed in alcohol-xylene series and stained with Haematoxyli-Eosin dye. The sections were examined microscopically for histopathological changes in the hippocampal zone.

14. STATISTICAL ANALYSIS

The statistical analysis was carried by one way ANOVA followed by Dunnet's ―t‖ test. P values <0.05 (95% confidence limit) was considerd statistically significant, using Software Graph pad Prism 6.0

15. TABLES AND GRAPH

Table 5: Phytochemical screening of *Caryota urens*

S.NO	CONSTITUENTS	REMARKS
1.	Alkaloids	Present
2.	Carbohydrates	Present
3.	Protein	Absent
4.	Steroids	Absent
5.	Phenols	Present
6.	Tannins	Present
7.	Flavonoids	Present
8.	Gums and Mucilage	Absent
9.	Glycosides	Absent
10.	Saponins	Absent
11.	Terpenes	Present
12.	Sterols	Absent

Table 6: Effect of HAECU in acute toxicity study

S.No	Treatment	Dose	Weight of animal (g)		Signs of Toxicity	Onset of Toxicity	Reversible or Irreversible	Duration
			Before test	**After test**				
1.	HAECU	2000mg/kg	25	28	No signs of toxicity			
2.	HAECU	2000mg/kg	20	22		Nil	Nil	14 days
3.	HAECU	2000mg/kg	20	22				

Table 7: Effect of HAECU in Open field

| S.no	Groups	No of head dipping	No of line crossing
1	Control	47.00 ± 1.41	128.34 ± 3.42
2	Negative control	$25.80 \pm 1.74^{a***}$	$89.60 \pm 3.07^{a***}$
3	Standard	$39.20 \pm 1.35^{b***}$	$122.25 \pm 4.49^{b***}$
4	HAECU 200 mg/kg	$31.80 \pm 2.39^{b**}$	$102.80 \pm 2.85^{b**}$
5	HAECU 400 mg/kg	$35.60 \pm 1.32^{b**}$	$110.22 \pm 1.11^{b***}$

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, $*p<0.05$, $**p<0.01$, $*p<0.001$

Graph 1: Effect of HAECU in head dipping
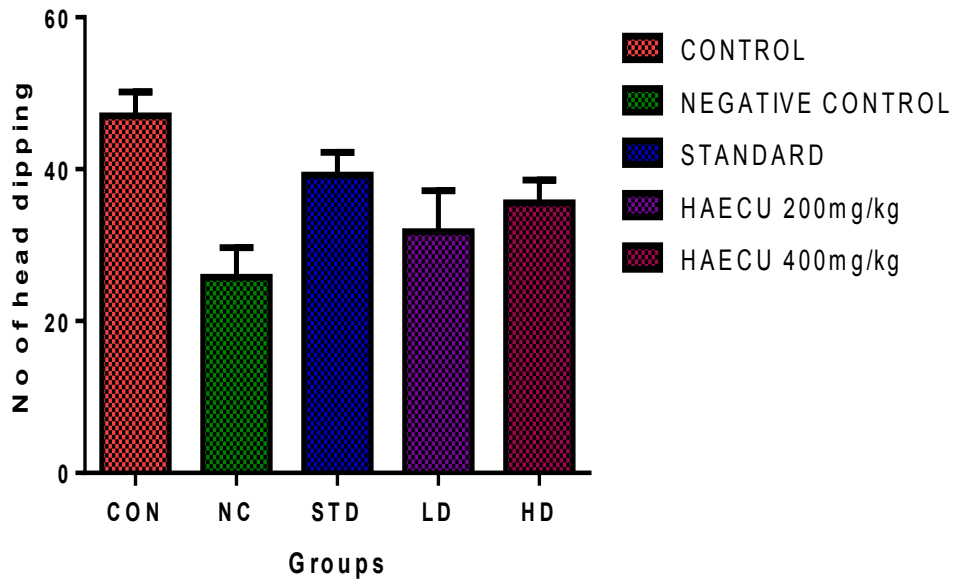
Graph 2: Effect of HAECU in line crossing
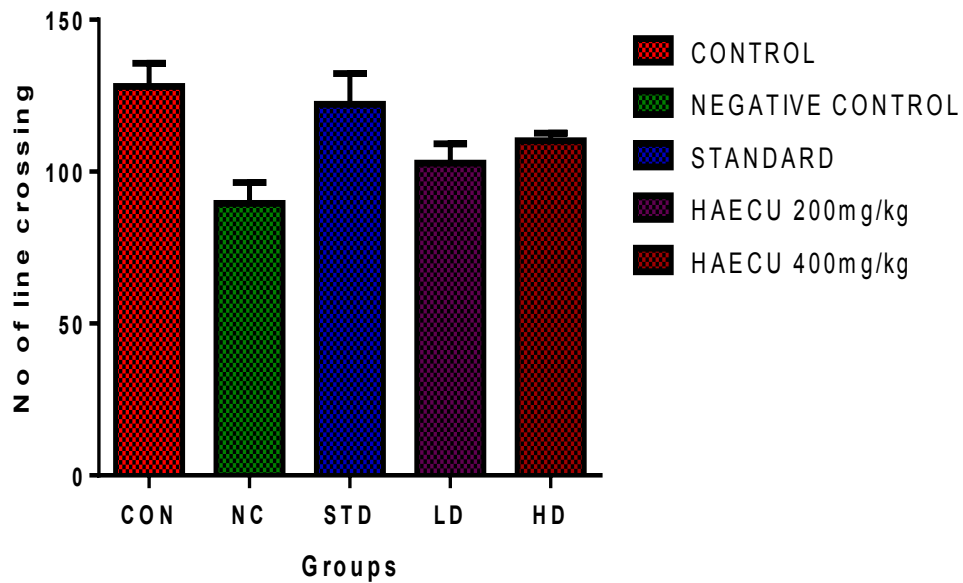
Table 8: Effect of HAECU in Closed field

| S.no	Groups	Activity scores
1	Control	407.2±7.31
2	Negative control	309.6±3.89[a]***
3	Standard	392.4±2.94[b]***
4	HAECU 200 mg/kg	360.4±2.94[b]***
5	HAECU 400 mg/kg	378.2±1.56[b]***

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, *p<0.001

Graph 3: Effect of HAECU in Closed field
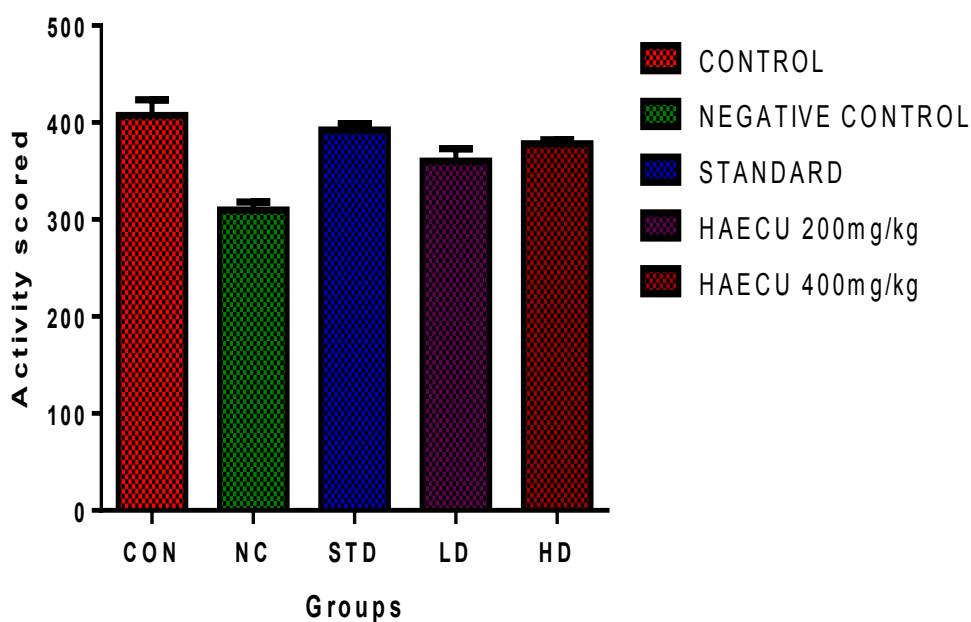
Table 9: Effect of HAECU in Passive avoidance

| S.no	Groups	Latency (secs)
1	Control	43.83 ± 1.24
2	Negative control	$26.50\pm0.76^{a***}$
3	Standard	$39.50\pm0.53^{b***}$
4	HAECU 200 mg/kg	$32.00\pm1.39^{b**}$
5	HAECU 400 mg/kg	$35.33\pm1.30^{b***}$

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, $*p<0.05$, $**p<0.01$, $*p<0.001$

Graph 4: Effect of HAECU in Passive avoidance
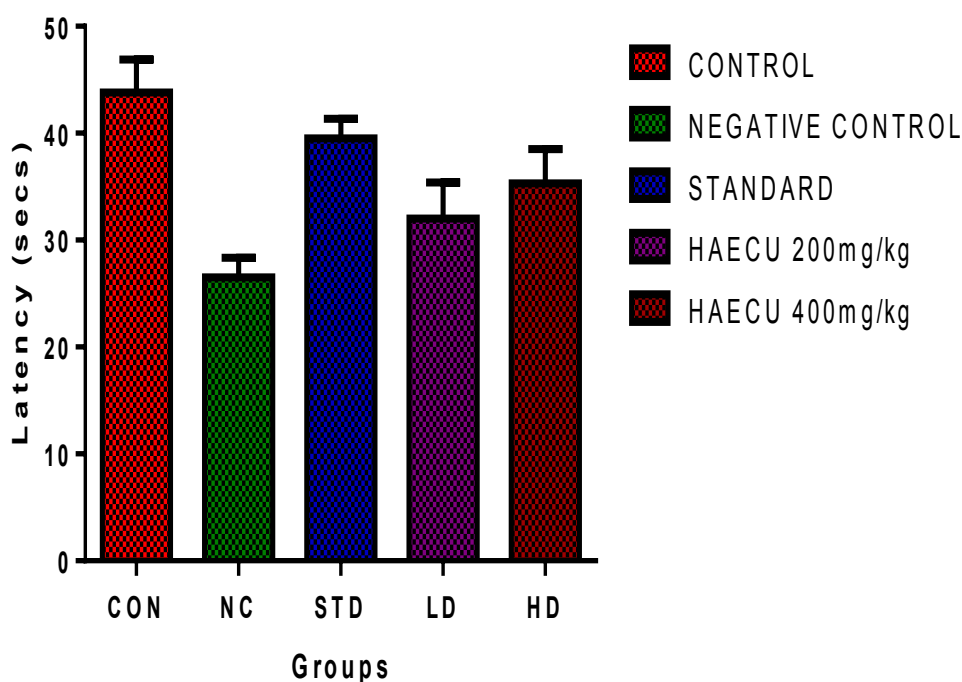
Table 10: Effect of HAECU in Y maze

S.no	Groups	% Alterations
1	Control	48.17
2	Negative control	30.27[a]***
3	Standard	44.40[b]***
4	HAECU 200 mg/kg	36.71[b]**
5	HAECU 400 mg/kg	39.65[b]***

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, *p<0.001

Graph 5: Effect of HAECU in Y maze
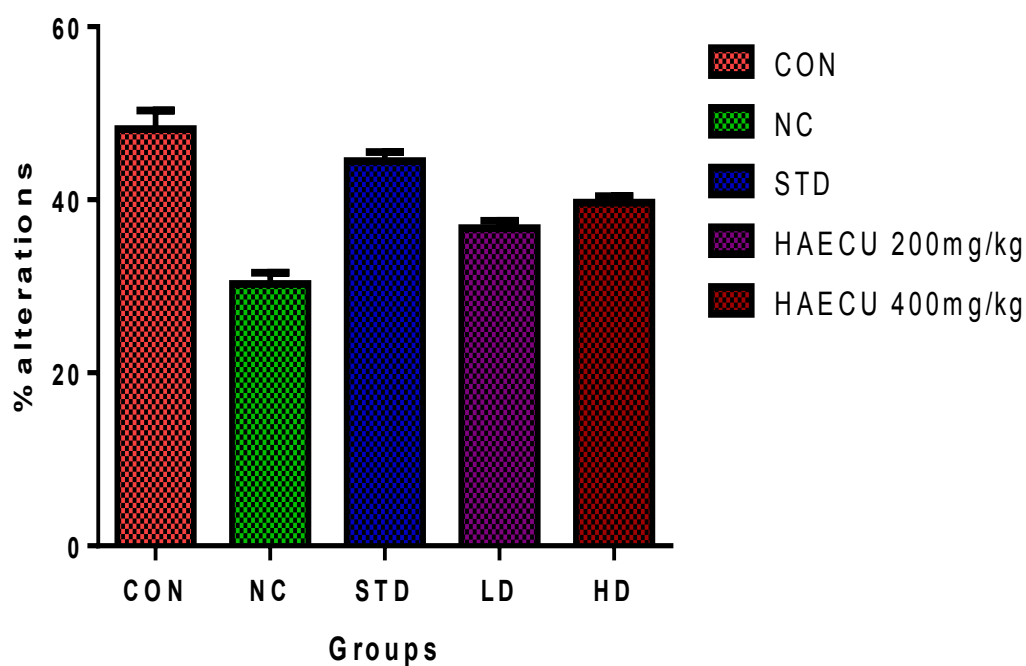
Table 11: Effect of HAECU in MSW

| S.no	Groups	Escape latency(sec)
1	Control	12.00±0.96
2	Negative control	36.00±2.20[a*]
3	Standard	12.83±1.44[b***]
4	HAECU 200 mg/kg	19.17±1.57[b***]
5	HAECU 400 mg/kg	15.33±1.25[b***]

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV

Statistical significance test for comparison was done by one way ANOVA followed by

Dunnet's 't' test. ,   ns- Non significant, *p<0.05, **p<0.01, *p<0.001

Graph 6: Effect of HAECU in MWM
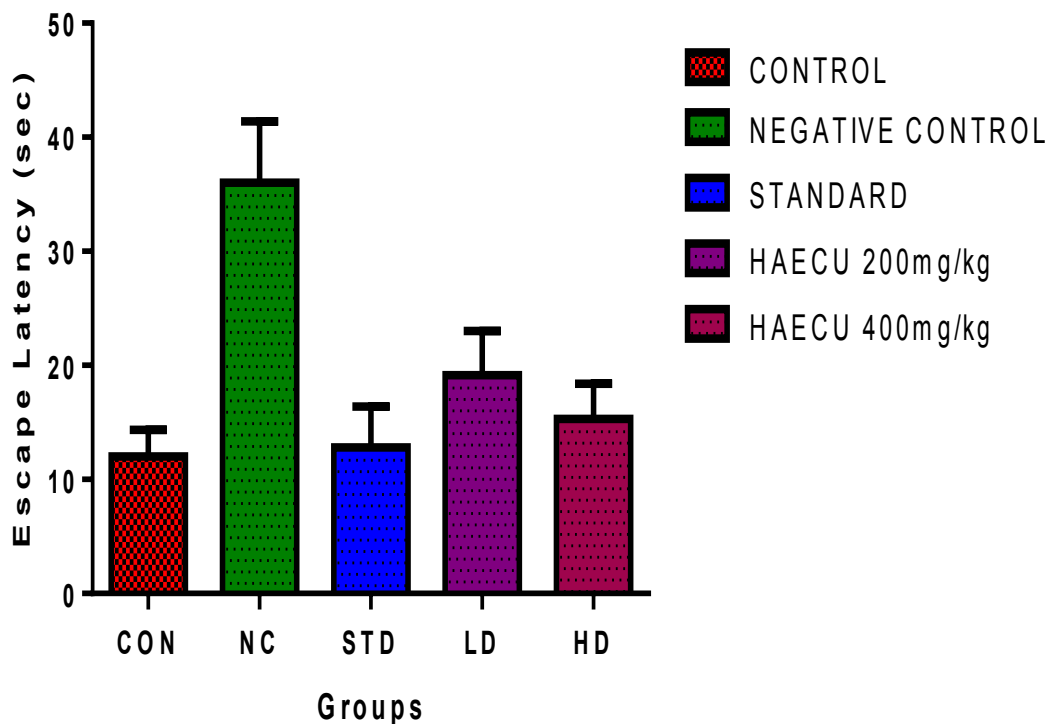
Table 12: Effect of HAECU in Dopamine

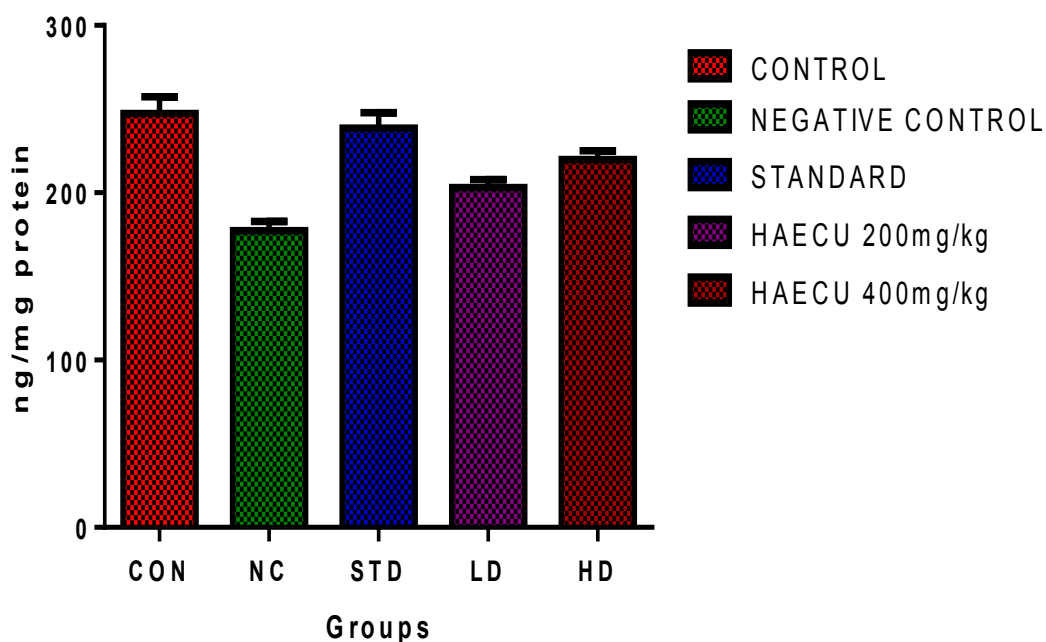| S.no	Groups	ng/mg wet tissue
1	Control	432.1±13.45
2	Negative control	303.0±4.51[a***]
3	Standard	385.6±3.80[b***]
4	HAECU 200 mg/kg	352.9±2.44[b**]
5	HAECU 400 mg/kg	367.5±4.60[b***]

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *$p<0.05$, **$p<0.01$, *$p<0.001$

Graph 7: Effect of HAECU in Dopamine
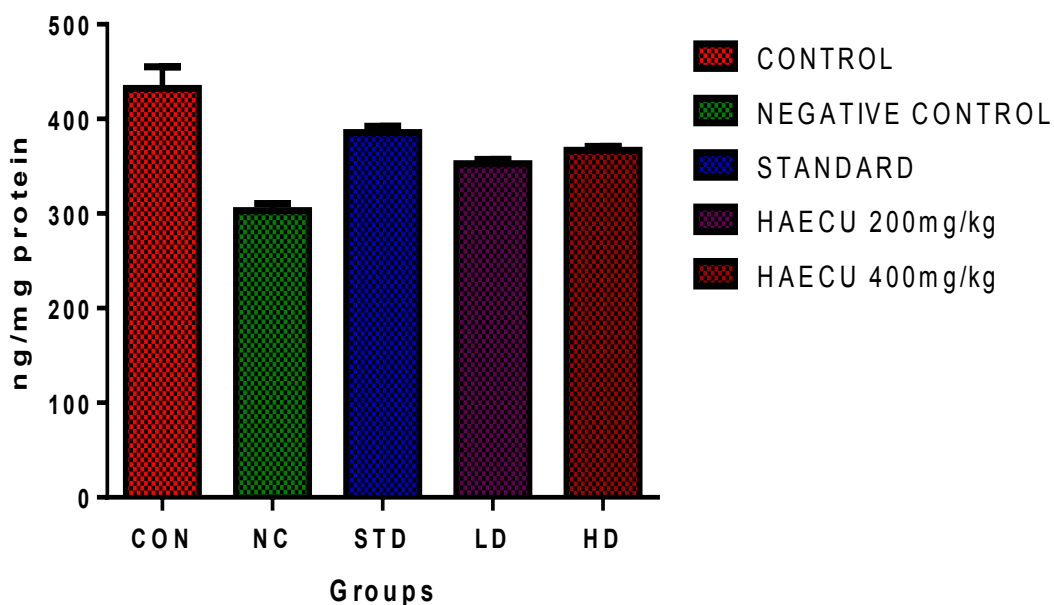
Table 13: Effect of HAECU in Serotonin

| S.no	Groups	ng/mg tissue
1	Control	247.33±5.86
2	Negative control	177.48±3.15^{a***}
3	Standard	238.56±5.46^{b***}
4	HAECU 200 mg/kg	203.52±2.72^{b**}
5	HAECU 400 mg/kg	220.00±2.92^{b***}

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, *p<0.001

Graph 8: Effect of HAECU in Serotonin

Table 14: Effect of HAECU in Acetylcholinesterase

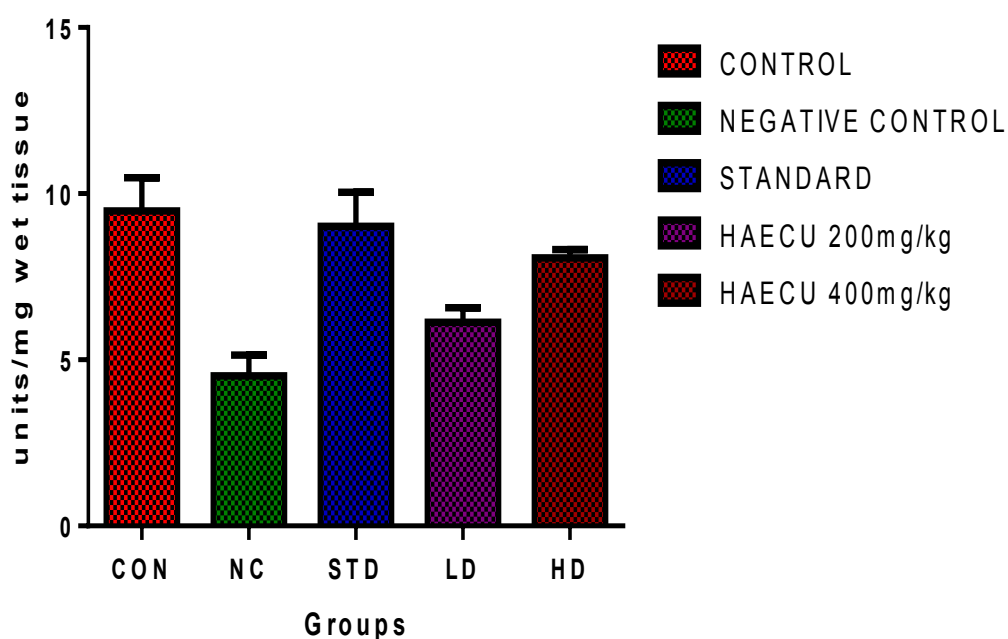| S.no	Groups	nmoles/mg wet tissue
1	Control	24.53±0.67
2	Negative control	32.40±0.9[a***]
3	Standard	18.31±0.81[b***]
4	HAECU 200 mg/kg	22.43±0.50 [b***]
5	HAECU 400 mg/kg	20.25±0.72 [b***]

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, $*p < 0.05$, $**p < 0.01$, $*p < 0.001$

Graph 9: Effect of HAECU in Acetylcholineesterase
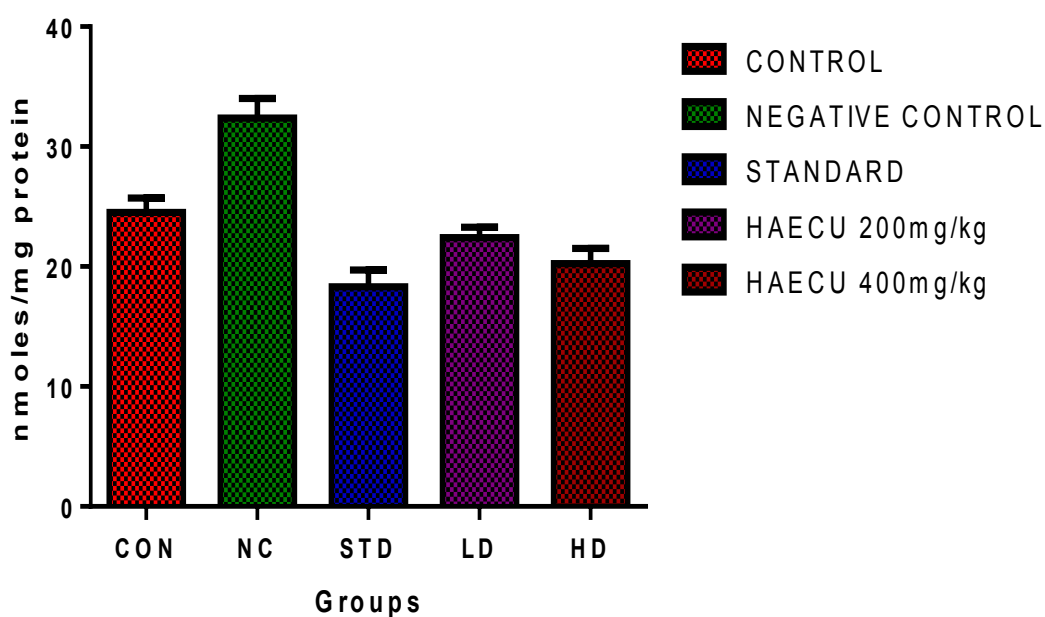
Table 15: Effect of HAECU in Super oxide (SOD)

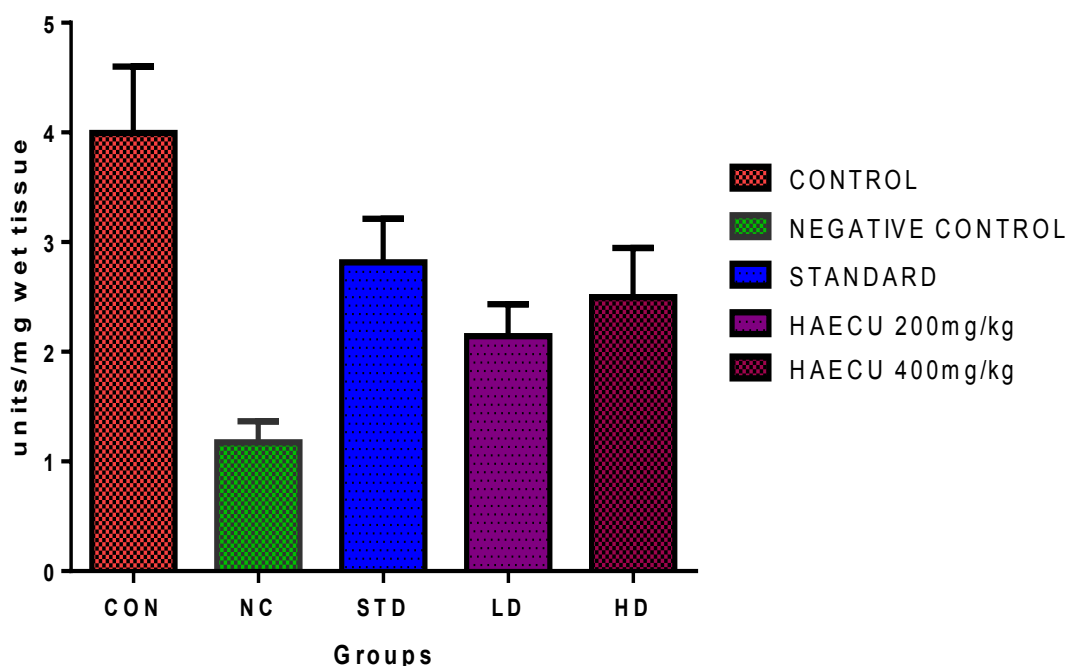| S.no	Groups	Units/mg wet tissue
1	Control	9.48±0.40
2	Negative control	4.51±0.25^{a***}
3	Standard	9.02±0.41^{b***}
4	HAECU 200 mg/kg	6.13±0.17^{b**}
5	HAECU 400 mg/kg	8.06±0.10^{b***}

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *$p<0.05$, **$p<0.01$, *$p<0.001$

Graph 10: Effect of HAECU in Super oxide (SOD)

Table 16: Effect of HAECU in Catalase

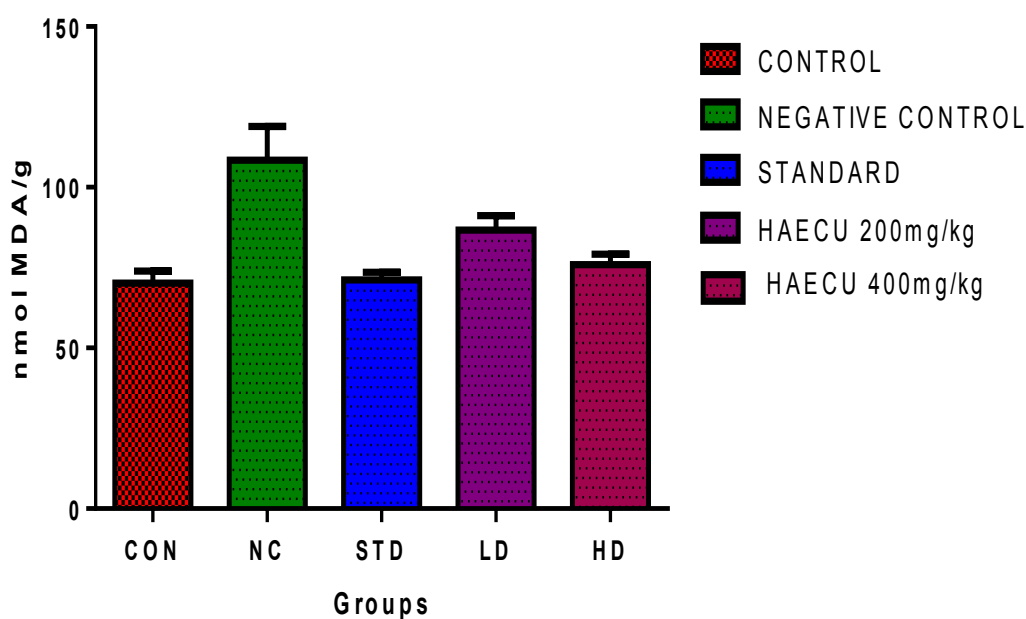| S.no	Groups	Units/mg wet tissue
1	Control	3.99±0.24
2	Negative control	1.17±0.07[a***]
3	Standard	2.81±0.16[b***]
4	HAECU 200 mg/kg	2.14±0.11[b***]
5	HAECU 400 mg/kg	2.49±0.18[b***]

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, *p<0.001

Graph 11: Effect of HAECU in catalase

Table 17: Effect of HAECU in Lipid Peroxide

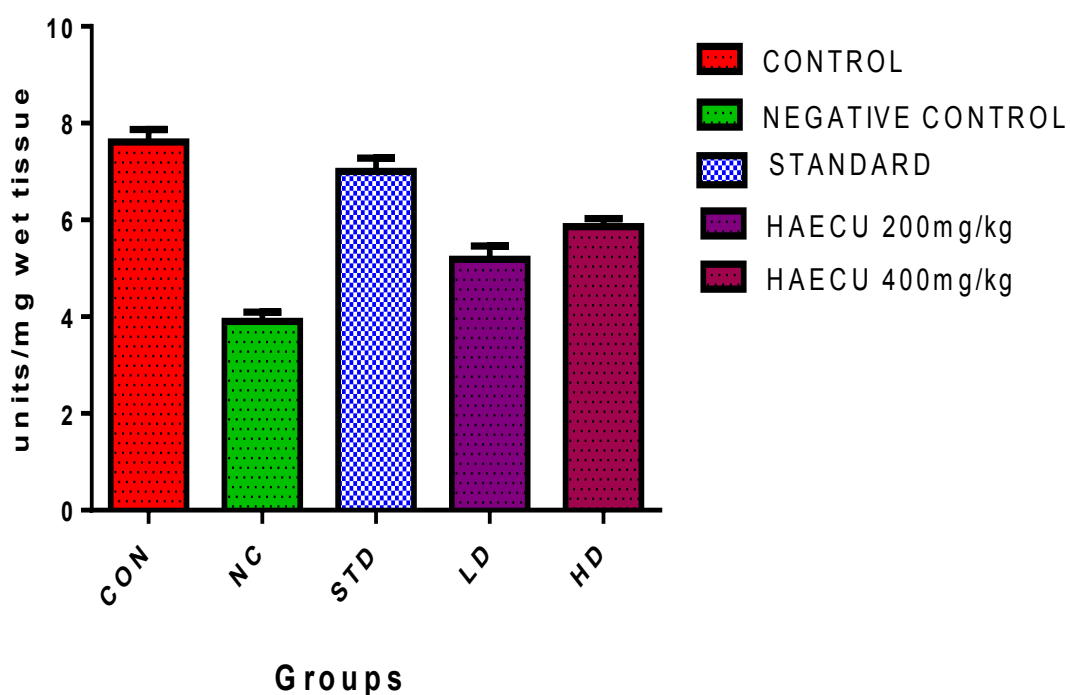| S.no	Groups	Units/mg wet tissue
1	Control	70.17±1.52
2	Negative control	108.50±4.24[a]***
3	Standard	71.20±0.96[b]***
4	HAECU 200 mg/kg	86.64±1.86 [b]***
5	HAECU 400 mg/kg	75.88±1.33 [b]***

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, *p<0.001

Graph 12: Effect of HAECU in Lipid Peroxide

Table 18: Effect of HAECU in Gpx

| S.no	Groups	Units/mg wet tissue
1	Control	7.61±0.26
2	Negative control	3.90±0.18[a]***
3	Standard	7.02±0.27[b]***
4	HAECU 200 mg/kg	5.18.±0.27 [b]***
5	HAECU 400 mg/kg	5.86±0.16 [b]***

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, *p<0.001

Graph 13: Effect of HAECU in Gpx

Table 19: Effect of HAECU in Grd

S.no	Groups	Units/mg wet tissue
1	Control	3.96±0.28
2	Negative control	1.88±0.20[a]***
3	Standard	3.85±0.19[b]***
4	HAECU 200 mg/kg	2.85±0.22 [b]**
5	HAECU 400 mg/kg	3.57±0.23 [b]***

Values are represented in Mean ± SEM, n=6

Comparison: a- Group I vs Group II, b- Group II vs Group III and Group IV

Statistical significance test for comparison was done by one way ANOVA followed by Dunnet's 't' test.

ns- Non significant, *p<0.05, **p<0.01, *p<0.001

Graph 14: Effect of HAECU in Grd
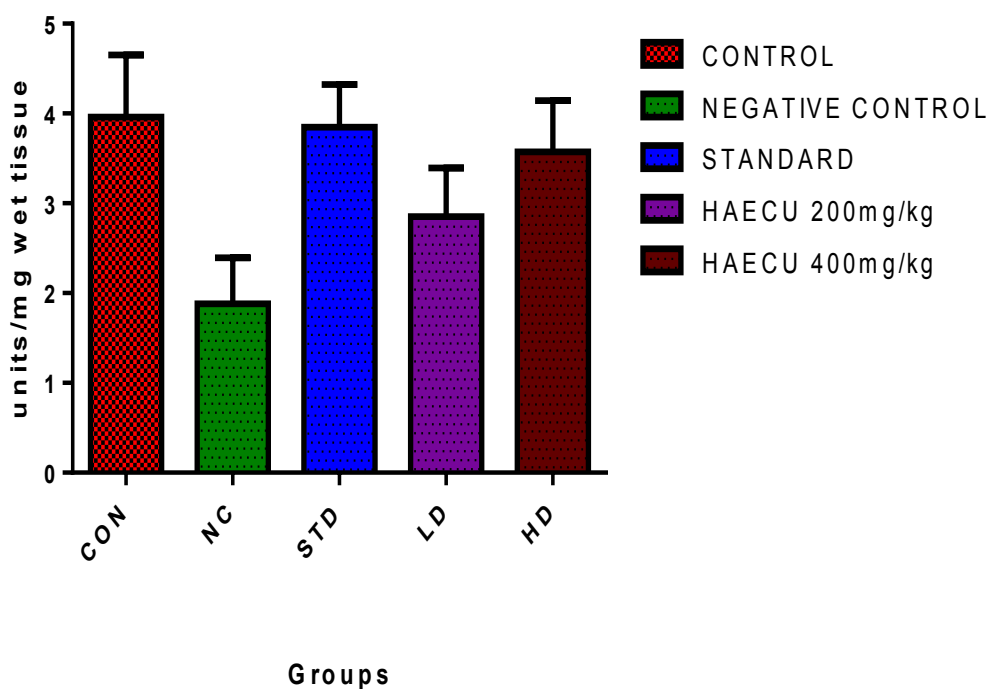
16. RESULTS .

16.1 PRELIMINARY PHYTOCHEMICAL ANALYSIS

The preliminary phytochemical analysis on HAECU revealed the presence of various phytoconstituents including alkaloids, carbohydrates, tannins, flavonoids, gums, mucilage, etc. which are given in Table 5.

16.2.ACUTE ORAL TOXICITY STUDIES

The acute oral toxicity was done according to OECD 423(acute toxic class method) guidelines. A single administration of starting dose of 2000mg/kg of body weight p.o. of HAECU was administered to the three female mice and observed for three days. There was no change in the body weight before and after treatment of the experiment and no sign of toxicity were observed. Observations are shown in table 6

16.3. HABITUATION STUDIES

16.3.1. Effect of HAECU on open field activity

The Group II animals showed significant decrease in head dipping and line crossing when compared with Group I animals ($p<0.001$). Treatment with HAECU (200 and 400mg/kg) showed increased head dipping and line crossing behaviour statistically ($p<0.01$ and $p<0.001$ for Groups IV and V respectively) and Group III also showed increased head dippings and line crossings behaviour significantly ($p<0.001$) when compared with Group II. Results are given in Table 7 and plotted in Graph 1 and 2.

16.3.2. Effect of HAECU on closed field activity

There was a significant ($p<0.001$) decrease in the activity scores produced by Group II animals when compared with Group I animals. Treatment with HAECU (200 and 400mg/kg) and the standard drug showed significant ($p<0.001$, $p<0.001$ and $p<0.001$ for Group III, IV and V respectively) increase in the activity scores when compared with Group II animals. Results are given Table 8 and plotted in Graph 3.

16.4. ASSESMENT OF LEARNING AND MEMORY

16.4.1. Effect of HAECU on Step down Passive Shock Avoidance test

The Step Down Latency (SDL) of Group II animals were significantly decreased ($p < 0.001$) when compared with Group I animals. Treatment with HAECU (200 and 400mg/kg) and standard drug ($p < 0.001$, $p < 0.01$ and $p < 0.001$ for Group III, IV and V respectively) showed significant increase in step down latency when compared with Group II. The increase in SDL indicates increase in short term memory. Results are given Table 9 and plotted in Graph 4.

16.4.2. Effect of HAECU on Y maze task

The percentage of alteration was significantly decreased in Group II when compared with Group I animals significantly ($p < 0.001$). Treatment with HAECU (200 and 400mg/kg) showed significantly increased in the percentage of alteration, ($p < 0.01$ and $p < 0.001$ for Group IV and V respectively) when compared to Group II animals. Standard drug also ($p < 0.001$) showed significantly increased in the percentage of alteration when compared Group II animals. Results are given Table 10 and plotted in Graph 5.

16.4.3. Effect of HAECU on Morris water maze task

The escape latency of Group II animals were significantly increased when compared with Group I animals ($p < 0.05$).). Treatment with HAECU (200 and 400mg/kg) and standard drug showed significant decrease ($p < 0.001$, $p < 0.001$ and $p < 0.001$ respectively) in the escape latency onto the hidden platform when compared with Group II animals. The decrease in escape latency indicates memory retention and non spatial working memory. Results are given Table 11 and plotted in Graph 6.

16.5.INVITRO BIOCHEMICAL ESTIMATIONS (NEUROTRANSMITTER LEVELS)

16.5.1. Effect of HAECU on Dopamine

The brain dopamine level in Group II animals were significantly decreased ($p < 0.001$) when compared with Group I animals. Treatment with HAECU (200 and 400mg/kg) and standard drug showed ($p < 0.01$, $p < 0.001$ and $p < 0.001$ respectively) significant increase in dopamine level on comparison with Group II animals. Results are given Table 12 and plotted in Graph 7.

16.5.2. Effect of HAECU on serotonin

The serotonin level in the brain of Group II animals were decreased significantly (p<0.001) when compared with Group I animals. Treatment with HAECU (200 and 400mg/kg) significantly increased the serotonin level (p<0.01 and p<0.001) when compared with Group II animals. Standard drug also (p<0.001) showed significantly increased level of serotonin when compared Group II animals. Results are given Table 13 and plotted in Graph 8.

16.5.3. Effect of HAECU on Acetylcholinesterase

The level of AChE in Group II animals showed (p<0.001) significant increase when compared with Group I animals. Treatment with HAECU showed significant decrease (p<0.01 and p<0.001 for Group IV and V animals respectively) in the AChE level when compared with Group II animals. Group III (p<0.001) showed significant reduction in AChE activity when compared with Group II animals. Results are given Table 14 and plotted in Graph 9.

16.6 EFFECT ON ANTIOXIDANT ENZYMES

16.6.1.Effect of HAECU on Superoxide Dismutase

The SOD in the brain of Group II animals were decreased significantly (p<0.001) when compared with Group I animals. Treatment with HAECU (Group IV and V) and Group III showed (p<0.01, p<0.001 and p<0.001) significant increase in SOD on comparison with Group II animals. Results are given Table 15 and plotted in Graph 10.

16.6.2. Effect of HAECU on Catalase

The Catalase in the brain of Group II animals were decreased significantly (p<0.001) when compared with Group I animals. Treatment with HAECU (Group IV and V) and Group III showed (p<0.001, p<0.001 and p<0.001) significant increase in Catalase on comparison with Group II animals. Results are given Table 16 and plotted in Graph 11.

16.6.3. Effect of HAECU on Lipid peroxidase

The Lipid peroxidase in the brain of Group II animals were decreased significantly (p<0.001) when compared with Group I animals. Treatment with HAECU (Group IV and V) and Group III showed (p<0.001, p<0.001 and p<0.001) significant decrease in Lipid

peroxidase on comparison with Group II animals. Results are given Table 17 and plotted in Graph 12.

16.6.4. Effect of HAECU on Glutathione peroxidase

The Glutathione peroxidase in the brain of Group II animals were decreased significantly ($p < 0.001$) when compared with Group I animals. Treatment with HAECU (Group IV and V) and Group III showed ($p < 0.001$, $p < 0.001$ and $p < 0.001$) significant increase in Glutathione peroxidase comparison with Group II animals. Results are given Table 18 and plotted in Graph 13.

16.6.5. Effect of HAECU on Glutathione reductase

The Glutathione reductase in the brain of Group II animals were decreased significantly ($p < 0.001$) when compared with Group I animals. Treatment with HAECU (Group IV and V) and Group III showed ($p < 0.01$, $p < 0.001$ and $p < 0.001$) significant increase in Glutathione reductase comparison with Group II animals. Results are given Table 19 and plotted in Graph 14.

16.7 ASSESMENT OF HISTOPATHOLOGICAL CHANGES

It was observed that there was decrease in density of neuronal cells and disrupted in the normal distribution of neuronal cells in hippocampal region in Group II animals with respect to Group I animals. Treatment groups (Group III, IV and V) exhibited improved neuronal configuration than Group II. Group IV, V showed significant improvement in the density of neuronal cells and hippocampal regions of brain when compared with neuronal loss in negative control group (Group II). Whereas Group III showed improved in the density of neuronal cells and hippocampal regions. Amount of amyloid plaques and tau tangles in Group III, IV, V are less when compared to Group II. Histopathological pictures are shown in FIG 8 to 12
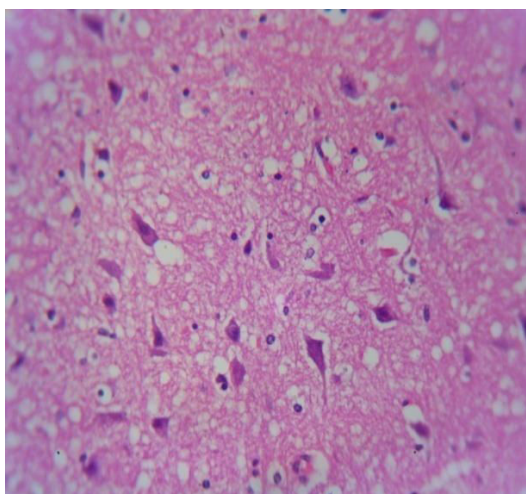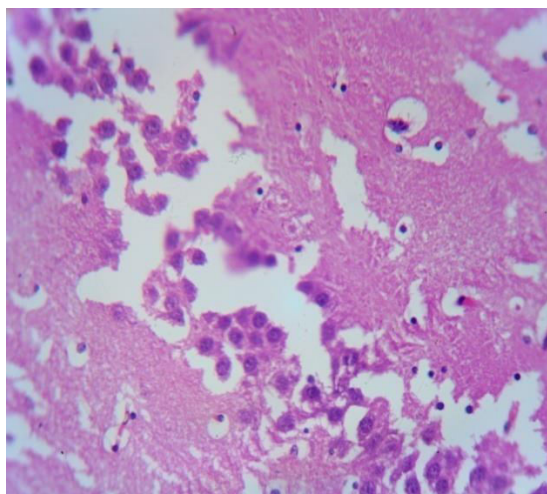
Fig 8: Control
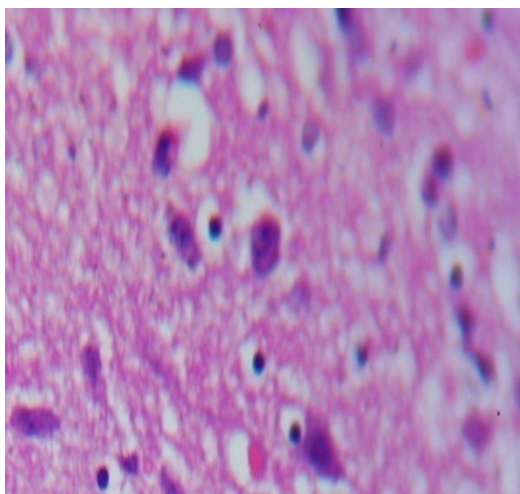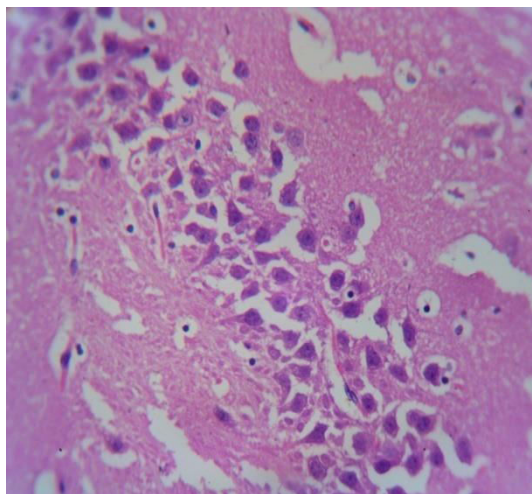
Cortex   Hippocamus

Fig 9: Negative control

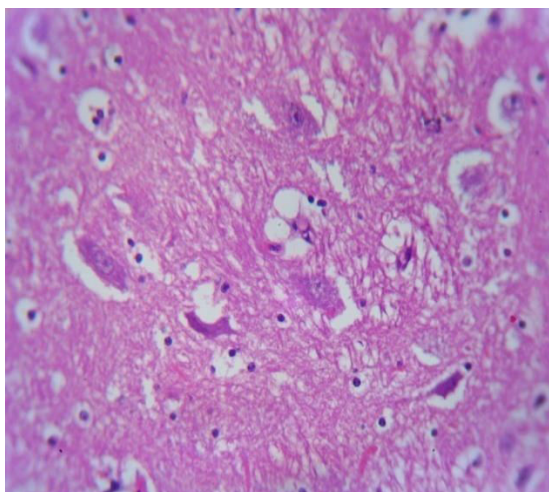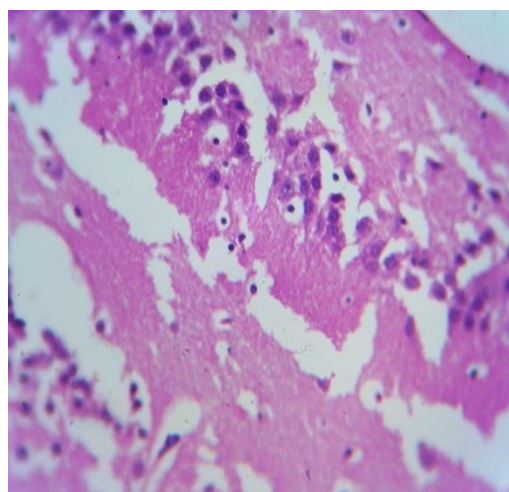Cortex   Hippocampus

Fig 10: Standard

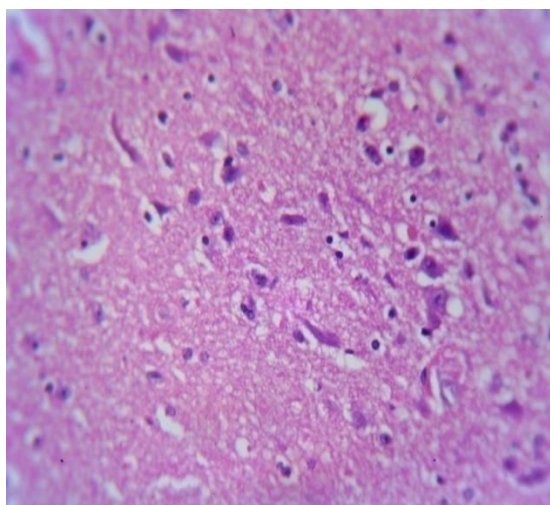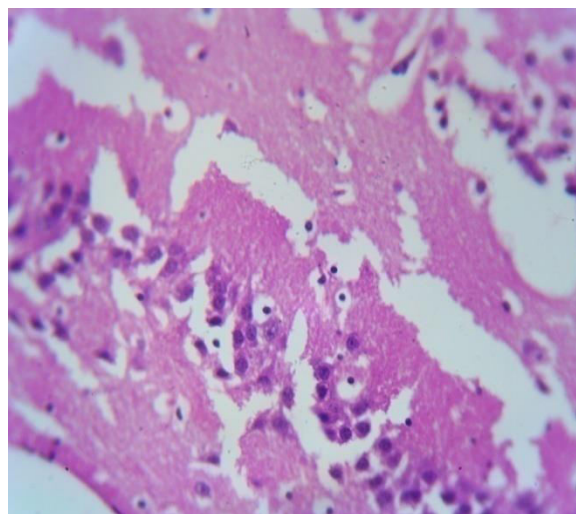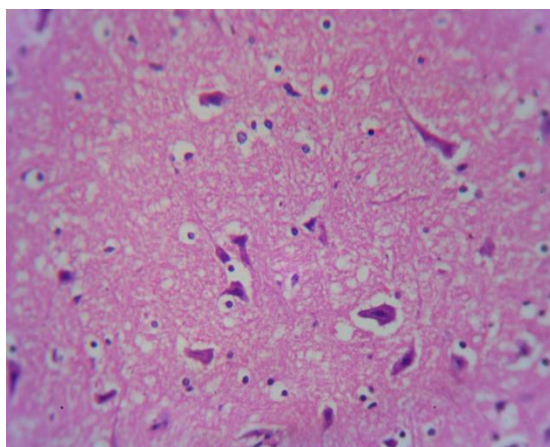Cortex   Hippocamus

Fig 11: Low dose

Cortex   Hippocamus
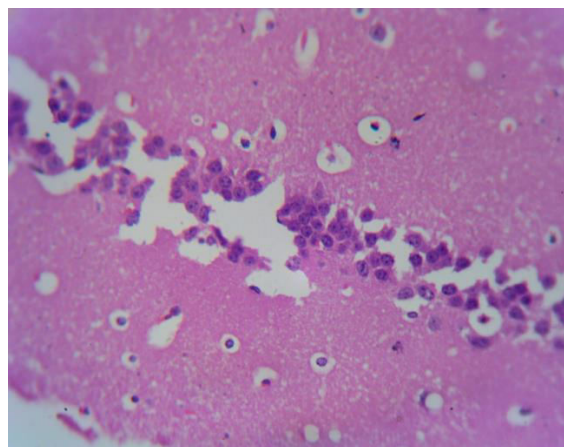
Fig 12: High dose

Cortex   Hippocamus

17. DISCUSSION

Alzheimer's disease (AD) is now the most common cause of dementia . The incidence of AD increases with age . Impairment of short-term memory usually is the first clinical feature. When the condition progresses, additional cognitive abilities are impaired, as the ability to calculate, and use common objects and tools. Acetylcholine esterase inhibitors are the only agents approved by the Food and Drug Administration (FDA) for the treatment of AD. All other agents prescribed for the treatment of AD are used on an off- label basis.

As per previous studies *caryota urens* has excellent antioxidant property, hence it is believed to have actions on CNS disorders and neurodegeneration diseases.

The present study has revealed the ameliorative effect of HAECU on STZ induced Alzheimer's disease in mice. STZ induced impairment of memory was assessed by using various behavioral parameters like Passive avoidance task, Y maze task, and Morris water maze test. It was found that treatment with HAECU protect cognitive deficits in STZ induced Alzheimer's disease.

Spatial learning in the open field habituation was approached to access learning and memory. The decrease in response to a normal environment after repeated exposures to the familiar environment is referred to spatial habitual learning. Recurrent exposure produces a decrease in the exploratory initiatives, which is implicative of memory pertaining to a specific feature of that environment. Exploratory activities may be reduced on subsequent contact with open field. In the result of this study reduced by the group of animals treated with HAECU indicated increased spatial habitual learning and sleep deprivation decreased spatial habitual learning.

Passive avoidance behavior based on negative reinforcement was used to examine the level of memory. An electric shock is as reinforcement during training sessions for 15 sec in the Step Down Latency (SDL) was recorded. SDL was defined as the time taken by the mouse to step down from the wooden platform to grid floor with its entire paw on the grid floor. SDL is increased as form of long term memory where STZ induced animals showed decreased SDL. Treatment with HAECU at test dosages showed improvement in long term memory as index of increase in SDL.

Y maze task is one of the simplest versions of spontaneous alteration task which is used to measure spatial working memory. The ability to alternate requires that the mice know

which arm they have already visited. Normal mice are expected to exhibit an alteration percentage of 60-70. Pretreatment with amnesic agents prior to trials may reduce spontaneous alteration percentage and agents possessing memory enhancing effects are expected to reverse these changes. According the animals which were STZ induced had a reduced spontaneous alteration but animals treated with HAECU produced a significant increase in alteration which was comparable to the untreated control which in turn indicates the increased spatial working memory of the animals.

Morris water maze task represents more specific for spatial memory. The essential feature f this technique is that mice are placed into large circular pool f water and can escape into a hidden platform. Thus, the platform offers no local cues to guide escape behavior and the mouse can escape from swimming by climbing on to the platform apparently learns the spatial location of the platform any starting position at the circumference of the pool. The only spatial cues are those outside water tank are primarily visual cues. Thus, the versatility of the task makes it a widely acceptable experimental model for the assessment of cognitive tests. Typically, STZ induced animals exhibited an increase time for escape latency indicating loss of visual cues to escape n to the platform. Such a diminished cognition was reversed by the administration of the HAECU at the specified dosage levels and exhibited escape latency (EL), indicating the well- developed spatial memory inspite of STZ induced Alzheimer's disease.

Both nicotinic and muscarinic cholinergic receptors are involved in cognitive and memory functions and several studies have suggested their roles in dementia. Marked cholinergic deficit is a hallmark of the pathogenesis of AD and various drugs including AchE inhibitors have designed to target this deficit, initially, cholinergic deficit was thought to be a muscarinic nature, but recent study show a specific loss of nicotinic acetyl choline receptors and marked loss of cholinergic neurons. There was a significant reduction in the level of acetylcholine esterase in the animals treated with HAECU and increased Ach regulate the impaired memory by STZ induction.

Serotonin is the critical neurotransmitter modulating short term habituation in Aplysia. In general, hippocampal depletions deleteriously affect habituation in the open field, but rodents also commonly exhibit decreased locomotor and exploratory activity.

The formation of new memories is thought to require the hippocampus and adjacent medial temporal lobe, but the final storage of memories is widely distributed by neocortical

network. Lesion studies have suggested that there is a wide distribution of neocortical memory traces encoded in the strength of synaptic connections among neurons across large areas of the neocortex. Although, glutamate and GABA receptors play a major role in learning and memory, serotogenic effects have also been detected and on the region implicated in memory storage are richly innervated by the serotogenic system.

It has been suggested that antioxidant might contribute to the prevention of AD. The superoxide dismutase (SOD) constitutes a mutually supportive team of defense against reactive oxygen species. The most remarkable effect of HAECU is increased activity of SOD in hippocampus. Treatment with HAECU preserved the reduced SOD to that of the normal control.

Usually increased level lipid peroxide causes oxidative stress. The HAEECU has better action on lipid peroxide , since the levels of lipid peroxide is reduced in the treatment groups.

Glutathione (GSH) is the major non-protein thiol antioxidant in mammalian cells and is considerd the main intracellular redox buffer. GSH protects cellular protein thiols against irreversible loss, thus preserving protein function The administration of HAECU significantly preserved the activity of GSH, GPx in the hippocampus to similar level observed in normal control mice. The restoration of activities of GSH, GPx by HAECU might promote scavenging of free radicals using recycled GSH from GSSG.

.

18. CONCLUSION

The selected dose 200mg/kg and 400mg/kg of *caryota urens* showed significant action in memory and learning processes but higher dose 400mg/kg showed better action than lower dose 200mg/kg.

The present study relieved the action of *caryota urens* on streptozotocin induced alzheimer's disease on mice model. From the results it can be concluded that *caryota urens* remarkable effect in memory enhancement and oxidative stress. Further studies are required for the identification of molecular level action and individual phytoconstitutent that may responsible for CNS action.

REFERENCE:

1. Bassam Abdul Rasool Hassan. Medicinal Plants (Importance and Uses). *Analytica Acta Rasool Hassan, Pharmaceut* Anal *Acta 2012, 3:10*

2. Plant medicine. *http//*www.medicinehunter.com/about *plant medicine*

3. M. Umadevi.Traditionally Used Anticancer Herbs In India. *Journal of Medicinal Plants Studies Year: 2013, Volume: 1, Issue: 3 First page: (56) Last page: (74)*

4. Alzheimer's disease.*http//www.wikipedia.org/wiki/Alzheimers_disease*

5. Memory .*http//www.wikipedia.org/wiki/Memory*

6. Amnesia .*http://en.wikipedia.org/wiki/Amnesia*

7. Rubinsztein DC .What is Neurodegenerative disease?*JPND Research. Retrived February 7, 2015. 46. (October 2006).*

8. Wenk GL .The roles of intracellular protein degradation pathways in neurodegeneration. *Nature 443(7113): 780-6 47. (2003).*

9. Wenk GL (2003). Neuropathologic changes in Alzheimer's disease. *J Clin Psychiatry Pg64 Suppl 9:7-10. PMID 12934968. 48.*

10. Estrada Sanchez AM, Mejia-ToiberJ, MassieuL .Parkinson's mechanism discovered. *HIMI Research news June 22, 2006. 49. (April 2008).*

11. Excitotoxic neuronal death and the pathogenesis of Huntington's disease. *Arch. Med. Res. 39(3):265-76*

12. Amyotrophic Lateral Sclerosis. U.S. Department Of Health And Human -Services Public Health Service National Institutes of Health

13. NahidJivad, Zahra Rabiei.A review study on medicinal plants used in the treatment of learning and memory impairments.*Asian Pacific Journal of Tropical Biomedicine.4.2014APJTB-2014-0412.2014*

14. Gooch MD and Stennett DJ. Molecular basis of Alzheimer's disease. *Am J Health-Syst Pharm. 1996: 53; 1545-1547.*

15. World Health Organization (WHO)*Active ageing: a policy framework.* Geneva: WHO; 2002. WHO/NMH/NPH/02.8.

16. Kalaria RN, Maestre GE, Arizaga R, Friedland RP, Galasko. D, Hall K, World Federation of Neurology Dementia Research Group. Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. *Lancet Neurol*2008; *7* : 812-26.

17. Harold D. Foster .Text book of *What Really Causes Alzheimer's Disease.*

18. Pandey Shivanand. A Review on Alzheimer's disease: Its Cause, Symptoms, And Treatment At World Wide.*International Journal of Applied Biology and Pharmaceutical Technology Volume: I: Issue-1 May-July -2010*

19. AsmaShahedaShaik .Alzhemier's Disease.Pathophysiology and treatment. *International Journal of Pharma and Bio Sciences V1(2)2010*

20. World health organization.www.who.int/alzheimer's disease

21. Dementia. *http://www.alz.org/in/dementia-alzheimers-en.asp*

22. Alzheimer's statistics . http//*www.newscientist.com*

23. Alzheimer's in India *www.downtoearth.org.in/news/why-india-has-the-worlds-lowest-rate-of-alzheimers-disease-9366*

24. Fusheng yang, Gisella P, Aynun *N.* Use of Curcumin . *Journal of Biological Chemistry*, *Vol 280, No 11*

25. Allan S. Vann. Evaluating Prescription Drugs Used to Treat: Alzheimer's Disease Consumer Reports Best Buy Drugs. *JAMDA 14 (2013) 525e527*

26. Dr.Larry chambers. Signs of Alzheimer's disease.*http//www.Alzheimer Society of Canada.com. pg no 57*

27. Kelly Bethune. Text bookof Diagnosis and Treatment of Alzheimer's Disease: Current Challenges . 1990;68:267-289

28. ADEAR. Alzheimer's disease Education and Referral Center. Alzheimer's disease Unravelling the Mystery. Plaques and Tangles: The Hallmarks of Alzheimer's disease. *http://www.alzheimers.org/* unraveling/06.html

29. Fotuhi, M. (2003).*The memory cure*. New York: McGraw-Hill.

30. Encyclopedia of Phosphorylation.*http://en.wikipedia.org/wiki/phosphorylation*

31. Iraizoz, I., Guijarro, J.L., Gonzalo, L.M., and de Lacalle, S. Neuropathological changes in the nucleus basalis correlate with clinical measures of dementia. *ActaNeuropathologica*(Berl) 98(2), 186-196.(1999).

32. Cutler, N.R., and Sramek, J.J.*Understanding Alzheimer's disease.*University Press of Mississippi. (1976).*Lancet*, 2(8000), 1403.(1996)

33. Davies, P., and Maloney, A.J.F. Selective loss of central cholinergic neurones in Alzheimer's disease.*Lancet*, *2(8000), 1403. (1976).*

34. Toghi, H., Abe, T., Hashiguchi, K., Saheki, M., and Takahashi, S. (1994).Remarkable reduction in acetylcholine concentration in the cerebrospinal fluid from patients with Alzheimer's type dementia.*Neuroscience Letters*, *177, 139-142*

35. Kay, A.D., Milstein, S., Kaufman, S., Creasey, H., Haxby, J.V., Cutler, N.R., and Rapoport, S.I. Cerebrospinal fluid bioptein is decreased in Alzheimer's disease. *Archives of Neurology, 43(10), 996-999. (1986).*

36. Joyce, J.N., Myers, A.J., and Gurevich, J.E. Dopamine D2 receptor bands in normal human temporal cortex are absent in Alzheimer's disease.*Brain Research*, 784(1-2), 7-17. (1998).

37. MalteButzlaff, Evgeni Ponimaskin. The role of Serotonin Receptors in Alzheimer's disease. *Opera Medica&Physiologica 1 ● 91-100 ● January 2016*

38. Treatment for alzheimer's. *http://www.alz.org/treatment*

39. SaloniTanna. Priority Medicines for Europe and the World "*A Public Health Approach to Innovation*" 2004

40. Anil Kumar Singhal , Medicinal plants with a potential to treat Alzheimer and associated symptoms *International Journal of Nutrition, Pharmacology, Neurological Diseases | May-August 2012 | Vol 2| Issue 2*

41. Mary-LetitiaTimiras M.D.Alzheimer's Disease. Overlook Hospital, summit, New Jersey.Evans DA. Milbank Q. 1990;68:267-289

42. Grünblatt E, Salkovic-Petrisic M, Osmanovic J, Riederer P, Hoyer S Brain insulin system dysfunction in streptozotocinintracerebroventricularly treated rats generates hyperphosphorylated tau protein. *J Neurochem 101:757–770, 2007*

43. Hou Y. Zhou L, Yang QD, Du XP, Li M, Yuan M, Zhou ZWChanges in hippocampal synapses and learning-memory abilities in a streptozotocin-treated rat model and intervention by using fasudil hydrochloride. *neuroscience.2011.10.030. Epub 2011 Oct 25*

44. Hoyer S , Lannert H , Long-term abnormalities in brain glucose/energy metabolism after inhibition of the neuronal insulin receptor: implication of tau-protein.*J Neural Transm Suppl. 2007;(72):195-202*

45. Prickaerts J, Blokland A, Honig W, Meng F, Jolles JSpatial discrimination learning and choline acetyltransferase activity in streptozotocin-treated rats: effects of chronic treatment with acetyl-L-carnitine.*Brain Res. 1995 Mar 13;674*

46. Prickaerts J, Fahrig T, Blokland A. Cognitive performance and biochemical markers in septum, hippocampus and striatum of rats after an i.c.v. injection of streptozotocin: a correlation analysis.*Behav Brain Res.* 1999 Jul;102(1-2):73-88.

47. Buffie J. Clodfelder-Miller, Anna A. Zmijewska, Gail V.W. Johnson and Richard S. Jope.Tau Is Hyperphosphorylated at Multiple Sites in Mouse Brain In Vivo After Streptozotocin-Induced Insulin Deficiency Diabetes .*NCBI 2006 Dec; 55(12): 3320-3325*

48. Fang Fang, Ning Yan, Zhanhui Feng, Xiangqin Liu, Zheng Xiao. Alzheimer's disease by Aβ1-40, aluminium and growth factor β1 /*Aging and Neurodegeneration,Vol. 1 (1): 15-19, September 20*

49. Szu-Ming Chen, Chi-Chen Fan, Ming-ShiuanChiue, Chi Chou. Hemodynamic and Neuropathological Analysis in Rats with Aluminum Trichloride-Induced Alzheimer's Disease. *Plos one. December 2013 | Volume 8 | Issue 12*

50. R.Kanan , D.Sivaraman, P.Muralidharan.Neuroprotectiveefeect of hydroalcoholic extract of areca cathechu on beta amyloid induced congnitive dysfunction in mice, *int.J.res. Ayurveda pharm. 4(5),sep- oct 2013*

51. AdhamR ,GehanY,chorine A, ismail. Neuroprotective role of vitamin D3 in colchicine-induced Alzheimer's disease in rats. *Alexandria journal of medicine.21*

52. Pradhipkumar , streptozotocin induced alzheimer's disease like changes and underlying neural degeneration and regeneration mechanism, *Neural Regen Res. 2015 Jul; 10(7): 1050–1052*

53. Karen Duff .text book of *Transgenic Mouse Models Of Alzheimer Disease*

54. Prickaerts, J., De Vente, J., Honig, W., Steinbusch, H., Ittersum M.M.V., Blokland, A.,Steinbusch, H.W., 2000.‖ Nitric oxide synthase does not mediate neurotoxicity after an ICV. injection of streptozotocin in the rat. *J. Neural. Transm. 107, 745–766*

55. Szkudelski, T.The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res. 50, 336–346 ., 2001*

56. The IUCN red list of threatened species *www.iucn.com*.

57. *Agroforestry Database: a tree reference and selection guide, version 4.0 pg 19*

58. Murshid.V.V.Evaluation Of Central Nervous System Activity Of *Caryotaurens*Fruit Extract On Various Animal Models, Rajiv Gandhi university, Bangalore. July 2013

59. P. Ranasinghe, G.A.S. Premakumaral, C.D. Wijayarathna and W.D. RatnasooriyaAntioxidant Activity of *Caryotaurens*L. (*Kithul*) Sap.*Tropical Agricultural Research Vol. 23 (2):117– 125 (2012)*

60. G. E. M. Wimalasiri , Antioxidant and anti-diabetic properties of *Caryotaurens*(*Kithul*) flour. *Procedia Food Science 6 (2016) 181 – 185*

61. A. Charles, M. Joseph, V. Alex Ramani. Qualitative phytochemical screening, anti-oxidant and anti-microbial activity studies on ethanolic flowers extract of caryotaureneslinn. *IJABPT , Volume: 2: Issue-3: July-Sept -2011*

62. Vaishnavi R and Suneetha V. Phytochemical analysis on *Caryotaurens*(fishtail palm) fruit from VIT university campus for pharmaceutical use.*Der Pharmacia Lettre, 2013, 5 (3):71-75*

63. Hasan M.H. Muhaisen. Chemical Constituents from the Base Leaves of Caryotaurens.IOSR Journal of Applied Chemistry (IOSR-JAC), Volume 5, Issue 5 (Sep. – Oct. 2013), PP 05-12

64. Amit Kumar Srivastav, Raghvendra Singh, S. Manimegalai and V. Devi RajeswariIdentification of Flavonoids in Methanolic Extract of Caryotaurens(Fish Tail Palm): A Phytochemical Screening Involving Structure Analysis by FTIR Spectroscopy.*Research Journal of Phytochemistry 9 (3): 127-136, 2015*

65. Md. Sahab Uddin, Md. Fakhrul Hasan, Abdullah Al Mamun, In Vitro Estimation Of Antioxidant Activity Of *Caryotaurens*fruits. IAJPS *2015, 2 (11), 1486-1490*

66. Oecd Guideline For Testing Of Chemicals - Acute Oral Toxicity – Acute Toxic Class Method

67. C.K.Kokate, A.P.Purohit, S.B.Gokhale.Text book of Pharmacognosy47[th]edition. August 1, 2007.

68. Yanxingchein, zhihouliang, Julie Blanchard. A non transgenic mouse model (i.c.v STZ) of alzheimer's disease. *Mol neurobiol.2013 apr, 47(2)*

69. Preparation of ACSF. *www.janelia.org*

70. H.Vogels. Jochen mass, Alexander. Screening of central nervous system drugs.

71. Turner . Depressant of central nervous system screening in pharmacology. 1st edition

72. Morris R, Development of a water maze procedure for studying spatial learning in the rat. *J Neuroscience Methods, 1984, 11:47-60*

73. Nunez. Morris Water Maze Experiment. *Journal of visualized Experiments*, *2008; (19): 897*

74. Saitoh, A.M., Yamada, M. Yamada, S. Kobayashi, N. Hirose, K. Honda and J. Kamei, 2006. ―Rho associated kinase path way inhibition produces anxiety-related behaviors in mice‖. *Psychopharmacology, 188: 10-2*

75. Olakunle James Onaolapo1, AdejokeYetundeOnaolapo2, Tolulope Josiah. Elevated Plus Maze and Y-Maze Behavioral Effects of subchronic, Oral Low Dose Monosodium Glutamate in Swiss Albino Mice. *IOSR Journal of Pharmacy and Biological Sciences. . Volume 3, Issue 4 (Sep-Oct. 2012)*

76. VinuthaB,Prashanth D, salma K. screening of selected medical plants for acetylcholineterase inhibitory activity. *Journalof ethanopharmacology.2007*

77. Margret Schlumfjf, Walter Lichtensteiger, Heinrich Langemann, A FluorometricMicromethodFor The Simultaneous Determination Of Serotonin, Noradrenaline And Dopamine In Milligram Amounts Of Brain Tissue. *Biochemical Pharmacology. Vol. 23, pp. 2337-2446.*

78. Misra H.P, Fridovich I. *Biochemistry*, 1967, 15:681

79. Aebi H. Methods of enzymatic analysis, ed. Newyork, *Academic press, 1972, 2:674.*

80. Ohkawa H, Ohisi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *AnnalBiochen, 1979; 95: 351-358.*

81. Beutler E, Duron O, Kelly B.M. Improved method for the determination of blood glutathione. *J Lab Clin Med, 1963; 61:882-8*