"EFFECT OF LISINOPRIL AND PERINDOPRIL IN SODIUM NITRITE AND ETHANOL INDUCED ANTEROGRADE AMNESIA IN MALE WISTAR RATS"

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REG. NO. BO0114002

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Dr. A. P. HOGADE$_{M.D.,}$
Professor and Head,
Department of Pharmacology & Pharmacotherapeutics,
J. N. Medical College,
Belagavi-590010
Karnataka, India.

Dr. (Mrs.) N.S.MAHANTASHETTI$_{M.D.,}$
Principal,
J. N. Medical College,
Belagavi-590010
Karnataka, India.

Date:
Place: Belagavi

Date:
Place: Belagavi

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Nehru Nagar, Belagavi-590 010, Karnataka-India

Website : http/www.jnmc.edu
E-Mail : Principal@jnmc.edu

☎ Office :+91-(0)831 2471350
FAX :+91 (0)831-2470759

Ref. No. : JNMC/PG/

Date :

To,

REG. NO. BO0114002

Postgraduate Student,
Department of Pharmacology,
2014 Batch,
J. N. Medical College,
Belagavi.

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Department of Pharmacology,
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ABBREVIATIONS

5HT	:	5-hydroxy tryptamine
ACCF	:	American College of Cardiology Task Force
ACE	:	Angiotensin Converting Enzyme
ACEI	:	Angiotensin Converting Enzyme Inhibitor
Ach	:	Acetyl choline
AD	:	Alzheimer's disease
AHA	:	American Heart Association
AMP	:	Adenosine monophosphate
AMPA	:	Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AMPK	:	Adenosine monophosphate-activated protein kinase
Ang I	:	Angiotensin I
Ang II	:	Angiotensin II
ANOVA	:	analysis of variance
AChE	:	Acetylcholinesterase
AChEI	:	Acetylcholinesterase Inhibitors.
ADAMS	:	Aging Demographics, and Memory Study
antiChE	:	Anticholinesterase
aPKC	:	activated Protein Kinase c
APP	:	Amyloid Precursor Protein
AT_1	:	Angiotensin 1 receptor
AT_2	:	Angiotensin 2 receptor
ATP	:	Adenosine triphosphate
BACE	:	Beta secretase

BBB	:	Blood Brain Barrier
BDNF	:	Brain derived neurotrophic factor
BP	:	Blood Pressure
BuChE	:	Butyrylcholinesterase
cAMP	:	cyclic Adenosine Monophosphate
CBP	:	CREB Binding Protein
CHD	:	Coronary heart disease
CNS	:	Central Nervous System
CPCSEA	:	Committee for the Purpose of Control & Supervision on Experiments on Animals
CREB	:	cAMP response element-binding protein
CRP	:	C reactive protein
CVD	:	Cardio Vascular Disease
CYP	:	cytochrome
DLB	:	Dementia of Lewy bodies
DM	:	Diabetes Mellitus
ELT	:	Escape Latency Time
FDA	:	Food and drug administration
GABA	:	Gamma amino butyric acid
GFR	:	Glomerular Filtration Rate
GSH	:	Glutathione
GSSG	;	oxidized Glutathione
HMG CoA	:	3-hydroxy-3-methyl-glutaryl coenzyme A (HMG CoA)
HTN	:	Hypertension
H_2O_2	:	Hydrogen Peroxide

IAEC	:	Institutional animal ethics committee
IDE	:	Insulin degrading enzyme
IL	:	Interleukin
LTM	:	Long term Memories
LTP	:	Long term Potentiation
MAO-I	:	Monoamine oxidase inhibitor
MAPK	:	Mitogen activated protein kinase
MCI	:	Mild cognitive impairment
mTOR	:	mechanistic Target Of Rapamycin
nACHRs	:	Nicotine acetylcholine receptors
NADP	:	Nicotinamide Adenine dinucleotide phosphate
NADPH	:	reduced Nicotinamide Adenine dinucleotide phosphate
NaNO2	:	Sodium nitrite
NEP	:	Neutral Endopeptidase
NMDA	:	N-methyl-d-aspartate
NMDA-R	;	N-methyl-d-aspartate Receptor
NO	:	Nitric Oxide
NSCs	:	Neural Stem Cells
PFC	:	pre-frontal cortex
PERTIENT	:	Perindopril Thrombosis Inflammation Endothelial Dysfunction and Neurohormonal Activation
PROGRESS	:	Perindopril Protection Against recurrent stroke study.
PPAR	:	Peroxisome proliferator-activated receptor gamma coactivator 1alpha
pKa	:	pH of dissociation constant of acid

PTSD	:	Post Traumatic Stress Disorder
Rac1	:	Ras-related C3 botulinum toxin substrate 1
RAS	:	Renin – Angiotensin System
RAAS	:	Renin – Angiotensin – Aldosterone System
SEM	:	Standard Error of Mean
STM	:	Short Term Memories
STEM	:	ST- segment elevation myocardial infraction
SPSS	:	Statistical package for social sciences
SSRIs	:	Selective serotonin reuptake inhibitors
TS	:	Time Spent
WHO	:	World Health Organization

ABSTRACT

Objective:

The present study was planned to study the effect of lisinopril and perindopril in sodium nitrite and ethanol induced oxidative stress and apoptotic model of anterograde amnesia in male Wistar rats.

Materials and Methods:

Nine groups (n=10), of healthy male Wistar rats (150-180 grams) were used for the study. The induction of anterograde amnesia was done by sodium nitrite and ethanol. The paradigm used was Morris water maze where a hidden platform was kept for the rats to escape to. The rats were trained to locate a hidden platform by releasing them into water for four times a day for four consecutive days. The acquisition of this task was measured by noting the time taken to escape to the platform. On the fifth day of the study, retrieval of this learnt task was measured by noting their time taken to search for the missing hidden platform. These two data measured were then compared amongst test groups and amnesia induced groups. Data was expressed as mean ± standard error of mean. On the 5th day (Retrieval trial) only vehicle (distilled water i.p) was administered to the groups.

Results:

Lisinopril and perindopril completely ameliorated the induction of anterograde amnesia by sodium nitrite. Perindopril showed significant decreased in mean ELT in ethanol induced anterograde amnesia but lisinopril did not show any significant difference in respect to ethanol.

Conclusion:

The study drugs in the present study ameliorated chemical hypoxia induced anterograde amnesia pointing towards a possible anti-oxidant role of them. These drugs can therefore potentially inhibit oxidative stress induced neurodegeneration especially in the ageing population at the commonly prescribed clinical doses. Additionally, perindopril may be exerting antiapoptotic action that might attribute to more beneficial effect in amnesia. Further studies are needed to substantiate these findings.

Key words: anterograde amnesia, lisinopril, perindopril, sodium nitrite, ethanol Morris water maze.

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INTRODUCTION

Amnesia commonly known as loss of memory is a pathological state of forgetfulness. It is a disturbance in the function of conscious memory for recent episodes and experiences. Retrograde amnesia is the inability to recall experiences that occurred before the onset of the amnestic state. While, anterograde amnesia is the inability to store, retain, and recall new knowledge.[1,93]

Amnesia is the most common presenting symptom of neurodegenerative dementia which has an insidious onset and progresses gradually. Elderly population is the predominantly affected group in neurodegenerative dementia, with anterograde amnesia being more common.[1] Moreover an increase in life expectancy has increased the proportion of elderly in the population and hence the burden of amnesia. This has resulted in significant social and occupational impairment in patients as well as in caregivers.[2]The global burden of amnesia is on a rise owing to an increase in human life expectancy. It has been estimated that approximately 3.2 million out of 70 million elderly people have dementia in India which is expected to be doubled by 2030 while the financial burden would triple by then.[8]

Various etiologies have been hypothesized in the pathogenesis of amnesia like amyloid cascade, tau protein, inflammatory, oxidative stress, apoptosis, vascular, cholesterol, diabetes & abnormal insulin signaling etc.[3] These are being explored as various targets in the management of amnesia. Most of these pathologies were considered irreversible as dead neurons could neither be regenerated nor replaced. However, recent research on neurogenesis has documented neural precursor stem cells in brain and that neurogenesis in hippocampus plays an important role in learning and memory.[11]

Currently, pharmacotherapy for the prevention and treatment of amnesia in neurodegenerative disorders is aimed at protecting from excitotoxicity (N-Methyl-D-aspartate antagonists) or supplementing neuronal transmission of viable neurons (Acetylcholinesterase inhibitors) or selectively improving efficiency of higher telencephalic integrative activities (nootropics – piracetam). However, these drugs are unable to arrest the pathology of the disease and have significant adverse effects which has limited their use.[4,5,6] Therefore, there is a need for more efficacious and safer drugs in the fight against amnesia. Interestingly, many drugs such as statins, Non-steroidal anti-inflammatory drugs, metformin, omega 3 fatty acids, vitamins etc. are being tested for their effects on amnesia.[2,4]

Hypertension (HTN) is one of the major risk factors for development of dementia in Alzheimer's disease and vascular dementia. Previous study have shown that there is typical association between decreased risk of dementia and use of antihypertensive agents.[91] Nonetheless, control trials of regularly used classes of anti-HTN drugs, like diuretics, calcium channel blockers and beta blockers have concluded mixed results with regard to their protective effects on the incidences of dementia. Meta-analysis have found that decrease in cognitive impairment or dementia has no significant association with blood pressure reduction.[7]Thus, it brings up the issue whether mechanism independent of blood pressure lowering accounts for the variable protective effects on cognition. In humans however, the effect of commonly used anti HTN drugs- angiotensin converting enzyme inhibitors (ACEIs) on cognition are controversial.[7]Aprevious meta-analysis concluded that antihypertensive treatment could only decrease the risk of vascular dementia but not Alzheimer dementia or cognitive decline.[8]

ACEIs (lisinopril and perindopril) are used clinically as effective and safe antihypertensive and vascular protective agents. Although these agents have been widely used in treatment of HTN and congestive heart failure, there are only a few preclinical and clinical studies in which ACEIs have been shown to reduce the incidence of dementia or slow down the rate of cognitive decline in patients with HTN.[7] Furthermore, the significance of ACE inhibitor usage in neurodegenerative diseases is still unknown.[9]

It has also been demonstrated that stimulation of the Renin-Angiotensin system (RAS) causes activation of inflammatory cytokines that may play a major role in neurodegenerative disorders.[12] Interestingly it is noted that ACEI (lisinopril) in certain studies is known to possess anti-inflammatory and anti-oxidant properties.[10] Similarly perindopril is also been reported to exert anti apoptotic, anti-inflammatory, antioxidant, antithrombotic, and profibrinolyticactions.[90]

However, there is scarcity of literature regarding use of these drugs in treatment of amnesia. There has been no study directly demonstrating anti-oxidant effect of lisinopril and perindopril in central nervous system. This study therefore intends to explore the direct effects of lisinopril and perindopril on experimentally induced anterograde amnesia in male Wistar rats.

OBJECTIVE

The present study was conducted to investigate:

1. The effect of lisinopril& perindopril in sodium nitrite induced oxidative stress model of anterograde amnesia in male Wistar rats.
2. The effect of lisinopril and perindopril on ethanol induced apoptotic model of anterograde amnesia in male Wistar rats.

REVIEW OF LITERATURE

Memory, the ability to hold information and review it at later time, is a biologically fundamental function essential for survival. Furthermore, memories are our identity: who we are is result of memories, which guide our thoughts and decisions, and impacts our emotional reactions.[14] Memories exist in numerous forms and rely on distinct neural systems. Memory cannot be explained and understood as a "unitary function or system". Rather memory is categorized as "contents, temporal characteristics and processes".[14]

Classification of memory on basis of time

Memory can be classified as "ultra-short-term, short-term, and long-term memory".[14]

1.Ultra-short-term memory / sensory memory: refers to ability to retain sensory information of large amount that people encounter daily for very brief period of time of about milliseconds and it immediately decays. Sensory memory can classified into 3 types: "echoic memory, iconic memory and haptic memory".

a. Iconic memory that can retains information, which is assembled through sight,
b. Echoic memory that can retains information which is assembled through auditory stimuli and
c. Haptic memory that can retains data which is assembled through touch.[16]

2. Short-term memory is holding of information in the conscious awareness for short period of time (usually for a few seconds) and is performed by working memory.[14] Working memory can be divided into four major elements that process information:

a. The episodic buffer -which integrates information from different sources and stores them.
b. The phonological buffer- which consolidates new words and stores it.
c. The central executive- responsible for attention control,
d. The visuospatial sketchpad -which creates and maintains a visuospatial representation.[15]

3. Long-term memories (LTM) refers to information held for long-lasting periods, sometimes for an entire lifetime, even if the information is removed from conscious awareness but which is retrievable after longer periods of time (Fig 1)

STMs and LTMs can also be differentiated based on their biological mechanisms: STMs relies on existing networks and posttranslational modifications. While, the LTMs is accompanied by structural and functional changes of neural networks that require de novo gene expression.[14,94]

Classifications of memoryon basis of content

Memory can be further classified according to its contents. Larry R. Squire and Endel Tulving. were able to classify content-based memory under two most influential headings, Declarative memories and Non-Declarative memory Fig 1

Declarative memory- these memories can be verbally formulated or declared; can be reproduced explicitly and are conscious. While, non-declarative memories normally are the ones that cannot be declared or verbalized, implicit and unconscious. [19,94]

Declarative memory constitutes of:

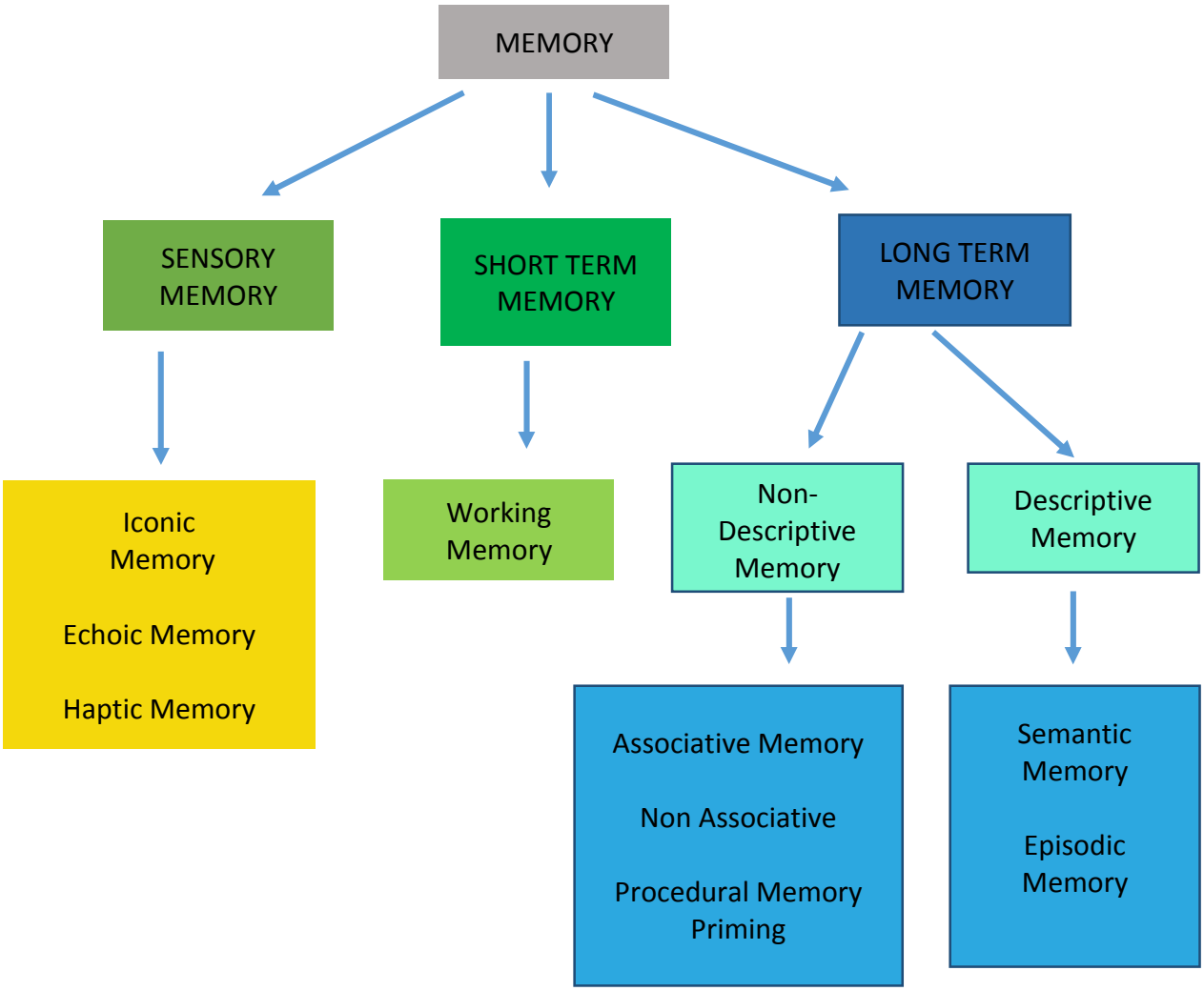
- Knowledge of experiences or procedural learning- It is demonstration of skills which have been acquired by learning and practicing it many times (e.g. motor skills)

- Factual knowledge or semantic memory- retrieval of semantic memory is accompanied by a feeling of 'knowing' For example, I used my semantic memory to comprehend this paragraph which was based on my facts about grammar and sentence formation
- Knowledge of events /episodic memory (time related events).

Nondeclarative memory is the memory which indirectly guides our behavior. It constitutes of:

- Non-associative memory -sensitization and habituation
- Associative memory -classical and operant conditioning
- Priming -a primary stimulus that influence a secondary one[13,19]

Fig 1. Classification of Memory



Memory Processes

For permanent information to be formed multiple steps in information processing is required. Before the formation of permanent memory, the first step is perception of information through sensory organs (registration). Once the information is perceived the next step is pre-processing or also known as encoding. The perceived information is converted into construct to be stored in brain, so that it can be retrieved later. The process of laying down of information begin with attention, which is regulated by thalamus. If the information perceived is memorable it causes rapid neuronal firing, which makes the information more intense, more chances of memory being encoded. In encoding, hippocampus play crucial role by analyzing the pre-processed information and ultimately deciding if the information can be lodged into an already existing data networks and associated with earlier contents (i.e.consolidation) Fig 2. The hippocampus temporarily stores acquired information which is very vulnerable to interferences. The type and number of triggers needed for retrieval of memory is based on the dominance, complexity, regency and frequency of former usage of "to-be-remembered".[17] Finally, every time stored data is retrieved, it is re-encoded.

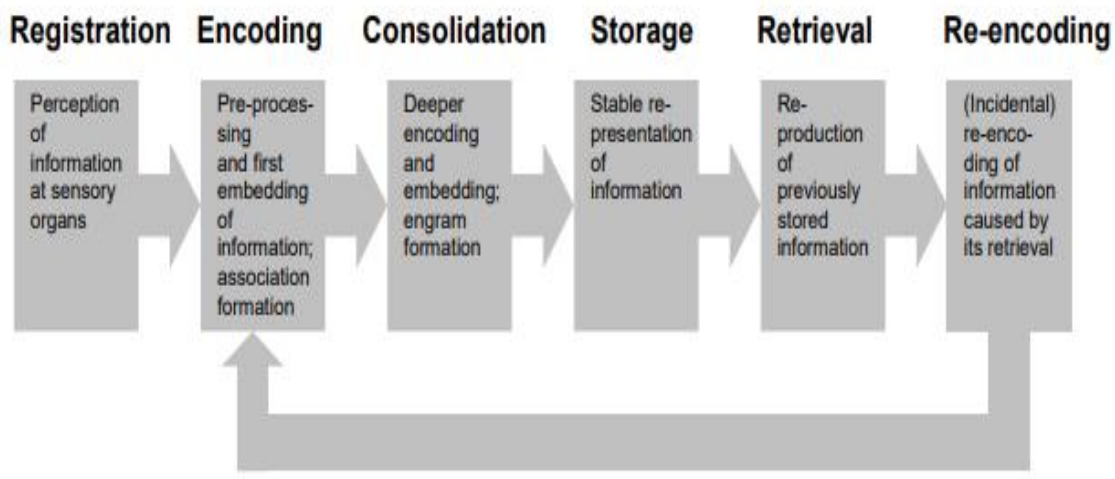


Fig 2. Process of memory formation

Neuroanatomy

The aspects of Memory are stemmed from a lifelong and postmortem study of an interesting case of a man named Henry Molaison.

Henry Molaison, a 29-year-old motor winder, had several episodes of generalized seizures from age16, which were resistant to anticonvulsant therapy. Failing to locate a focal cause, his doctor decided to do a radical surgery (August 1955) by dissecting out his bilateral medial temporal lobe including the hippocampal and other adjacent structures including most of the amygdaloid complex and entorhinal cortex.

Though his operation was uneventful, and he recovered from his seizures, doctors noticed a new symptom. At post operation assessment he gave the date as March 1953,and his age as 27. He was not able to recognize the staff members of hospital or find his way to the bathroom, and he could not recall day-to-day events of his stay in the hospital. He suffered from severe anterograde amnesia. There was also a partial retrograde amnesia as he did not remember anything up to 19 months before his operation. His early memories were seemingly normal and there was no impairment of personality or general intelligence (he could solve crossword puzzles). Also, his ability to form working memories was intact as he could learn new motor skills, though being not able to remember learning them (he was found capable of learning a hand eye coordination skill of mirror drawing over a period of days.[16,17&18]

This influential case exhibited that memory was a “distinct cerebral function”, which can be separated from other perceptual and cognitive abilities. Similarly, surgical lesions of the medial temporal lobe in monkeys, which approximated the damage sustained by patient Henry M, have shown to reproduce similar observations of impairment in human memory. Both monkeys and humans were able to retain their

skills, habituation and classic conditioned learning and other task of non-declarative memory but impaired on tasks of declarative memory. These observations identified that the medial temporal lobe with its structures and connections plays an important role in declarative memory.[19]The medial temporal lobes contain the hippocampus, a brain structure with an elaborate S-shaped curve that inspired imaginative pathologists to name it after the Greek mythological creature for "sea horse." Within the curves of the hippocampus are different neurons folded over on each other, working together to cement the foundations of new memories.

Involvement of hippocampus in memory

Hippocampus plays crucial part in storage of memory, its role in memory was recently identified. In a patient who had permanent circumscribed cognitive impairment after global ischemia revealed involvement of bilateral hippocampal lesion in his neuropathological imaging. This observation suggested that damage to the hippocampus itself is sufficient to produce a "clinically significant and long-lasting memory impairment" .[15,20] Studies have suggested that the largest area that is triggered in retrieval is the posterior medial temporal lobe of hippocampus.[20]

Involvement of Diencephalon

Involvement of diencephalon in amnesia though established centuries ago it was recently accepted that damage to medial diencephalic region is sufficient to cause severe amnesia. Mediodorsal thalamic nucleus and mamillary nuclei are two structures most frequently involved structures in relation to memory. The idea that injury to the mammillary nuclei impairs memory was concluded by the findings in alcoholic Korsakoff's syndrome. Other areas responsible for memory storage are the

amygdala (memory for emotionally disturbing or aversive experiences)[20] and the pre-frontal cortex (for working memory).[22] The hippocampus temporarily stores acquired information which is very vulnerable to interferences.

The molecular and cellular substrates in memory was identified by Lomo in 1966, in his study he described "a cellular model of experience-dependent plasticity-long-term potentiation (LTP)".[23] The term long term potentiation is involved in memory consolidation, it is a process which allows a synapse to increase in strength as increasing numbers of signals (excitatory postsynaptic current) are transmitted between the two neurons. NMDA receptors are known to trigger an influx of calcium that can induce LTP.

The Two steps involving LTP

1. **Strengthening of pre-existing connections** occurs by translocation of a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors to post-synaptic membrane
   - NMDA-R activation by glutamate results in Ca++ - calmodulin-dependent phosphorylation of pre-existing AMPA glutamate receptor and translocation to post synaptic membrane.
   - There is also release of arachidonic acid and Nitric Oxide (NO), retrograde messengers, which acts presynaptically to sustain synaptic activity.

Formation of new synaptic connections - this requires both translation and transcription

- This result following protein kinase APKA-a cAMP-dependent protein kinase) and mitogen activated protein kinase (MAPK) activation which further activates the transcription factor cAMP response-element binding protein (CREB).[23]
- CREB regulates a transcription cascade ultimately involved in a process that yields synapse-specific structural changes[23]

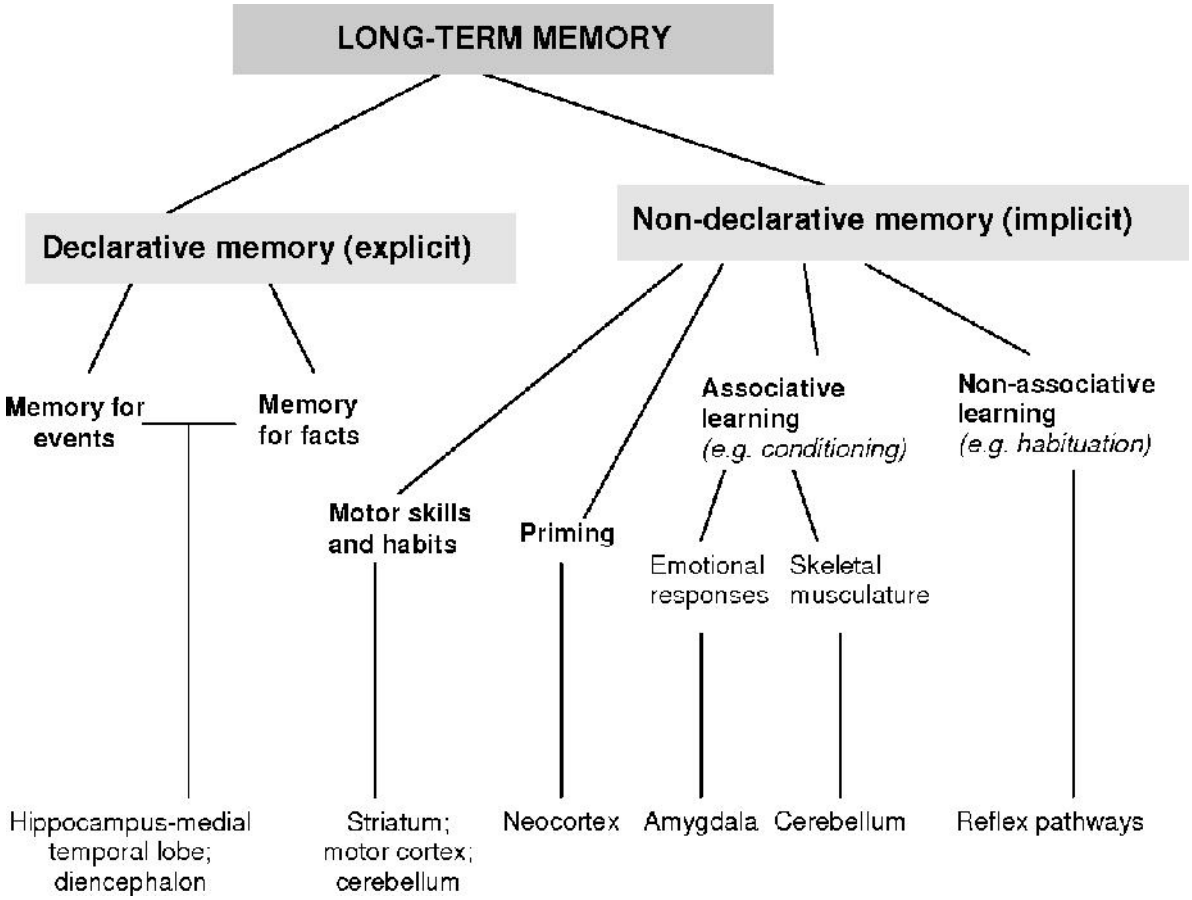


Fig 3 Neuroanatomical regions involved in memory

Neurochemistry of Cognition

The building blocks of all cells and the mechanisms by which they communicate with each other are central to the study of cognition. The complexity of the brain is readily apparent at the macroscale level and mesoscale level, with ~100 billion neurons and ~100,000 miles of connectivity fibers.[24] However, at the microscopic level there is an equally impressive intracellular and intercellular complexity. Cognitive and behavioural neurological syndromes may often be due to a chemical, a receptor, or other neurochemical perturbation. Most of the identified neurotransmitters in the CNS either primarily affect cognition or secondarily affect the primary systems. Neurotransmitters like Glutamate and GABA systems are implicated in the functioning of neural networks that mediate learning and other cognitive functions through neuronal processes such as long-term potentiation. These networks are shown to be modulated by the ascending monoaminergic systems, including dopamine, noradrenaline, and serotonin, mediating effects of arousal and motivation on cognition. Additionally, possible roles for neuropeptide neurotransmitters are identified, including those in social cognition.[25]Based on various physiological, biochemical and behavioral criteria, a number of neuropeptides have also been implicated in cognitive functions such as learning and memory. These peptides include corticotropin releasing factor, urocortin, neuropeptide Y, vasoactive intestinal polypeptide, neurotensin, galanin, opioid peptides, nociception, oxytocin and angiotensin. The three systems which have been perceived to have the greatest role include the NMDA-glutamate system, the cholinergic system and the GABAergic system.[26,27]

Memory Disorders

The Memory disorders are a group of disorders that involve loss of memories ranging to cognitive impairment to finally resulting in dementia.

Forgetting is a term generally used to refer to the 'apparent' loss of information already encoded and stored in long-term memory. It may be due to interference, retrieval problems and motivated forgetting**,** Nonetheless, forgetting if is sufficiently severe indicates a pathologic condition. Even with a serious problem with forgetting patient can lead a non-disabled life by learning new material and recalling of memory can be improved by proper cueing. Forgetting in large amount of people could be due to primarily a functional (clinical or neuropsychologic) disturbance. Unlike forgetfulness, amnesia is almost always a pathologic symptom; with few exceptions, it is the product of a structural process involving a restricted neuroanatomic location.[28]

Amnesic syndrome: The amnesic syndrome can be defined as an abnormal mental state in which memory and learning are affected out of all proportion to other cognitive functions in an otherwise alert and responsive patient[29]. Patients with amnesia are capable of holding a limited amount of information in mind for a very brief period of time, but with increased retention interval or increased interference, their recall and recognition of the information inevitably fails. Although amnesia is characterized by a pervasive and devastating memory loss, some components of memory remain intact.[29] Patients with Alzheimer's disease and other dementias may have a severe amnestic condition as part of the clinical picture; however, alterations of language, cognition, and personality are also present. Rather, these persons do not have a true amnestic syndrome. The syndrome can be caused in several ways: head

injuries (or any damage to hippocampus, fornix, mammillary bodies, anterior thalamic nuclei, [30] vascular accidents, infections, substance abuse (Korsakoff's syndrome), or degenerative neurological disease processes (e.g. Alzheimer's) .[31]Amnesia is divided into psychological and biological amnesia.

Psychological Amnesia

Infantile Amnesia - the loss of ability in adults to recall early episodic memories that occurs in childhood and which are rapidly forgotten. This may be due to the underdevelopment of the infant brain, which would preclude memory consolidation, or deficits in memory retrieval.[32]

Dream Amnesia – Freud considered dreams to be expressions of forbidden sexual or aggressive urges. These urges can produce strong guilt or anxiety if we became aware of them in ourselves. Thus, their expression in dreams is hidden behind the disguise of the actual content of the dreams.[33]

Defensive Amnesia- It is also known as Post Traumatic Stress Disorder (PTSD) develops in people exposed to traumatic events associated with devastating fear/ helplessness/ horror. It is important to note that not all individual develop PTSD, it is only in constitutionally susceptible individuals. PTSD develops in patients who re-experiencing the traumatic effect (in the form of flashbacks and nightmares). The other symptoms consist of generalized emotional numbing and avoidance, and hypervigilance.[34] Other factors like genetics and early life experience, may increase an individual's susceptibility for developing psychopathology following traumatic experience.

Biological Amnesia

Transient Global Amnesia - Transient global amnesia is variant of amnestic syndrome in which amnesia is completely reversible in most patients. The etiology of transient global amnesia is poorly understood. This is a profound memory problem with no loss of consciousness. It comes suddenly without any obvious cause, lasting for few hours to days before memory becomes normal again. This type is called "global" as there is loss of most of stored memory as well as lack of formation of new memories. It is mostly due to temporary alteration of the blood flow to the brain.[33]

Substance Induced Amnesia - Normal neuronal processes responsible for reinforcement, motivated behavior, and plasticity are terminated by abusive drugs that may lead to maladaptive and anomalous behavior. Substance like alcohol, cannabis can be responsible for substance induced amnesia. A person under the influence of alcohol may have amnesia for the events occurring at that time due to disruption of encoding and storage processes by the effects of alcohol on brain. Maladaptive drug-related patterns of behavior may additionally enhance motivational strength of the drug or reduced motivational strength of other naturalistic rewards. A more severe type of amnestic syndrome is Korsakoff's syndrome, which is characterized by loss of recent memory and confabulation of facts which the patient is unable to remember.[33]

Diseases of Brain: These include cerebral infections, head injuries, strokes, brain tumors, multiple sclerosis, disorders of metabolism and toxic chemicals and neurodegeneration (includes Alzheimer's disease, Lewy body dementia etc.). The type of amnesia in the various conditions depends on the region of the brain involved. All amnestic patients have anterograde amnesia and vary in the severity and extent of their retrograde amnesia. Retrograde losses of memory in amnesia are usually

temporally graded in that they are most severe for time periods closest to the onset of amnesia.

Burden of Memory Disorder

The global burden of dementia disorder has been seriously underestimated. According to WHO report Geneva 2018 "Every 65 seconds someone in the United States develops Alzheimer's disease".[34]

Globally India houses 2nd most number of individuals suffering from dementia, but most people with dementia do not receive a diagnosis or support. India along with China has the highest number of older people, where geriatric services are underdeveloped and talking mental health issues carries stigma. Worldwide at least 44 million people are living with the global health crisis and the figures are likely to be doubled by 2030.[35]

In 2018, prevalence of Alzheimer's dementia in American's was estimated approximately around 5. 7 million individuals of all ages. Out of this number people who are of age 65 and older were 5.5 million and approximately 200,000 individuals were less than 65years, who suffered from younger-onset Alzheimer's. [34]

Women are expected to bear the worst of impact in India, as elderly women in country experience the higher life expectancies than their male counterparts, while also encountering neglect as they age. 2016 Lancet study on health life expectancy revealed that while women in India have overall life expectancy than men, women also spend more years in ill health and disability than men.[36]

More women than men have dementia and Alzheimer's. About two-thirds of Americans with Alzheimer's are women. Around 5.5 million people aged 65 and above with Alzheimer's in the United States, 3.4 million are women and 2.0 million

are men. Based on estimates from the Aging, Demographics, and Memory Study (ADAMS), among people aged 71 and older, 16 percent of women have Alzheimer's or other dementias compared with 11 percent of men. The prevailing view is that this discrepancy is because women's live expectancy is more than men, and aging is the greatest risk factor for Alzheimer's.[34]

Of top 10 causes of death that cannot be prevented, declined the progression or cured Alzheimer adds to this list , and it is becoming a common cause of death among geriatric population in India., with increase in the life expectancy there is increased risk of developing Alzheimer dementia.

Impact of dementia is not only a burden of patient but also affect the lives of caregiver and the society. Friends, family members and other care giver who aid the older adults amounts up to approximately 83 percent.[34] Nearly 48 percent of caregivers who provide help to older adults do so for dementia patients. The responsibilities of caring for someone with dementia often fall to women. Approximately two-thirds of caregivers are women and daughters account to over one third of dementia caregivers. It is seen that among care providers wives provide care more for husband than vice versa. On average, female caregivers spend more time taking care of patients than male caregivers. According to a poll conducted to survey the care providers in 2014 by Alzheimer's Association Women and Alzheimer's Poll, among both men and women, it was noted that 67 percent were women. Two and half many women have been reported living with the person with dementia full time.[34]

The costs of health care and long-term care for individuals with Alzheimer's or other dementias are substantial, and dementia is one of the costliest conditions to society. Total payments in 2018 (in 2018 dollars) for all individuals with Alzheimer's or other dementias was estimated at $277 billion.[34]

Causes of Amnesia

Amnesia has several root causes; most are traceable to brain injury or brain damage which is known as neurological or organic amnesia. Possible causes of neurological amnesia include:

- Stroke - Brain inflammation (encephalitis) resulting from infection with a virus such as herpes simplex virus or as an autoimmune reaction to cancer somewhere else in the body (paraneoplastic limbic encephalitis),
- Long-term alcohol abuse leading to thiamine (vitamin B-1) deficiency (Wernicke-Korsakoff syndrome),
- Tumors in areas of the brain that control memory
- Seizures -Electro convulsive therapy, a procedure in which electrical currents are passed through the brain, sometimes used to treat certain mental illnesses.[37]
- Certain medications, such as benzodiazepines.
- Head injuries, such as those sustained in car accidents, can lead to confusion and problems remembering new information,
- Depression, bipolar disorder, or schizophrenia when symptoms have not been well controlled.
- The most common causes are - neurodegenerative disorders like Alzheimer 's, vascular dementia, Lewy body dementia and frontotemporal dementia.[37]

Neurodegenerative brain disease are more common causes of amnesia. The most common cause of neurodegenerative disease is still Alzheimer's disease.[34,38] The typical pathological features in AD include neurofibrillary tangles, which develop within neurons Amyloid β senile plaques, which develop between neurons in brain. These microscopic changes are believed to be an integral part of the cause,

development and course of the illness. toxic amyloid plaques from peptides due to the abnormal processing of amyloid precursor protein (APP) into toxic forms of Aβ peptides. Thus, it is a disorder in which toxic Aβ peptides accumulate to form a deposition of amyloid plaques that aggravate various inflammatory processes.[39] One theory is that inflammation around the plaques destroys neighboring neurons and leads to further formation of neurofibrillary tangles by hyperphosphorylation of tau proteins by the same amyloid cascade. Healthy neurons have a support structure called microtubules that act like tracks and guide nutrients and molecules down along the length of axons from the body of the cell. Tau protein function to stabilize these microtubules. Chemically changed tau protein undergoes hyperphosphorylation forming neurofibrillary tangles. These hyperphosphorylated tau link together to form microtubular filaments. The severity of dementia is thought to be directly related to the density of the hyperphosphorylated filaments. These compromise the microtubular function destroying the neuron. When such abnormal processes affect cholinergic neurons, there is a decrease in synaptic levels of acetylcholine (ACh) levels.[39]

Vascular Dementia is other most common cause of dementia in the elderly. Vascular dementia results from vascular disease like hemorrhagic brain injury, microinfarcts, microbleeds, macro-infarcts and ischemia these can disrupt structural cognitive networks, The decrease in blood flow to the brain may be due to direct occlusion from a blood clot or secondary to chronic illnesses such as hypertension, diabetes, and dyslipidaemia.

These insults alter cerebral hemodynamics such as hypoperfusion, disrupted cerebrovascular autoregulation, neurovascular decoupling. Hemorrhagic and ischemic strokes is leading factors for vascular dementia, stroke survivor is commonly affected by post-stroke dementia or cognitive impairment. Other risk factors that directly

associate with Cardiovascular Disease (CVD) like smoking, diabetes, hypertension and hyperlipidemia, and cardiac disease, can also cause cognitive impairment.[41]Symptomatic treatments are available for these diseases and they delay the course of the disease and improve the quality of life. But it is equally important to understand the risk factors of dementia to effectively plan preventive interventions. Risk factors can be divided as modifiable and non-modifiable. Timely mitigation of the modifiable risk factors would benefit a lot to the patients.[38]

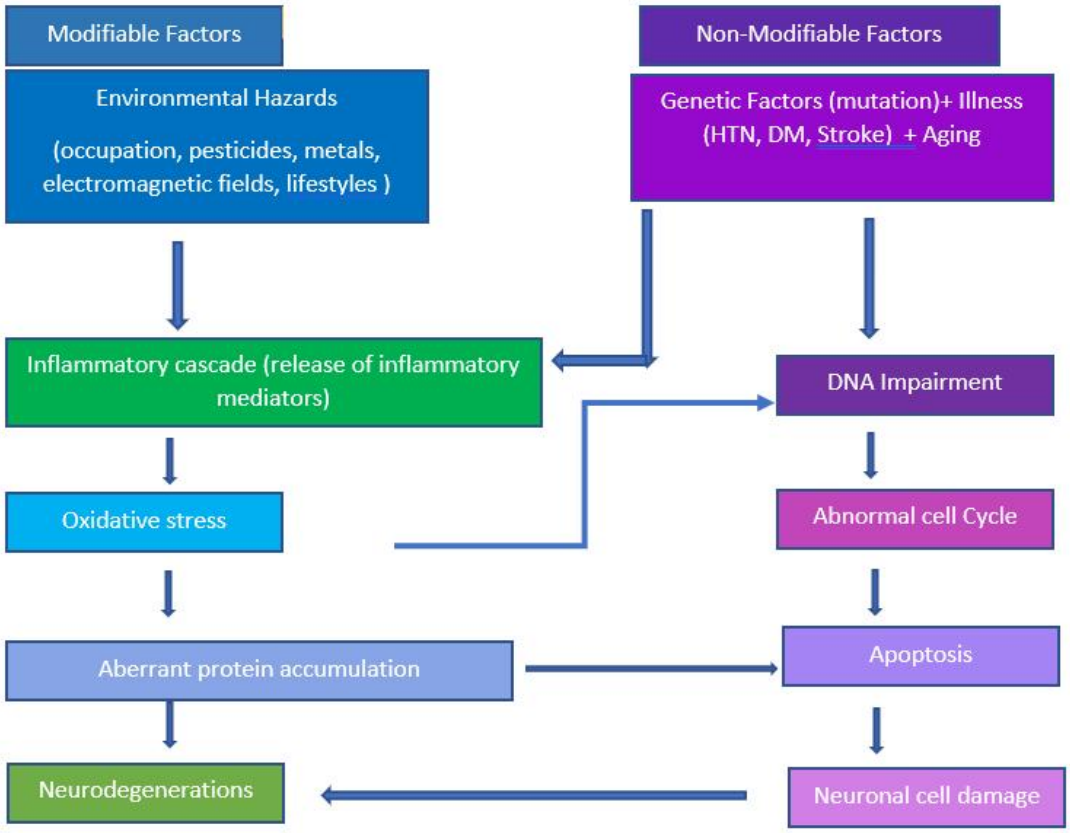


Fig 4: Various modifiable and non-modifiable factors responsible for neurodegeneration.

Pharmacotherapy of Amnesia

The idea that the information survives in the context of the pathology is to change the paradigm of amnesia and to instigate the search for therapeutic strategies to make seemingly lost memories available again, instead of simply preventing the memory loss in the first place. Such treatments would have wide-ranging utility, as amnesia is a common symptom of many different brain disorders. Management of amnesia starts with identifying the form of amnesia retrograde or anterograde amnesia. Efforts to find newer therapies therefore should be aimed to halt the progress of the disease-causing amnesia.[41] No medications are currently available for treating most types of amnesia[42].

Pharmacotherapy of the amnesia is presently limited to either shielding from excitotoxicity (NMDA antagonists) or supplementing neuronal transmission of viable neurons (Acetylcholinesterase inhibitors) or specifically enhance efficiency of higher telencephalic integrative exercises (nootropics – piracetam).[43,44]Currently, there are no approved drugs which could modify the disease pathology.[41] Majority of approved drugs against cognitive impairments are aimed at restoring the cholinergic neurotransmission.[45]

Acetylcholinesterase inhibitors: (AChEI) The *cholinergic hypothesis* was put forward following the observation that cognitive deterioration results from a progressive loss of cholinergic neurons with decline in acetylcholine levels in brain. Therefore, the mainstay of treatment till date has been the utilization of drugs that affect the cholinergic transmission either by inhibiting the synaptic Ach degradation or by enhancing cholinergic neurotransmission.[39] Tacrine was the first AChEI that was approved for the management for over 15 years ago. Due to its poor tolerability and risk of hepatotoxicity tacrine is no longer recommended. Second-generation

AChEIs are now FDA approved for the treatment of mild-to-moderate dementia in AD, as well as Lewy body dementia and vascular dementia.

The agents preferred are

- Donepezil is FDA approved acts by selectively inhibiting AChE,
- Rivastigmine, FDA approved and acts by inhibiting both AChE and butyryl cholinesterase (BuChE)
- Galantamine also FDA approved: is known to selectively inhibits AChE and also modulate nicotinic acetylcholine receptors (nAChRs).[40]
- Huperzine A, a Chinese herb with reversibly and selectively acetylcholinesterase inhibition activity is currently under investigation. [97]

N-methyl-D-aspartate (NMDA) Antagonist: Excitotoxicity occurs when there is overstimulation of glutamate receptors or excessive exposure to the neurotransmitter glutamate, causing neuronal damage or death. The death is due to overload/ overactivation of NMDA- glutamate receptors leading to excessive influx of calcium ions which causes activation of proteases; recruiting free radicals, oxidants and causing lipid peroxidation. In contrast, physiological NMDA receptor activity is required for normal neuronal function, the total block of NMDA receptor activity would be clinically unacceptable. Therefore, drugs that selectively block NMDA receptors without immensely disrupting normal function. Drugs like memantine are used. Other drugs like calcium channel antagonists and protease inhibitors reduced the Ca influx and further excitotoxicity.[39]

Dimebolin (Dimebon, latrepirdine) weak inhibitor of butyryl cholinesterase and acetylcholinesterase, also weakly blocks NMDA receptor signaling pathway.

Trial results showed that dimebolin had no significant benefits over placebo which lead to discontinuation of its development.[40]

Abnormal Proteins (Amyloid β, Tau Protein)

Vaccination against Aβ42 has proved highly efficacious in mouse models of AD, helping clear brain amyloid and preventing further amyloid accumulation. However, similar approach in human trials, was associated with life-threatening complications, like meningoencephalitis.[40]

Phase II of active immunization vaccine suspended following cases of meningoencephalitis

Passive immunization with monoclonal antibodies against Aβ 42 has been tried in mild to moderate AD.

Passive immunization: Bapineuzumab – in phase trials no improvement in decline in cognitive function.

Two phase III trials failed to show beneficial effects of Solanezumab but may be effective in early AD.

Gantenerumab and crenezumab are currently in phase II trials.[39,40]

β and γ secretase inhibitors: Secretase inhibitors they inhibit β and γ secretase, which responsible for production of amyloid precursor protein and later β amyloid. The first two placebo control trials of γ secretase inhibitors tarenflurbil and semagacestat were negative. Semagacestat may have accelerated cognitive decline compared to placebo and had risk of skin cancer. Avagacestat γ secretase inhibitor is under Phase II trial that was found to worsen cognitive function.[40]

Tau kinase inhibitors: Tau kinase inhibitor like lithium is known to reduce tau hyperphosphorylation and prevention of neurofibrillary tangles. Clinical evidence are encouraging but large scale, clinical trials are still required.[39]

Tau aggregation inhibitors: Inhibits tau aggregation by increasing their proteolytic degradation. TRX-0237 a tau aggregation is still ongoing clinical trial.[39]

HMG-CoA reductase inhibitors: (statins) Along with depleting cholesterol which inhibits APP and β-amyloid also reduced oxidative stress thereby prevent further neurodegeneration. Cochrane review found insufficient evidence to inhibitors (statins). [39, 40]

Metal chelators: They dissolve amyloid plaques, zinc and copper are involved in the formation of amyloid plaques. More experimental trails on their safety and efficacy are required.[39]

Oxidative stress and inflammation – There are various factors that can lead to accumulation of oxidative stress like ageing, excitotoxicity, protein misfolding, hypoxia, mitochondrial dysfunction etc. Oxidative stress is both, the cause and effect of inflammation which lead to neuronal death.[43] various pharmacological agents are being developed against this oxidative stress(free radical scavengers, anti-inflammatory drugs, drugs reversing vascular dysfunction, etc.).[46]

- **<u>Antioxidants and Anti-inflammatory</u>**

Curcumin (extract of turmeric) has antioxidant as well as anti-inflammatory properties. Clinical trials are underway. Curcumin acts by reducing APP and β amyloid plaques.Vitamin E, folic acid, ginseng and omega 3, are being used in AD but their clinical benefit needs further evaluation.[39,40]

Alpha-tocopherol – still under investigation in preventing neurodegenerative disorders.

Antibiotics, like tetracyclines and rifampicin reduce inflammatory cytokines and interfere with plaque development. More data on efficacy are required randomized control trail for minocycline underway.[39]

Estrogens: In a prospective observational study it was seen that use of estrogen replacement therapy appeared to protect about 50% against development of AD in women. Sadly, a prospective placebo-controlled study with combined use of estrogen-progesterone in asymptomatic postmenopausal women increase the prevalence of dementia. Additionally, no benefit has been found in the treatment of AD with estrogen alone.[39, 40]

Ginkgo biloba: A controlled trial of an extract of Ginkgo biloba found modest improvement in cognitive function in subjects with AD and vascular dementia. Unfortunately, a comprehensive 6-year multicenter prevention study using ginkgo found no slowing of progression to dementia in the treated group.[39]

- **Apoptosis inhibitors**
  - Acetyl-L-carnitine – investigational.[47]
- **Drugs improving cerebral metabolism**
  - Pyritinol - claimed to increase glucose transport across BBB.[44]
  - Citicoline - claimed to increase cerebral metabolism and blood flow.[44]
- **Drugs improving vascular dysfunction**
  - Dihydroergotamine – selectively improves cerebral blood flow[44]. Has been recommended for MCI and dementia.
  - Gingko biloba extracts – antagonise platelet aggregation, improves cerebrovascular circulation,andproduces improvement in cognitive performance.[39]

- Pentoxifylline, suloctidil, vincamine, and calcium channel blockers (e.g., nimodipine) are the other investigational drugs.[42]

1. **Drugs enhancing memory formation**

   - **Pirebedil –** a dopaminergic agonist useful in cerebral circulatory insufficiency, claimed to improve memory, concentration and vigilance.[44]
   - **Nootropic drugs**
     - Piracetam –improves efficiency of cognitive functions by probably improving blood flow.[44]

2. **Drugs recruiting neural stem cells and promoting neurogenesis**

Neurodegeneration was considered as an irreversible process. But the demonstration of neurogenesis in the adult mammalian brain has challenged the dictum. Hippocampus & lateral ventricles are regions of brain concerned with memory and olfaction respectively. It has been demonstrated that new neurons are generated at sub granular layer of dentate gyrus of hippocampus and sub ventricular layer of lateral ventricle. These new neurons come from neuron precursor stem cells which can be stimulated both at physiological and pathological conditions.[48]

Drugs Used In Present Study:

Lisinopril and Perindopril are dicarboxyl-containing Angiotensin converting enzyme inhibitors (ACEI) structurally related to enalapril.

History of ACEI

The discovery of an orally inactive peptide from snake venom established the important role of ACEIs in regulating blood pressure. This led to the development of Captopril, the first ACEI. When the adverse effects of Captopril became apparent new derivatives were designed. Then after the discovery of two active sites of ACE: N-domain and C-domain, the development of domain-specific ACE inhibitors began.The development of the nonapeptide teprotide (Glu-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro), which was originally isolated from the venom of the Brazilian pit viper Bothrops jararaca, greatly clarified the importance of ACE in hypertension. However, its lack of oral activity limited its therapeutic utility.[49, 50]

Mechanism of Action of ACEIs

ACEIs act by interfering in RAS system. ACEIs are competitive inhibitors of angiotensin-converting enzymes. They compete with Angiotensin I for binding to ACE and inhibits enzymatic proteolysis of Angiotensin I (Ang I) into active Angiotensin II (Ang II). By inhibiting the conversion Angiotensin, I into Angiotensin II they decrease the production of Ang II which lowers the BP and increases natriuresis, this effect is seen due to decrease Ang II subsequently causes reduction in aldosterone secretion, which decrease sodium reabsorption in collecting duct and decrease potassium excretion. ACEIs also enhances plasma renin activity likely due to a loss of feedback inhibition mediated by ATII on the release of renin and/or stimulation of reflex mechanisms via baroreceptors.[56] ACE enzyme has numerous

substrates, by inhibiting ACE other than Ang II different effects can also be observed, like increased in bradykinin levels. ACE causes breakdown of bradykinin, ACEIs potentiate bradykinin levels by inhibiting ACE. Bradykinin regulates vasodilator which makes further decrease in BP., although this mechanism may predispose angioedema. Additionally, ACEIs cause increase in natural stem cell regulators N acetyl- seryl- aspartyl- lysyl- proline, which may contribute to the cardioprotective effects of ACE inhibitors by 5 folds. [52,53]

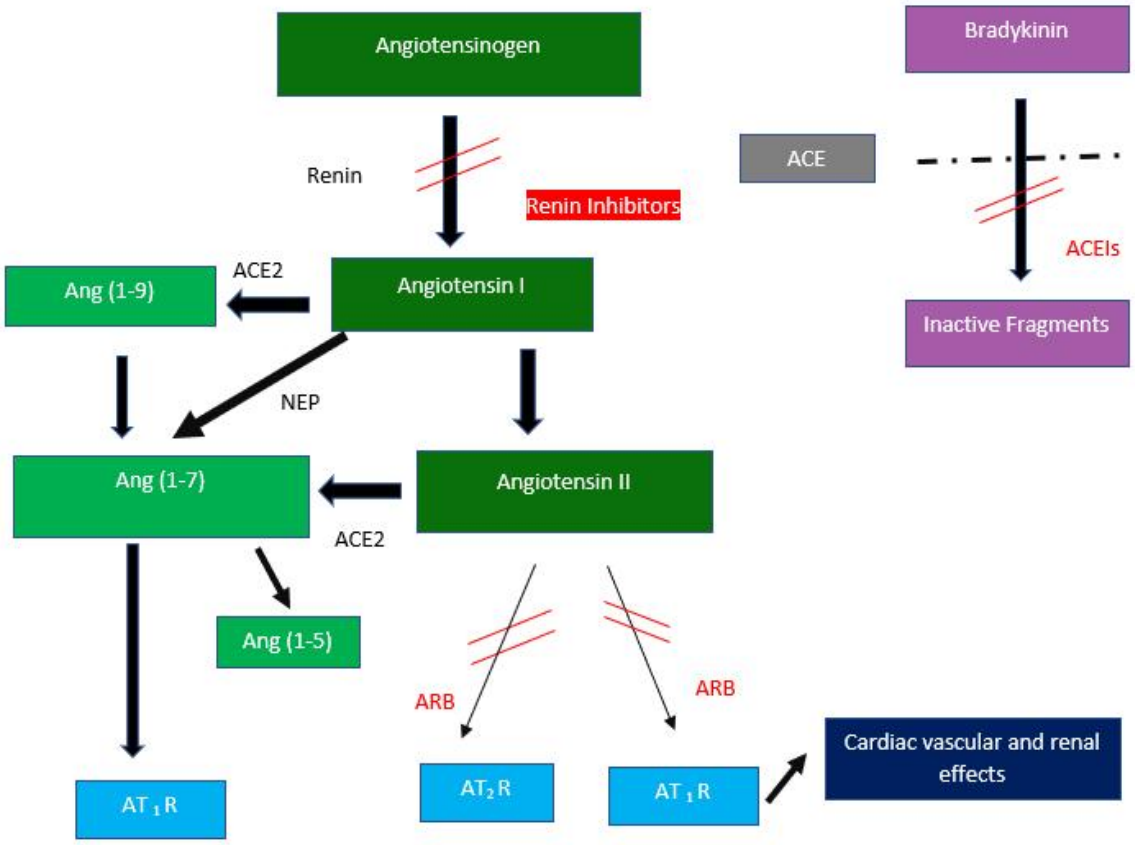


Fig 5: Mechanism of Action of Angiotensin Converting Enzymes Inhibitors. Ang I – Angiotensin I,Ang II – AngiotensinII, ACE- Angiotensin converting enzyme, ACEI-Angiotensin converting enzyme inhibitor, AT- Angiotensin binding receptors. NEP- Neutral-endopeptidase.

Lisinopril:

Lisinopril is classified as an angiotensin converting enzyme inhibitor and has been available for nearly 3 decades. It has some key features that make it different from enalapril and captopril;

1) it is hydrophilic

2) it has a long half-life and

3) it is not broken down by the liver.[53]

Lisinopril is approved by the Food and Drug Administration (FDA) for the management of hypertension in adult and pediatric patients six years and older and as adjunctive therapy in the treatment of heart failure. It is also FDA-approved for the treatment of ST-segment elevation myocardial infarction (STEMI) within 24 hours in hemodynamically stable patients to improve survival.

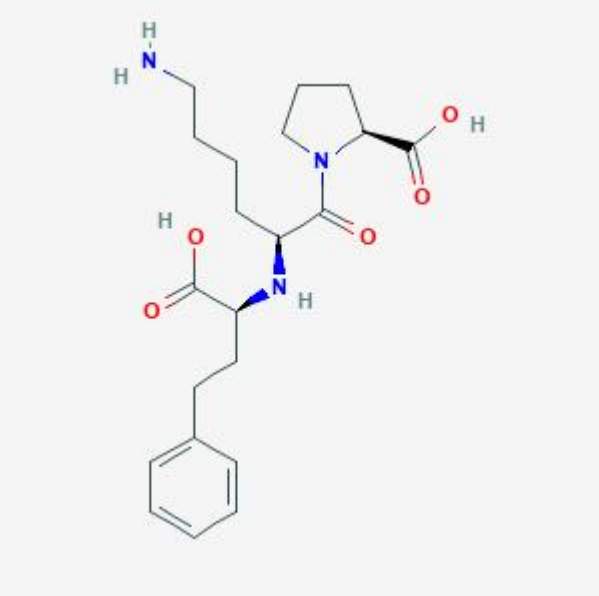


Fig 6: Structural formula of Lisinopril

Pharmacokinetics:

Lisinopril absorption is unchanged by food and is excreted unchanged in the urine. It does not have good bioavailability after oral intake - ranging from 10-30%. The time to peak concentration can vary from 6-8 hours. The drug does not bind to albumin or other proteins and its distribution in patients with heart failure is poor.[4,5& 6]

In general, it is recommended that the administration of lisinopril should be adjusted for patients in whom the glomerular filtration rate (GFR) is less than or equal to 30 mL/min.

In adults, the usual dosage ranges from 2.5 to 40 mg per day depending on the indication. For adolescents and children greater than or equal to 6 years, the initial dose is 0.07 to 0.1 mg/kg once daily with a maximum initial dose of 5 mg/day and increments of 1- to 2-week intervals. The maximum pondered dose is 0.6 mg/kg/day or 40 mg/day.[4,5]

According to the recent *2017 Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults*, among patients in whom pharmacological therapy is indicated, ACE inhibitors are among the recommended first-line agents for the management of hypertension with a lisinopril starting dose of 10 mg up to 40 mg daily.[53]

Adverse Effects

The primary adverse effects of ACE inhibitors include hyperkalemia, dry cough, angioedema, hypotension, dizziness, and renal insufficiency. These effects may be more common in patients with renal, autoimmune, or collagen vascular diseases. The American Heart Association / American College of Cardiology task

Force (AHA/ACCF) recommends careful use in patients with cardiomyopathy with outflow obstruction, as they may lead to exacerbation of symptoms.

Historically, ACE inhibitors have been associated with an increase in morbidity and mortality in patients with aortic stenosis, however, recent randomized and placebo-controlled trials suggest that ACE inhibitors might be safe and might even provide some benefits in certain patients.[4,53]

Contraindications

- In patients with renal artery stenosis
- Patients with history of hereditary angioedema,
- Renal failure with prior lisinopril use,
- Patients having DM and taking alsikiren and
- Angioedema in patient taking neprilysin inhibitors (sacubitril/ valsartan) and mTOR inhibitors (sirolimus, everolimus etc.)
- It is pregnancy category class D due to its teratogenic effects (e.g., decreased fetal renal function, oligohydramnios, lung hypoplasia, skeletal malformations, death in the fetus/neonate, etc.), thus its use is contraindicated in pregnant women and/or fertile women without proper contraception. Manufacturers recommend against the use of lisinopril in breastfeeding woman because the amount secreted in breast milk and its effects in the breastfed infant is unknown.[53]

Toxicity

Since lisinopril metabolism depends on renal excretion, overdose management consists of general supportive care, including gastric emptying strategies with appropriate intravenous fluids, vasopressors, and hemodialysis. Maintenance of optimal blood pressure with fluids is critical in patients who are hypotensive. Some reports suggest the use of Ang II administration as an alternative supportive treatment for the treatment of ACE inhibitors overdose.

There is no antidote available for lisinopril.[53]

Perindopril

Perindopril is a angiotensin-converting enzyme (ACE) inhibitor, according recent AHA guidelines is a first line therapy in type I hypertension. Perindopril is available in two forms perindopril arginine and perindopril erbumine. Perindopril is a pro drug which is hydrolyzed by the liver to form its active metabolite perindoprilat. Perindoprilat is known to increase serum aminotransferase at low rate and in rare case cause acute liver injury.It is used in the treatment of mild to moderate essential hypertension, mild to moderate congestive heart failure and to reduce the cardiovascular risk of individuals with hypertension or post-myocardial infarction and stable coronary disease.[54]

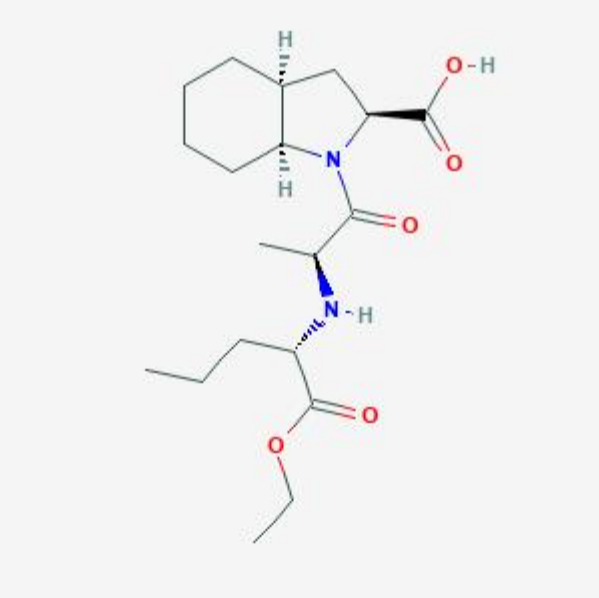


Fig 7: Structural formula of Perindopril.

Pharmacokinetics

Perindopril is a nonsulfhydryl prodrug that is metabolized via first pass effect (62%) and systemic hydrolysis (38%) to perindoprilat, its active metabolite, following oral administration. It is rapidly absorbed with peak plasma concentrations occurring approximately 1 hour after oral administration. Bioavailability is 65-75%. Following absorption, perindopril is hydrolyzed to perindoprilat, which has an average bioavailability of 20%. The rate and extent of absorption is unaffected by food. However, food decreases the extent of biotransformation to peridoprilat and reduces its bioavailability by 35%. Perindopril is extensively metabolized following oral administration, with only 4 to 12% of the dose recovered unchanged in the urine. Clearance of perindopril is 219 - 362 mL/min [oral administration] extensively metabolized,with only 4-12% of the dose recovered in urine following oral administration. Six metabolites have

beenidentified:perindoprilat, perindopril perindoprilat glucuronide, a perindopril lactam, and two perindopril at lactams. Only perindoprilat is pharmacologically active.[55] Peridoprilat and perindoprilat glucuronide are the two main circulating metabolites. Biological half-life of Perindopril, 1.2 hours; Peridoprilat, 30-120 hours. The long half-life of peridoprilat is due to is slow dissociation from ACE binding sites. Perindoprilat is 10-20% plasma bound.[4,5& 6]

Toxicity

Hepatotoxicity

Perindopril, like other ACE inhibitors is known to increase serum aminotransferase (<2%) at low rate and in rare case cause acute liver injury in controlled trials, although similar to results were seen with placebo. The elevations seen were temporary and rarely required change of dose. Though potential side effects of perindopril include jaundice and hepatic failure, clinically apparent cases of acute liver injury due to perindopril are not yet reported. Other ACE inhibitors have been associated with rare cases of clinically apparent liver injury, which raised after 2 to 12 weeks of initiating therapy. Few cases of immunoallergic reactions like fever, rashes and eosinophilia have been report and most patients do not develop autoantibodies. In addition, rare instances of severe acute hepatocellular injury, sometimes arising 1 to 4 years after starting therapy, have been linked to selected ACE inhibitors, but not specifically to perindopril.[58]

Adverse Effects:

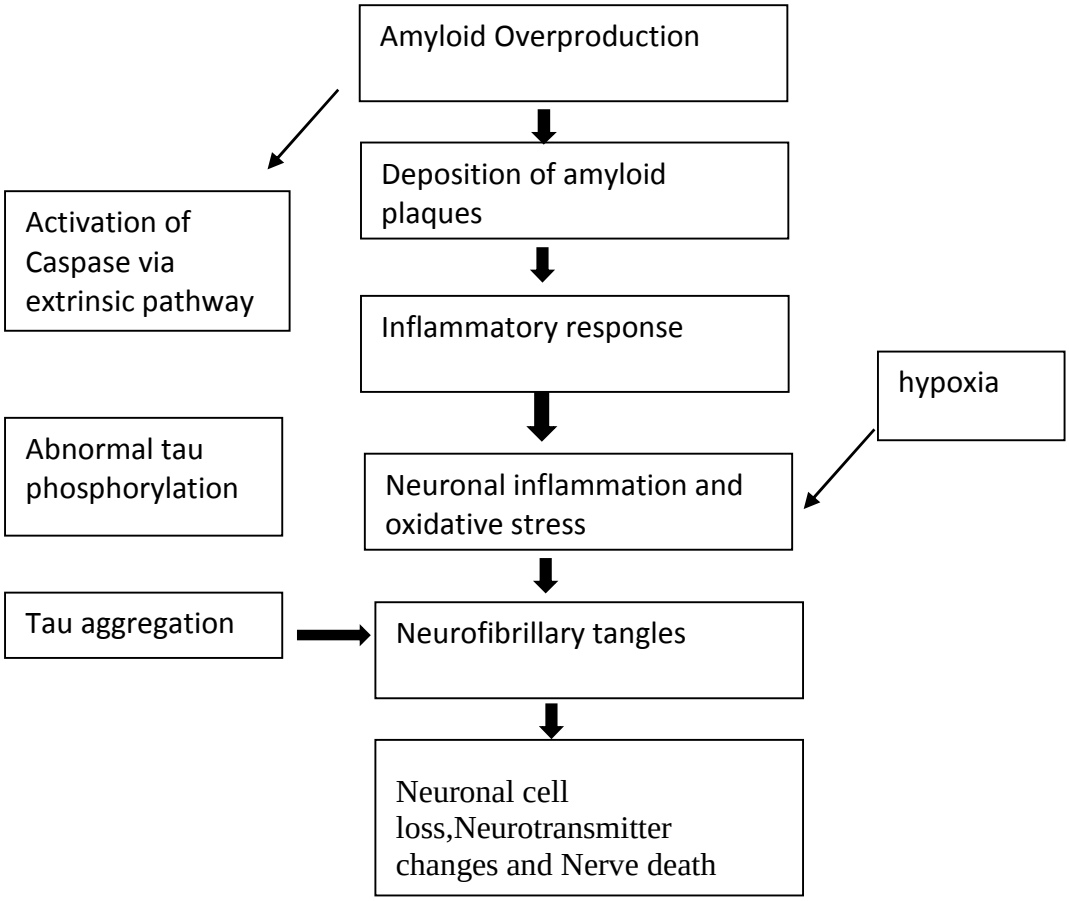
- Cough,
- Digestive symptoms like nausea vomiting
- Headache,
- Dizziness and
- Fatigue.

Contraindications

- In patients with hereditary or idiopathic angioedema
- In pregnancy, it's a category D class of drug
- Diabetic patients with concomitant use of aliskiren
- Renal artery stenosis
- Patients with hepatic impairment
- Kidney disease with reduced kidney function
- Angioedema in patient taking neprilysin inhibitors (sacubitril/ valsartan) and mTOR inhibitors (sirolimus, everolimus etc.)
- Patients with decreased brain blood flow problem.

Link Between Study Drug and Disease

Fig 8: Showing Pathogenesis of neuronal cell loss and neuronal death



Hyperphosphorylation of tau – Tau is a normal constituent of neurons. On hyperphosphorylation it causes instability in the cytoskeletal structure of neurons leading to neuronal death.[81,82 & 95]

Vascular dysfunction – Hypoxia due to insufficient circulation can itself lead to brain ischemia or cause metabolic derangements.[81,82 & 95]

Inflammation and Oxidants – These are the cause and effect of all the pathologic processes leading to neuronal death.[85,95]

Apoptosis - Aβ amyloid is known to induce neuronal apoptosis by activating caspases and p55. Activation of p55 is known to directly activate the apoptosis pathway[95,84]

The cholesterol pathway- Hypercholesterolemia stimulates β, γ secretases due to which more of insoluble β amyloid is formed rather than soluble α form.

The beta amyloid pathway – Excessive generation of β amyloid results in excessive deposition around nerves and later causing inflammatory and oxidative stress.[80,95]

Recently it is identified that brain has its own intrinsic RAS system, which is involved in memory and cognition. Specific mechanism as to how RAS modulates in memory and cognition is unclear. Previous Studies have shown that Ang II causes inhibition of cholinergic neurons, integrity of cholinergic system is essential in learning and memory. Additionally, RAS is involved in the activation of inflammatory cytokines that may play a role in degenerative dementia, the studies have shown that in patients with AD there is increased concentration of ACE, angiotensin II and angiotensin I receptors in cerebral cortex[66]. A study conducted in hypertensive rats reported that captopril but not hydralazinesignificantly attenuates the age-related impairment in learning and memory despite equal blood pressure control in the two groups.[60]

These results support the contention that the mechanism of preservation of learning and memory may not be primarily due to the blood pressure lowering effect of captopril. Hence it is hypothesized that centrally acting ACEIs which can cross blood brain barrier can be effective in preventing neurodegeneration caused by other mechanism other than lowering blood pressure.[59, 11]

Lisinopril and Perindopril as Antioxidant

ACE inhibitors in addition to reducing levels of levels of angiotensin II and potentiating bradykinin levels, it has other actions. Studies have reported that ACE inhibitors have important involvement in vascular oxidative stress. Majority of the vascular endothelium possess enzyme system that utilizes NADPH or substrates of NADPH, vascular cells like endothelial cells, fibroblasts and smooth muscles cells uses these NADPH or its substrates for production of superoxide anion and these systems are activated in response to Ang II. In an experimental as well as clinical studies suggested that activation of RAS in turn triggers NADPH oxidase in the arterial wall. In regard to this action of ACE few studies have suggested that ACEIs entitle unprecedented antioxidant strategy targeting oxidative stress at its sources. ACEIs by inhibiting ACE stimulation of vascular NADPH oxidase, thereby preventing the increased superoxide flux associated with activation of the renin-angiotensin system, superoxide reacts with Nitric Oxide (NO). In an experimental study it was reported that Ang II induces lipid peroxidation and further formation of peroxynitrite this can be prevented by ACEIs [59, 96]

Lisinopril and Perindopril as Anti Apoptotic

Perindopril has been is long acting ACEIs that is known to offer 24-hour blood pressure control. Studies have shown that perindopril also exhibit higher lipophilicity (crosses BBB rapidly) and has stronger affinity to ACE at local site, in regard to this perindopril causes local inhibition of the RAAS in tissues (heart, kidneys, brain, adrenal glands, blood vessel) and a greater selectivity for bradykinin binding sites as compared to other ACE inhibitors.

Perindopril has been reported to inhibit strongly the apoptosis of endothelial cells as compared to other ACE inhibitors. In human trials, treatment with perindopril has shown to reduce resistin levels in stable coronary heart disease (CHD) and also decreased levels of C-reactive protein (anti-inflammatory action), reduced monocyte chemo-attractant protein-1 (anti-atherosclerotic effect), lowered oxidized low-density lipoprotein(antioxidant property), lowered fibrinogen (antithrombotic effect) and increased plasminogen activator inhibitor-1(profibrinolytic effect).[93]

Perindopril Protection Against Recurrent Stroke Study (PROGRESS) showed that perindopril exerts pleotropic effects even in normotensive subjects; effects that were not related to decrease in BP. PROGRESS study also summarized that perindopril possess anti-inflammatory , anti-oxidant , anti – atherosclerotic, anti-thrombotic, anti – apoptotic, anti-fibrotic, NO stimulating and profibrinolytic effects which were are not equally exhibited with other ACE inhibitors. This greater pleiotropic effects with perindopril treatment may be attributed to greater tissue ACE binding and/or greater selectivity for bradykinin sites on angiotensin converting enzyme in comparison to other ACE inhibitors.[81,82&83]

Lisinopril being ACE inhibitor has generally benefits as it works at several distinct mechanisms, that include vasodilation, due to enhanced NO production that may improve bradykinin availability, rise in the levels of tissue plasminogen activator, increase in fibrinolysis, improvement in antioxidant action , better anti-remodeling property, endothelial function preservation and anti- atherosclerotic actions. Despite this, the binding affinity for ACE is higher for bradykinin than for angiotensin I which suggests that the ACE primarily functions to breakdown bradykinin. Therefore, both the active sites of ACE (the N-and C-terminals) are

required to interfere with both the conversion of Ang I to Ang II and breakdown of bradykinin. However, inhibition of conversion of Ang I to ANG II require action at C domains (carboxyl domain), whereas inactivation of bradykinin requires the activity of both terminal sites. Bradykinin is known to exert potent anti-apoptotic effect on the endothelium as well as cardiac myocytes. The Perindopril- Thrombosis, Inflammation, Endothelial Dysfunction and Neurohormonal Activation (PERTIENT) study showed that there was a significant increase in bradykinin levels CHD patients treated with perindopril.[81,82 & 83] Also, perindopril lead to a 31% reduction in apoptotic rate which was in part, mediated through stimulation of B_2 type of bradykinin receptors; because, the rate of apoptosis was elevated after addition of bradykinin B_2 receptor antagonist.[61,93]

I. <u>Screening methods for assessment of learning and memory</u>:

The process of memory formation is divided into three general stages[62].

Stage 1 - Acquisition (learning)—involves the initial perception of a new experience.

Stage 2 - Storage of this learning into a short-term memory which is transient and labile.

Stage 3 - Consolidation of this memory into a long-term memory.

<u>Behavioural models for the Evaluation of Learning & Memory processes can be classified into</u>[63]:

I. Exteroceptive Aversive Stimuli Models – External stimuli are used

1) **Behaviour on mazes**
2) **Passive avoidance**
3) **Active Avoidance**

II. Interoceptive Aversive Stimuli Models – Internal stimuli are used

1) **Electroshock-induced amnesia**
2) **Hypoxic stress-induced learning deficits**
3) **Pharmacological and discrimination assays**

Table 1: Following table is a brief synopsis of methods used in assessment of learning and memory:

Models	Parameters measured	Advantages	Limitations
Behavioural animal models[63]			
A. Exteroceptive aversive stimuli models			
Behaviour in mazes			
Morris water maze[64]	**Learning** • Escape latency time • Escape path length **Spatial Memory** • Index of retrieval • No. of crossings **Working memory**	1) High face, predictive validity & sensitivity 2) Open field test 3) Natural motivation 4) Rodents are natural swimmers 5) Decreases possible olfactory or visual bias.	Risk of hypothermia
Land mazes			
Elevated plus maze[65]	**Mainly assessment of working memory and spatial discrimination** • No. of correct entries • No. of incorrect entries	1. No risk of hypothermia 2. High face, predictive validity	1. Motivation needs to be given 2. No natural tendency 3. Olfactory cues
Barnes maze			
Radial arm maze			
Y Maze			
Passive avoidance	**Inhibition of learnt behaviour by an aversive stimulus**		
Step down avoidance	**Memory of learnt task** • Step down latency • Step through latency	1. High face, predictive validity & sensitivity	Highly aversive stimuli
Step through avoidance			
Uphill avoidance			
Active avoidance	**Animals have to predict the onset of aversive stimuli by other visual or audio clues**		
Shuttle box avoidance	**Memory** % of conditioned reflex retained over a no. of tasks done	1. High face, predictive validity & sensitivity	Highly aversive stimuli
Pole jumping apparatus			

<table>
<tr><td colspan="4">B. Interceptive aversive stimuli models</td></tr>
<tr><td>Electroshock induced amnesia</td><td><u>Electric shock induces retrograde amnesia of learnt task.</u></td><td rowspan="2">1. No conditioning or motivation required
2. Less of observer bias</td><td rowspan="2">1. Highly aversive stimuli
2. No assessment of working memory possible</td></tr>
<tr><td>Hypoxic Stress-induced Learning Deficits</td><td><u>Electric shock induces retrograde amnesia of learnt task.</u></td></tr>
<tr><td colspan="4"><u>Pharmacological methods of inducing amnesia</u></td></tr>
<tr><td><u>AGENTS USED</u></td><td colspan="3"><u>PRINCIPLE</u></td></tr>
<tr><td>Scopolamine induced amnesia</td><td colspan="3"><u>Amnesia is induced by inhibition of cholinergic transmission</u></td></tr>
<tr><td>Diazepam induced amnesia</td><td colspan="3"><u>Amnesia is induced by GABAergic inhibition and oxidative stress</u></td></tr>
<tr><td>Streptozocin induced amnesia</td><td colspan="3"><u>Amnesia is induced by restricting glucose metabolism by neurons</u></td></tr>
<tr><td>Colchicine induced amnesia</td><td colspan="3"><u>Induces neuronal death by inhibition of microtubules</u></td></tr>
<tr><td>Sodium nitrite induced amnesia</td><td colspan="3"><u>Induces chemical hypoxia in brain and oxidative stress</u></td></tr>
<tr><td>Ethanol Induced Amnesia[72]</td><td colspan="3"><u>Apoptosis in the brain and neural plasticity by reducing GABAergic inhibition and facilitating glutamatergic excitation</u></td></tr>
</table>

METHODOLOGY

Adult healthy male Wistar rats weighing between 150-180 g sourced from the central animal house of Jawaharlal Nehru Medical College Belagavi, were acclimatized for 10 days in 12:12 hr. light - dark cyclebefore experimentation. They were maintained on standard rat chow pellet (Amrut Brand) and water *ad libitum.* The study was approved by the IAEC (Institutional animal ethical committee) constituted as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), New Delhi.

Method of collection of data

The present study was an experimental design which involved the initial screening of the male Wistar rats. In this screening rats were allowed to swim in water for 2 minutes and were selected on the basis of their swimming ability. The selected rats were then randomly divided into 9 groups with 10 rats in each group (n=10).

Exclusion Criteria-

- Rats which failed to swim.
- Rats with a predominant tendency to float.
- Rats which failed to locate the platform on all occasions for two consecutive days.
- Rats with visual or motor impaired ability.

Table 2: Grouping of animals according to treatment schedule

Groups(G)	Drugs administered and their doses
I	Control (Distilled water ip)
II	Sodium Nitrite (50mg/kg ip)
III	Ethanol (1g/kg i.p)
IV	Sodium nitrite (50mg/kg)[69] + Lisinopril (4mg/kg) +
V	Ethanol (1g/kg) [68] + Lisinopril (4mg/kg)
VI	Lisinopril (4 mg/kg)
VII	Sodium nitrite (50mg/kg) + Perindopril (1.5 mg/kg)
VIII	Ethanol (1g/kg)[68] + Perindopril (1.5 mg/kg)
IX	Perindopril (1.5 mg/kg)

Experiment

To see the effect of Lisinopril and Perindopril on anterograde amnesia, the paradigm used was Morris water maze[70]. It comprises of a circular water tank comprised of 150 cm diameter and 50 cm height with non-reflecting interior surface. The interior surface was painted in a way that made the maze opaque when filled with opaque water. It was filled with water up to 30 cm at 25±5^{o} C. It was divided into four quadrants using threads. In it, a square (side 10 cm) platform having height of 29 cm was placed in the middle of one quadrant (goal quadrant – Qg). The position of the platform was kept fixed throughout the training and acquisition trials. The water was made opaque by dissolving 3 liters of fresh milk daily.

Principle of Morris water maze [63,64& 70]

Morris water maze is an established paradigm for assessing spatial learning and memory[61,70,71]. Spatial learning and memory formation is the function of hippocampus. Rats are natural swimmers and when put into water, they have a natural tendency to escape from it. In Morris water maze, this natural tendency to escape is provided by a hidden platform in the pool. Animals swim in an opaque pool of water to find an escape route when they stumble upon the hidden platform by trial & error. Upon subsequent release in that same pool, they quickly learn to find the location of the submerged platform with the use of variety of cues and strategies, including distal cues in the immediate environment. The time or path taken by the rats to find the platform is usually measured as a learning index. Should the platform be removed from the pool, they continuously search the hidden platform by the spatial memory formed. There are drugs which may inhibit or improve one of the above-mentioned strategies used by rats and are thus effectively evaluated.

Morris water maze has become the gold standard for hippocampal function assessment.

Principle Of Methods

Sodium nitrite Induced Oxidative Stress Model Of Anterograde amnesia

Sodium Nitrite Induced Amnesia: sodium nitrite influence learning and memory by causing various alteration in metabolism of brain. hippocampus is one of the vulnerable structures in brain to damage caused by oxidative stress. We know that hippocampus plays vital role in memory.[87]

Sodium nitrite causes chemical hypoxia by converting haemoglobin into methaemoglobin and increases the generation of free radicals likes reactive nitrogen species and reactive oxygen species. These free radicals utilize GSH and other antioxidants from the brain, thereby lowering their levels, these conditions comprise anti-oxidant defence leading to oxidative stress. Similarly causes protein oxidation and lipid peroxidation all these various steps lead to derangement of normal cellular metabolism, finally leading to cell damage. [86]

Ethanol Induced Apoptosis Model Of Anterograde Amnesia

Ethanol induced amnesia through various mechanism, but the major pathway is by apoptosis. Ethanol can cause increase in GABA activity, which in turn cause decrease in NMDA neuronal activity. This inhibition in neuronal activity is known to activate apoptotic neurodegeneration. Ethanol also increases mitochondrial membrane permeability thereby causing mitochondrial damage and triggering cell death. Ethanol also causes accumulation of proapoptotic proteins and decrease in antiapoptotic proteins. Ratio of anti-apoptotic proteins to proapoptotic proteins is reversed. It also increases cystotome C levels and cleaves caspase 3. Cytochrome c activates cleavage of caspases 3, cleaving of caspases 3 trigger the apoptotic pathway leading to cell death.[88]

The procedure for experiment is as follows: [61,70,71]

For first 10 days, rats were acclimatized to the experimental room and the investigator by repeated handling. Following every trial, rats were dried thoroughly to prevent any hypothermia.

Day 1 Training – rats were familiarized with the task and trials were not counted. The familiarization by the rats were confirmed when they learnt that there is an escape route from this aversive stimulus i.e. water.

Day 2-5 - **Acquisition trial:** Control and drug treated (on each day for four days) rats were released into water facing towards the wall in one of the quadrants (Q). They were subjected to 4 trials per day for four days with 5 minutes interval between each, with the subsequent trial occurring after finishing the ongoing trial with all the 10 rats for that same quadrant. During successive trials and successive days, starting points was changed every time as following (Table 3).

Table 3: Order of release of rats in specific quadrants.

Days	1st trial	2nd trial	3rd trial	4th trial
1	Q1	Q2	Q3	Qg
2	Q2	Q3	Qg	Q1
3	Q3	Qg	Q1	Q2
4	Qg	Q1	Q2	Q3

At first, the rats were trained to locate the hidden platform by 'hit and trial' method. They learnt the position of the hidden platform by using distal cues. Several distal cues were provided to the rats with strict adherence to their same fixity for all the days of the trial. Even the position of the investigator was fixed with respect to the distal cues.

Subsequently, they were allowed to escape to the platform and stay there for 20 seconds (to generate a spatial memory of the hidden platform with the help of

distal cues). The time required to escape to platform (Escape Latency Time- ELT) was noted and compared amongst different days and different groups. Rats unable to locate the platform within 120 seconds were guided to the platform by hand and again kept there for 20 seconds. Rats failing the task on consecutive trials for two successive days were excluded from the study.

Day 5 - **Retrieval trial:** On the sixth day, platform from goal quadrant was removed and rats (all groups were administered vehicle now) were evaluated for time spent in previously goal quadrant (Index of Retrieval). This was done only once and the farthest quadrant from the goal quadrant was chosen to release the rats. This quadrant was kept the same for all groups. The time spent in the previously goal quadrant was compared amongst control, amnesia induced, and drug treated groups. The human dose was converted to rat dose according to Paget's and Barne's conversion table.[73]

Dosing and Schedule of Drugs

1. 10ml/kg Distilled water (i.p) as vehicle was given 30 minutes before trial.
2. Sodium nitrite was administered in the dose of 50mg/kg i.p 30 minutes before the trials, Which was obtained from Department of Pharmacology J.N.Medical College Belagavi
3. Ethanol was administered in the dose of 1 g/kg[68] which was made from a 12.6% ethanol in 0.9% saline (vol/vol) stock solution, freshly for each experiment and then diluted to the required concentration. Ethanol was injected i.p. at a volume of 1 ml/kg, which was obtained from Department of Pharmacology J.N.Medical College Belagavi
4. Lisinopril was purchased from hospital pharmacy. Dose of 4mg /kg 0f rat was selected as this was equivalent to effective dose of Lisinopril used clinically in

humans for the management of Hypertension. The drug was dissolved in sterile water and then administered i.p. 6 hours prior to the trail.

5. Perindopril was purchased from hospital pharmacy. Dose of 1.5 mg /kg rat of was selected as this was equivalent to effective dose of Perindopril used clinically in humans for the management of Hypertension. The drug was dissolved in sterile water and then administered orally 3 hours prior to the trail.

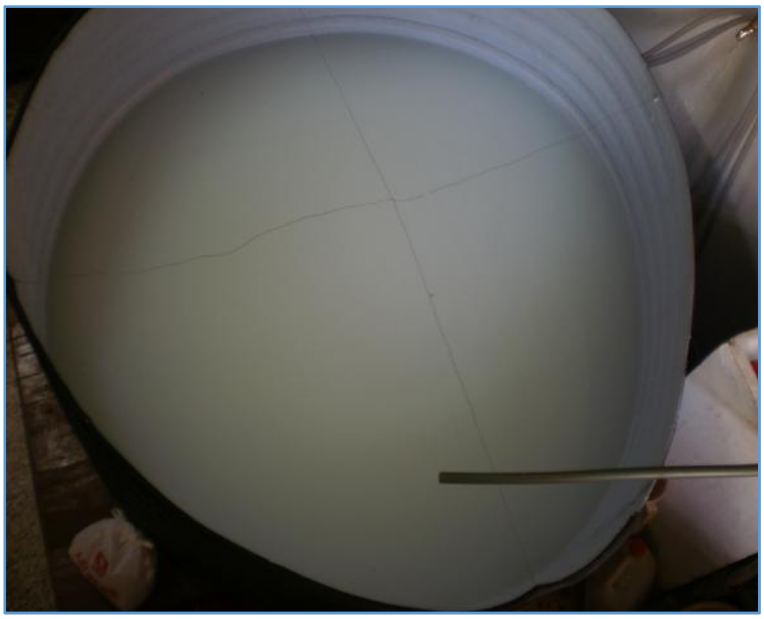
Sodium nitrite, Ethanol, Lisinopril and Perindopril were administered to the animals in acquisition trial on each day. Distilled water was administered to control group and to all groups before retrieval trial. Fresh pasteurized milk was purchased daily to make the water opaque.

After the experimental period, on 7th day all animals were euthanized using overdose of Thiopentone sodium by intraperitoneal route (90mg/kg for male Wistar rats) (as per the guidelines of CPCSEA).

Statistical analysis:

The data for all the groups was expressed as Mean ±SEM and was analyzed by using one-way ANOVA (Analysis of variance) of repeated measurement followed by *post hoc* Dunnett's and Bonferroni's using Graph Pad Prism 8.00 Software. p value ≤ 0.05 was considered statistically significant.

Image 1: Morris water maze



Morris water maze with 150 cm diameter and 50 cm height. It has been divided into four quadrants using threads. The water has been made opaque by adding fresh milk.

Image 2 – Depicting the location of hidden platform in the goal quadrant.

Rats first learn its location by hit and trial.

Image 3 – This shows the location of hidden platform in the goal quadrant. Mice first learn its location by hit and trial. Later they use cues to locate the platform in acquisition trial.

Image 4– Depicting the retrieval trial where the platform has been removed.

The rat is shown here searching for the removed platform in the goal quadrant.

RESULT

In the present study, the effect of lisinopril and perindopril on anterograde amnesia induced by sodium nitrite and ethanol in adult male Wistar rats was studied using Morris water maze. Ten rats were selected per group based on their ability to actively swim. Similar test conditions were followed for all groups.

Sodium nitrite induced oxidative stress model of anterograde amnesia

The Mean escape latency time (ELT) in seconds (s) was measured to assess learning and acquisition of the task of locating the submerged, invisible platform using Morris water maze. The mean ELT of control group which received normal saline on day 1 was 47.15 ± 6.539. Sodium nitrite group showed increase in mean ELT of 68.6 ± 3.907, which was statistically significant in comparison to control ($p< 0.05$). Mean ELT of lisinopril was 47.45 ± 5.037 (Table 4), which was almost equivalent to control group while, treatment with perindopril alone showed mean ELT of 9.825 ± 1.727 (Table 4) that was statistically significant as compared to control ($p<0.0001$) and sodium nitrite ($p<0.0001$). Mean ELT in sodium nitrite and lisinopril group was 50.6 ± 4.006 (Graph 1), which was less than the mean ELT in sodium nitrite group, though was not statistically significant. However, administration of perindopril in sodium nitrite induced amnesia recorded a mean ELT 14.8 ± 1.787 which was statistically significant as compared to sodium nitrite ($p = <0.0001$) and control ($p = <0.001$) group (Graph 1).

On day 2 of the trail mean ELT in control group and sodium nitrite group was 41.05 ± 7.685 and 65.05 ± 4.040 respectively. Mean ELT in lisinopril group was 47.03 ± 10.26 (Table 4), and 49.85 ± 11.43 in sodium nitrite and lisinopril group which were not statistically significant. Perindopril showed statistically significant

decrease on day 2 as compared to control ($p<0.01$) and sodium nitrite group ($p = <0.0001$) with mean ELT of 8.875 ± 1.481. Similarly, administration of perindopril along with sodium nitrite showed statistically significant decrease($p<0.0001$) as compare to sodium nitrite and control ($p<0.05$) with the mean ELT of 13.25 ± 1.991. (Table 4) (Graph 3).

On day 3 of the trail, mean ELT in control and sodium nitrite groups were 35.88 ± 7.267 and 53.7 ± 3.366 respectively (Graph5). Lisinopril alone and in combination with sodium nitrite did not show statistically significant difference as compared to control or sodium nitrite groups with mean ELTs of 43.79± 6.814 and 35.9 ± 4.686 respectively (Table 4). However, Perindopril alone showed a statistically significant decrease as compared to control ($p<0.01$) and sodium nitrite group ($p<0.0001$) with mean ELT of 6.95 ± 0.8683. Similarly, administration of perindopril with sodium nitrite showed statistically significant decrease as compared to control ($p<0.05$) and sodium nitrite ($p<0.0001$) with mean ELT of 12.15 ± 2.509. (Table 4) (Graph 5).

On day 4 of the trail, mean ELT in control group and sodium nitrite were 28.65 ± 4.594 and 53.1 ± 1.427 respectively. While, sodium nitrite group showed statistically significant compared to control ($p< 0.01$). Lisinopril alone and co-administered with sodium nitrite showed statistically significant difference as compared to sodium nitrite with their mean ELT of 25.70 ± 6.967 ($p < 0.05$) and 27 ± 7.901 ($p < 0.05$) respectively (Graph 7). Similarly, perindopril showed a statistically significant decrease as compared to control ($p<0.01$) and sodium nitrite ($p<0.0001$) with mean ELT of 3.95 ± 0.2068 (Graph 7). Similarly, administration of perindopril

with sodium nitrite showed statistically significant decrease as compared to sodium nitrite ($p<0.0001$) with mean ELT of 10.98 ± 1.869 (Table 4).

On 5th day retrieval trail was performed, where the platform was removed, and time spent in goal quadrant was taken into account. The mean time spent (TS) in the goal quadrant in control group was 77.95 ± 2.395 while, it was 52.40 ± 2.532 in sodium nitrite group which was statistically significant as compared to control ($p<0.01$). (Table 6). Lisinopril alone as well as in combination with sodium nitrite showed statistically significant increase in mean TS in goal quadrant as compared to sodium nitrite group with values of 83.45 ± 6.128 ($p<0.01$) and 80.00±9.424 ($p< 0.05$), respectively. Similarly, perindopril alone as well as in combination with sodium nitrite showed statistically significant increase in mean TS in goal quadrant as compared to sodium nitrite group with mean TS of 87.15± 5.300 ($p<0.001$) and 79.03 ± 6.10 ($p< 0.05$) respectively (Table 6) (Graph 9).

Lisinopril treated group showed gradual decrease in mean ELT from day 1 to day 4, Lisinopril and sodium nitrite treated group of rats showed gradual decrease in mean ELT from day 1 to day 4.

Perindopril treated group showed exceptional results, mean ELT was gradual decreased from day 1 to day 4, Perindopril and sodium nitrite showed gradual decrease in mean ELT from day 1 to day 4.

Ethanol induced apoptotic model of anterograde amnesia

On day 1 of the trial mean ELT of ethanol group was 68.65 ± 6.074, which was statistically significant ($p<0.05$) in compare to control. Lisinopril alone and in combination with ethanol did not show any statistically difference in compare to ethanol with mean ELT of 68.08 ± 8.882 of latter (Graph 2) (Table 5). However,

perindopril alone and in combination with ethanol showed statistically significant when compared to ethanol and control with mean ELT 9.825±1.727 ($p<0.0001$) and 14.98 ± 2.284($p<0.0001$) of respectively. Perindopril in combination with ethanol showed statistically significant compared to control ($p<0.05$). (Table 5) (Graph 2).

On 2 day mean ELT of ethanol was 64.2 ± 4.808 (Table 5). Mean ELT of lisinopril and ethanol was 45.68 ± 8.539, which was not statistically significant compared to ethanol. Lisinopril alone too had no statistically significance with ethanol However, perindopril alone and with combination with ethanol showed statistically significant compared to ethanol with the mean ELT of 8.875±1.481 ($p=<0.0001$) and 13.431 ± 1.898 ($p=<0.0001$), respectively (Table 5). Perindopril in combination with ethanol showed statistically significant compared to control ($p<0.05$) (Graph 4)

On day 3, mean ELT of ethanol was 58.11±6.484 it was statistically significant compared to control ($p<0.05$). Mean ELT of ethanol and lisinopril was 38.3 ± 7.502, which was not statistically significant compared to ethanol (Table 5). However, perindopril alone and with combination with ethanol showed statistically significant compared to ethanol with mean ELT of 6.95 ±0.8683 ($p=<0.0001$) and 12.8 ± 1.236 ($p=<0.0001$) (Table 5). Perindopril in combination with ethanol showed statistically significant compared to control ($p<0.05$) (Graph6)

On day 4 trial mean ELT of ethanol group was 50.9 ± 2.196 which was statistically significant to control group ($p<0.05$). Mean ELT of ethanol and lisinopril was 38.93 ± 10.07, which was not statistically significant compared to ethanol (Table 5)(Graph 8) However, perindopril alone and with combination with ethanol showed statistically significant compared to ethanol with mean ELT of 3.95 ± 0.2068($p=<0.0001$) and 11.8 ± 1.92 ($p=<0.0001$) (Table 5) (Graph 8).

On 5th day retrieval trail was performed, the mean time spent (TS) in the goal quadrant in ethanol group was 53.88 ± 3.626 which was statistically significant as compared to control ($p<0.05$). (Table 7). Lisinopril alone as well as in combination with ethanol did not show statistically significant as compared to ethanol or control group, with mean TS of 83.45 ± 6.128 and 46.03 ± 2.539 (Table 7) respectively. However, perindopril alone as well as in combination with ethanol showed statistically significant increase in mean TS in goal quadrant as compared to ethanol group with mean TS of 87.15± 5.300 ($p<0.01$) and 81.68 ± 5.289 ($p< 0.05$) respectively (Graph 10).

Lisinopril and ethanol treated group showed gradual decrease in mean ELT from day 1 to day 4. Perindopril and Ethanol also showed decrease in mean ELT from day 1 to day 4.

Table 4 -Effect of various treatment on sodium nitrite induced anterograde amnesia with mean escape latency time during acquisition trial in Morris water maze.

Groups n=9 in each group	Mean escape latency time(seconds) ± SEM			
	Day 1	Day 2	Day 3	Day 4
I. Control	47.15± 6.539	41.05 ± 7.685	35.88 ± 7.267	28.65 ± 4.594
II. $NaNO_2$	68.60 ± 3.907 ^	65.05 ± 4.040	53.7 ± 3.366	53.1 ± 1.427 ^^
III. $NaNO_2$ + Lisinopril	50.6 ± 4.006	49.85 ± 11.43	35.9 ± 4.686	27.7 ± 7.901 *
IV. Lisinopril	47.45 ± 5.037	47.03 ± 10.26	34.79 ± 6.814	25.70 ± 6.967 *
V. $NaNO_2$ + Perindopril	14.8 ± 1.787** ^^^	13.25 ± 1.991 ** ^	12.15 ± 2.509** ^	10.98 ± 1.869**
VI. Perindopril	9.825 ± 1.727** ^^^^	8.875 ± 1.481** ^^	6.95 ± 0.8683** ^^	3.95 ± 0.2068** ^^

Table 4 *- p <0.05, **- p<0.0001 compared to $NaNO_2$ group, ^-p<0.05, ^^-p<0.01, ^^^-p<0.001 ^^^^- p<0.0001 compared to control, (ANOVA followed by Post hoc Dunnett's test and Bonferroni's)

Table 5 -Effect of various treatment on ethanol induced anterograde amnesia with mean escape latency time during acquisition trial in Morris water maze.

Groups n=9 in each group	**Mean escape latency time(seconds) ± SEM**			
	Day 1	**Day 2**	**Day 3**	**Day 4**
I. Control	47.15± 6.539	41.05 ± 7.685	35.88 ± 7.267	28.65 ± 4.594
II. Ethanol	68.65 ± 6.074 ^	64.2 ± 4.808	58.11± 6.484 ^	50.9 ± 2.196 ^
III. Ethanol+Lisinopril	68.08 ± 8.882	45.68 ± 8.539	38.3 ± 7.502	38.93 ± 10.07
IV. Lisinopril	47.45 ± 5.037	47.03 ± 10.26	34.79 ± 6.814	25.70 ± 6.967
V. Ethanol + Perindopril	14.98 ± 2.284 * ^^^	13.431 ± 1.898 * ^	12.8 ± 1.236* ^	11.8 ± 1.92*
VI. Perindopril	9.825 ± 1.727* ^^^^	8.875 ± 1.481* ^^	6.95 ± 0.8683* ^^	3.95 ± 0.2068*^^

Table 5 *- p<0.0001 compared to ethanol group, ^ p<0.05, ^^-p<0.01, ^^^-p<0.001 ^^^^- p<0.0001 compared to control (ANOVA followed by Post hoc Dunnett's test and Bonferroni's)

Table 6 Effect of various treatment on time spent (s) in goal quadrant on 5th day in sodium nitrite induced anterograde amnesia during retrieval trial in Morris water maze.

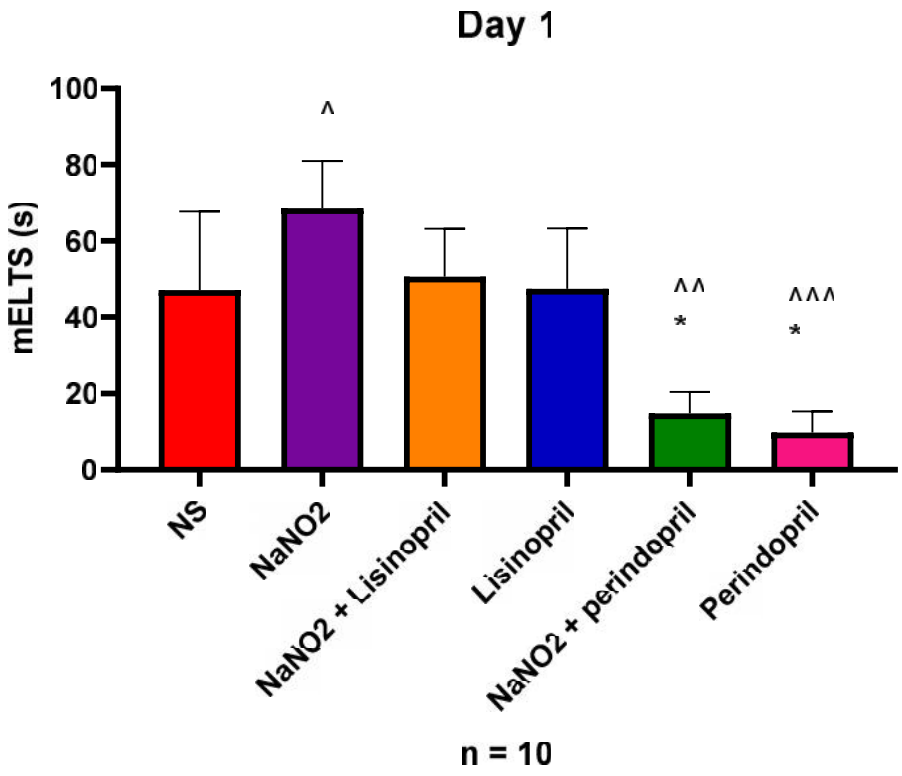
Groups (n=10 in each group)	**Time Spent (seconds)**
I. Control	77.95 ± 2.397
II. $NaNO_2$	52.40 ± 2.532 ^
III. $NaNO_2$ +Lisinopril	80.0 ± 9.424 *
IV. Lisinopril	83.45± 6.128**
V. $NaNO_2$ + Perindopril	79.03± 6.100*
VI. Perindopril	87.15 ± 5.300 ***

Table 6 *- p <0.05, **-p<0.01, ***-p<0.001 ****- p<0.0001 as compared to $NaNO_2$ group, ^-p<0.01 as compared to control. ANOVA followed by Post hoc Dunnett's test and Bonferroni's

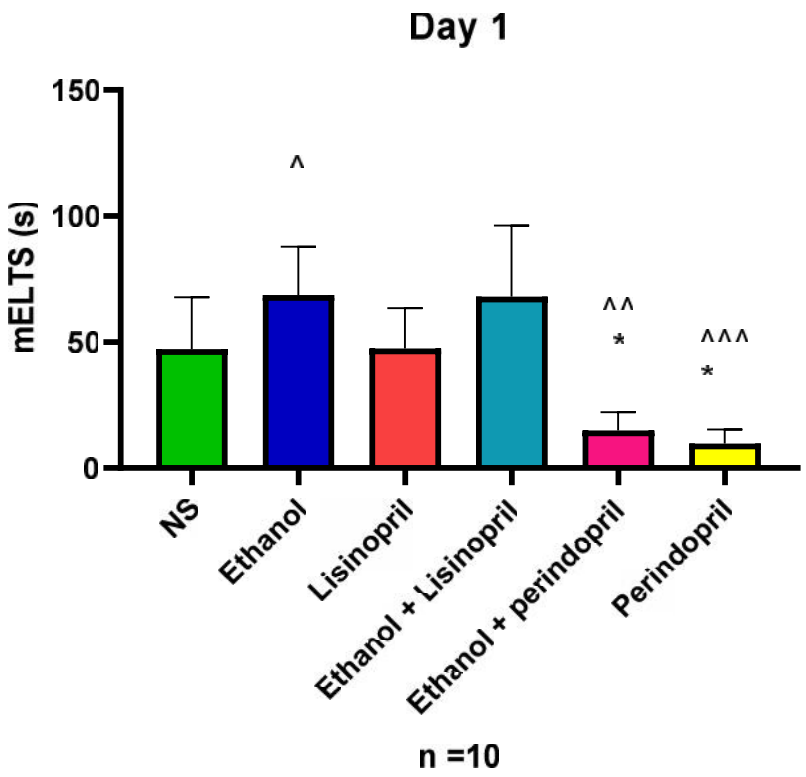
Table 7 Effect of various treatment on time spent (s) in goal quadrant on 5th day in ethanol nitrite induced anterograde amnesia during retrieval trial using Morris water maze.

Groups (n=10 in each group)	Time Spent (seconds)
I. Control	77.95 ± 2.397
II. Ethanol	53.88 ± 3.626 ^
III. Ethanol + Lisinopril	46.23 ± 2.539
IV Lisinopril	83.45± 6.128
V Ethanol + Perindopril	81.68± 5.289*
VII Perindopril	87.15 ± 5.300 **

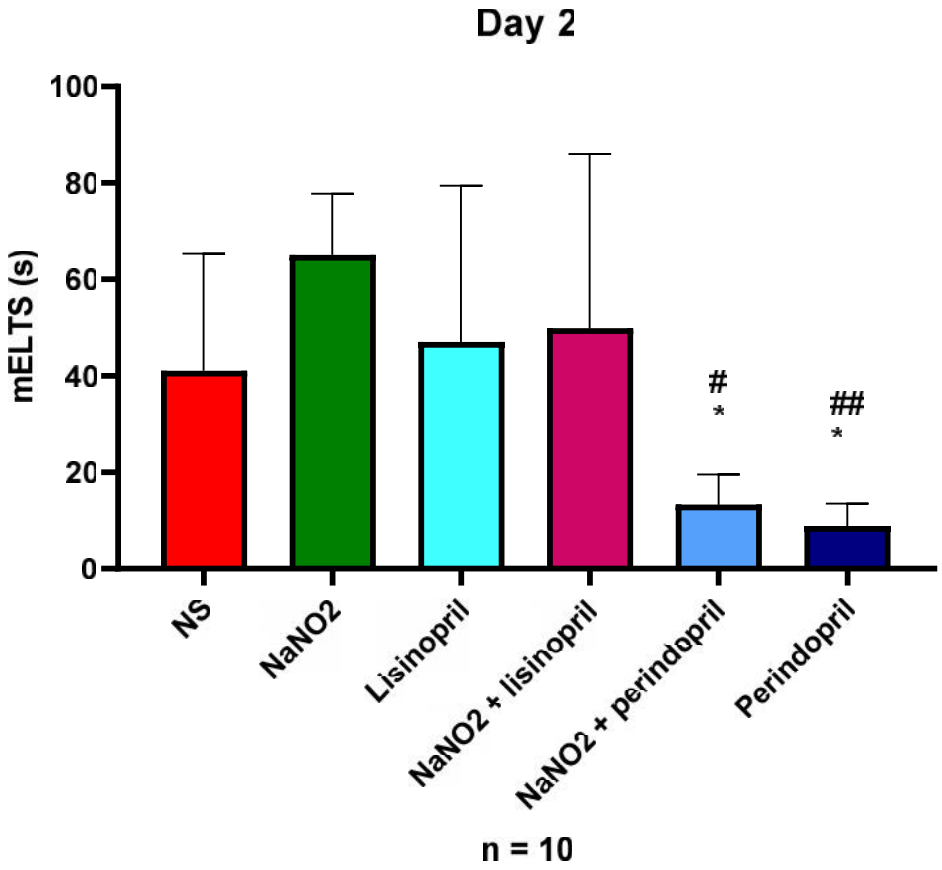
Table 7 *- p <0.05, **-p<0.001 as compared to Ethanolgroup, ^ p<0.05, as compared to control. ANOVA followed by Post hoc Dunnett's test and Bonferroni's



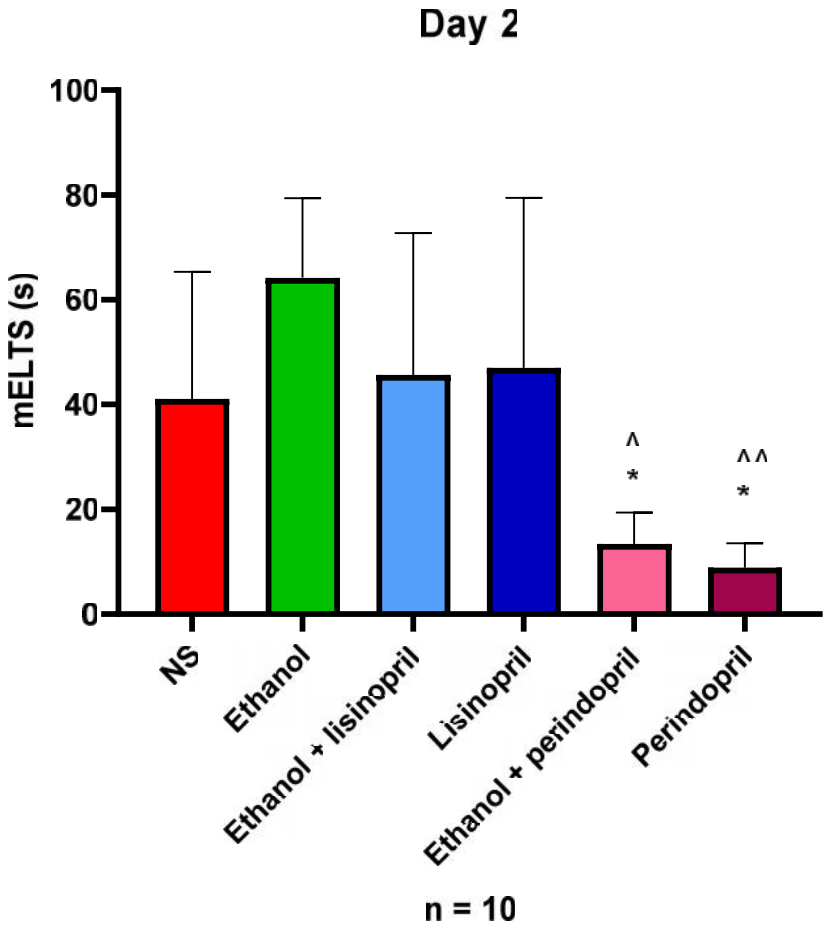
Graph 1 showing comparison of Mean Escape Latency Time (ELT) on day 1 of acquisition trial among sodium nitrite treated group. Post hoc analysis by Dunnett's Test and Bonferroni's Test. *-p<0.0001 compared to $NaNO_2$ and ^-p< 0.05, ^^- p< 0.001, ^^^-p<0.0001 compared to control.



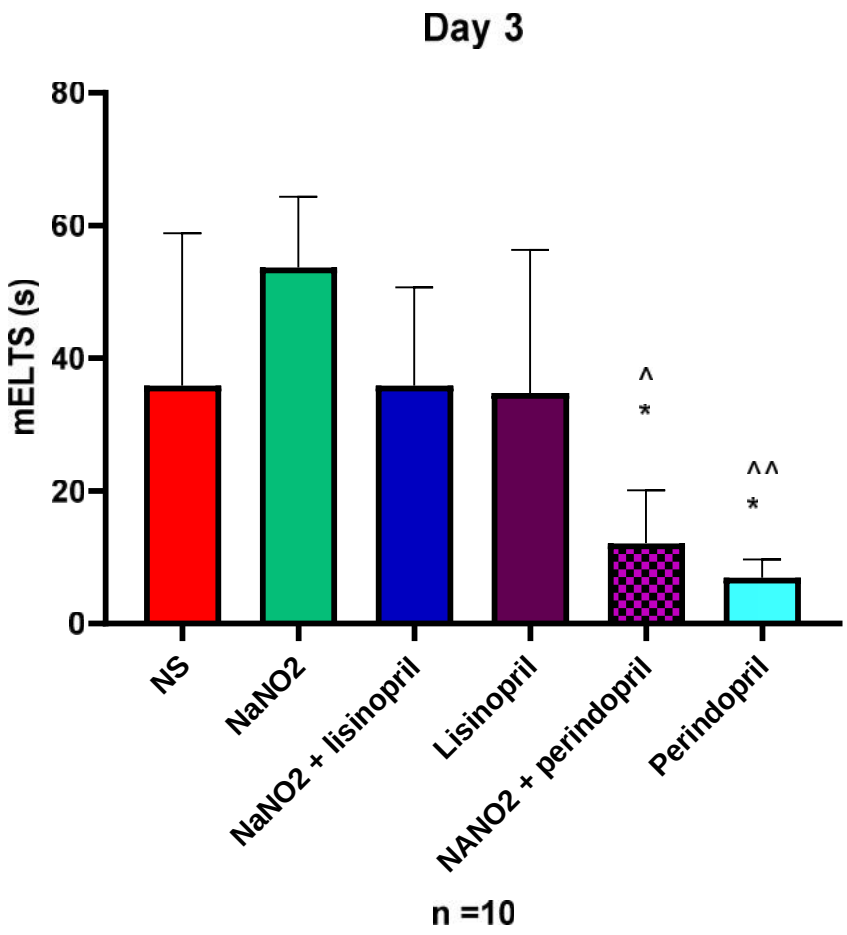
Graph 2 showing comparison of Mean Escape Latency Time (ELT) on day 1 of acquisition trial among ethanol treated group. Post-hoc analysis by Dunnett's Test and Bonferroni's Test. *-p<0.0001 in compared to ethanol ^-p<0.05, ^^-p<0.001, ^^^p<0.0001 compared to control



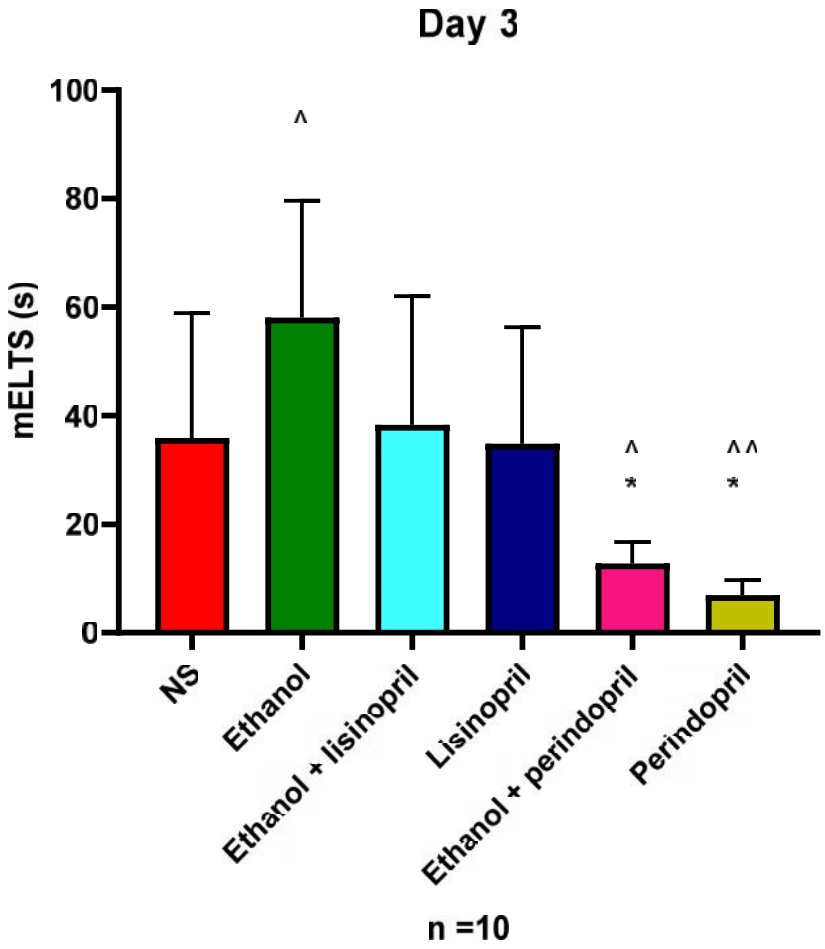
Graph 3 showing comparison of Mean Escape Latency Time (ELT) for on day 2 of acquisition trial among sodium nitrite treated group. Post hoc analysis by Dunnett's Test and Bonferroni's Test. *-p<0.0001 compared to $NaNO_2$ and #-p< 0.05, ##- p< 0.01 compared to control.



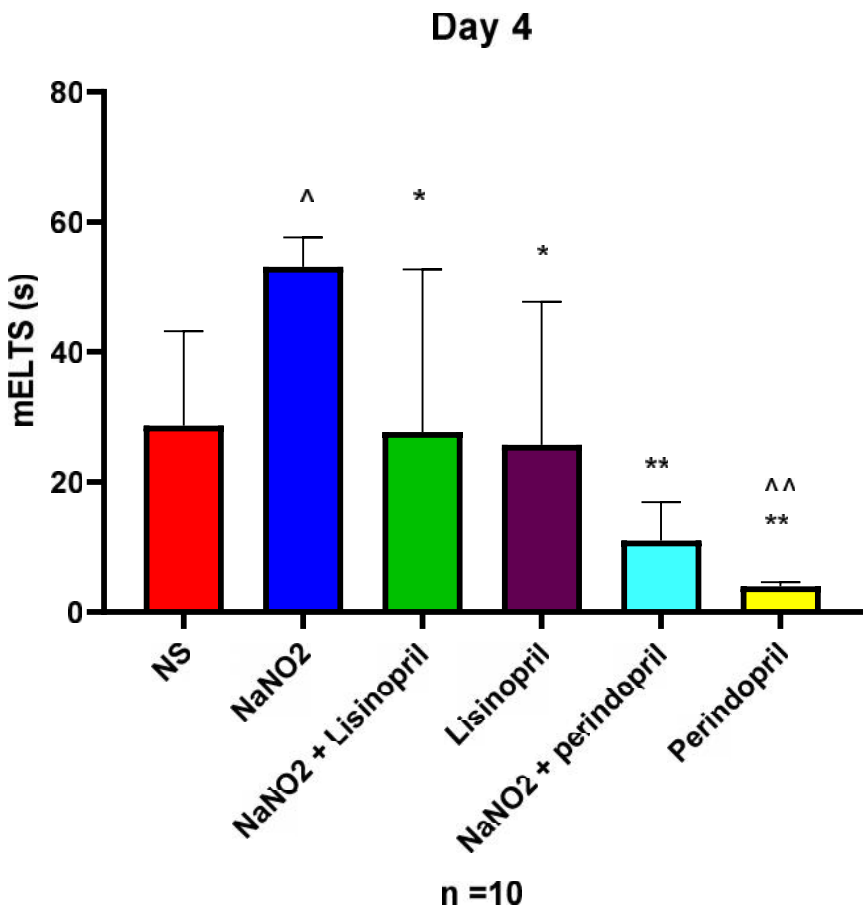
Graph 4 showing comparison of Mean Escape Latency Time (ELT) on day 2 of acquisition trial among ethanol treated group. Post hoc analysis by Dunnett's Test and Bonferroni's Test. *-p<0.0001 in compared to ethanol ^-p<0.05, ^^-p<0.01, compared to control.



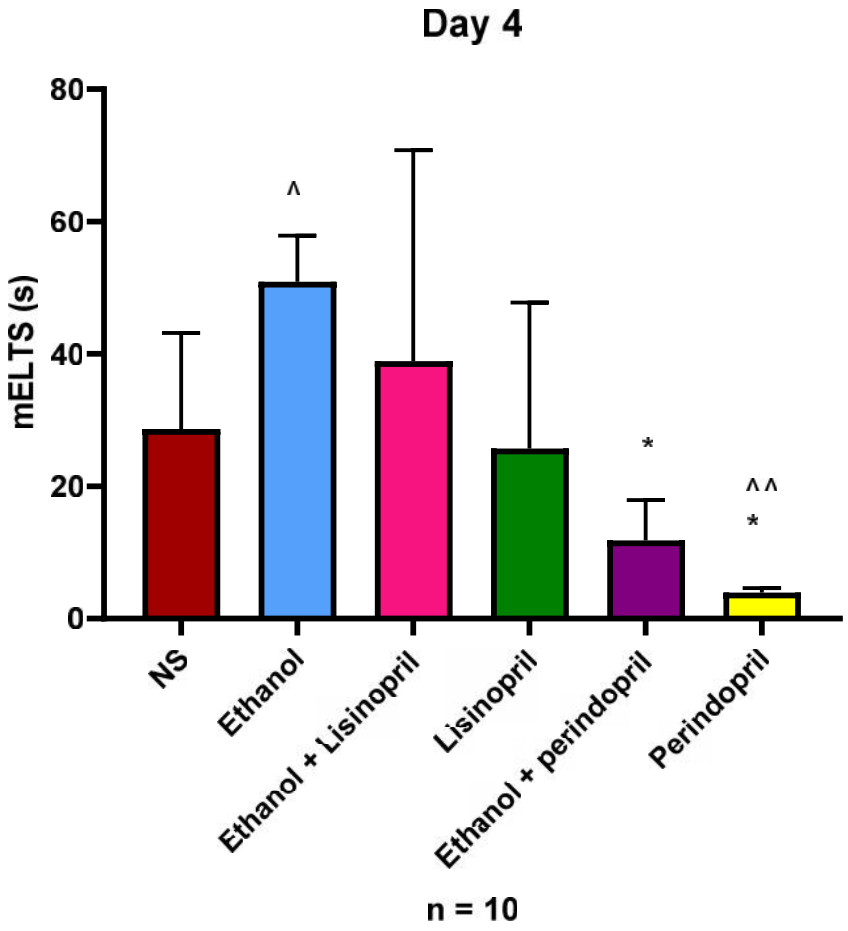
Graph 5 showing comparison of Mean Escape Latency Time (ELT) for on day 3 of acquisition trial among sodium nitrite treated group. Post hoc analysis by Dunnett's Test and Bonferroni's Test. *-p<0.0001 compared to $NaNO_2$ and ^-p< 0.05, ^^- p< 0.01 compared to control.



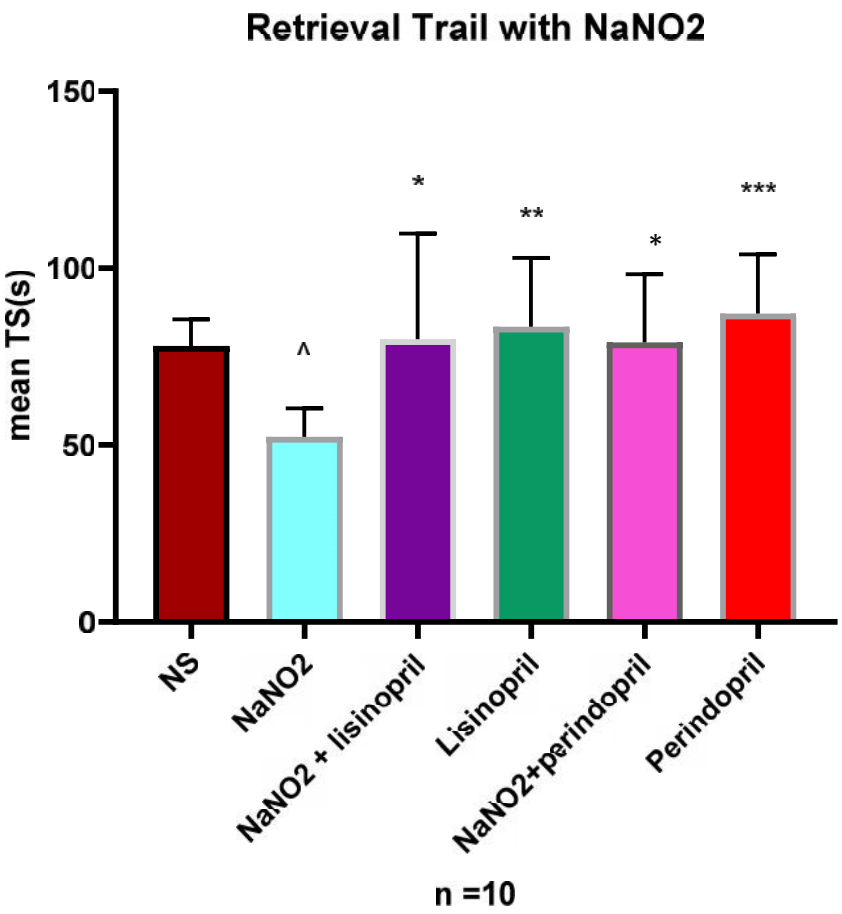
Graph 6 showing comparison of Mean Escape Latency Time (ELT) on day 3 of acquisition trial among ethanol treated group. Post hoc analysis by Dunnett's Test and Bonferroni's Test. *-p<0.0001 in compared to ethanol ^-p<0.05, ^^-p<0.01, compared to control.



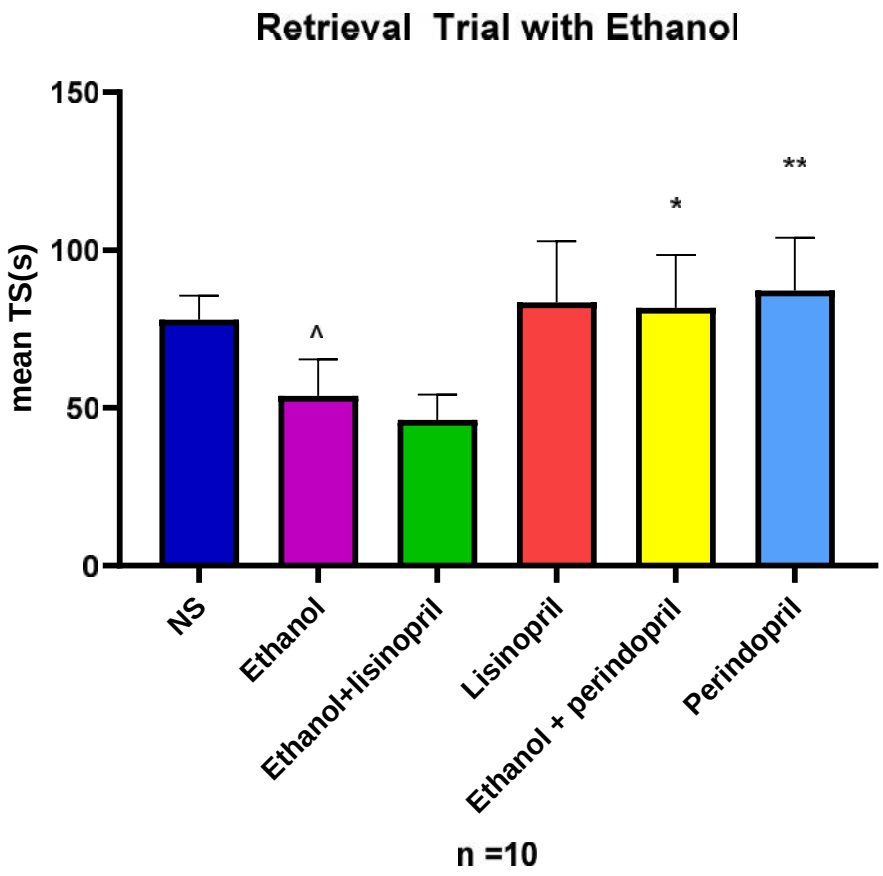
Graph 7 showing comparison of Mean Escape Latency Time (ELT) for on day 4 of acquisition trial among sodium nitrite treated group. Post hoc analysis by Dunnett's Test and Bonferroni's Test.*-p<0.05, *-p<0.0001 compared to $NaNO_2$ and ^-p< 0.05, ^^- p< 0.01 compared to control.



Graph 8 showing comparison of Mean Escape Latency Time (ELT) on day 4 of acquisition trial among ethanol treated group. Post hoc analysis by Dunnett's Test and Bonferroni's Test. *-p<0.0001 in compared to ethanol ^-p<0.05, ^^-p<0.01, compared to control.



Graph 9 showing comparison of mean time spent among sodium nitrite treated group in retrieval trial. Post hoc analysis by Dunnett's Test compared to control and Bonferroni's. *-p<0.05,-p<0.01, ***-p<0.001 compared to sodium nitrite. ^-p< 0.05.compared to control.**



Graph 10 showing comparison of index of retrieval among ethanol treated groups in retrieval trial. Post hoc analysis by Dunnett's Test compared to control and Bonferroni's *-p<0.05 and **-p<0.001 compared to ethanol and ^-p<0.05 compared to control

DISCUSSION

The objective of present study was to evaluate the effect of selected ACEIs - lisinopril and perindopril in clinical equivalent doses on learning and memory on sodium nitrite & ethanol induced anterograde amnesia in male Wistar rats using Morris water maze. A decrease in mean ELT during acquisition trial and increase in time spent in the goal quadrant during retrieval trail, suggesting normal acquisition and retrieval of memory was analyzed in the study.

Sodium nitrite by inducing chemical hypoxia causes hypoxic neuronal injury. The administration of sodium nitrite, results in conversion of hemoglobin to methemoglobin thereby reducing oxygen carrying capacity of blood. Sodium nitrite is also known to cause oxidative injury to neurons by forming peroxynitrates.[87]This causes lipid peroxidation, protein oxidation and lowers the cellular energy levels due to derangement of normal metabolism. All these factors contribute to cell damage. Sodium nitrite has been used to induce cognitive deficits in rats by administering sodium nitrite (50 mg/kg, i.p) while studying nootropic effect of various drugs. The dose sodium nitrite and ethanol have been selected on basis of literature.[89]

Ethanol induced amnesia through various mechanism, but the major pathway is by apoptosis. Ethanol can cause increase in GABA activity, which in turn cause decrease in NMDA neuronal activity. This inhibition in neuronal activity is known to activate apoptotic neurodegeneration. Ethanol also increases mitochondrial membrane permeability thereby causing mitochondrial damage and triggering cell death. Ethanol also causes accumulation of proapoptotic proteins and decrease in antiapoptotic proteins. Ratio of anti-apoptotic proteins to proapoptotic proteins is reversed. It also increases cystotome C levels and cleaves caspase 3. Cytochrome c activates cleavage

of caspases 3, cleaving of caspases 3 trigger the apoptotic pathway leading to cell death.[88]

The paradigm used was Morris water maze.[64,65] Morris water maze is a 'gold standard' for hippocampal function assessment. The structure of the hippocampus is conserved throughout the mammals, making the rat hippocampus suitable model for study of human hippocampal dependent processes. [71] Rats as all rodents have also a deep sense of spatial memory via which it can burrow holes and enter into our houses daily. Rodents are natural swimmers and have a natural tendency to escape water. Thus, this maze serves as a natural motivation for them. [71] The other advantages are that it decreases possible bias due to scent of previous animal and since it is an open field test, no direction specific behavior needs to be induced. [71] Rats are better performers as they have lesser tendencies to float.

In sodium nitriteinduced oxidativemodel of amnesia, Lisinopril alone showed significant increase in mean ELT and decrease in TS in goal quadrant during retrieval as compared to sodium nitrite group, suggesting that lisinopril has potential as cognitive enhancer.

Lisinopril effect showed in sodium nitrite induced anterograde suggest that lisinopril could overcome the effect of sodium nitrite. Sodium nitrite causes memory impairment by effecting mainly hippocampus and neocortex. Hippocampus is the part of the brain that is responsible for antegrade memory. Sodium nitrite by inducing chemical hypoxia causes hypoxic neuronal injury. The administration of sodium nitrite, results in conversion of hemoglobin to methemoglobin, this methemoglobin is responsible for cerebral hypoxia that initiates free radical such as peroxynitrate also increases hydrogen peroxides, it also causes protein oxidation and lipid peroxidation

which causes oxidative stress and damages hippocampus. Data collected from this study shows lisinopril significant overcomes anterograde amnesia induced by sodium nitrite. Previous studies have shown that lisinopril administration to spontaneously hypertensive rats reduced lipid peroxidation, by increasing the GSH content. GSH is the one of the important intracellular radical oxygen species scavengers. The study also documented that lisinopril plays active role in restoring GSH content and may act by circulating other cellular antioxidants.[13]Therefore, it can be postulated that lisinopril may act indirectly by scavenging free radicals and hence possess antioxidant effect. Few studies have also suggested that sodium nitrite produces vasodilation, which can cause increased production of angiotensin, that can cause simulation of brain RAS.[53] Activation of brain RAS has been reported to cause decline in cognitive function.[73] The protective effect of lisinopril in memory impairment could also be attributed to decreasing the RAS activity in the brain.However, lisinopril did not show any beneficial effect in ethanol induced anterograde amnesia which may rule out its anti- apoptotic activity.

Perindopril treated group showed exceptional results, indicating that pretreatment with perindopril not only has protective effect against sodium nitrite and ethanol but also has memory enhancing capabilities as perindopril showed better result than control group from day 1. Perindopril alone showed significant difference in retrieval trail compared to sodium nitrite and ethanol. Perindopril combined with sodium nitrite group and perindopril co-administered with ethanol group exhibited significant change during retrieval trail in comparison to sodium nitrite and ethanol.

Perindopril effect shown in comparison to control group suggest that perindopril enhance cognitive function. These observations can be co-related to other previous studies which showed that perindopril increased levels of reduced (GSH)

and oxidized glutathione (GSSG) in comparison to normal saline which was used as control. This study also showed that perindopril caused 50 % of inhibition of ACE in hippocampus.[80] ACE is known to have neurodegenerative effect. Perindopril also exhibited marked protective effect in sodium nitrite induced anterograde amnesia, which suggest that perindopril has potent antioxidant effect, Furthermore, a different study states that sodium nitrite causes reduction in GSH and similar antioxidant levels in erythrocytes, while, Perindopril is known to increase the levels of GSH and GSSG.[82] Therefore, it can be argued that perindopril may counter act sodium nitrite induced oxidative stress with this mechanism.

During the retrieval trial perindopril treated rats showed retrieval of the platform location. This retrieval of the task memory could have only happened after the rats learnt it and perindopril treated rats did show increased in time spent to search the hidden platform in the goal quadrant on fifth day.

Perindopril therefore ameliorated induction of amnesia by ethanol. This could mean perindopril might also have anti apoptotic activity. A trail conducted on patients with coronary artery disease, concluded that perindopril was superior to placebo and it showed that there were reduction in bradykinin breakdown levels and reduced endothelial apoptosis.[81]

An in-vitro study showed that perindopril has greater tissue affinity than other ACE inhibitors, and also possessed increased effect on bradykinin potentiation. In a different experimental model perindopril displayed greater bradykinin/angiotensin I selectivity ratio than other ACE inhibitors.[90]

In a study conducted on animal it was seen that perindopril even at lower dosage than that is required for reducing angiotensin II levels could increase the bradykinin levels. This data can be supported by the Perindopril-Thrombosis,

Inflammation, Endothelial Dysfunction and Neurohormonal Activation (PERTINENT) trial conducted in humans having cardiovascular heart disease. This study explained the efficacy of perindopril in reducing cardiovascular events by showing enhanced bradykinin/AngII binding site selectivity and decreased endothelial apoptosis.[81]

Perindopril showing better results than control can be explained on basis of its pharmacokinetic profile, perindopril has higher lipophilicity than other ACEIs and stronger tissue ACE binding capacity. Study have shown that perindopril has better pharmacokinetic profile and causes local inhibition of the RAAS in tissues such as the kidneys, heart, adrenal glands, brain, and blood vessels, and has much better selectivity for bradykinin binding sites compared with other ACEIs.

This study found definite role of perindopril and possible role of lisinopril in ameliorating induction of anterograde amnesia by sodium nitrite. An obvious next step would be demonstration of hypoxic insult to the brain by using pathologic and neuroimaging techniques and then exploring the effects of these drugs. Matson in his review has suggested the role of oxidative stress in neurodegeneration.[83] Oxidative stress even may be the earliest insult to neuronal damage.[84] Added to this insult is the process of ageing where there is an increase in reactive oxygen species and decreased ability to fight them. Perindopril and lisinopril which are usually started in midlife can then arrest the process of neurodegeneration at its start and bring down the prevalence of cognitive impairment.

Alternatively, lisinopril as anti-apoptotic needs to be re-evaluated. This study also suggests that the anti-oxidant role of perindopril and lisinopril and antiapoptotic effect of perindopril in arresting neurodegeneration will open new avenues in the management of number of neurodegenerative disorders of the brain.

CONCLUSION

In the current study, perindopril and lisinopril were explored for their effects on models of anterograde amnesia. Ethanol and sodium nitrite were used to induce anterograde amnesia in male Wistar rats. The paradigm used was Morris water maze, which is a gold standard for evaluating hippocampal spatial memory formation in rodents. Perindopril and lisinopril showed significant decrease in escape latency time (ELT) in acquisition trial when given before sodium nitrite. Similarly, perindopril and lisinopril treated rats showed significant retrieval of task memory when given before sodium nitrite in the retrieval trial. This suggests that both the drugs are effective in sodium nitrite induced oxidative stress induced amnesia model indicative of their antioxidant property. However, only perindopril showed significant effect in ethanol induced apoptotic model of anterograde amnesia model indicative of its anti-apoptotic property. Lisinopril had no effect in ethanol induced anterograde amnesia.

The findings of this study appear to be clinically relevant. It can therefore be concluded that ACEIs might help in overcoming cognitive impairment, independent of blood pressure lowering account. These drugs with their repertoire of pleiotropic effects might offer benefit in the management of neurodegenerative disorders and its symptoms.

SUMMARY

The objective of this study was to evaluate the role of selected ACEIs in clinical equivalent doses on learning and memory in sodium nitrite & ethanol induced anterograde amnesia in male Wistar rats using Morris water maze.

Perindopril when given before sodium nitrite, significantly decreased the escape latency time (ELT) of locating the hidden platform, in acquisition trial. Lisinopril too showed decrease in ELT with sodium nitrite. Similar effects were seen by both the drugs in retrieval of task memory in the retrieval trial. These results point towards possible anti-oxidant role of these drugs in overcoming hypoxia induced neuronal damage. Perindopril when treated before ethanol showed decrease in ELT in acquisition trail but not significant change in retrieval trail.This points towards possible anti apoptotic role of perindopril. Lisinopril however did not have any effect on ethanol induced anterograde amnesia.

With increasing age, there is increased risk of encountering neurodegenerative dementia, the most common reason being increased oxidative stress to neurons. Early treatment in reducing these risks at old age and taking preventive measure in dealing with their etiologies beforehand should be first step in encountering neurodegenerative dementia. Drugs that reduce oxidative processes, inhibit apoptosis and reduce inflammation may add benefit in the treatment of neurodegenerative disorders. However, the antioxidant and antiapoptotic role of perindopril and lisinopril needs further investigation before their clinical use as prophylactic drugs in neurodegenerative disorders.

BIBLIOGRAPHY

1. FauciSF, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL et al. Harrison's Principles of Internal Medicine. 17th ed. New Delhi. Mcgraw Hill Publications. 2012;162-70.

2. Qiu C, Kivipelto M, von Strauss E. Epidemiology of Alzheimer's disease: occurrence, determinants, and strategies toward intervention. Dialogues ClinNeurosci. 2009;11(2):111–28.

3. ZebaMohandas E, Rajmohan V, Raghunath B. Neurobiology of Alzheimer's disease. Indian J Psychiatry. 2009; 51: 55-61.

4. BruntonL,Chabner B, Knollman B; Goodman & Gilman's The Pharmacological Basis of Therapeutics;12thed New Delhi. McGraw Hill Publications. 2011; 609-28.

5. Tripathi K. D. Essentials of Medical Pharmacology. 6thed. New Delhi. Jaypee Brothers medical Publishers. 2010; 469-74.

6. Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. Rang and Dale's Pharmacology. 7thed. New York. Elsevier. 2007; 481-84.

7. Kaycee M, Sink M, Xiaoyan L, Williamson J, Kritchevsky S, Yaffe K, et al Angiotensin Converting Enzyme Inhibitors and Cognitive Decline in Older Adults with Hypertension: Results from the Cardiovascular Health Study. Arch Intern Med. 2009; 169(13): 1195–202.

8. Dementia–Public Health Priority 2012 World Health Organization. Available from whqlibdoc.who.int/publications/2012/9789241564458_eng.pdf. Last Accessed on 27th Oct 2014.

9. Gopalkumar R, Szabo ST, Alxopoulos GS, Zamas AS Strategies for dementia prevention: latest evidence and implications. TherAdv Chronic Dis. 2017: 8(8-9) 121–36.
10. Polizio AH and Pena C. Lisinopril as an antioxidant in hypertension? antioxid redox signal. 2007; 9(3): 393-7.
11. Augusto-Oliveira M, Arrifano GPF, Malva JO, Crespo-Lopez ME. Adult Hippocampal Neurogenesis in Different Taxonomic Groups: Possible Functional Similarities and Striking Controversies. Cells. 2019;8(2):125.
12. Girouard H and Iadecola C.Neurovascular coupling in the normal brain and in hypertension, stroke, and Alzheimer disease. JAppl Physiol.2006; 100:328-35.
13. Greene AJ, Spellman BA, Dusek JA, Eichenbaum HB and Levy WB. Relational learning with and without awareness: Transitive inference using nonverbal stimuli in humans. Memory & Cognition 2001;29 (6): 893-902.
14. Bisa R, Travaglia A and Alberini C The neurobiological bases of memory formation: from physiological conditions to psychopathology. *Psychopathology.* Authors manuscript,2015 October; 47(6): 347–356.
15. Fujiwara E . Brain and behaviour in functional retrograde amnesia Bielefeld (Germany): Bielefeld University, 2004.

https://www.researchgate.net/publication/36448904_Brain_and_behaviour_in_functional_retrograde_amnesia (last accseeed 2019, 20 Feb.)

16. Camina E and Guell Francisco The Neuroanatomical, Neurophysiological and Psychological Basis of Memory: Current Models and Their Origins. Front. Pharmacol. 2017 June; 438(8):1-16.

17. MacKay, D. G. & James, L. E. Aging, retrograde amnesia, and the binding problem for phonology and orthography: a longitudinal study of "hippocampal amnesic" H.M. Aging, Neuropsychology, and Cognition. 2002;9: 298-333.

18. Shah B, Pattanayak RD, Sagar R. The Study of Patient Henry Molasion and What it taught Us Over Past 50years; Contribution to Neuroscience. Journal of Mental Health and Human Behavior, September 2014;19(2) :92-5.

19. Morgan Z and Squire LR. Neuroanatomy of Memory. Annu. Rev. Neurosci. 1993; 16:547-63.

20. Squire LR, Ojemann JG, Mienzin FM, Petersen SE, Videen TO, and Raichlem ME. Activation of the hippocampus in normal humans: A functional anatomical study of memory. Proc. Natl. Acad. Sci. 1992; 89: 1837-41.

21. Gabrieli JDE. Cognitive Neuroscience of Human Memory. Annu Rev Psychol. 1998; 49:87-115.

22. Chudasama Y and Robbins TW. Dopaminergic Modulation of Visual Attention and Working Memory in the Rodent Prefrontal Cortex. Neuropsychopharmacology. 2004; 29: 1628–36.

23. Tully T, Bourtchouladze R, Scott R, Tallman J. Targeting the CREB pathway for memory enhancers. Nat Rev Drug Discov. Apr 2003 ;2(4): 267-77.

24. Alexander G Murley, James B Rowe, Neurotransmitter deficits from frontotemporal lobar degeneration. Brain.2018 May;141(5) :1263–85.

25. Robbins WT. Neurochemistry of cognition. London. Oxford University Press;2016. Doi10.1093/med/9780199655946.001.0001 (accessed on 21 Feb 2019).

26. Stone TW and L Gail Darlington LG. The kynurenine pathway as a therapeutic target in cognitive and neurodegenerative disorders. Br J Pharmacol. 2013 Jul;169(6): 1211-27.
27. Sarter M, Bruno JP and Parikh V. Abnormal Neurotransmitter Release Underlying Behavioral and Cognitive Disorders: Toward Concepts of Dynamic and Function-Specific Dysregulation. Neuropsychopharmacology. 2007 Jul;32(7):1452-61.
28. Palmer E. Transient Global Amnesia and the Amnestic Syndrome. Medical Clinics of North America.1986 Nov; 70(6):1361-1374.
29. Fradera A and Kopelman MD. Disorders of memory. Brain. 2002 Oct;125(10):2152-90.
30. Aggleton JP and Saunders RC. The relationships between temporal lobe and diencephalic structures implicated in anterograde amnesia. Memory. 1997; 5(12): 49-71.
31. Brown, A. S., Consolidation Theory and retrograde amnesia in Humans, Psychon Bull Rev. 2002 Sep;9(3):403-25.
32. Alberini CM, Travaglia A . Infantile Amnesia: A Critical Period of Learning to Learn and Remember. Journal of neurosciences, 2017 Jun 14;37(24):5783-95.
33. Morgan CT, King AR, Weisz RJ and Schoplar J. Introduction to psychology. 7th ed. New Delhi. Tata Mcgraw Hill Publications. 2004; 208-212.
34. Tipps ME, Raybuck JD, and Lattal KM. Substance abuse, memory, and post-traumatic stress disorder. Neurobio learn Mem. 2014 July; 0:87-100.
35. Gaugler, J, Bryan J, Johanson T, Marin A and Weuve J.2018 Alzheimer's Disease Facts and Figures. Alzheimer's Dement. 2018;14(3):367-429. Available at: https://alz.org/ (Last Accessed 23 Feb 2019).

36. Sheikh S. Memory's Last Breath; Living with Alzheimer's in India. News 18 [Newspaper on Internet]. 2018 July 29; [citied 2019 Feb 23]. Available from: https://www.news18.com/news/india/memorys-last-breath-living-with-alzheimers-in-india-1827271.html (Last Accessed 23 Feb 2019).
37. Alzheimer's Disease Looms as Elderly Population in India Rises. The Tribune [Newspaper on Internet]. 2017 Sep 20; [citied 2019 Feb 23]. Available from: https://www.tribuneindia.com/news/health/alzheimer-s-disease-looms-as-elderly-population-in-india-rises/470185.html. (Last Accessed 23 Feb 2019).
38. Amnesia. Mayo clinic [document on internet]. Mayo Foundation for Medical Education and Research. 2017 Aug 25: [citied 2019 Feb 23]. Available from: https://www.mayoclinic.org/diseases-conditions/amnesia/symptoms-causes/syc-20353360. (Last Accessed 23 Feb 2019).
39. Shaji KS, Girish N, Srikala B, Dias A, Varghese M, Joteeswaran AT. The Dementia India Report 2010. Prevalence, impact, costs and services for dementia. ARDSI, New Delhi.http://ardsi.org/downloads/ExecutiveSummary.(Last Accessed 23 Feb 2019).
40. Bishara D, sauer J and Taylor D. The pharmacological management of Alzheimer's disease. Progress in Neurology and Psychiatry. 2015 Aug :14(1):16 – 22.
41. Jameson JL, Fauci SF, Braunwald E, Kasper DL, Hauser SL, Longo DL, et al . Harrison's Principles of Internal Medicine. 20th ed. [monograph on the Internet] New Delhi. : The McGraw-Hill Companies Inc.; 2018 [cited 2019 Feb 23] Available from: http://www.accesspharmacy.com. (Last Accessed 23 Feb 2019).

42. Luis CO and Rayan TJ. United States of Amnesia: rescuing memory loss from diverse conditions. Dis Model Mech. 2018 May 1; 11(5): 33-55.

43. Hong-Qi Y, Zhi-Kun S, Sheng-Di C. Current advances in the treatment of Alzheimer's disease: focused on considerations targeting Aβ and tau. TranslNeurodegener. 2012 Oct 30;1(1):21.

44. Rang HP, Dale MM, Ritter JM., Flower RJ, Henderson G. Rang and Dale's Pharmacology. 7th ed. Elsevier. 2007; 476-91.

45. Tripathi K.D. Essentials of Medical Pharmacology. 7thed. New Delhi. Jaypee Brothers medical Publishers. 2010; pp 486-491.

46. Blandina P, Efoudebe M, Cenni G, Mannaioni P, and Passani MB. Acetylcholine, Histamine, and Cognition: Two Sides of the Same Coin. Learn Mem. 2004 Jan;11(1):1-8.

47. Keller JN, Schmitt FA, Scheff SW, Ding Q, Chen Q, Butterfield DA et al. Evidence of increased oxidative damage in subjects with mild cognitive impairment. Neurology. 2005; 64:1152–56.

48. Zanelli SA, Solenski NJ, Rosenthal RE, Fiskum G. Mechanisms of ischemic neuroprotection by acetyl-l-carnitine. Ann NY Acad Sci. 2005;1053: 153–61.

49. Zhao C, Deng W, Gage FH. Mechanisms and Functional Implications of Adult Neurogenesis. Cell. 2008 February; 22:645–660.

50. Acharya K.R, Sturrock ED, Riodan JK, Ehlers MR. ACE revisited: A New Target for Structure-Based Drug Design. Nat Rev Drug Discov. 2003; 2 (11): 891–902.

51. Redelinghuys, P.; Nchinda, A.T.; Sturrock, E.D. Development of Domain-Selective Enzyme Inhibitors. New York Academy of Science.2005; 1056: 160–75.

52. Brown NJ and Vaughan DE, Angiotensin-Converting Enzyme Inhibitors. AHA Journals, 1998;97:1411-420.

53. Goodman, Louis S., Laurence L. Brunton, Bruce Chabner, and Björn C. Knollmann. Goodman & Gilman's Pharmacological Basis of Therapeutics. 12th Ed New York: McGraw-Hill. 2011;721-744.

54. Olvera LE, Terrell JM. Lisinopril. [Updated 2019 Jan 20]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2018 Jan [citied 2019 Feb 23] Available from: https://www.ncbi.nlm.nih.gov/books/NBK482230/.(Last accessed 23 Feb 2019)

55. Perindopril. National Center for Biotechnology Information. PubChem Compound Database; CID=107807, https://pubchem.ncbi.nlm.nih.gov/compound/107807 (accessed Feb. 23, 2019).

56. Todd PA and Fitton A. Perindopril: A Review of its Pharmacological Properties and Therapeutic Use in Cardiovascular Disorders. Drugs. 1991; 42:90-114.

57. Natesh R, SchwagerSL,EvansH,SturrocED,andAcharya KR .Structural Details on the Binding of Antihypertensive Drugs Captopril and Enalaprilat to Human Testicular Angiotensin I-Converting Enzyme. Biochemistry.2004; 43(27):8718-24.

58. Drug Record Perindopril, Liver tox. Clinical and research information on drug induced liver injury. https://livertox.nih.gov/Perindopril.htm [Last accessed on 2019 Feb 23].

59. Mansourian FW, Chang Y, et al. Insulin-degrading enzyme regulates the levels of insulin, amyloid beta-protein, and the beta-amyloid precursor protein intracellular domain in vivo. ProcNatlAcadSci U S A. 2003;100(7):4162-7.

60. Rao YM, Lakshmi IA and Saroja M. Effect of ACE Inhibitors on Anti-oxidant Status in Stretozotocin induced Diabetic Rats. Asian Journal of Pharmaceutical and Clinical Research.2011;4(1) 134-137.

61. Wyss JM, Kadish I, van Groen T. Age-related decline in spatial learning and memory: attenuation by captopril. ClinExpHypertens. 2003;25(7):455–474.

62. Dincolantonion JJ, Lavie JC and Keefe HJ. Not All Angiotensin-Converting Enzyme Inhibitors Are Equal: Focus on Ramipril and Perindopril Postgraduate Medicine. 2013 July 4; 125 (4): 1941-9260.

63. Tully T, Bourtchouladze R, Scott R, Tallman J. Targeting the CREB pathway for memory enhancers. Nat Rev Drug Discov. 2003 Apr;2(4): 267-77.

64. Narwal S, Saini DR, Kumari K, Singh G, Negi RS et al. Behavior & Pharmacological Animal Models for the Evaluation of Learning & Memory Condition. Indo Global Journal of Pharmaceutical Sciences.2012; 2(2): 121-129.

65. Morris R. Developments of a water maze procedure for studying spatial learning in the rat. Journal of Neuroscience Methods. 2006;1(2): 848-858.

66. Itoh J, Nabeshima T and Kameyama T. Utility of an elevated plus-maze for the evaluation of memory in mice: effects of nootropics, scopolamine and electroconvulsive shock. Psychopharmacology (Berl). 1990; 101(1): pp 27-33.

67. Deb, D, Bairy, L, Nayak V &Rao M. (2015). Comparative Effect of Lisinopril and Fosinopril in Mitigating Learning and Memory Deficit in Scopolamine-Induced Amnesic Rats. Advances in Pharmacological Sciences. 2015 July; 14:1-11.

68. Jawaid T, Jahan S, Kamal M. A comparative study of neuroprotective effect of angiotensin converting enzyme inhibitors against scopolamine-induced memory impairments in rats. J Adv Pharm Technol Res. 2015;6(3):130-5.

69. Raoufi, N, Moshfegh, A, Piri, M, Shahin MS &Nami M. Activation of the Nitric-Oxide System in Nucleus Accumbens Inhibits the Nicotine Reversal Effects upon Ethanol-Induced Amnesia. Journal of Advanced Medical Sciences and Applied Technologies. 2016; 2: 151-161.

70. Tayyaba Z, Hasnain SK, Hasan N. Evaluation of the oral hypoglycemic effect of Trigonellafoenum-graecum L. (methi) in normal mice. Journal of ethnopharmacology. 2001 June; 75: 191-95.

71. Bromley-Brits K, Deng Y, Song W. Morris Water Maze Test for Learning and Memory Deficits in Alzheimer's Disease Model Mice. J Vis Exp.2011 Jul 20;(53):2920.

72. Maei HR, Zaslavsky K, Teixeira CM and Frankland PW. What is the most sensitive measure of water maze probe test performance? Front IntegrNeurosci. 09 March 2009; 3(4):1-9.

73. Moreno CM, Ignacio C, Burke P & Middleton F. Molecular Aspect of Alcohol and Nutrition. 1st ed.Elsevier Publication 2016 ; 45-61.

74. MN Ghosh. Fundamentals of experimental pharmacology. 6th ed. Kolkata: Hilton and company; 2015. 171-178.

75. Kopelman MD. Disorders of memory. Brain. 2002 Oct; 125(10): 2152-90.

76. Mohandas E, Rajmohan V, Raghunath B. Neurobiology of Alzheimer's Disease. Indian Journal of Psychiatry. 2009; 51:55-61.

77. Reed JM and Squire LR. Retrograde Amnesia for Facts and Events: Findings from Four New Cases. Journal of Neuroscience .15 May 1998; 18 (10): 3943-954.

78. Singh B, Sharma B, Jaggi AS, Singh N. Attenuating effect of lisinopril and telmisartan in intracerebroventricularstreptozotocin induced experimental dementia of Alzheimer′s disease type: Possible involvement of PPAR-g agonistic property. J Renin Angiotensin Aldosterone Syst. 2013; 14:124-36**.**

79. Jackson L, Eldahshan W, Fang S and Ergal A. Within the Brain : the Renin Angiotensin System. Int J Mol Sci. 2018 March;19(3):876.

80. Rashid H, Mahboob A and Ahmed T. Role of Cholinergic receptor in memory retrieval depends on gender and age of memory .Behav Brain Res. 2017 Jul 28;331:233-240.

81. Maashoody T, Zal F and Rastegar K. Perindopril may improve the hippocampal reduced glutathione content in rats. Adv Pharm Bull. 2014 Jun; 4(2): 155–159.

82. Ferrari R. Treatment with Angiotensin Converting enzymes inhibitors :insight into perindopril cardiovascular protection. Eur heart j. 2008 sep ;10: G13- G20.

83. Dinicolantonio JJ, Lavie CJ, O'Keefe JH. Not all angiotensin -converting enzyme inhibitors are equal; focus on ramipril and perindopril. Postgrad Med.2013 Jul;125(4);154-6.

84. Mattson MP. Apoptosis in Neurodegenerative disorders. Nat Rev Mol Cell Biol. 2000 Nov;1(2): 120-9.

85. Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj EK *et al*. Oxidative damage is the earliest event in Alzheimer disease, J. Neuropathol. Exp. Neurol. 2001 Aug; 60(8):759–767.

86. Menigoz A, Ahmed T, Sabanov V, Pinto S, Kerselars S, Segal A *et al*. TRPM4-dependent post-synaptic depolarization is essential for the induction of NMDA receptor-dependent LTP in CA1 hippocampal neuronsPflugers Arch. 2016; 468: 593–607.
87. Ansari, F.A., Ali, S.N., Mahmood, R., Sodium nitrite-induced oxidative stress causes membrane damage, protein oxidation, lipid peroxidation and alters major metabolic pathways in human erythrocytes. Toxicology in Vitro. 2015 July; 29:1878–86.
88. Hlinak Z, Krejcí I, Hondlik J and Yamamoto A . Behavioral consequences of sodium nitrite hypoxia in male rats: amelioration with alaptide treatment, Methods Find. Exp. Clin. Pharmacol. 1990 July ;12 (6): 385-393.
89. Naseer IM, Ullah I, Narasimhan ML, Lee HY, BressanRA ,Yoon GH *et al.* Neuroprotective effect of osmotin against ethanol-induced apoptotic neurodegeneration in the developing rat brain. Cell Death & Disease. 2014 march ;53(5):1150-63.
90. Bhattacharya SK. Nootropic Effect of BR-16A (Mentat), a Psychotropic Herbal Formulation, on Cognitive Deficits-induced by Prenatal Undernutrition, Postnatal Environmental Impoverishment and Hypoxia in Rats. Indian Journal of Experimental Biology. 1994; (44)1: 45-48.
91. Ferrari R. Treatment with Angiotensin Converting enzymes inhibition in cardiovascular disease: evidence with perindopril. Expert Rev CardiovascTher. 2005; 3:15-29.
92. Dinicolantonio JJ, Lavie CJ and O'keefe JH. Not all angiotensin-converting enzymes inhibitors are equal: focus on ramipril and perindopril. Postgrad Med. 2013; 125(4): 154-68.

93. Tadic M, Cuspidi C and Hering D. Hypertension and cognitive dysfunction in elderly: blood pressure management for this global burden. BMC Cardiovascular Disorders. 2016; 16:208.

94. Norris D. Short-term memory and long-term memory are still different. Psychol Bull. 2017;143(9):992–1009.

95. FauciSF, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL et al. Harrison's Principles of Internal Medicine. 17th ed. New Delhi. Mcgraw Hill Publications. 2012;2536-49.

96. MünzelTandKeaney FJ Are ACE Inhibitors a "Magic Bullet" Against Oxidative Stress? Circulation AHA. 2001; 104:1571-74.

97. Stahl SM. Essential Psychopharmacology-Neuroscientific basis and practical applications. 2nd ed. New York. Cambridge university press. 2000; pp 459-498.

ANNEXURE I

ETHICAL CLEARANCE CERTIFICATE

K.L.E.UNIVERSITY'S

JAWAHARLAL NEHRU MEDICAL COLLEGE,
Nehru Nagar, Belgaum – 590 010. Karnataka, India
CENTRAL ANIMAL HOUSE

Phone: (0831) 2471701 (O) / 2471350
Fax No. 0831 – 2470759
Website : http: //www.jnmc.edu
E.Mail: domejnmc@sancharnet.in

MDC/AH/ 2014-15 Date : 23-12-14.

CERTIFICATE

This is to certify that the M.Sc / M.D / Ph.D / Research project entitled : "Effect of Lisinopril and Perindopril in Sodium nitrite and Ethanol induced Anterograde Amnesia in Male Wistar Rats- An Experimental Study". Submitted by : REG. NO. BO0114002 ., Dept. of Pharmacology, JNMC.

Has been approved by the Institutional Animal Ethical Committee meeting held on 23/12/14 vide Resolution No. 5/4.

Animals sanctioned : WISTAR RATS : 90 (M) .

Signature :

Chairman / Mem. Secretary, IAEC, JNMC.

Main Nominee CPCSEA IAEC, JNMC

Name :

Dr. S.N. Sambrekar
Chairman / Mem. Secretary, IAEC, JNMC.

Dr. S. Ramachandra Setty
Main Nominee CPCSEA IAEC, JNMC