THE EFFECT OF SIMVASTATIN AND ROSUVAStATIN ON
RELIEVING THE SYMPTOMS OF ALZHEIMER’S
DISEASE IN A MOUSE MODEL

BY

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DECLARATION

I, Alice Kanyemba, certify that this dissertation is my original work and has been prepared in accordance with guidelines of the Master of Science in Clinical Pharmacology program, University of Zimbabwe. I further attest that this work has not been submitted, in part or in full, for any other degree at any university and/or any publication.

Signature_______________________________________________
Date__________________________

I, having supervised and read this dissertation, I am satisfied that this is the original work of the author in whose name it is being presented. I confirm that the work has been completed satisfactorily for presentation in the examination.

Name of Supervisor______________________________________________
Signature________________________________________________________
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Date_______________________

Chairman of the Department of Clinical Pharmacology, University of Zimbabwe

Signature________________________________________________________
Date_______________________
DEDICATION

I dedicate this to everyone who has stood by me and loved me at various stages throughout my life. I thank you for allowing me to be what I can.
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ABBREVIATIONS

Aβ - β amyloid protein
ACh - Acetylcholine
AD - Alzheimer’s disease
ADCLT - Alzheimer Disease Cholesterol Lowering Treatment
AAR - Alternate Arm Returns
BBB - Blood Brain Barrier
CSF - Cerebrospinal fluid
CNS - Central Nervous System
CLASP - Cholesterol Lowering Agents To Slow Progression Of Alzheimer’s Disease study
DSM V - Diagnostic and Statistical Manual of Mental Disorders 5
DI - Discrimination Index
FDA - Food And Drug Administration Agency
LLAs - Lipid Lowering Agents
LEAD_e - The Atorvastatin/Donepezil in Alzheimer Disease Study
MCI - Mild Cognitive Impairment
NFTS - Neuro-fibrillary tangles
NOR - Novel Object Recognition test
SAR - Spontaneous Arm Returns
SAP - Spontaneous alternation percentage
STM - Short Term Memory
ABSTRACT

**Background:** Alzheimer's disease (AD) is the most common form of dementia affecting nearly 36 million people globally. None of the currently approved drugs prevent or even slow the course of the disease.

**Objective:** To investigate effects of simvastatin and rosvastatin in relieving symptoms of AD in mice.

**Methods:** The design was experimental using a mice model. A total of 72 Balb, male mice aged 8-12 weeks were divided into 9 groups with 8 mice in each group. Scopolamine 1mg/kg/day was given intraperitoneally over a period of 2 weeks to induce AD. Study groups were control, simvastatin 5mg/kg/day, simvastatin 10mg/kg/day, simvastatin 20mg/kg/day, rosvastatin 10mg/kg/day, rosvastatin 20mg/d/day, rosvastatin 40mg/kg/day and donepezil 5mg/kg/day administered by oral gavage for 2 weeks. Y-maze to assess short term memory and Novel Object Recognition test to assess cognition, short-term, intermediate and long-term memory were administered starting 24 hours after the last dose of the experimental drugs. Parameters assessed with Y-maze were total arm entries, same arm entries, alternate arm entries, percentage spontaneous arm alternations. Data were analysed using One way or Two way ANOVA with an appropriate post hoc where required. An a priori alpha level of 0.05 was set for all statistical analysis.

**Results:** All 8 mice in simvastatin 20mg/kg group died therefore this group was excluded from statistical analysis. There was no significant difference in the parameters used in the Y-maze test across all treatment groups. Total Arm Returns (F=0.5708, d.f. = 6; 28, p=0.9396), Same Arm Returns (F= 0.4762, d.f= 6; 28, p=0.820), Alternate Arm Returns (F=0.4272, d.f 6,28, p= 0.8545) and percentage spontaneous alternation ( F=0.2911, d.f= 6,28, p= 0.9361). Similarly, no significant difference were noted in the time spent exploring familiar objects across the experimental groups (F=0.4578, d.f=6;28, p=0.7453). However, significant differences were noted on the time spent exploring novel objects across the treatment groups (F=5.755, d.f= 6,28, p= 0.0005). The discrimination indices were found to be different between groups (F= 8.031, d.f= 6.28, p< 0.001; Kruscal-Wallis test $\chi^2= 20.46; p=0.0023$.

**Conclusion:** Repeated dosing of simvastatin and rosvastatin had no effect on memory in mice. No dose response relationship in reversing symptoms of AD with these statins was observed.
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RESEARCH TOPIC: THE EFFECT OF SIMVASTATIN AND ROSUVA STATIN ON RELIEVING THE SYMPTOMS OF ALZHEIMER’S DISEASE IN A MOUSE MODEL
CHAPTER 1: INTRODUCTION

Alzheimer’s disease (AD) was first characterised by Louis Alzheimer in 1907. It is a gradually progressive dementia affecting cognition, behaviour and functional status. It is the most common form of dementia and is a degenerative brain disease of unknown cause that usually starts in the middle age or old age. Neurons are the chief cells destroyed by AD. In the brain, neurons connect and communicate with synapses where tiny bursts of chemicals called neurotransmitters carry information from one cell to another. AD destroys this process and eventually destroys synapses and kills neurons, damaging the brain’s communication network. AD results in progressive memory loss, impaired thinking, disorientation and changes in personality and mood. In advanced cases, AD is accompanied by a profound decline in cognitive and physical functioning. Although drugs may reduce AD symptoms for a time, the disease is eventually fatal.

1.1 PROBLEM STATEMENT AND RESEARCH SIGNIFICANCE

According to Alzheimer’s Drug Discovery Foundation, nearly 36 million people globally are believed to be living with Alzheimer’s or other dementias. AD is predicted to affect 1 in 85 people globally by 2050. The prevalence of AD in people aged 85 or older is 85%. Furthermore, life expectancy is increasing in developing countries with an anticipated rise in AD patients as well. Data on the prevalence and economic burden of AD in developing counties, including Zimbabwe, is scarce.

None of the currently approved drugs prevent or even slow the course of AD. Current AD drugs are expensive. The economic and social costs are staggering. It is the third most expensive illness in the United States after heart disease and cancer, and the majority of
medical and caregiving expenses are left to the patients' families and to government programs. In 2005 in US, annual Medicare costs were estimated at $91 billion and Medicaid costs for institutionalized AD patients were estimated at $21 billion. According to Alzheimer’s Association, the total national cost of AD in the same country in 2013 was estimated at $203 billion. This figure is expected to rise to $1.2 trillion by 2050\textsuperscript{2}. Also from the same source, with life expectancy and the number of AD cases increasing, the cost of AD is projected to quadruple over the next 50 years. The potential financial burden of this disease on the healthcare system could reach crisis proportions in the near future unless more effective avenues are developed to provide care for these individuals, to prevent the disease from occurring, or to slow its progression. The US Food and Drug Administration (FDA) has approved 5 medications to treat symptoms of AD. Donepezil, galantine, rivastigmine and tacrine are cholinesterase inhibitors. Memantine, is an NMDA (N-methyl-D-aspartate) receptor antagonist, which works by regulating the activity of glutamate, a chemical messenger involved in learning and memory. On average, the 5 approved Alzheimer’s drugs are effective for about 6-12 months for about half of the individuals who take them.

Repurposing or revisiting drugs is an exciting approach because we can study drugs that are already approved for use by physicians to treat other diseases. Current AD drugs only help mask the symptoms but do not treat the underlying disease. A breakthrough AD drug would treat the underlying disease and stop or delay the cell damage that eventually leads to worsening of symptoms.

In AD, B-amyloid protein (AB) is deposited in the form of extracellular plaques and previous studies have determined that AB generation is cholesterol dependent. Due to the role of statins in cholesterol reduction, it is biologically plausible they may be efficacious in the
treatment of AD and dementia\textsuperscript{3}. Use of statin therapy in established AD is a relatively unexplored area. Direct evidence of statin effects on treating dementia is lacking. However, numerous studies have examined the role of statins in the prevention of dementia and most studies have shown that statin use decreases occurrence of dementia. While evidence from retrospective case control studies suggests a beneficial role of statins in prevention of AD, a similar benefit has not been established in prospective cohort studies or clinical trial\textsuperscript{4}. Animal studies and observational clinical studies have also indicated that statins might be effective in treating certain neurological diseases, in particular, multiple sclerosis, AD and ischaemic stroke.\textsuperscript{5} The studies concluded that statins significantly reduced risk of any dementia.\textsuperscript{5} One study showed that widely used cholesterol lowering drugs, simvastatin and lovastatin reduced intracellular and extracellular levels of AB40 and AB42 peptides in primary cultures of hippocampal neurons and mixed cortical neurons.\textsuperscript{6}

There is need to reduce the economic, physical and social impact of AD in terms of prophylaxis and treatment of the disease. Due to the inconsistencies in literature and the knowledge gaps, more research is needed on the use of the statins in AD hence this study. Simvastatin and atorvastatin were chosen because relatively few studies (and conflicts) was on them and they are 2 of the 3 statins registered in Zimbabwe and are therefore easily available.
CHAPTER 2: LITERATURE REVIEW

2.1 EPIDEMIOLOGY

Alzheimer’s disease is the most common form of dementing illness, and the prevalence increases with each decade of life. Onset can be as early as age 40 years, resulting in the arbitrary age classifications of early onset (age 40–64 years) and late onset (age 65 years and older). Increasing age is the greatest risk factor for AD. The prevalence of AD increases exponentially with age, affecting approximately 7% of individuals aged 65 to 74 years, 53% of those aged 75 to 84 years, and 40% of persons aged 85 years and older. Alzheimer’s Drug Discovery Foundation states that nearly 36 million people globally are believed to be living with Alzheimer’s or other dementias. AD is projected to affect 1 in 85 people globally by 2050. The prevalence of AD in people aged 85 or older is 85%. Furthermore, the prevalence of AD is anticipated to continue increasing in developing countries in tandem with the increase in life expectancy. Data on the prevalence and economic burden of AD in developing countries, including Zimbabwe, is scarce.

Genetic inheritance is also a significant risk factor, although other factors may contribute. Factors determining age of onset and rate of progression remain largely undefined. Survival following AD diagnosis is typically 4 to 6 years. AD is the fifth leading cause of death for those aged 65 years and older in the United States. As noted by Brunnstrom 2009 and Englund 2009, AD may not cause death directly. The most common cause of death was noted to be pneumonia, possibly resulting from eating problems in the terminal stage of the disease.
The exact aetiology of AD is unknown; however, several genetic and environmental factors have been explored as potential causes of AD. Dominantly inherited forms of AD account for less than 1% of cases.\(^2,8,9\) Williamson et al. stated that more than half of early-onset, dominantly inherited cases of AD can be attributed to alterations on chromosomes 1, 14, or 21. The majority and most aggressive early-onset cases are attributed to mutations of a gene located on chromosome 14, which produces a protein called presenilin 1. A structurally similar protein, presenilin 2, is produced by a gene on chromosome 1. Both presenilin 1 and presenilin 2 encode for membrane proteins that may be involved in amyloid precursor protein (APP) processing. Scientists have identified more than 160 mutations in presenilin genes, and these mutations appear to result in reduced activity of secretase, an enzyme important in \(\beta\)-amyloid peptide (\(\beta\)AP) formation. APP is encoded on chromosome 21. Only a small number of early-onset familial AD cases have been associated with mutations in the \(APP\) gene, resulting in overproduction of \(\beta\)AP or an increase in the proportion of \(\beta\)AP ending at residue 42.\(^9\)

Genetic susceptibility to sporadic, late-onset AD is thought to be primarily linked to the apolipoprotein E (\(APOE\)) genotype.\(^9,10,11\) Thus far, the contribution of other candidate genes appears to be minor, although AD may be a heterogeneous disease resulting from complex interactions among multiple susceptibility genes and environmental factors. The gene responsible for the production of \(APOE\) is located on chromosome 19. There are three major subtypes or alleles of \(APOE\) (e.g., \(*2, *3,\) and \(*4\)). Humans inherit one copy of the \(APOE\) gene from each parent. Inheritance of the \(APOE*4\) allele is believed to account for much of the genetic risk in sporadic AD. The mechanism through which \(APOE*4\) confers an increased risk is unknown, although \(APOE*4\) is associated with other factors that may contribute to AD
pathology, such as abnormalities in mitochondria, cytoskeletal dysfunction, and low glucose usage.⁹ According to the same source, the risk for AD is 2- to 3-fold higher in individuals with one APOE*4 allele and 12-fold higher in individuals with two APOE*4 alleles compared to those with no APOE*4 alleles. Moreover, onset of symptoms occurs at a relatively younger age as compared with patients having zero or only one copy of APOE*4 in their genotype.¹¹ The strength of association is not the same across all races however.¹² Although inheritance of the APOE*4 allele increases the risk of AD, it is not diagnostic or even essential for disease presence.

A number of environmental factors are associated with an increased risk of AD, including age, decreased reserve capacity of the brain (reduced brain size, low educational level, and reduced mental and physical activity in late life), head injury, Down syndrome, depression, herpes simplex, and risk factors for vascular disease (hypercholesterolemia, hypertension, atherosclerosis, coronary heart disease, elevated homocysteine, obesity, and diabetes).²,¹² Whether these vascular risk factors are true causal risk factors for AD contributing to AD pathology, or whether they result in cerebrovascular pathology that, in turn, contributes to the symptoms of AD, remains to be established. AD may develop in individuals over the course of decades suggesting that AD is a disease most people are in the process of developing throughout adulthood.¹⁴,¹⁵

2.2 PATHOPHYSIOLOGY / PATHOGENESIS

The signature lesions in AD are neuritic plaques and neurofibrillary tangles (NFTs) located in the cortical areas and medial temporal lobe structures of the brain.¹²,¹³ Along with these lesions, degeneration of neurons and synapses, as well as cortical atrophy occurs. Plaques and
NFTs may also be present in other diseases, even in normal aging, but at least in younger demographics there tends to be a higher burden of plaques and NFTs in AD-affected subjects than there is in age-matched controls. Several mechanisms have been proposed to explain changes in the brain that result in the symptoms of AD, including misfolding of proteins (βAP aggregation and deposition leading to the formation of plaques and hyper-phosphorylation of tau protein leading to NFT development); synaptic failure and depletion of neurotrophin and neurotransmitters; and mitochondrial dysfunction (oxidative stress, impaired insulin signalling in the brain, vascular injury, inflammatory processes, loss of calcium regulation, and defects in cholesterol metabolism).

AD destroys neurons and impairs the communication between neurons required for normal brain function. In advanced cases, AD is accompanied by a decline in cognitive and physical functioning. This is marked histologically by the degeneration of brain neurons (especially in the cerebral cortex) and by the presence of neurofibrillary tangles and plaques containing beta-amyloid. Neuritic plaques and neurofibrillary tangles are the pathologic hallmarks of AD.

2.3 TYPES OF DEMENTIA

Common types of dementia in late life include the following

- Alzheimer’s disease
- Vascular dementia
- Lewy body dementia
- Fronto-temporal dementia including Pick’s disease
• Reversible causes of dementia (e.g. normal pressure hydrocephalus, thyroid dysfunction, Vitamin B12 deficiency, depression)

2.4 THEORIES OF AD

There are several theories or hypotheses suggested to explain the pathophysiology of AD. These include the cholinergic, amyloid, tau and oxidative stress hypotheses.

2.4.1 The Cholinergic Hypothesis

The current mainstay of treatment as well as this study is based on this hypothesis. It states that memory impairment in AD results from depletion of acetylcholine (ACh), an excitatory chemical messenger that regulates learning and memory in the brain. The discovery of vast cholinergic cell loss led to the development of a cholinergic hypothesis linked to the pathophysiology of AD. Cholinergic cell loss was noted as the source of memory and cognitive impairment in AD. Multiple neuronal pathways are destroyed in AD. Neuronal damage can be seen in conjunction with plaque structures. Widespread cell dysfunction or degeneration results in a variety of neurotransmitter deficits, with cholinergic abnormalities being the most prominent. Loss of cholinergic activity correlates with AD severity. In late AD, the number of cholinergic neurons is reduced, and there is loss of nicotinic receptors in the hippocampus and cortex. Presynaptic nicotinic receptors control the release of acetylcholine, as well as other neurotransmitters important for memory and mood, including glutamate, serotonin and norepinephrine. Consequently, it was presumed that increasing cholinergic function would improve symptoms of memory loss. Others argue that this is
flawed for two reasons. First, cholinergic cell loss appears to be a secondary consequence of Alzheimer’s pathology, not the disease-producing event; second, cholinergic neurons are only one of many neuronal pathways destroyed in AD.\(^9\)

### 2.4.2 Amyloid Cascade Hypothesis

Neuritic plaques (also termed amyloid or senile plaques) are extracellular lesions found in the brain and cerebral vasculature. Plaques from AD brains largely consist of a protein called β-amyloid protein (Aβ).\(^1\) Aβ peptides consisting of 36 to 43 amino acids are produced via processing of a larger protein, APP. Aβ\(_{42}\) is less common than other Aβ peptides, but is prone to aggregation and plaque formation. The amyloid cascade hypothesis states that there is an imbalance between the production and clearance of Aβ peptides resulting in aggregation that causes accumulation of Aβ and ultimately leading to AD.\(^15\) The hypothesis has substantially evolved since it was initially proposed. While Aβ sequestered in plaques was at first believed to represent the critical toxic species, more recent versions of the hypothesis assume Aβ that is not sequestered in plaques actually drives the disease.\(^15\)

Even so, the amyloid cascade hypothesis seems most applicable in cases of early-onset, autosomal dominant AD. However, such cases comprise less than 1% of AD, and it is not clear whether it is reasonable to etiologically extrapolate to the late-onset form (which afflicts the vast majority of those affected). Whether individuals with late-onset AD also carry genetic variations that promote a primary Aβ amyloidosis remains to be shown.\(^12\)
2.4.3 Tau hypothesis

It postulates that a chemical change occurs in a protein called tau that keeps microtubules stable.\textsuperscript{2} Tau protein provides structural support to microtubules, the cell's transportation and skeletal support system.\textsuperscript{15} Microtubules are crucial structures to the cell’s internal transport system. In AD, tau protein is modified causing neurons’ microtubules to pair with other tubules forming tangles. Hippocampus and cerebral cortex in persons with AD are composed of abnormally hyper-phosphorylated tau protein. Ultimately, the microtubules disintergrate, blocking messengers involved in memory such as Ach. This contributes to bring death leading to memory loss. The density of the neurofibrillary tangles (NFTs) correlates well with the severity of the dementia, because they are a hallmark of neuronal death.\textsuperscript{15}

2.4.4 Oxidative stress hypothesis

Oxidative stress occurs when there is an imbalance between free radicals and their scavengers (antioxidants). Free radicals are unstable oxygen agents capable of reacting with other molecules such as DNA, proteins, lipids leading to changes in structure and function.\textsuperscript{1}

Other hypotheses proposed to explain AD pathogenesis include mitochondrial dysfunction, and postmenopausal loss of oestrogen in women. Each of these mechanisms may contribute to AD pathogenesis, but the extent of the contribution is uncertain. A single common mechanism for producing AD does not exist. Regardless of the source, however, the features remain the same: degeneration of neurons in higher brain areas; accumulation of NFTs and
neuritic plaques; profound destruction of cholinergic pathways; and an insidious dementia, slowly progressive until death.

2.5 SIGNS AND SYMPTOMS OF AD

The onset of AD is almost imperceptible, without abrupt changes in cognition or function. Deficits occur progressively over time, affecting multiple areas of cognition.\textsuperscript{2,12} For treatment and assessment purposes, Alzheimer's symptoms can be divided broadly into two basic categories: cognitive symptoms and non-cognitive (behavioural) symptoms. Cognitive symptoms are present throughout the illness, whereas behavioural symptoms are less predictable.

The patient may have vague memory complaints initially, or the patient's significant other may report that the patient is "forgetful." Cognitive decline is gradual over the course of illness. Behavioural disturbances may be present in moderate stages. Loss of daily function is common in advanced stages\textsuperscript{12}

Cognitive symptoms include memory loss (poor recall and losing items), aphasia (circumlocution and anomia), apraxia, agnosia, disorientation (impaired perception of time and unable to recognize familiar people), impaired executive function. Non-cognitive symptoms are depression, psychotic symptoms (hallucinations and delusions), behavioural disturbances (physical and verbal aggression, motor hyperactivity, uncooperativeness, wandering, repetitive mannerisms and activities, and combativeness).\textsuperscript{2} Functionally, inability to care for self (dressing, bathing, toileting, and eating) may be noted.
2.6 MANAGEMENT OF AD

Laboratory tests are needed to rule out vitamin B$_{12}$ and folate deficiency, hypothyroidism. In addition, blood cell counts, serum electrolytes, liver function and thyroid function tests are needed. Other diagnostic tests which may aid diagnosis include CT or MRI scans.

AD is still primarily a clinical diagnosis. The patient's history and examination should suggest that cognitive decline from a previously higher baseline has occurred. Until recently the only way to confirm a clinical diagnosis of AD was through direct examination of brain tissue at autopsy or biopsy, although the recent development of in vivo plaque detection methods may change this. Several criteria have been developed for the detection and diagnosis of dementia, including the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision (DSM-V-TR)* criteria, the Agency for Healthcare Research and Quality (AHRQ) Guidelines, the American Academy of Neurology Guidelines, the National Institute of Neurological Disorders and Stroke (NINDS) criteria, and the National Institute of Neurological Communicative Disorders and Stroke (NINCDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA) Criteria.

Almost any medication can contribute to cognitive impairment in vulnerable individuals, but certain classes of medication are more commonly implicated. Benzodiazepines and other sedative hypnotics, anticholinergics, opioid analgesics, antipsychotics, and anticonvulsants have been associated with cognitive impairment.$^{16,17}$ NSAIDs, histamine H$_2$-receptor antagonists, digoxin, amiodarone, antihypertensives, and corticosteroids have been implicated in cases of delirium.$^{16}$ Because medications are a reversible cause of cognitive symptoms, medication review and management are essential.

It has long been recognized that aging individuals experience changes in cognitive function. Mild cognitive impairment (MCI) constitutes a syndromic designation that categorizes
patients with cognitive complaints insufficient to interfere with life.\textsuperscript{18} Persons diagnosed with MCI carry a 10\% to 15\% chance per year of progressing to an AD diagnosis. A logical extension of this is that what clinicians are actually seeing in most people with MCI is the initial manifestation of a progressive degenerative dementia that will eventually meet AD diagnostic criteria.\textsuperscript{20} However, it is important to note that not everyone meeting MCI criteria does or will have AD.

\section*{2.6.1 TREATMENT OF AD}

The primary goal of treatment in AD is to symptomatically treat cognitive difficulties and preserve patient function as long as possible. Secondary goals include treating the psychiatric and behavioural sequelae that occur as a result of the disease. Current AD treatments have not been shown to prolong life, cure AD, or halt or reverse the pathophysiologic process.

Non-pharmacologic therapy interventions are the current primary interventions for management of AD, and medications should be used in the context of multimodal interventions. Behavioural and psychiatric symptoms are among the most challenging and distressing symptoms of the disease and may be the determining factor in a family's decision to seek institutional care. Symptoms such as sleep disturbances, wandering, urinary incontinence, agitation, and aggression in patients with dementia are best managed using behavioural interventions rather than medications whenever possible.\textsuperscript{20, 21}

Pharmacotherapy of non-cognitive symptoms is primarily empiric, with side-effect profiles used as a guide in selecting the appropriate treatment because of limited clinical data.\textsuperscript{33} Psychotropic medications with anticholinergic effects should be avoided because they may actually worsen cognition and interfere with cholinesterase inhibitor therapy.\textsuperscript{1} Other side
effects in the elderly include sedation, medication-induced postural instability, and extrapyramidal side effects, which can decrease the clinical usefulness of traditional psychotropic agents.

The Food and Drug Administration (FDA) agency has approved 5 medications to treat symptoms of AD. These work mainly by two mechanisms. Firstly are cholinesterase inhibitors which work by slowing down the disease activity by preventing the breakdown of acetylcholine (ACh). Acetylcholine is believed to be important for memory and thinking. Examples of cholinesterase inhibitors include donepezil, galantine, rivastigmine and tacrine.

The second mechanism involves regulation of glutamate activity at the NMDA receptor (N-methyl-D-aspartate). Glutamate is neurotransmitter involved in learning and memory. Memantine, an agonist at NMDA receptors, protects brain cells against excess glutamate release by cells damaged by AD and other neurological disorders. Attachment of glutamate to cell surface “docking sites” called NMDA receptors permits calcium to flow freely into the cell. Over time this leads to chronic overexposure to calcium, which can speed up cell damage. Memantine prevents this destructive chain of events by partially blocking the NMDA receptors. Cholinesterase inhibitors and memantine are used to treat cognitive symptoms of AD.

2.7 STATINS AND AD

Statins interfere with substances important for memory formation such as cholesterol and coenzyme Q10 as well as dolichols, biochemicals that transport genetic instructions from DNA in order to help manufacture specific proteins. Dolichols play an important role in cellular processes and influence all the hormones involved with your emotions and moods.
Insufficient dolichol causes the entire process of neurohormone production to be altered in a way that could affect both mood and memory.\(^1\)

At the same time, statins damage mitochondrial DNA and brain cells. In the brain, glial cells manufacture the cholesterol the brain needs for cognitive function. Statins stop the glial cells from producing cholesterol in the same way they stop liver from producing it. The result is that the brain doesn’t get enough cholesterol to function effectively.\(^1\)

Lovastatin, simvastatin and cerivastatin (no longer available in the US) are able to cross the blood–brain barrier (BBB) (lipophilic statins) into the central nervous system (CNS), while others such as atorvastatin, pravastatin, rosuvastatin and fluvastatin do not. Simvastatin is a lipophilic statin, and crosses the blood-brain barrier more readily than less lipophilic statins such as pravastatin, fluvastatin, and rosuvastatin.\(^6, 22\) The statins are thought to reduce the amount of Aβ peptides by reducing cholesterol from the blood and/or the cerebrospinal fluid (CSF). By reducing the amount of Aβ peptides, they may reduce the incidence or the progression of AD.\(^5\) Some believe statins which do not cross the BBB should be preferred as those that cross the BBB may increase the rate of neuronal death by decreasing cholesterol beyond what is needed for neuronal formation.

Use of statin therapy in established AD is a relatively an unexplored area. Direct evidence of statin effects on treating dementia is lacking. However, numerous studies have examined the role of statins in the prevention of dementia and most studies have shown that statin use decreases occurrence of dementia. Animal studies and observational clinical studies have also indicated that statins might be effective in treating certain neurological diseases, in particular, multiple sclerosis, AD and ischaemic stroke.\(^5\) Similarly, evidence from
retrospective case control studies suggests a beneficial role of statins in prevention of AD. However, a similar benefit has not been established in prospective cohort studies or clinical trials. The studies concluded that statins significantly reduced risk of any dementia.\textsuperscript{22, 6}

In AD, β-amyloid protein (Aβ) is deposited in the form of extracellular plaques and previous studies have determined that Aβ generation is cholesterol dependent. Elevated Aβ level is regarded as a risk factor for AD. Because of the effect of statins in cholesterol reduction, it is biologically plausible that they may be efficacious in the treatment of AD and dementia.\textsuperscript{23}

### 2.7.1 Clinical studies

Alzheimer Disease Cholesterol Lowering Treatment (ADCLT) 2005 included 63 patients who were treated with atorvastatin 80mg or matching placebo. No benefit of statins in AD symptoms was noted.\textsuperscript{23} Cholesterol Lowering Agent to Slow Alzheimer’s disease Study (CLASP) 2008 was a multicentre, randomised, double blind, placebo controlled trial of simvastatin to slow progression of AD. Four hundred patients from 40 centres were used. Participants were given 20mg simvastatin or placebo for 6 weeks followed by 40mg of simvastatin or placebo for 18 months. Results of study were not available online.\textsuperscript{24}

The Atorvastatin/Donepezil in Alzheimer’s Disease (LEAD\textsubscript{4}) study was a randomized controlled trial (RCT) evaluating the efficacy and safety of atorvastatin in patients with mild to moderate AD. This was an international, multicentre, double-blind, randomized, parallel-group study. Subjects had mild to moderate probable AD (Mini-Mental State Examination score 13–25), were aged 50–90 years, and were taking donepezil 10 mg daily for ≥3 months prior to screening. Entry low-density lipoprotein cholesterol levels (LDL-C) were >95 and <195 mg/dL. Patients were randomized to atorvastatin 80 mg/day or placebo for 72 weeks followed by a double-blind, 8-week atorvastatin withdrawal phase. Co-primary endpoints were changes in cognition (Alzheimer's Disease Assessment Scale-Cognitive Subscale
[ADAS-Cog]) and global function (Alzheimer's Disease Cooperative Study Clinical Global Impression of Change [ADCS-CGIC]) at 72 weeks. A total of 640 patients were randomized in the study. There were no significant differences in the co-primary endpoints of ADAS-cog or ADCS-CGIC or the secondary endpoints. Atorvastatin was generally well-tolerated. This study provided Class II evidence that intensive lipid lowering with atorvastatin 80 mg/day in patients with mild to moderate Alzheimer disease (aged 50–90), taking donepezil, with low-density lipoprotein cholesterol levels between 95 and 195 mg/dL over 72 weeks does not benefit cognition (as measured by Alzheimer's Disease Assessment Scale-Cognitive Subscale) \((p = 0.26)\) or global function (as measured by Alzheimer's Disease Cooperative Study Clinical Global Impression of Change) \((p = 0.73)\) compared with placebo.\(^{25}\)

One study investigated the effect of atorvastatin on memory in patients with high blood pressure, a condition known to result in cognitive impairment. Researchers found no significant difference in memory and psychomotor functions (the relationship between cognitive function and physical movement) between patients receiving atorvastatin and those not receiving the drug.\(^{23}\) Another study by Alberto Serrano-Pozo. 2010 titled “Effects of Simvastatin on Cholesterol Metabolism and Alzheimer Disease Biomarkers” indicated that simvastatin treatment can affect brain cholesterol metabolism within 12 weeks, but did not alter molecular indices of AD pathology during this short-term treatment. It was a 12-week open-label trial with simvastatin 40 mg/d for 4 weeks and then 80 mg/d for 8 weeks in 12 patients with AD or amnestic mild cognitive impairment and hypercholesterolemia.

### 2.7.2 Animal studies

In a study evaluating the effect of statins on Aβ40 and Aβ42 in guinea pigs, simvastatin and lovastatin reduced intracellular and extracellular levels of Aβ40 and Aβ42 peptides in
primary cultures of hippocampal neurons and mixed cortical neurons. Likewise, the same study showed that guinea pigs treated with high doses of simvastatin showed a strong and reversible reduction of cerebral Aβ42 and Aβ40 levels in the CSF and brain homogenate.

Milind and Nirmal (2007) investigated the beneficial effects of atorvastatin and simvastatin on cognitive dysfunction of rats. A total of 38 groups of rats were used after inducing amnesia with scopolamine and high fat diet. The study highlighted the ameliorative role of statins in experimental amnesia with possible involvement of their cholesterol dependent as well as cholesterol independent actions.

Georgieva-Kotetaroa investigated the effect of 90 day treatment with atorvastatin and rosuvastatin on learning and memory processes of rats without brain damage. Male Wistar rats were treated orally for 90 days with atorvastatin and rosuvastatin in a dose 10 and 20 mg/kg body weight in parallel with vehicle treated group. After this period learning ability and memory retention were evaluated. The study concluded that atorvastatin (10 and 20 mg/kg body weight) and rosuvastatin (10 mg/kg body weight) improve cognitive function in rats after 90 day treatment.

An investigation on the effects of a late subchronic rosuvastatin treatment (0.2 or 2.0 mg/kg) for 21 days, on iron- and age-induced cognitive deficits by Rafael (2010) showed that rosuvastatin improved recognition memory deficits associated with iron loading and aging, providing evidence that statins may be considered for the treatment of age-associated cognitive decline.
In addition, lipid lowering agents (LLAs) may slow cognitive decline in AD and have a neuro-protective effect.\textsuperscript{30} It is also accepted that statins have anti-inflammatory effects that are independent of their ability to lower cholesterol. The anti-inflammatory effects of statins may be beneficial in neurologic disease.\textsuperscript{5}

Based on the studies mentioned above, it’s safe to say the jury is still out on whether statins improve memory or prevent Alzheimer’s. Studies of statins show highly variable outcomes, making it difficult to draw firm conclusions. Several confounding factors among the studies, including differing blood-brain barrier permeabilities among statins, the stage in AD at which statins were administered, and the drugs’ pleiotropic metabolic effects, all of which possibly contributed to the substantial variability observed to date. It has been recommended that future human studies of this important therapeutic topic (1) take the blood-brain barrier permeabilities of statins into account when analysing results, (2) include specific analyses of the effects on low- and high density lipoprotein cholesterol, and, most important, (3) conduct statin treatment trials solely in patients with mild AD, who have the best chance for disease modification.

2.8 METHODS OF INDUCING DEMENTIA IN MICE

2.8.1 Transgenic mice model

During the past decades, numerous stable transgenic mouse strains have been developed. Transgenic mouse models can mimic a range of Alzheimer’s disease-related pathology. The models have contributed significant insights into the pathophysiology of beta-amyloid toxicity, particularly the effects of different beta-amyloid species and the possible pathogenic role of beta-amyloid oligomers. They are also useful in preclinical testing of potential
therapies. None of the models however fully replicates the human disease. Furthermore, the models address only the familial form of the disease, which barely represents 5% of AD cases.\textsuperscript{31} The mice are also costly.

\subsection*{2.8.2 Aluminium Chloride model}

Neurotoxicity from exposure to aluminium causes impairment of learning memory and cognition. Concentrations of aluminium in the brain of AD patients are significantly high.\textsuperscript{32} Long term administration of soluble aluminium salt worsens their learning ability together with diminished cholinergic function. Aluminium and other metals including zinc, copper and iron influence the oligomerisation and conformational changes of β-amyloid protein. It also induces appearance of tangle-like structures similar to the NFTs found in the brains of AD patients. The rats become lethargic.

\subsection*{2.8.3 High fat diet}

High fat diet increase disease neuropathology and/or cognitive deficits in AD models. Hypercholesterolaemia increases the risk and accelerates AD.

\subsection*{2.8.4 Scopolamine model}

Scopolamine (hyoscine) is a competitive antagonist of muscarinic acetylcholine receptors approved for use mainly in the prevention of nausea and vomiting associated with motion sickness.\textsuperscript{1}
Scopolamine produces similar memory deficits seen in the elderly and thus has been used as a psychopharmacological model drug of CNS ageing. The hippocampus is sensitive to drugs that cause memory loss (amnestic drugs) such as scopolamine which was used in this study. The drug, however, cannot induce the full range of deficits seen in patients with AD but is cheap and readily available. This model was chosen as current study is based on the cholinergic theory.

2.9 ANIMAL BEHAVIOURAL, LEARNING AND MEMORY MODELS

Table 1: Some behavioural tasks used in learning and memory in animal models

<table>
<thead>
<tr>
<th>Task</th>
<th>Parameter studied</th>
<th>Region of brain involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-maze spontaneous alternation</td>
<td>Spatial working memory</td>
<td>Hippocampus</td>
</tr>
<tr>
<td>Novel Object Recognition</td>
<td>Spatial displacement and recognition memory</td>
<td>Hippocampus and pre-frontal cortex</td>
</tr>
<tr>
<td>Morris water maze</td>
<td>Spatial working memory</td>
<td>Hippocampus</td>
</tr>
<tr>
<td>Radial arm maze</td>
<td>Spatial learning task</td>
<td>Hippocampus and pre-frontal cortex</td>
</tr>
<tr>
<td>Active/ passive avoidance</td>
<td>Natural tendency of avoidance</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from Yamada M. et al. 2011

2.9.1 Y-maze spontaneous alternation

For spontaneous alternation behaviour (SAB), all arms of the maze (A, B and C) will be open. For efficient alternation, the mouse must remember from which arm they came and explore the third arm finalizing a complete alternation. A spontaneous alternation is defined as successive entries into each of the three arms on overlapping triplet sets e.g. ABC, CAB, BAC, numbers of arm entries and sequence of entries. A mouse whose short-term memory
is impaired cannot recall which arm it has just visited and therefore exhibits decreased spontaneous alternation.

2.9.2 Novel Object Recognition (NOR)

The test is based on the natural preference of mice to explore a new object in a familiar environment. Initially, the animal is exposed to two identical objects and gets to explore these for a while. Then, one of the identical objects is replaced by a novel one. When the subject “remembers” the previous exposure to the familiar object, it will explore the novel object to a greater extent. If exploration of both objects is similar, this can be translated as a deficit in memory. The task requires no external motivation or punishment. Y-maze was used as the initial short term memory test and NOR was used as the confirmatory test as well as to assess long-term memory.

2.10 STUDY RATIONALE

Given the inconsistency in the literature regarding the effect of statins on dementia, there is need to carry out more studies in order to shed more light on the nature of the relationship between statin use and dementia. Simvastatin and rosuvastatin were chosen for this study because little data is available concerning the drugs in association with treatment of dementia. In addition, no studies have been done directly comparing the two. Furthermore, they are 2 of the 3 statins registered in Zimbabwe and are therefore easily available.

2.11 RESEARCH QUESTION

What are the effects of simvastatin and rosuvastatin in relieving the symptoms of Alzheimer’s disease in a mice model?
2.12 AIM

To investigate effect(s) of simvastatin and rosuvastatin in relieving symptoms of AD in mice.

2.13 SPECIFIC OBJECTIVES

1. To investigate effects of repeated dosing of simvastatin (5, 10, 20mg/kg body weight) on memory in scopolamine induced Alzheimer’s disease in mice.

2. To investigate effects of repeated dosing of rosuvastatin (10, 20, 40mg/kg body weight) on memory in scopolamine induced Alzheimer’s disease in mice.

3. To investigate if there is any dose response relationship in reversing symptoms of Alzheimer’s disease with simvastatin at the same doses.

4. To investigate if there is any dose response relationship in reversing symptoms of Alzheimer’s disease with rosuvastatin at the same doses.

2.14 HYPOTHESES

1) To investigate effects of repeated dosing of simvastatin on memory in scopolamine induced Alzheimer’s disease in mice.

\[ H_0 \] Repeated dosing of simvastatin has no effect on memory in scopolamine induced Alzheimer’s disease in mice.

2) To investigate effects of repeated dosing of rosuvastatin on memory in scopolamine induced Alzheimer’s disease in mice.
H₀ Repeated dosing of rosuvastatin has no effect on memory in scopolamine induced Alzheimer’s disease in mice

3) To investigate if there is any dose response relationship in reversing symptoms of Alzheimer’s disease with simvastatin

H₀ There is no dose response relationship in reversing symptoms of Alzheimer’s disease with simvastatin

4) To investigate if there is any dose response relationship in reversing symptoms of Alzheimer’s disease with rosuvastatin

H₀ There is no dose response relationship in reversing symptoms of Alzheimer’s disease with rosuvastatin
CHAPTER 3: METHODOLOGY

3.1 STUDY DESIGN
The design was experimental using mice model of Alzheimer’s disease.

3.2 Animal Husbandry
A total of 72 male Balb/c mice aged between 8 and 12 weeks were used. Animals were kept in mice cages with a 12:12 light:darkness cycle. They were given free access to water and commercially prepared mouse food. Wood shavings in cages were changed twice a week to keep environment dry and free from infections.

3.3 INDUCTION OF ALZHEIMER’S DISEASE
Scopolamine 1mg/kg/day intraperitoneal was administered over a period of 2 weeks to induce AD in mice. Mice were monitored for side effects of scopolamine like drowsiness, seizures which could have affected their parameters as well as behavioural tests. Mice were weighed on alternate days as a way of picking up problems early.

3.4 DRUGS
Scopolamine 1mg/kg/day was administered intraperitoneally over a period of 2 weeks to induce AD in mice. Statins and donepezil were administered 24hrs after induction of dementia. Rosuvastatin, simvastatin and donepezil were given at doses stated above by oral gavage over 2 weeks. Doses were chosen based on previous animal studies. Rosuvastatin was chosen as it is the most efficacious and does not cross the BBB while simvastatin is a lipophilic statin which readily transverses the BBB. Simvastatin and atorvastatin effects on cholesterol are comparable with very little differences between the two. However, studies with simvastatin are much fewer.
All drugs were obtained from locally available formulations. Scopolamine used was manufactured by Pharmacare Limited Batch 90F1211 Expiry February 2015, Rosuvastatin from Glenmark Pharmaceuticals Batch 05140200 Expiry January 2016, Simvastatin from Ipca Laboratories Batch V0044004 Expiry February 2016 and Donepezil from Actavis Batch F35800 Expiry 2016.

3.5 PROCEDURES AND TECHNIQUES

Healthy adult Balb\_c male mice, aged between 8 to 12 weeks were obtained from Government Veterinary Services. Experimental groups comprised of 8 mice each

(i) Normal controls receiving distilled water (vehicle control)
(ii) Scopolamine 3m/kg/day (Alzheimer’s model)
(iii) Scopolamine + simvastatin 5mg/kg/day
(iv) Scopolamine + simvastatin 10mg/kg/day
(v) Scopolamine + simvastatin 20mg/kg/day
(vi) Scopolamine + atorvastatin 10mg/ kg/day
(vii) Scopolamine + atorvastatin 20mg/kg/day
(viii) Scopolamine + atorvastatin 40mg/kg/day
(ix) Scopolamine + donepezil 5mg/kg/day

Data collection instruments were chosen after reviewing other animal studies which used mice. Behavioural tests were done from 24 hours after last drug dose. Y-maze with three identical arms labelled A, B and C respectively was used. Each arm was 19 cm long, 5cm wide and 14,5cm high and oriented at an angle of 120 degrees from the other two. This method assesses short term memory and is sensitive to various parameters of exploratory
behaviour. The mouse was placed at the end of one arm and allowed to move freely through the maze for a single five minute session. The number of arm entries serves as an indicator of locomotor activity. Alternation was defined as consecutive entries by a mouse into the three different arms. An arm entry was judged to be completed when the hind paws of the mouse were completely placed in an arm. Between each session, any faeces were cleared from the maze, and the maze floor was cleaned with 70% alcohol to remove any olfactory cues. The sessions were recorded by a video. The percentage of spontaneous alternation was determined using the following equation:

\[
\% \text{SAP} = \frac{[\text{Actual alternation} - \text{(Total alternations)}]}{[\text{Possible alternations} - (\text{total number of arm entries}) - 2]} \times 100
\]

Novel Object Recollection (NOR) test was used to assess cognition memory as well as short, intermediate and long-term memory. The test was done in an open field or arena made of plastic which was 43cmx31cmx16cm. Two different kinds of objects were used. The task consisted of three phases: habituation, familiarisation and test phase. During habituation, the mice explored an empty arena for 5 minutes. 24 hours after habituation, animals were exposed to the familiar arena with two identical objects placed at an equal distance. The next day, the animals were allowed to explore the open field for 5 minutes each in the presence of a familiar object and a novel object (plastic cube) to test long term recognition memory. The location of the object was counter-balanced so that one-half of the mice in each group saw the novel object on the left side of the arena, and the other half saw the novel object on the right side of the arena to eliminate bias of sides. Object recognition was distinguished by more time spent interacting with novel object (“recognition memory”). A mouse was scored as exploring when its head was oriented towards the object within a distance of 2cm or when the
nose was in contact with the object. Sessions were recorded on video as well. The discrimination index was determined using the following equation

\[ DI(\%) = \frac{Tn}{(Tn+Tf)} \times 100 \]

Mice were sacrificed at the end of the study with an overdose of chloral.

3.6 ETHICAL APPROVAL

Ethical approval was obtained from Joint Research and Ethical Committee of the University of Zimbabwe Ref number 176/14
CHAPTER 4: RESULTS

Each experimental group had 8 mice. All 8 mice in the simvastatin 20mg/kg group died, 2 died in the rosuvastatin 10mg/kg group and 1 in rosuvastatin 20mg/kg group during the last week of statin administration. One death was recorded in the rosuvastatin 40mg/kg group and two in the donepezil 5mg/kg group on day 4 and 5 of scopolamine administration, respectively.

Summary of results

4.1 Total Arm Entries

Table 4.1 Y-maze Total Number of Arm Entries per 5 minute session

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control</th>
<th>Sim 5mg</th>
<th>Sim 10mg</th>
<th>Ros 10mg</th>
<th>Ros 20mg</th>
<th>Ros 40mg</th>
<th>DPZ 5mg</th>
</tr>
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<tbody>
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<td>11</td>
<td>8</td>
<td>9</td>
<td>8</td>
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<td>7.60</td>
<td>8.60</td>
<td>8.60</td>
<td>8.20</td>
<td>8.00</td>
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<tr>
<td>Standard deviation</td>
<td>1.58</td>
<td>1.48</td>
<td>1.48</td>
<td>1.51</td>
<td>1.14</td>
<td>1.78</td>
<td>1.58</td>
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</table>
One way ANOVA showed that the total number of arm entries was not statistically significant across the treatment groups, \( F=0.5708; \) d.f. = 6; 28; \( p=0.9396 \). Similar results were obtained with Kruskal-Wallis test \( \chi^2=3.34; \) \( p=0.7649 \).
### 4.2 Same Arm Entries (SAR)

**Table 4.2 Y-maze Same arm entries per 5 minute session**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control</th>
<th>Sim 5mg</th>
<th>Sim 10mg</th>
<th>Ros 10mg</th>
<th>Ros 20mg</th>
<th>Ros 40mg</th>
<th>DPZ 5mg</th>
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<tr>
<td>Standard deviation</td>
<td>1.58</td>
<td>1.48</td>
<td>1.14</td>
<td>1.51</td>
<td>3.66</td>
<td>1.79</td>
<td>1.58</td>
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</table>
One way ANOVA was insignificant and the same arm entries showed no difference among groups (F= 0.4762; d.f= 6.28; p=0.820). Kruscal-Wallis test was also insignificant (χ²=2.526; p= 0.8202)
4.3 Alternate Arm Entries/ Returns (AAR)

Table 4.3 Y-maze Alternate arm entries per 5 minute session

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control</th>
<th>Sim 5mg</th>
<th>Sim 10mg</th>
<th>Ros 10mg</th>
<th>Ros 20mg</th>
<th>Ros 40mg</th>
<th>DPZ 5mg</th>
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<tbody>
<tr>
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<td>5</td>
<td>5</td>
<td>6</td>
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<td>6</td>
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<tr>
<td>Standard deviation</td>
<td>1.22</td>
<td>0.71</td>
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<td>1.00</td>
<td>0.71</td>
<td>1.58</td>
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</table>
There was no difference in number of alternate arm entries among groups with one way ANOVA (F=0.4272; d.f 6,28; p=0.8545). Kruscal-Wallis was also statistically insignificant ($\chi^2=3.062; p=0.8011$).
### 4.4 Spontaneous Alternation Performance (%SAP)

**Table 4.4 Percentage spontaneous alternation performance**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control</th>
<th>Sim 5mg</th>
<th>Sim 10mg</th>
<th>Ros 10mg</th>
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<td>15.43</td>
<td>14.88</td>
<td>15.95</td>
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</table>
Figure 4.4 Percentage Spontaneous Arm Alternations

One way ANOVA was not statistically significant as it showed no differences in percentage spontaneous alternation (F=0.2911; d.f= 6.28; p= 0.9361). Kruskal-Wallis test collaborated this result $\chi^2 = 1.687; p=0.9361$. 
4.5 Time spent exploring familiar object (Tf₁/Tf₂)

Table 4.5 Time in seconds spent exploring familiar object

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal 1</th>
<th>Animal 2</th>
<th>Animal 3</th>
<th>Animal 4</th>
<th>Animal 5</th>
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<th>Standard deviation</th>
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<tr>
<td>Ros 10mg</td>
<td>62</td>
<td>51</td>
<td>46</td>
<td>57</td>
<td>53</td>
<td>53.8</td>
<td>6.06</td>
</tr>
<tr>
<td>Ros 20mg</td>
<td>54</td>
<td>62</td>
<td>48</td>
<td>73</td>
<td>58</td>
<td>59.0</td>
<td>9.38</td>
</tr>
<tr>
<td>Ros 40mg</td>
<td>54</td>
<td>47</td>
<td>54</td>
<td>66</td>
<td>61</td>
<td>56.4</td>
<td>7.30</td>
</tr>
<tr>
<td>DPZ 5mg</td>
<td>50</td>
<td>53</td>
<td>61</td>
<td>54</td>
<td>48</td>
<td>53.2</td>
<td>4.97</td>
</tr>
</tbody>
</table>
Figure 4.5 Time spent exploring familiar object (either F₁ or F₂)

Two way ANOVA was insignificant p >0.05 indicating that times spent exploring familiar object were similar in all groups.
### 4.6 Time spent exploring novel object (Tn)

**Table 4.6 Time in seconds spent exploring novel object**

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal 1</th>
<th>Animal 2</th>
<th>Animal 3</th>
<th>Animal 4</th>
<th>Animal 5</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>72</td>
<td>93</td>
<td>84</td>
<td>69</td>
<td>84</td>
<td>80.23</td>
<td>9.81</td>
</tr>
<tr>
<td>Sim 5mg</td>
<td>64</td>
<td>53</td>
<td>64</td>
<td>82</td>
<td>78</td>
<td>68.20</td>
<td>11.76</td>
</tr>
<tr>
<td>Sim 10mg</td>
<td>44</td>
<td>52</td>
<td>49</td>
<td>58</td>
<td>51</td>
<td>57.74</td>
<td>5.07</td>
</tr>
<tr>
<td>Ros 10mg</td>
<td>58</td>
<td>63</td>
<td>53</td>
<td>64</td>
<td>63</td>
<td>60.2</td>
<td>4.66</td>
</tr>
<tr>
<td>Ros 20mg</td>
<td>52</td>
<td>64</td>
<td>62</td>
<td>83</td>
<td>56</td>
<td>68.87</td>
<td>11.95</td>
</tr>
<tr>
<td>Ros 40mg</td>
<td>61</td>
<td>53</td>
<td>42</td>
<td>54</td>
<td>58</td>
<td>62.00</td>
<td>7.23</td>
</tr>
<tr>
<td>DPZ 5mg</td>
<td>56</td>
<td>57</td>
<td>82</td>
<td>65</td>
<td>71</td>
<td>72.00</td>
<td>10.76</td>
</tr>
</tbody>
</table>
The times spent exploring novel object were found to be different among groups with One-Way ANOVA (F=5.755; d.f= 6,28; p= 0.0005). Bonferroni’s multiple comparisons performed at the 0.05 significance level found that there was no significant difference between control and donepezil and simvastatin 5mg groups. However, the rest of the groups were significant and had p values less than 0.05 when compared with control group.
### 4.7 Percentage of Discrimination Index (DI %)

**Table 4.7 Percentage Discrimination Index (DI %)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal 1</th>
<th>Animal 2</th>
<th>Animal 3</th>
<th>Animal 4</th>
<th>Animal 5</th>
<th>Mean</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.46</td>
<td>34.50</td>
<td>31.14</td>
<td>42.50</td>
<td>38.60</td>
<td>46.72</td>
<td>4.78</td>
</tr>
<tr>
<td>Sim 5mg</td>
<td>42.85</td>
<td>49.52</td>
<td>40.74</td>
<td>36.92</td>
<td>43.88</td>
<td>49.60</td>
<td>4.61</td>
</tr>
<tr>
<td>Sim 10mg</td>
<td>56.00</td>
<td>46.93</td>
<td>51.00</td>
<td>51.66</td>
<td>57.00</td>
<td>51.32</td>
<td>4.08</td>
</tr>
<tr>
<td>Ros 10mg</td>
<td>51.66</td>
<td>44.73</td>
<td>44.46</td>
<td>47.10</td>
<td>45.68</td>
<td>50.19</td>
<td>2.95</td>
</tr>
<tr>
<td>Ros 20mg</td>
<td>50.94</td>
<td>49.20</td>
<td>43.63</td>
<td>46.79</td>
<td>50.87</td>
<td>49.31</td>
<td>3.10</td>
</tr>
<tr>
<td>Ros 40mg</td>
<td>46.95</td>
<td>47.00</td>
<td>56.25</td>
<td>55.00</td>
<td>51.26</td>
<td>52.64</td>
<td>4.35</td>
</tr>
<tr>
<td>DPZ 5mg</td>
<td>47.16</td>
<td>48.18</td>
<td>42.65</td>
<td>45.37</td>
<td>40.33</td>
<td>52.87</td>
<td>3.25</td>
</tr>
</tbody>
</table>
The discrimination indices were found to be different between groups with one way ANOVA ($F= 8.031; d.f= 6.28; p< 0.001$). Kruscal-Wallis test $\chi^2 = 20.46; p=0.0023$. Tukey’s post hoc performed at the 0.05 significance level was significant in control vs simvastatin 10mg ($M=40.35, SD=9.7$), control vs rosuvastatin 10mg ($M=39.3, SD=8.4$), control vs rosuvastatin 20mg ($M=39.4, SD=11.4$), control vs rosuvastatin 40mg ($M=41.4, SD=9.6$), simvastatin 5mg vs simvastatin 10mg ($M=48.4, SD=14.4$) and simvasatatin 5mg vs rouvastatin 40mg ($M=49.9, SD=10.9$).
CHAPTER 5: DISCUSSION AND CONCLUSION

The objectives of this study were to investigate effects of repeated dosing of simvastatin and rosuvastatin on memory in scopolamine induced AD in mice. The study also aimed to find out if there was any dose-response relationship in reversing these effects.

Parameters to assess short-term memory namely, total number of arm entries, same arm entries, alternate arm entries and spontaneous arm alternations percentage were all not statistically significant. No differences were noted in the total number of arm entries per group indicating that the animals had comparable locomotor activity. A mouse must remember from which arm it came from and explore other arms. A mouse with impaired short-term memory cannot recall which arm it has just visited and therefore shows decreased spontaneous alternation. Results showed that effect on memory was almost the same and statins did not show a significant effect on short-term memory.

Long-term memory assessed by time spent exploring familiar object as well as time spent with novel object had different results. Time spent exploring familiar object was statistically insignificant indicating that the effect on long-term memory was comparable among groups. No drug was shown to have a greater effect. The results concur with a study by Alberto Serrano-Pozzo et al in 2010 which also found no significant difference in memory and psychomotor function between patients receiving simvastatin and those not receiving the drug. The study was a 12-week open-label trial with simvastatin 40 mg/d for 4 weeks and then 80 mg/d for 8 weeks in 12 patients with AD or amnestic mild cognitive impairment and hypercholesterolemia. It also indicated that simvastatin treatment can affect brain cholesterol metabolism within 12 weeks, but did not alter molecular indices of AD pathology during this short-term treatment period of 12 weeks.
However, time spent exploring the novel object was statistically significant. Naturally, the mice were expected to spend more time with the novel object as they ‘remember’ previous exposure to the familiar object. Many parts of the brain are involved in this task including the hippocampus, septum, basal forebrain and prefrontal cortex. If exploration of both objects is similar, this can be translated to a deficit in memory. Results showed greater exploration of novel object in all groups except in simvastatin 10mg/kg and rosuvastatin 40mg/kg which suggests that effect on long-term memory could be dose–related; higher doses of statins could have a negative effect on memory. This was noted in control vs simvastatin 10mg, control vs rosuvastatin 10mg, Control vs rosuvastatin 20mg, Control vs rosuvastatin 40mg, simvastatin 5mg vs simvastatin 10mg and simvastatin 5mg vs rosuvastatin 40mg groups. This is in contrast to an animal study by Rafael et al in 2010 which noted that rosuvastatin 0,2 or 2mg/kg administered for 21 days improved recognition memory deficits associated with iron loading and aging. Less exploration of novel object in the rest of the groups suggests a deficient long-term memory which suggests that the statins had no effect on memory and show no benefit in AD. This finding concurs with the LEAD study which noted that atorvastatin 80mg/day in patients with mild to moderate AD, taking donepezil, did not benefit cognition or global function. Another study by Alberto Serrano-Pozo et al (2010) indicated that simvastatin 40mg/d for 4 weeks and then 80mg/d for 8 weeks in 12 patients with AD or amnestic mild cognitive impairment and hypercholesterolemia treatment can affect brain cholesterol metabolism within 12 weeks. However, this did not alter molecular indices of AD pathology during that short-term treatment.

The study had several limitations that are important to consider for future research. The duration of study was 6 weeks. This influenced the duration of induction of dementia and giving of test drugs. Longer periods of administration could have produced different results.
In addition, inclusion of other tests in analysis like brain histology, measuring cholesterol levels would have helped validating the results. Use of more behavioural tests such as Morris Water Maze would also have enabled further analysis of the behaviours observed.

**Conclusion**

Repeated dosing of simvastatin and rosuvastatin had no effect on memory in mice. No dose response relationship in reversing symptoms of AD with these statins was observed.
REFERENCES


