THE EFFECT OF WILD YAM *(Dioscorea villosa)* TINCTURE ON MEMORY AND LEARNING IN MICE

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER IN SCIENCE IN MEDICAL PHYSIOLOGY

BY

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H56/70004/2013

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2018

DECLARATION

This thesis is my original work and has not been presented for a degree or any other award in any other University

Signature……. Date……..

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H56/70004/2013

Supervisors' Approval:

This thesis has been submitted for examination with our approval as University Supervisors

1. Signature……

   Date…….

   Prof. K. Thairu, MBChB, FRCP, PhD

2. Signature……..

   Date…….

   Prof. N.B. Patel, PhD

DEDICATION

This thesis is dedicated to my family and all those who supported and encouraged me in the course of my studies.

ACKNOWLEDGEMENTS

I thank the almighty God for the inspiration and knowledge to carry out this research study.

I wish to acknowledge my supervisors Prof. Kihumbu Thairu and Prof. Nilesh Patel for their guidance, support and close supervision.

I acknowledge provision of the gavage needle, pure diosgenin for calibration and the wild yam tincture used in the experiment provided by Prof. K Thairu.

I acknowledge the use of the T-maze apparatus and food reward for the experimental animals provided by Prof. N. Patel.

I wish to acknowledge Prof. J Midiwo from the Department of Chemistry (UON) for carrying out the chemical assay of the wild yam tincture and the Department of Biochemistry for use of their animal cages during my study.

I acknowledge technical support from Mr. Joseph Mwaniki, Mr. D.K. Wafula, Mr. Nzivo and Mr. David Horo from the department of Medical Physiology. I also acknowledge support from my colleague Mr. Eliphas Gitonga for statistical analysis of my research data.

Special thanks to my dear husband Simon Kamenchu Ringera for his unwavering moral and financial support for my entire research study without which this project would not have been accomplished.

I acknowledge greatly the support of my children James, Carole and Peter Kamenchu for IT services rendered to me in the course of my research study.

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GLOSSARY OF TERMS AND ABBREVIATIONS

Ach: Acetylcholine

ACTH: Adrenocorticotropin

AD: Alzheimers Disease

ADH: Antidiuretic Hormone

CGAIR: Consultative Group on International Agricultural Research

CNS: Central Nervous System

COX: Cyclooxygenase

DHEA: Dehydroepiandrosterone

DHEAS: Dehydroepiandrosterone Sulphate

(GSH-Px) $_{(MDA)}$: Glutathione Peroxidase

HCV: Hepatitis C Virus

LTM: Long Term Memory

LTP: Long Term Potentiation

LWDH: Liuwei Dihuang

MS: Multiple Sclerosis

PMS: Premenstrual Syndrome

RAM: Random Access Memory

ROS: Reactive Oxygen Species

SAM: Senescence Accelerated Mice

SOD: Super Oxide Dismutase

STM: Short Term Memory

TNF: Tumor Necrosis Factor

U.V: Ultra Violet

VaD: Vascular Dementia

Vit: Vitamin

WHO: World Health Organization

WM: Working Memory

ABSTRACT

In this work, the effect of orally administered tincture of wild yam (*Dioscorea villosa*) on learning and memory in mice at different ages (young mature, middle aged and elderly) has been studied. To the best of the researcher's knowledge, this kind of study has not been done before. The wild yam tincture was administered to the test animals by oral gavage. FELASA guidelines were adhered to in all the experiments. Forty male swiss albino mice were at first randomly allocated into two age cohorts (n=20): ages 3 – 6 months (young mature) and 10 – 14 months (middle age). Each cohort was further divided into a control and test group (n=10 each). The old age cohort evolved when the middle-aged animals reached old age (18 – 24 months) after eight months of the experiment period. The animals were subjected to learning and memory tests. using the T-Maze. **Long term memory (reference memory)** in the mice was assessed using a left – right discrimination task in a T- maze apparatus. **Short term memory (**working memory in rodents**)** was assessed using T-maze forced alternation task. **Behavioral flexibility** was tested by the reversal learning while **memory retention** was assessed by the delayed alternation task. For each test four parameters were evaluated: Correct Response (%), Latency (sec), Distance Travelled (cm) and by number of Omission Errors. In this study the effective food reward (bait) used was a breakfast cereal "Kellogg's Honey Loops". Data was analyzed using STATA version 11 software, using independent t-test and results presented as mean ± standard error of means (SEM). The significance level was $P<0.05$. **Motivation** was measured by reduction in omission errors. The test and control mice showed no significant difference in memory tests before wild yam tincture was administered. After administration of wild yam tincture the young mature and middle age test mice showed increased learning ability and improved memory: **The old age mice showed improved learning and motivation. The young mature, middle age and old age test mice showed statistically insignificant difference from the control mice in behavioral flexibility. The middle age and old age mice showed enhanced learning and better memory than the control mice. The middle age mice showed enhanced memory retention. Old age mice showed no significant memory retention.** This study recommends that further studies be undertaken to explore the benefits of wild yam in human volunteers considering that wild yam root tincture. capsules and creams and other root preparations are already widely used as food supplements in Chinese and Western Alternative/Complimentary medicine for other purposes rather than the enhancement of memory and learning. Despite the seriousness of Age-Related Dementia and its accompanying severe memory loss.

CHAPTER ONE: INTRODUCTION

1.1 Memory and Aging

Memory is central to our ability to attend and perform our daily life activities and correctly function in society. Loss in memory occurs as part of the aging process and memory deficits related to age have been significant sources of morbidity in any human population (Fjell et al., 2014). Dementia is one of the main causes of disability in older people (WHO, 2013). Memory loss is associated with the occurrence of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (Fjell et al., 2014). As the brain undergoes the aging process it becomes susceptible to neurodegeneration which results in memory and motor function deficits. The average weight of the normal human brain decreases steadily with age. The number of synapses decrease with aging and consequently nerve cells and neuropil is lost. This may explain decreased memory function in older individuals and declining scores in tests of memory. (Anderton 2002).

The gradual and progressive decline in memory during aging is associated with a decrease in hippocampal volume in humans, non-human primates and rodents (Picq et al., 2012).

According to the World Health Organization (WHO) the prevalence of age related health problems is becoming an important public health concern. Cognitive impairment and dementia are increasing globally and are predicted to increase proportionately more in developing countries where four fifth of the people older than 60 years live. With increasing longevity in Africa the greatest economic burden is due to dementia and its associated memory deficits (Global Health 2012). It is estimated that 35.6 million people are currently living with dementia worldwide and the number will double every 20 years reaching 115.4 million in 2050 (WHO 2013).

Cognitive functions such as long term memory and working memory are impaired by the aging brain (Buckner, 2004). Research studies indicate that behavioral and neuronal deficits occur with aging and some of the changes observed include a decrease in calcium balance (Landfield and Eldridge, 1994) and a decline in receptor system responsiveness including the dopaminergic (Joseph et al., 1990; Levin and Cepeda, 1998) muscarinic (Egashira, Tokoyama and Yamenaka, 1996; Joseph et al., 1990) adrenergic (Gould and Bickford, 1997) and the opioid receptor

systems (Kornhuber et al., 1996). These neuronal deficits may lead to changes in cognitive (Bartus, 2010) and motor behavior (Joseph, Bartus and Clody et al., 1983).

Because of the great importance of memory in human affairs research studies must explore ways and means of alleviating age related cognitive and behavioral deficits. Age related changes associated with learning and memory also occur commonly in rodents (Alireza et al., 2007).

Many research studies have been carried out regarding the main active ingredient in wild yam (diosgenin). However diosgenin has been falsely marketed as a hormonal replacement therapy to alleviate menopausal or Pre Menstrual Syndrome (PMS) symptoms. It is also said to have the ability to mimic certain hormones especially progesterone. Most of these claims however remain scientifically unproven. Studies done on humans and also in the laboratory suggest that wild yam has no hormonal effects. The steroidal saponin, diosgenin in wild yam can only be converted to the hormone progesterone in the laboratory but not in the mammalian body. (Marker and Krueger, 1940). Commercially available products of wild yam have been shown to improve mood, memory and sleeping habits.

The present study investigated the effect and mechanisms of action of wild yam tincture on memory and learning in three cohorts of mice at different age phases (young mature, middle age, and old age). T – maze procedures were used to assess memory and learning ability.

1.2 Chemical composition of wild yam

The dried root and rhizome of wild yam is used in commercial preparations classified as nutraceuticals (i.e. they contain a naturally balanced combination of active constituents) (Afoakwa and Sefa-Dedeh, 2001).

The chemical constituents of wild yam comprise of steroidal saponins (diosgenin,dioscin), phytosterols (sitosterol, stigmasterol, taraxerol), alkaloids (dioscorine), polyphenols, flavonoids, taninns, starch, vitamins (e.g. Vitamin B – complex) and minerals such as *K, P, Na, Mg, Fe, Zn, Se, Si, Cr, Co, B, Mn, Cu.* Diosgenin, a steroidal saponin is a major active constituent in yam tubers and has led to substantial research studies on its actions.

Edible yams provide carbohydrates, proteins and minerals. They provide energy (≈ 120 calories per 100 g) and comprise mainly of complex carbohydrates and fiber.

Edible yams are also a good source of Vitamin $B-complex$ e.g. B_1, B_2, folic acid, B_5 and B_3. They contain considerable amounts of antioxidants and vitamin C (≈ 20% per 100g body weight). Yams contain small levels of vitamin A and Beta carotene (Annigan, 2017).

Yams are a rich source of minerals especially potassium and phosphorous. Other minerals include copper (Cu), Iron (Fe), Magnesium (mg) and Calcium (Ca) (Annigan, 2017).

Yams are used in diets for tiredness, fatigue, depression, anxiety or stress. They are good food in osteoporosis and in diseases caused by the loss of myelin and multiple sclerosis.

STEROIDAL SAPONINS: (http://dx.doi.org/10.1590/S0103-50532007000500030

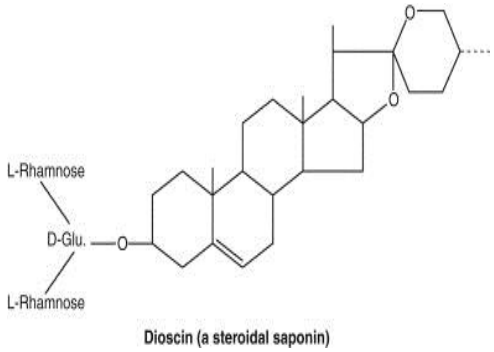


Figure 1: Dioscin (Steroidal Saponin)

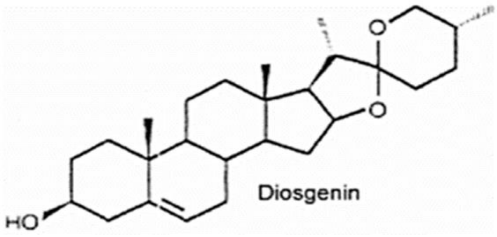


Figure 2: Diosgenin (Steroidal Saponin)

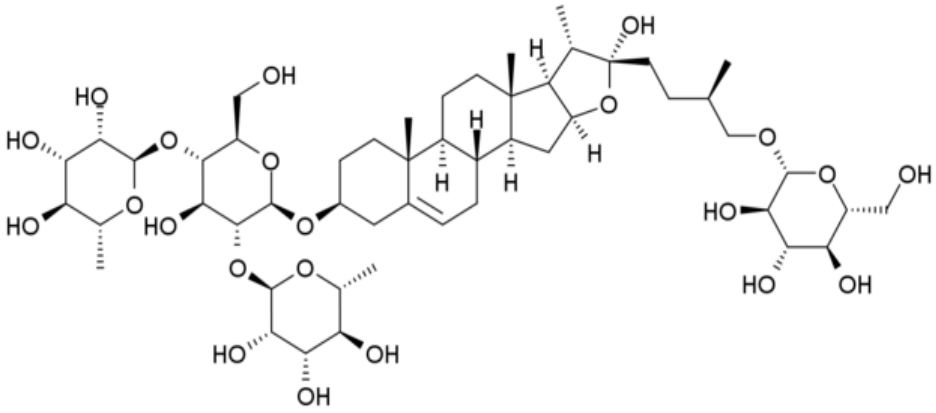


Figure 3: Protodioscin

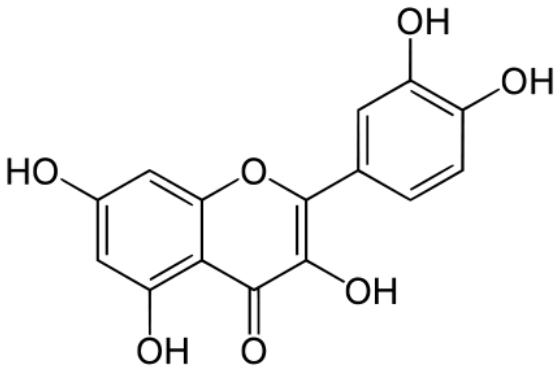


Figure 4: Polyphenol

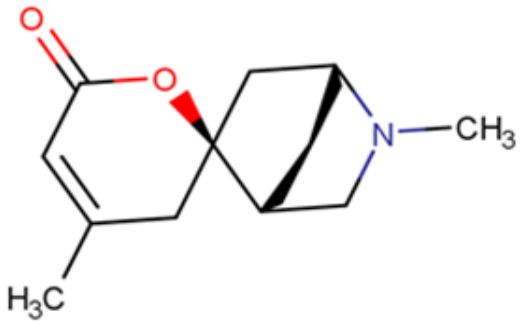


Figure 5: Dioscorine (an alkaloid)

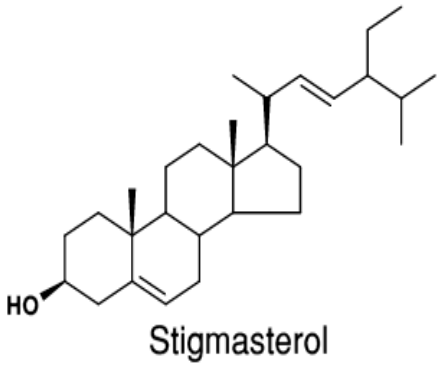


Figure 6: Stigmasterol (a phytosterol)

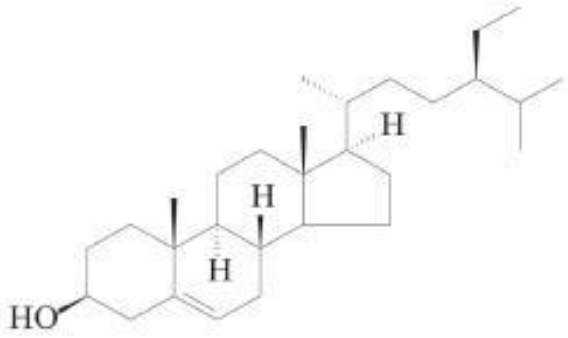


Figure 7: β sitosterol (a phytosterol)

Steroidal saponins are the main active constituents in yam (Liu et al., 1995; Hu et al., 1996, 1997). In nature they occur as glycosides and show several physiological activities which include hypoglycemic (Kato et al., 1995) hypocholesterolemic (Malinow, 1985; Sauvaire et al.,1991), hemolytic (Santos et al.,1997; Zhang et al.,2011), antithrombotic(Zhang et al.,2011; Peng et al.,1996) antiviral (Aquino et al.,1991) and antineoplastic activities (Hu et al.,1996,1997; Ravi Kumar et al.,1979; Sung et al.,1995).

Flavonoids in wild yam are potent antioxidants some of which are more powerful than vitamins C and E. Beta carotene (pro vitamin A) shows several biological activities which include antioxidant action, immune booster and a potent source of vitamin A and a useful substance in night vision and also in neurodegenerative diseases such as Alzheimer's disease (AD) (Turner et al., 2005).

Polyphenols are organic compounds that occur naturally in various plant species (>800 species) identified to date. These compounds are found mainly in cereals, vegetables, fruits and also in

beverages. They consist of large multiples of phenol structural units and they are reactive species towards oxidation. Polyphenols have been shown to have diverse physiological actions in the human body including suppression of tumor growth, protection of UV radiation, free radical scavenging and enhancing brain health and protection against dementia (Spagnuolo et al., 2016).

Studies have shown that the antioxidant activity of the polyphenols helps protect the DNA molecule and also reduces the inflammatory response in the body (Spagnuolo et al., 2016). Polyphenols delay the onset of dementia and as a result decrease the potential risk for neurodegenerative diseases. Presence of polyphenols in dietary supplements decreases the progression of dementia due to their neuroprotective effects (Spagnuolo et al., 2016)

Wild yam contains various minerals which are associated with several health benefits in the human body including conduction of nerve impulses, energy production, muscle contraction {potassium (K), phosphorus (P)}, immune system boosters {zinc (Zn)}, influencing attention span {iron (Fe)} an essential part of haemoglobin (Hb) and red cell formation. Enhanced body use of insulin by Chromium (Cr), normal communication and function among nerve cells {calcium (Ca), magnesium (Mg)} and activation of B-vitamins (P), co-factor for the anti-oxidant enzyme super oxide dismutase (Mn), production of red blood cells, proteins such as haemoglobin (Hb), elastin and collagen (Cu), (Umapathy M. 2009; Annigan J. 2017).

The presence of B-vitamins in wild yam may enhance various nutritional health benefits. Some of these benefits are described as follows:

- **Vit B1 (Thiamine):**

Promotes healthy nerves, improves energy levels, alertness and mood. Thiamine may enhance memory in Alzheimer's disease although adequate scientific evidence is not available. It is used to treat psychosis related to alcohol withdrawal. It also enhances sleep and energy levels in the elderly (> 65 years) (Turner et al., 2005).

- **Vit B2 (Riboflavin)**

Boosts antioxidant activity hence protects many body tissues. It is essential for tissue maintenance, repair and may be important for the health of nerves. It increases energy supplies to brain cells and hence reduces frequency and severity of migraine headaches. In combination with other B vitamins (B6 and B3), it may help protect against a broad range of nerve and other

ailments including AD, epilepsy, numbness and tingling, Multiple Sclerosis (MS), anxiety, stress and fatigue (Turner et al., 2005).

- **Vit B3(Niacin, Nicotinic acid)**

Health benefits associated with Niacin include release of energy from carbohydrates, control of blood sugar, keeping the skin healthy and maintaining the nervous and digestive systems (Turner et al., 2005).

Wild yam is popularly promoted erroneously as a natural alternative to oestrogen therapy and a source of steroidal compounds such as progesterone and DHEA (Dihydroepiandrosterone).

However research has known that diosgenin cannot be synthesized or converted into the above byproducts in the human body. This can only be done in the laboratory (Marker R.E and John Krueger, 1941). It is also marketed as wild Mexican yam or Chinese yam (*D.Villosa* or *D.Barbasco*) and these products contain large amounts of antioxidants and relatively substantial amounts of diosgenin.

CHAPTER TWO: LITERATURE REVIEW

2.1 GENERAL DESCRIPTION OF WILD YAM

Botanical and Common Names

Family: Dioscoreaceae

Dioscorea villosa (China root, Colic Root, Devil's Bones, Rheumatism Root, Yuma)

Dioscorea opposite syn.*D. balatas* (Shan yao, Chinese yam)

The wild yam plant is native to North and Central America but it is also grown in the tropics and temperate parts of the world. There are many species of dioscorea (over 600 spp.) and twelve species of them are considered edible types. The medicinal parts of wild yam include the rhizome, the outer bark and the root. The plant maybe described as a deciduous perennial climbing vine which consists of green leaves that are heart-shaped and small green flowers.

The yam tuber is usually brown in colour, cylindrical in shape and also twisted. The plant can climb to a height of twenty feet (20 ft).

Figure 8: Wild Yam

Adapted from: **http://naturalremediesforpms.com/wild-yam.html**

2.2 CULTIVATED YAM SPECIES

Traditional yam tuber types commonly grown in various parts of the world

- *Dioscorea rotundata (White yam):* Native to Africa

Adapted from: http://worddirection.com/the-potentials-of-pre-storage-curing-and-nanobiotectnology-in-the-control-of-postharvest-losses-of-yam-dioscorea-rotundata-poir-tuber/

- *D. cayenensis (yellow yam):* Native to Africa

Adapted from: http://www.ourfood.nl/ingr/grfr_krsp/dioscorea-yam/gele-yam.html

- *D. opposita*

Adapted from: https://www.ebay.com/itm/5-Chinese-Yam-Seeds-Dioscorea-Opposita-Organic-Vegetables-/161614761926

- *D. bulbifera (the aerial potato):Found in Africa and Asia*

- *D. trifida (the cush-cush yam):* Native to Guyana,[S.America]

Adapted from: https://www.pfaf.org/User/Plant.aspx?LatinName=Dioscorea+trifida

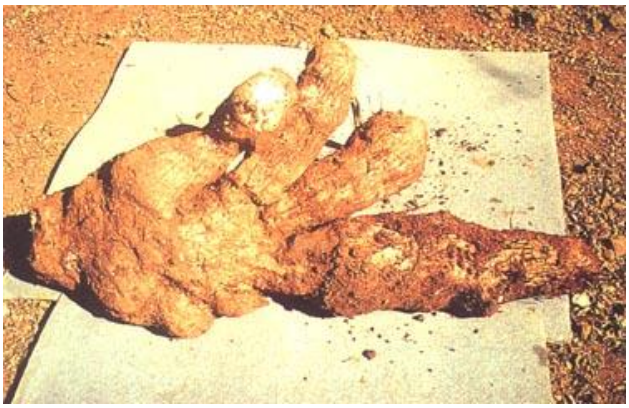
- *D. esculenta (The lesser yam):* Native to South East Asia

Adapted from: https://www.flickr.com/photos/stephenbuchan/2414058089

- *D. alata (Winged yam, water yam)*

Adapted from: https://plants.ifas.ufl.edu/plant-directory/dioscorea-alata/

- *D. minutiflora* (Yam commonly grown in Central Kenya)



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- *D. dumetorum (the bitter yam)*

Adapted from : https://www.researchgate.net/figure/B-Whole-and-Cross-section-of-D-dumetorum-trifoliate-yam_fig2_303910814

D. rotunda the white yam and D. *cayenensis* the yellow yam are native to Africa. These cultivated yam tubers are very important for dietary purposes although most of the yam species are considered poisonous. The toxic yam types can be made edible by soaking, boiling and drying. Yam is consumed in many parts of the world and is a staple food in Africa and also in other parts of the world such as West Indies, South America and the Pacific Islands. There are approximately 200 varieties of yam tubers grown in Africa.

Cultivation of yams in Africa and Asia started many years ago as reported in the Yalta conference and thereafter continued in other countries. Cultivated yam varieties in Africa were lost during periods of war and famine (late 1900s) but development of new varieties has been in progress (CGAIR 1994)

Yams are nutritionally a good source of protein, carbohydrates, minerals and they can be preserved or stored for several months without loss of their nutritional value (CGAIR, 2006). In the traditional society (Africa, Pacific Islands) yams were associated with ceremonies and festivals. Studies have been done that suggest edible yam has positive effects on memory and cognitive ability. However, more research needs to be done (Yang, 2017).

2.3 TRADITIONAL USES

Many of the Dioscorea species are used as both food and medicine.

The African yam species contain various natural toxic substances (e.g. dioscorine) and therefore cannot be consumed in their raw forms.

Wild yam products have been used in many parts of the world e.g. Central America to relieve pain associated with menstruation, child birth and ovarian disorders. The plant was also used by the Maya & Aztecs to relieve pain (Chevallier, 2000).

2.4 MEDICINAL USES

There is emerging evidence for application of phytochemicals in medicine.

A Chinese medicinal Herb (Dioscorea, Rd-SHAN YAO) improves memory function in Alzheimer's disease (AD), dementia and memory impairment (Furthwine, 2012).

Dioscorea relieves pain due to its anti-inflammatory action (Lima, 2013) and promotes fast healing in nerve injuries due to formation of neurofactors and regeneration of nerve cells in diabetic neuropathy by increased formation of nerve growth factor (Furthwine, 2012).

Dioscorea yam tubers show biological activities against various metabolic disorders (Ulbricht et al., 2007; Raju & Rao, 2009).

Studies suggest the following effects;

- Cholestrol lowering (Cayern and Dvornik, 1979).
- Decreased adipocyte lipid accumulation, attenuates inflammatory process (Uemura et al., 2010).
- Anti-diabetic, enhanced insulin dependent glucose uptake (Uemura et al., 2010).
- Modulation of liver function and therapeutic control of liver disease (Kosters et al**.,** 2005**;** Nibbering et al**.,** 2001).

In vivo studies suggest anti-cancer activity by diosgenin in the following organ sites; colon, breast and lung (Raju et al., 2004; Malisetty et al., 2005; Miyoshi et al., 2011; Yan et al., 2009).

In vitro studies also suggest anticancer activity of purified diosgenin in the colon, breast, prostate, cervix, liver, bone/blood, larynx and skin (Raju et al., 2004; Chiang et al., 2007; Chen et al., 2011; Corbiere et al., 2003; Legar et al., 2004 a; Legar et al., 2004 b).

A study by Turchan-Cholewo et al., 2006, reported that diosgenin has the potential to reduce the risk of dementia in drug abusers with HIV infection.

In vitro studies reported that diosgenin inhibits the replication of hepatitis C virus (HCV) of low concentrations.

Oxidative Stress & Inflammation

Aging and its related cognitive decline is influenced mainly by oxidative stress and reactive oxygen species (ROS) (Olanow, 1992; Valko et al., 2007). Excessive production of ROS or its inadequate neutralization leads to accumulation in the cell and as a result leads to damage of the cellular macro molecules such as proteins, lipids and DNA. Formation of ROS is a significant step in neuronal death in various age-related neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (De luliis et al., 2006). The ROS oxidizes neuronal cellular macromolecules leading to cell death. Oxidative damage is said to influence the aging process and its related cognitive deficits hence spatial learning and memory could be reversed or retarded by antioxidant supplementation (Socci et al., 1995). Nutritional and herbal supplements have been used to change behavioral and neuronal deficits brought about by aging. Scientific studies and evidence suggest that cognitive decline may be influenced by inflammatory reactions and processes that occur in the central nervous system (CNS) (Shukitt Hale, 1999).

These inflammatory events increase glial fibrillary acidic protein expression (Rozovsky, Finch and Morgan, 1998) increase production of tumor necrosis factor (TNF) (Chang HN, Wang SR, Chiang SC, et al., 1996), increase interleukin-6 (IL-6) concentrations in old mice (Spaulding CC, Walfors RL, Effros, 1997; Volpato S, Guralnik JM, Ferrucci L, et al., 2001) and upregulation of C-reactive protein (Kushner, 2001).

Interaction of reactive oxygen species generating agents and cytokines during aging has been proposed (Manev and Uz, 1999). Neuronal functional deficits and glial cell-neuron interactions are initiated by both extracellular signals and reactive oxygen species (Rosenman et al., 1995; Stella et al., 1997; Woodroofe, 1995). Research studies suggest that administration of potent

inflammatory agents such as lipopolysaccharides to central neurons produce alterations in working memory and spatial learning (Hauss-Wergrzyniak et al., 1999; Yamada et al., 2009).

Research suggests that oxidative stress in aging maybe decreased by the consumption of fruit and/or vegetable extracts. These dietary supplements contain high levels of antioxidants which reduce cellular susceptibility to oxidative stress (Joseph et al., 2005). Rats fed on a diet of spinach performed far better on a memory and a learning test (Bickford, 2000). These observations are supported by research studies carried out on dogs whose performance on cognitive tests was enhanced as a result of diet enriched with anti-oxidants.

A study by researchers at Tufts University-Boston (USA) on rats also suggests that antioxidants could reverse age related cognitive deficits. The study suggests that rodents performed better in negotiating mazes and signals improved for short term memory (Joseph et al., 2003). From numerous studies, researchers hypothesize that antioxidants enhance the permeability of cellular membranes to nutrients and important chemical substances. (Joseph et al., 1998)

Lack of acetylcholine is associated with dementia and Alzheimer's disease. Food supplements rich in antioxidants have been shown to enhance cognitive function which may otherwise decline due to aging, dietary patterns and genetic deficiencies.

Elderly rats fed on food rich in antioxidants (blueberries) showed improvement in short term memory and motor coordination.

The major qualitative categories of human memory:

a. **Declarative memory**: This is one type of long term memory which refers to those memories that are available to consciousness and can be expressed as remembered images, sounds and events (Purves et al., 1997). Declarative memory refers to "knowing what" and it is also referred to as explicit memory because the information is stored explicitly and can be retrieved at will. These memories are learned experiences through practice and repetition. They comprise of sensory motor actions that are carried out by the body in an automatic fashion. These memories are encoded by different parts of the brain found within the medial temporal lobe. Declarative memories are consolidated and stored in the cerebral cortex (Tramoni et al., 2011).

b. **Non-declarative memory:** This incudes motor skills and other related functions that are acquired and retrieved without having to "think about it".

They are also referred to as procedural memory and consists of "knowing how" and these memories are not generally available to consciousness once learned. These memories are not easily forgotten because they are formed as a result of practice and repetition. These memories are mediated by brain regions namely the motor cortex, cerebellum and the basal ganglia (Squire and Zola, 1996).

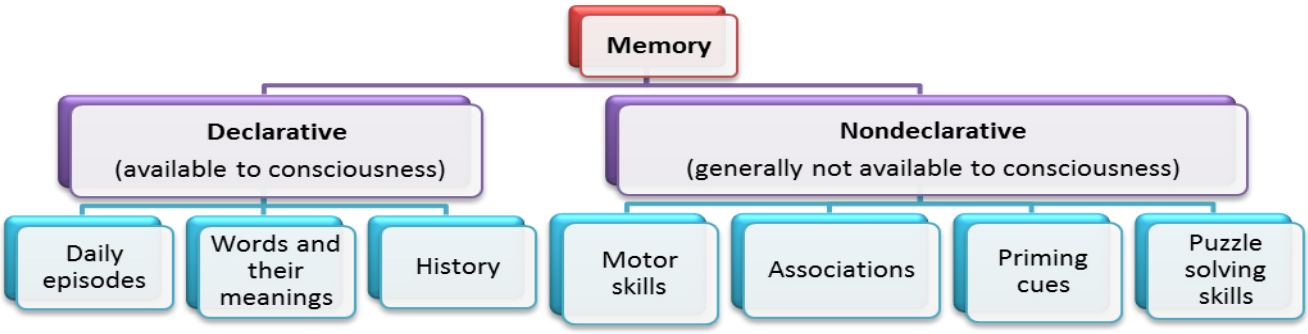


Figure 9: The major qualitative categories of human memory

Source: Dale Purves et al., 1997

The major temporal categories of human memory:

Memory can be classified in accordance with time over which it is effective. This includes the following:

a. **Sensory memory (Registration memory):** This form of memory is not strictly considered a memory function but represents a transient process by which perceptions enter the memory system (Golob et al., 2008).

b. **Immediate memory:** This is the initial stage of short term memory storage in which an experience is held in mind for ≈ 30 seconds up to a few minutes. This memory acts as a register with a large capacity for verbal, visual and tactile modalities. (Schneegans and Bays, 2018)

c. **Short Term Memory (STM):** The ability to hold information in an active state where it is available within a duration of minutes to weeks. STM can be transferred into long term memory through a process of consolidation mediated by the medial temporal lobe. This memory form occurs in the prefrontal lobe cortex where it can be accessed and utilized

for daily tasks. A particular advantage of short term memory is its ability to be tested in rodents. STM is also referred to as primary memory (Baddeley A, 1997).

d. **Working memory (WM):** A distinct sub-type of short term memory where information is held temporarily in order to carry out cognitive tasks on a daily basis. This memory type has a small capacity which can hold 5 – 7 items at a time and depends on control of attention and mental effort. Working memory involves basic life tasks such as reading, problem solving, rationalization and navigation (Diamond, 1999).

- **Working memory in primates and rodents**

Working memory has been studied widely in humans and primates. In the animal literature there is no distinction between short term memory, long term memory and working memory. The latter term (WM) has been used to describe short term memory in animals. There is no evidence to suggest the existence of independent multiple memory systems in primates and rodents. (Bratch et al., 2016)

In humans independent multiple memory systems exist and working memory is considered to operate under executive control. (Bratch et al., 2016) However, rodents realize memory in a manner similar to humans (Diamond, 2013). Experimental data suggests that both spatial and olfactory memories in the rats operate under an independent working memory system. (Bratch et al., 2016).

Most tests of working memory in non-human animals are actually tests of simple STM (i.e. they assess none of the processing aspects of working memory that actually distinguishes it from short term memory (Louis, 2003). Rats like humans have a working memory (the brains RAM).

e. **Long Term Memory (LTM):** This memory type is also referred to as reference memory and has the ability to hold information for days, weeks or a lifetime. Information in LTM maybe stored permanently depending on its importance and entails transfer of information initially acquired to a more permanent form of storage. The recall and encoding of information in the LTM is carried out by specific systems in the medial temporal lobe of the brain and especially in the hippocampus (Nell et al., 1997). Declarative and non-declarative memories are different types of LTM. These memory

types are distinct and operate through different processes in the brain and these observations are evidenced by studies of amnesic patients (Robert A. Bjork 1975).

Long term memory represents knowledge required to complete a task in a given trial session e.g. where an animal is expected to locate a food reward (bait) in a maze (Deacon & Rawlins 2006).

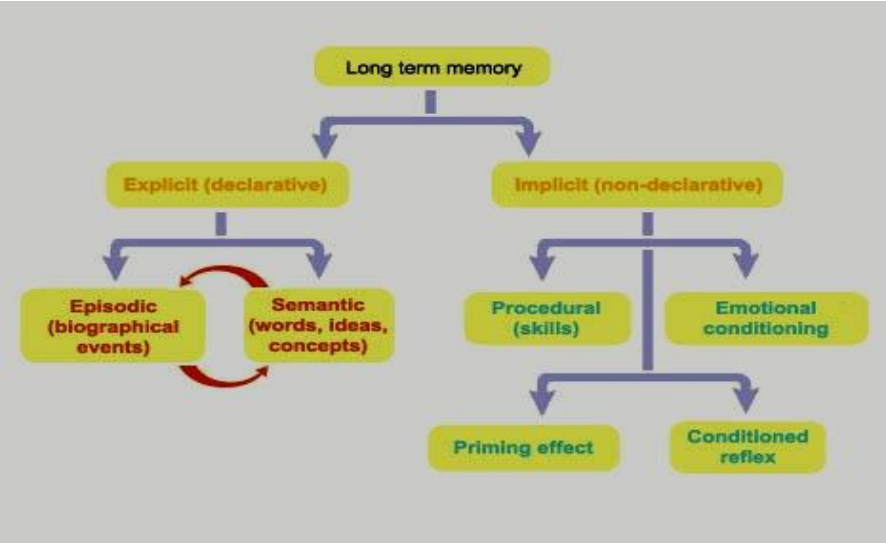


Figure 10: Major components of long term memory

Courtesy of: https://www.pinterest.com/pin/265008759298158131/

f. **Spatial Learning and Memory:** These are neurological functions that allow us to remember things associated with our environment. It entails gathering information regarding ones environment and space orientation (D'Hooge, DeDeyn 2001). Spatial working memory is important for an animal particularly rodents in carrying out a given maze task. Rodents spatial memory is required to identify the location of a bait (food reward) in a maze. Humans and animals perceive spatial memories in form of a cognitive map within both the short term and long term memory (Bisby, and Burgess 2014). Research suggests that the hippocampus and the surrounding medial temporal lobes are important for spatial representation and also for episodic memory (Bisby, and Burgess 2014).

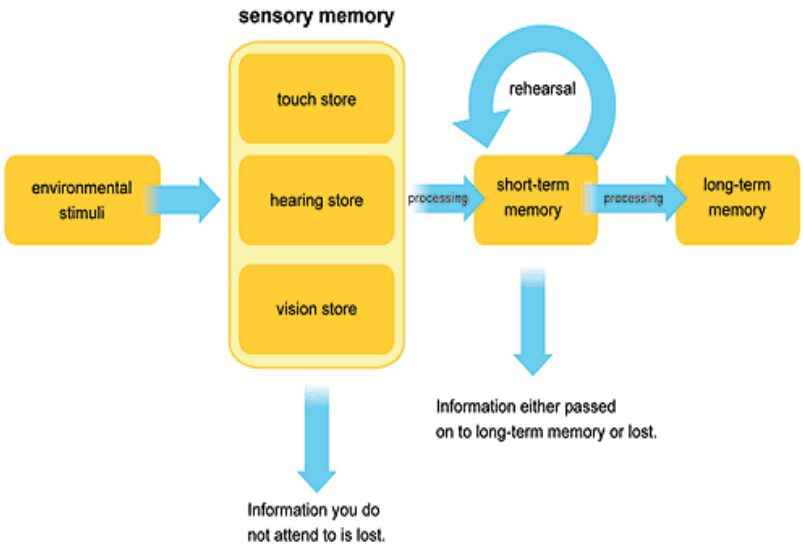


Figure 11: The major temporal categories of human memory

2.5 PHYSIOLOGY OF MEMORY AND LEARNING

Learning and memory are closely associated and consist of processes such as encoding, attention and recall of events. Memory is formed as a result of electrical activity within the nervous system while learning is the ability to acquire knowledge and information through experience. It can be measured by the probability of a certain behavioral response towards a given stimulus. Rewards and punishments are significant aspects of learning and also the environment within which these events occur (Horner et al., 2015).

Memory represents a permanent form of information that has been acquired or learned. Memory formation occurs as a result of changes in synaptic transmission within a given neural system. These changes are also influenced by previous neural activities and occur at both the cellular and molecular levels. Experiences can result in the formation of new synapses. Different types and aspects of memory are processed by distinct cortical and non-cortical areas of the brain (D' Hooge 2001).

All types of memory occur due to learning and hence determine the behavioral response by the individual animal or human being. Memory is a cognitive process and components that constitute its formation include perception, orientation, encoding, storage and recall (Hasselmo et al., 2006 and McGaugh 2000)

Memories fade away and when they escape retrieval they are considered forgotten, (i.e. information is lost over time) (Pederson, 2001).

Memory Phases

Memory formation consists of learning, consolidation and retrieval. Learning can occur during acquisition trials in a controlled environment and memory traces are hence formed. However memory traces undergo labile changes which may modify the original information (McGayl, 1996, 2000; Izquierdo et al., 1989, 1999, 2000; Dudai, 2000).

The memory trace is physically stored during the process of consolidation. Recall or retrieval of memory confirms its formation and storage and the mechanism that mediates this process is the facilitation of cholinergic transmission (Hasselmo M, McGaughy J. 2000). Mild or severe disorders in memory that are associated with aging hinder the storage, retention and recollection of memories. Most disorders such as Alzheimers disease, Huntingtons disease or traumatic head injuries are exacerbated by the effects of aging.

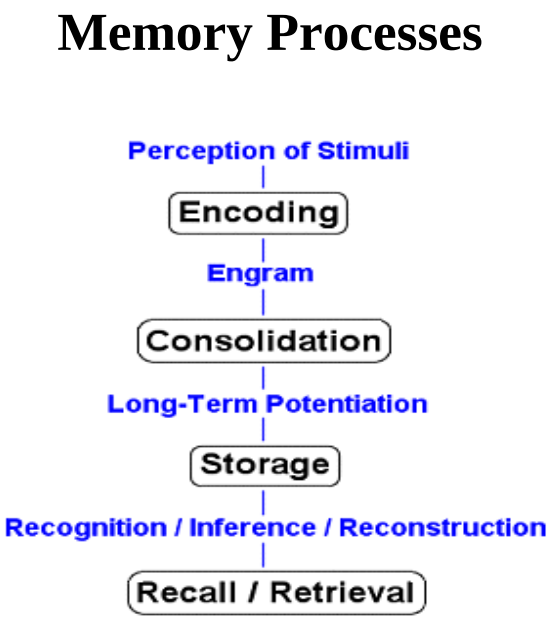


Figure 12: Memory processes

Diagram by: Luke Mastin

Forms of learning

- **Habituation (Negative memory)**

Habituation is a form of learning which occurs after repetition of a neutral stimulus. The organism or subject stops to respond to a stimulus that is no longer biologically relevant. Therefore habituation is a type of negative memory in which the brain ignores information of no consequence. Habituation therefore results from inhibition. It is an example of non-associative learning i.e. the organism does not associate the stimulus with a reward or punishment (Glanzman et al., 1997). Studies done in large snail Aplysia demonstrate habituation an effect which is converted to facilitation. Habituation effect in the sensory terminal is due to progressive closure of Ca^{++} channels in the terminal membrane (Kandel and Klein, 1978).

Facilitation effect is due to:

(i) Serotonin release at the facilitator synapse on the sensory pre-synaptic terminal.

(ii) Serotonin binds to its receptors in the presynaptic membrane and activates adenylcyclase resulting in the formation of Cyclic AMP (cAMP). (Glanzman et al., 2012)

(iii) The Cyclic AMP (Camp) acts on a protein kinase resulting in phosphorylation of a protein that is part of the K^{+} channels in the sensory terminal membrane. This blocks the channels for K^{+} conductance and the blockage can last for several minutes.

- **Sensitization (Positive Memory)**

This is a form of learning process where a repeated stimulus is associated with either a pleasant or unpleasant stimulus resulting in a progressive increase in response. In sensitization the brain stores memory traces of the information which results from facilitation or upregulation of the synaptic pathways (Bailey et al., 1983; Kandel, 1979).

Physiological Basis of Memory (Mechanisms)

2.5.1 Short Term Memory (STM)

In the Short Term Memory information is retrieved and processed at any point in time. The ability to remember can result from temporary chemical or physical changes that can persist for up to several weeks.

Neuropsychological data suggests that the hippocampus acts mainly in encoding and recall of information (Hannula and Ranganath, 2008). Studies by Gibbs et al., (2008) suggests that the formation of short term memory involves hyperpolarization of post tetanic afferent neurons which maybe inhibited by accumulation of potassium ions at synaptic junctions.

However studies by Krencik et al. (2015) proposes a model which involves the interaction of excitable neurons and astrocytes. The process involves electrical and chemical transmission by neurons through synapses resulting in the flow of calcium ions (Ca2+) from astrocytes to neuronal synapses. The neuron astrocyte interaction and the corresponding calcium ion waves consequently influence synaptic activity in short term memory.

Working Memory (WM)

Functions of working memory are mediated in the frontal lobes of the cerebral cortex. Distinct regions of the brain deal with specific information such as encoding for location, color, shape and size of objects. In this form of memory information is processed and stored at the same time. Neurotransmitters are significant in the operation of working memory and dopamine is essential to working memory. Attention focusing is essential for many memory based skills (Goldman et al., 1996).

2.8.2 Long Term Memory

Mechanisms for long term memory propose transfer of information from short term memory to a more permanent storage after undergoing processing in both the hippocampus and the medial temporal lobe. After existence in the short term memory, memories fade away or are consolidated into long term memories. (Radwanska et al., 2011). Consolidation of short term memory is influenced by various factors such as hormonal levels and neuro-transmitters at specific synaptic sites. Other factors include motivation and attention focus. Chemical substances

that affect memory include Acetylcholine (*Ach*), Glutamate, Noradrenaline which are said to enhance memory consolidation High levels of GABA (Gamma-aminobutyrate) tend to decrease consolidation of Short Term Memory. Other hormones such as vasopressin (ADH), adrenocorticotropin (ACTH) affect memory retention (Radwanska et al., 2011)

Opioid peptides influence learning especially where the experiences involve a painful or unpleasant stimulus. This is achieved by decreased motivation for learning as a result of inhibited emotions such as fear and anxiety both of which are components of an unpleasant or painful experience (Vander et al., 1994).

Memories can be consolidated very rapidly and they can be retained over extended periods particularly because of the unlimited capacity of long term memory.

Consolidation of memory involves cellular or molecular changes within the neurons and these changes constitute the memory trace. These changes result from electrical activity initiated in the neurons during short term memory (Radwanska et al., 2011; Vander et al., 1994).

2.8.3 Model for Memory Consolidation (LTP)

One model for memory consolidation is Long Term Potentiation (LTP). It consists of increased synaptic strength due to stimulation of chemical synapses and patterns of electrical activity. This activity results in increased signal transmission within the nerve cells.

LTP is significant at excitatory synapses in the Central Nervous System (CNS) and its activity is important in the early stages of learning which occur in the hippocampus (Vander et al., 1994).

LTP is considered the most studied form of synaptic plasticity. However, there are other mechanisms that influence storage of information in the brain such as activity-dependent LTD (Malenka and Bear, 1994).

Long term depression (LTD) has opposite effects and produces a persistent decrease in synaptic strength. Synaptic activity can also result in long lasting efficacy in the post synaptic cell and the effects may last for a duration of minutes, hours or weeks.

Beattie et al., (2000) proposes a molecular mechanism, a mirror image of LTP which results from low frequency synaptic stimulation and produces an NMDA (N-Methyl-D-Aspartate) receptor dependent LTD.

Calcium ion dependent protein phosphatases are essential and a reduction of AMPA receptors occur at the synaptic sites in the hippocampus. Synapses in the cerebellum also undergo a form of LTD which involves activation of G – protein coupled and the protein kinase C (PKC) and the mediated loss of AMPA receptors (Cho et al., 2000).

The above cerebellar activity through fiber-Purkinje cells has been suggested to play a role in motor learning. Changes or modification in synaptic strength leads to encoding of memories and LTP is considered the major cellular mechanism by which memory and learning occur

Enriched environments e.g. one with toys, social situations, physical challenges improve long term memory in experimental animals. The enriched environment induces dramatic changes in the brain particularly the cerebral cortex, hippocampus and cerebellum (Mohammed et al., 1998)

Enriched environments have the ability not only to alter the number of neuroglia cells in the CNS but also the branching pattern of dendrites and increased spines on neuronal processes. This phenomenon is called plasticity and is associated with fast learning in animals. Plasticity can occur during middle or old age and this suggests that neuronal connections and patterns continue to change throughout life (Mohammed et al., 1998).

2.8.4 Location of Memory

The hippocampus plays a temporary role and is important in the early phases of learning. Thereafter declarative memories are stored in other areas of the brain such as the basal ganglia.

Other structures such as the cerebellum, pons and thalamus are essential for procedural learning (non declarative) and the amygdala is involved in learning associated with pain or unpleasant experiences (Anderson, 1982).

2.8.5 Plasticity of central synapses

No single mechanism can explain all forms of memory. Even within a single organism a variety of types of memory exist and a variety of mechanisms underlie them (Tomonori et al., 2014). Synapses are the physical sites of many if not all forms of memory storage in the brain. Synapses alter the processing capabilities of a neural circuit in interesting and useful ways. The synaptic strength (i.e. the mean amplitude of the post-synaptic response) of many synapses may depend on the previous activity. The sensitivity of a synapse to its past activity can lead to a long term change in its future effectiveness which is all we need to build memory into a neural circuit.

It has been very difficult to demonstrate that specific forms of memory use particular types of synaptic plasticity and correlation of memory with synaptic plasticity remains a coveted goal of current research (Nell et al., 1997).

2.6 JUSTIFICATION

There is emerging evidence for application of phytochemicals in medicine in spite of the wide use of conventional pharmaceutical products. Medicinal herbs, plants and foods have been used for a long time to slow cognitive decline in aging and to manage age related memory deficits (Giancarlo, 2006).

Scientific studies and reports suggest that the most important active ingredient in wild yam root (diosgenin) has potential to enhance brain function. The effect of pure diosgenin on memory has been demonstrated but the effect of wild yam on memory needs to be investigated further. (Tohda et al., 2017).

The World Health Organization (WHO) estimates that up to 80% of people globally still rely on traditional remedies for their medicines resulting in the increasing demand for medicinal plants. About 85% of traditional medicines involve the use of plant extracts (Ekor, 2014). Wild yam products have been used and are still being used widely by women as a natural alternative to hormone replacement therapy for menopausal symptoms. It is estimated that at least 80% of Africans prefer traditional medicine for the treatment of common ailments (Elujoba et al., 2005)

2.7 HYPOTHESES

- **Null Hypothesis (Ho):**
  - Wild yam tincture (*Dioscorea Villosa*) has no effect on memory and learning in mice.
- **Alternate hypothesis (HA):**
  - Wild yam tincture (*Dioscorea Villosa*) has an effect on memory and learning in mice.

AIM: To investigate the effect of wild yam tincture on memory and learning in mice.

2.8 OBJECTIVES

- **General Objective:** To investigate the effect of wild yam tincture on memory and learning in male mice of different ages.
- **Specific Objectives:**

1. To determine the effect of wild yam tincture on long term memory in mice.
2. To determine the effect of wild yam tincture on short term memory in mice.
3. To determine the effect of wild yam tincture on behavioral flexibility in mice.
4. To determine the effect of wild yam tincture on memory retention in mice.

CHAPTER THREE: MATERIALS AND METHODS

3.1 WILD YAM TINCTURE:

Mexican wild yam tincture (D. *villosa*) obtained from Healthy U, Sarit centre-Nairobi. Manufacturer: Nature's Laboratory – North Yorkshire Y022 4NH, U.K. Root Tincture: 1:3 45%.

Wild yam tincture was used because it was easily dispensed to mice using a gavage needle. Other available preparations such as root powders and creams were not found suitable for administration to mice.

The chemical analysis was done according to the following method:

1. **Extraction of** *Dioscorea villosa* **(wild yam) root tincture:**

1000 ml of the *D. villosa* was extracted with 1000 ml of distilled ethyl acetate to obtain the crude extract. Separation was done using a funnel to obtain the aqueous layer which was then evaporated using a rotary evaporator under reduced pressure to obtain 1 gm of the crude product.

2. **Isolation of Diosgenin:**

Crude extract of *D. villosa* (1g) was dissolved in a mixture of 1:1 CH_2Cl_2/MeOH (Dichloromethane/methanol) then transferred into a round bottomed flask containing 1g of silica gel.

Homogenization was done using rotary evaporator under reduced pressure to give a dry and uniformly adsorbed extract. The adsorbed extract was then spread on the bench to dry and then the ground powder using a mortar and pestle. The powder was carefully loaded into a small column packed with 10g of silica gel under *n*-hexane solvent.

Elution was done with increasing polarities of n-hexane/$CHCl_3$ (Chloroform) then CH_2Cl_2/MeOH (Dichloromethane/ methanol). 100 ml of the elute were collected and evaporated. Analytical Thin Layer Chromatography (TLC) plates spotting showed the compound was eluted at 100% CH_2Cl_2 (Dichloromethane) and 1% CH_2Cl_2/MeOH (Dichloromethane/ methanol).

3. **Percentage yield of Diosgenin:**

Weight of the crude extract=1g=1000mg

Weight of the diosgenin crystals=1mg

% yield= 1/1000 X 100= 0.1%

Dosage of tincture

Doses of tincture vary widely according to the strength of the preparation.

The dosage of the yam tincture used in our experiment was calculated on proportional weight basis using the manufacturer's recommended human food supplement dose of 800mg of wild yam tincture twice daily for a 70kg man.

A standard dosage of 0.5mls /day/mouse of wild yam tincture was determined and administered to the test animals by oral gavage for 30 days. The dosage was calculated on the basis of volume: weight ratio for a 70kg man (Human dosage range (40 – 50 mls/day).

3.2 T-MAZE

It is an apparatus shaped like a T and can be used to test learning and memory in rodents.

T – maze procedures were used to test spatial learning, short term memory and long term memory in male mice.

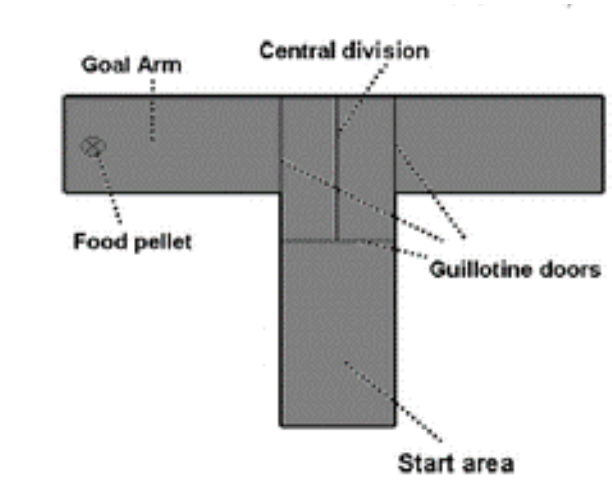


Figure 13: T – Maze

Adapted from: https://neurowiki2012.wikispaces.com/Animal+models+and+learning+paradigms

Three cohorts of mice (A, B and C) were subjected to the T-maze task before and after the test animals were given wild yam tincture by mouth through gavage. Both the test and control animals underwent training and test sessions (10 sessions per animal per day).

3.3 EXPERIMENTAL ANIMALS

Forty male Swiss albino mice obtained from Department of Public Health, Pharmacology and Toxicology, University of Nairobi were used. At the beginning of the experiment the animals were randomly allocated into two age cohorts (n=20); ages 3-6 months (mature adult) and 10-14 months (middle age) and each cohort was further divided into a control and test group (n=10) each.

The old age group was not available at the beginning of the experiment but some of the animals used during their middle age had reached old age (18-24 months) in the course of the experimental period which lasted 8 months. The sample size was determined using the G – power analysis method. The sample size is also supported by previous similar animal studies.

The animals weighed 20-30gms and were fed *ad libitum* on commercial pellets (by Unga Farm Care Africa LTD) provided water supply and kept at standard laboratory conditions in accordance with FELASA (Federation of European Laboratory Animal Science Associations) guidelines. The animals were housed in separate cages in the department of Medical Physiology, animal unit. Exclusion criteria included any animal that was clinically unwell. One week acclimatization period was allowed for the animals before experimentation. The mouse was chosen as the experimental animal due to its close genetic make up to that of the human species.

Male mice were used because they have nearly constant hormonal levels contrary to females; and estrous cycling affects reference memory in rodents (Pompil et al., 2010). All the experiments were performed during the same time period (9.00 am to 6.00 pm).

Figure 14: Experimental Mice Cohort A

Figure 15: Experimental Mice Cohort B

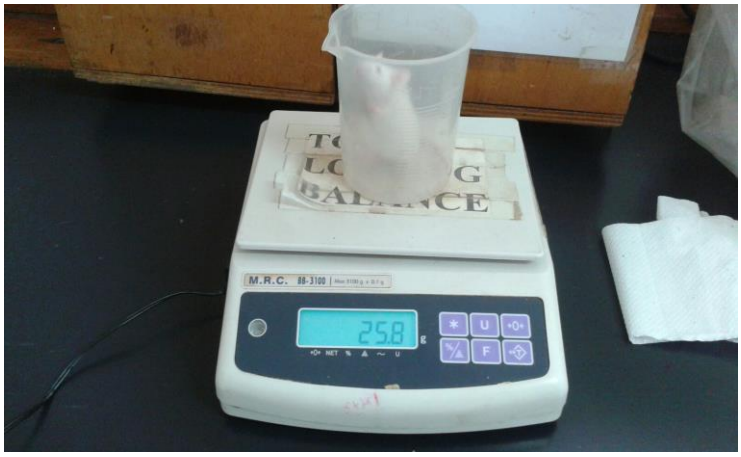


Figure 16: Experimental mouse being weighed using a weighing balance

Figure 17: Researcher weighing experimental mouse

Figure 18: Researcher administering oral gavage to experimental mouse

Table 1: Age groups of mice cohorts during experimental period (Aug 2015-April 2016)

	Cohort			
	A	**B**	**C**	**MEMORY TEST**
MONTH	(3–6 months) Mature Adult	(10–14 months) Middle Age	(18–24 months) Old Age	-
SEPT. 2015	√	√	O	-
OCT.2015	√	√	O	-
NOV.2015	√	√	O	-
DEC.2015	√	A* + B	B*	L/R/D – Task (L T M)
JAN.2016	√	A* + B	B*	-
FEB.2016	√	A* + B	B*	L/R/D – Task (L T M)
MAR.2016	O	A* + B	B*	REVERSAL TEST (Behavioral Flexibility)
APRIL 2016	O	A* + B	B*	**1.**Forced Alt Task (STM)
				2.Delayed Alt Task (Memory retention)

KEY

- A – Young mature adult cohort
- B – Middle age cohort
- A* Cohort A mice in middle age group
- B* Cohort B mice in old age group
- LTM : Long Term Memory
- STM : Short Term Memory
- L/R/D : Left Right Discrimination

This study investigated the effect of wild yam tincture on memory and learning in three cohorts of male mice at different age phases:

3.3.1 Cohort A: Young mature Adult (3-6 months)

Human Age Equivalent (20-30years)

This age group represents mice that are mature and have not undergone senescence. Beyond six months mice may exhibit some age related change. This age group therefore can be considered the reference for any significant age change.

3.3.2 Cohort B: Middle Age (10-15 months)

Human Age Equivalent (38-47years)

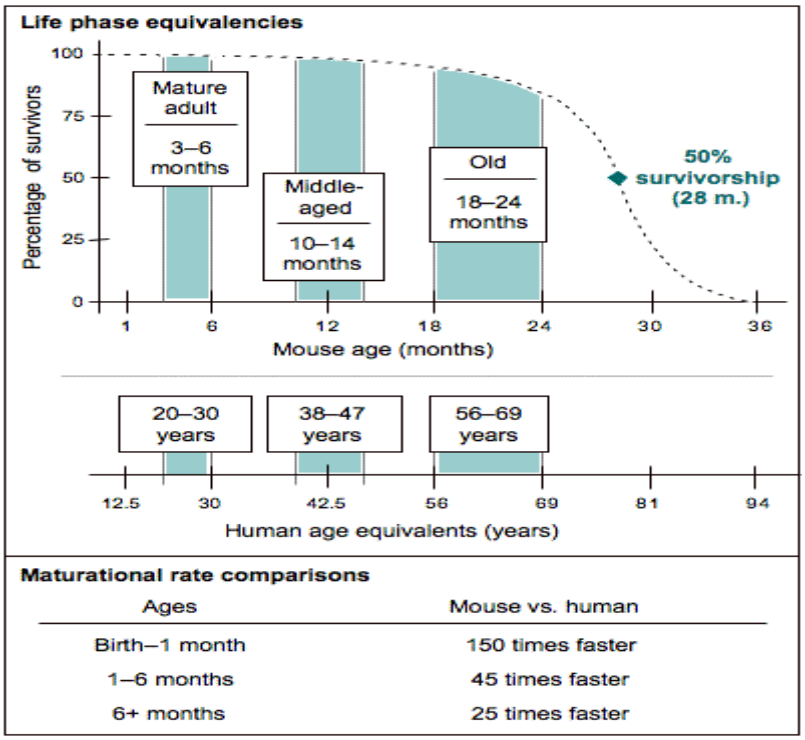
This age group represents animals with changes in some biomarkers of aging in mice. This age phase maybe an indicator of whether age related changes are progressive or appear only in old age for the first time.

3.3.3 Cohort C: Old Age (18-24 months)

Human Age Equivalent (56-69 years)

This age group represents mice with significant age related changes in the biomarkers of aging in most animals. Senescent changes are associated with this period in almost all biomarkers.

The upper age limit is influenced by the particular genotype of mice. Mouse age in human years is calculated on the basis of an age online conversion tool which equates one mouse year to 0.05 human years (Dutta et al., 2015). The three age groups were fed on wild yam tincture by oral gavage and their respective controls served as vehicle treated controls for the same period of time. The above age groups were tested for spatial learning and cognitive memory.



Maturational rate comparisons

Ages	Mouse vs. human
Birth–1 month	150 times faster
1–6 months	45 times faster
6+ months	25 times faster

Figure 19: Life phases in mice

Representative age ranges for mature life history stages in C57BL/6J mice; comparison to human beings. (Adapted from Figure 20-3: Flurkey K, Currer JM, Harrison DE. 2007. The Mouse in Aging Research.

3.4 EXPERIMENTAL PROTOCOL

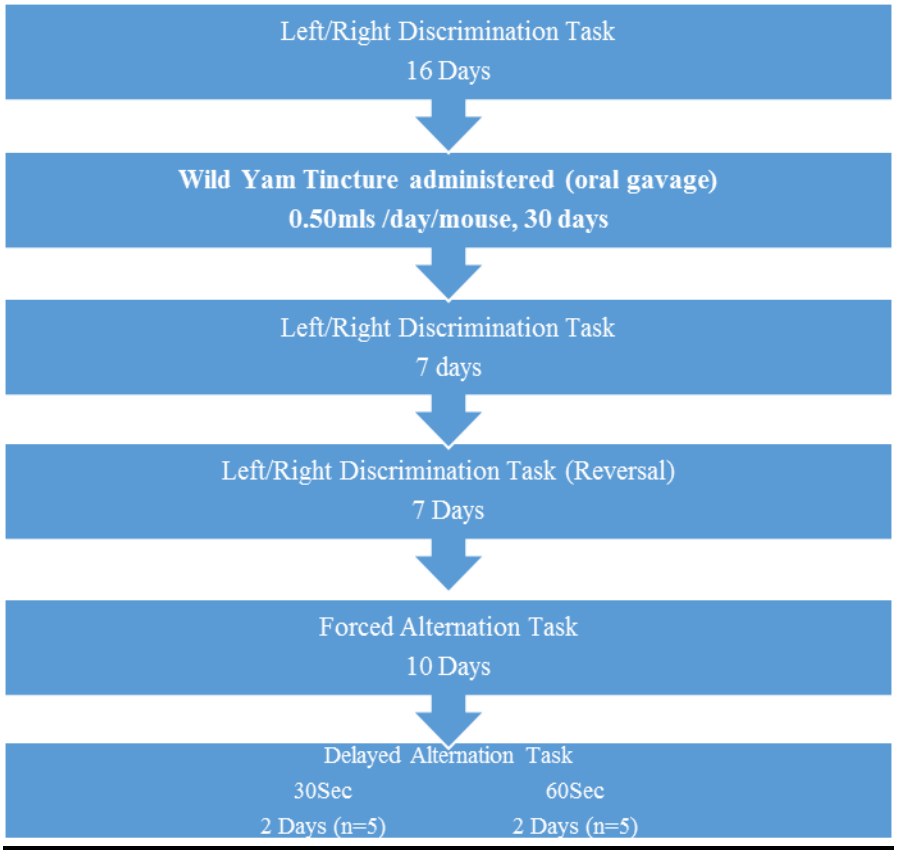


Figure 20: Experimental protocol for memory and learning tests

Courtesy of Nature Protocols 2006

Memory Tests

- **T – maze left-right discrimination task**
  - Test was used to assess long term memory
- **T – maze forced alternation task**
  - Test was used to assess short term memory
- **Reversal learning test**
  - Test was used to assess behavioral flexibility
- **T – maze delayed alternation task**
  - Test was used to assess retention of memory

Experimental Procedure

Habituation

The researcher held the animals gently, picked them up and put them down slowly in their cages several times at intervals of five minutes for the animals to get used to the researcher's touch.

Mice were fed with rodent food pellets and water supply in their respective cages.

One month before the pre-training sessions mice were weighed daily and fed with food pellets which were rationed to allow the animals maintain 85% of their free feeding body weight. The mice were also provided with Kellogg's honey loops (food reward) daily in their home cages to habituate the animals to its taste.

Habituation to the T – maze apparatus and pre-training

Pieces of honey loops were placed inside the T – maze and all mice in a cage were allowed to explore the maze freely and eat the food reward for a duration of three minutes.

After habituation mice were subjected to pre-training sessions daily for five days. Training entailed placing the mouse in the start location arms of the T – maze and allowing it to enter in one of the baited arms to locate and eat the food reward.

Each mouse in a cohort of ten received a trial run in succession before the first animal started its first trial. Ten trials per animal were run in a daily session.

If the mouse entered the baited arm (goal arm) and ate the honey loops, within 1 – 2 minutes it was then removed and returned to the start location for subsequent runs. Equal numbers of left and right runs were given.

After the pre-training sessions, the mice were subjected to various memory tests.

Left-right discrimination task

Procedure

The test and control mice were given a free choice run of ten trials in a session per day for 16 days before administration of wild yam tincture.

The mouse was placed at the start goal of the T – maze and allowed to run forward and choose to enter either of the right or left arms of the maze. One arm of the maze was baited with the food reward (the goal arm).

If the mouse entered the baited arm and ate the food reward the response was scored as a correct response and if the animal did not eat the reward within 30 seconds the response was considered an omission error.

The animal was thereafter returned to the start goal for subsequent runs.

During the trial period four parameters were scored for each mouse in each cohort [correct response (%), Latency (sec), Distance travelled (cm), Omission errors].

The group average for each of the above parameters was determined.

After 16 days of the above trial sessions the test animals were administered with wild yam tincture for 30 days and thereafter they were subjected to the left right discrimination task for 7 days.

Left-right discrimination task (Reversal)

The procedure was similar to the left-right discrimination task described above except the food reward was placed in the T – maze arm that was previously unbaited.

After each trial the four parameters mentioned above were scored and the average score for the group was determined at the end of each session.

In the above reversal task the trials were done for a period of seven days as per the protocol.

Forced alternation task

In the above task each trial consists of a forced choice run followed by a free choice run in the maze.

Procedure

The mouse was placed in the start location of the maze and the door of the right arm of the maze was closed (blocked) while the left arm remained open and baited with the food reward.

The mouse therefore was "forced" to enter the left arm and eat the food reward.

If the mouse entered the arm and did not eat the reward within 30 seconds the response was considered an "omission error".

The mouse was thereafter returned to the start location for a free choice run.

Doors to both the left and right arms of the maze were opened and the mouse allowed to make a choice between the two arms of the maze.

If the mouse chose the arm it was forced to enter in the first run (i.e. the left arm) the response was considered an "error" response and hence the mouse was confined within that arm for 10 seconds as a penalty. Thereafter the mouse was returned to the start goal for subsequent trials.

However if the mouse entered the right arm (i.e. the arm that was opposite the one visited in the forced choice run) the response was considered a correct response and the mouse received a reward. If the mouse did not consume the reward within 30 seconds the response was considered an "omission error" and scored as such.

The test and control mice underwent 10 consecutive trials per session for 10 days.

The group average was then determined for each of the four parameters in each cohort.

At the end of each session the animals were returned to their respective cages and the apparatus cleaned to prevent a bias based on olfactory cues.

Delayed alternation task

This test was carried out to assess retention of short term memory in mice for a period of 4 days. The procedure was similar to the forced alternation task outlined above except that time delays were inserted between the forced choice run and the free choice run.

A delay of 30 seconds and 60 seconds was inserted between the sessions (i.e. after the forced choice run the animal was delayed for 30 seconds before it was released to undergo the free choice run). This was also repeated for 60 seconds. The trial sessions were done in two days for each delay period.

The test animals were divided into two groups (n=5) for each delay period.

The parameter scored in this test was the "correct response“ only.

3.5 MEMORY TESTS

Parameters of measurement in memory tasks:

In the memory tasks, four parameters were measured namely: **correct response;** a measure of the number of times a mouse enters the correct maze arm (goal arm) across a session of ten trials. This parameter evaluates the level of learning ability of the rodent. **Latency;** the amount of time in seconds that the mouse takes to eat the food reward during a trial session. This parameter evaluates the spatial learning ability and performance of the animal. **Distance travelled;** the total distance in centimeters that the mouse travels during a trial session and it assesses the animal's spatial learning ability. **Omission errors;** measures the number of times the mouse fails to eat the food reward within 30 seconds during a trial session and the errors are a reflection of the opportunity the animal had to locate the food reward in the goal arm. An increase in omission errors suggests delayed learning acquisition and tends to increase during the initial trial sessions. The four parameters are hence an index of spatial learning ability and overall performance of the animal in the T- maze. Correct response, latency and cumulative distance are co-related hence are measures of the animals spatial learning ability and performance (Shoji, H., et al., 2012, Deacon & Rawlins 2006). Differences in latency and distance travelled maybe attributed to factors such as motivation, levels of training and habituation, learning strategies and behavioral flexibility (Shoji, H., et al., 2012). Consideration of correct responses and omission errors may indicate the learning ability and level of performance of the rodent (Shoji, H., et al., 2012). For each parameter an average value for the group was calculated at the end of ten consecutive trials per session.

3.6 DATA AND STATISTICAL ANALYSIS

Data generated from the study were entered into STATA version 11 statistical software and were analyzed using independent t – test. Results were expressed as means ± standard error of means (SEM). Differences were considered to be significant if $P < 0.05$.

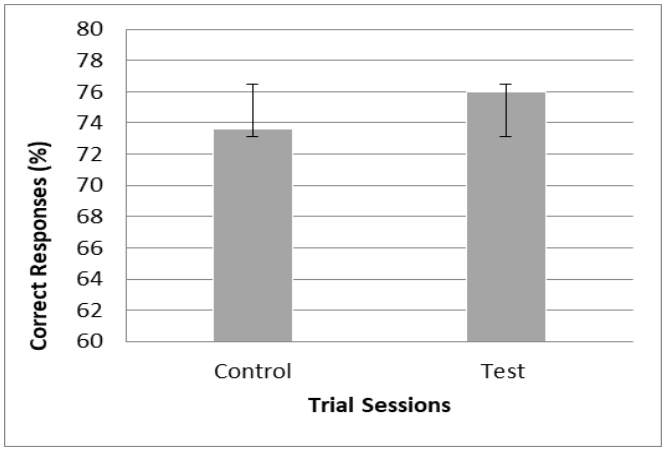
CHAPTER FOUR: RESULTS

4.1 Extract yield

The weight of the crude extract of wild yam tincture (*D. Villosa*) was determined and the main active ingredient diosgenin was isolated through a solvent system of Dichloromethane/methanol (CH_2CL_2/MeOH).

4.2.1 T-maze left-right discrimination task

Mice received ten trials daily for 16 days and the average percentage of correct responses for the group was determined. There were no significant differences in the percentage of correct responses between the control mice and test mice before administration of wild yam tincture [**73.6 ± 1.5% (c) Vs. 76 ± 1.1% (t) , t=1.24** p=**0.2232**]. A graphical representation of the results is shown in Figure 21.



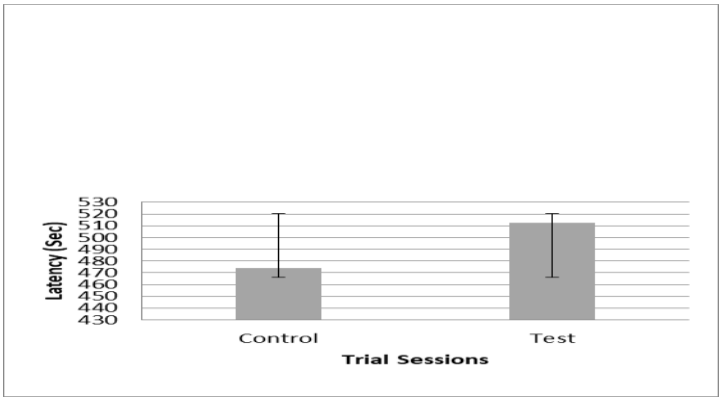
Cohort A

(Young Mature Adult)

Figure 21: Bar graph showing correct responses before administration of wild yam tincture

4.2.2 T-maze left-right discrimination task

Mice received ten trials daily for 16 days and the average latency for the group was determined. There were no significant differences in latency between the control mice and test mice before administration of wild yam tincture [**474.3 ± 17.5 sec (c) Vs 512.6 ± 18.8 sec (t), t=1.5 p = 0.1469**]. A graphical representation of the results is shown in Figure 22.



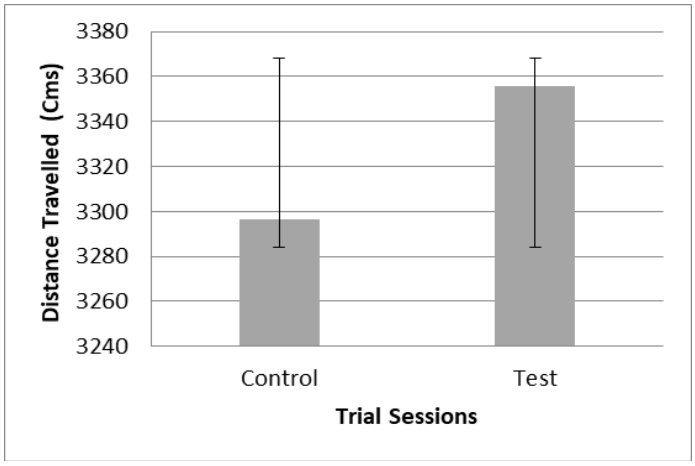
Cohort A

(Young Mature Adult)

Figure 22: Bar graph showing latency before administration of wild yam tincture

4.2.3 T-maze left-right discrimination task

Mice received ten trials daily for 16 days and the average total distance travelled by the group was determined. There were no significant differences in the distance travelled between the control mice and test mice before administration of wild yam tincture [**3324.3 ± 13.9 cm (c) Vs 3357 ± 23.8 cm (t), t=1.20, p=0.2563**]. A graphical representation of the results is shown in Figure 23.



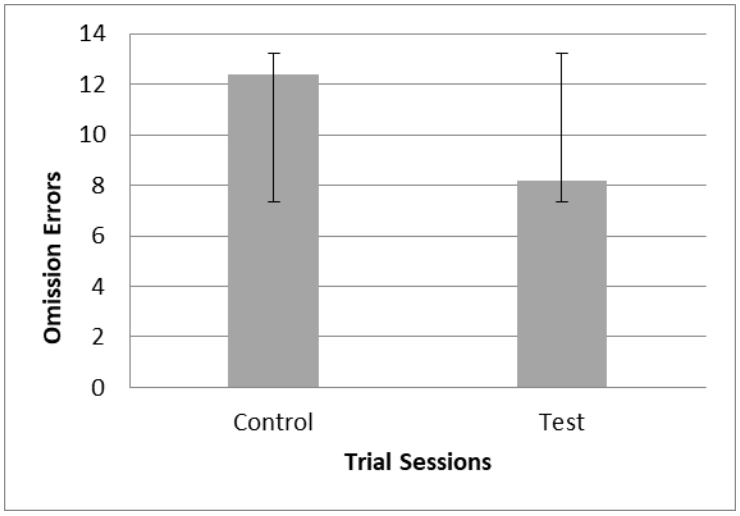
Cohort A

(Young Mature Adult)

Figure 23: Bar graph showing distance travelled before administration of wild yam tincture

4.2.4 T-maze left-right discrimination task

Mice received ten trials daily for 16 days and the average number of omission errors for the group was determined. There were no significant differences in the number of omission errors between the control mice and test mice before administration of wild yam tincture [**4.7 ± 1.0 (c) Vs 3.9 ± 0.7 (t), t= 0.7039, p=0.4950**]. A graphical representation of the results is shown in Figure 24.



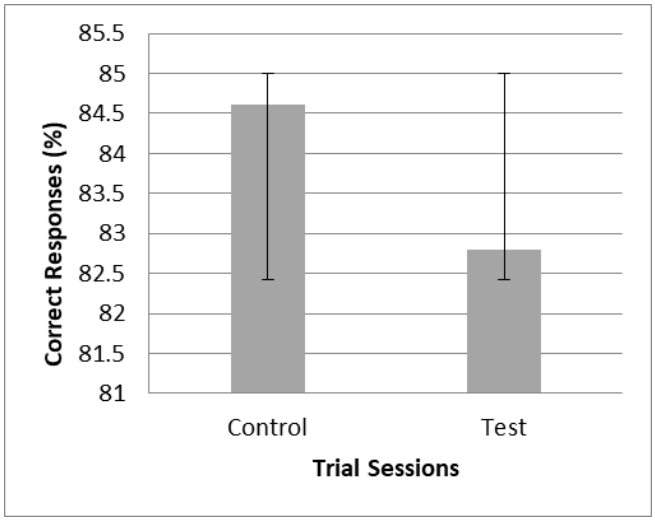
Cohort A

(Young Mature Adult)

Figure 24: Bar graph showing omission errors before administration of wild yam tincture

4.3.1 T-maze left-right discrimination task

Mice received ten trials daily for 16 days and the average percentage of correct responses for the group was determined. There were no significant differences in the percentage of correct responses between the control mice and test mice before administration of wild yam tincture [**84.6 ± 1.9 % (c) Vs. 82.8 ± 1.8 % (t), t= -0.6937, p= 0.4932**]. A graphical representation of the results is shown in Figure 25.



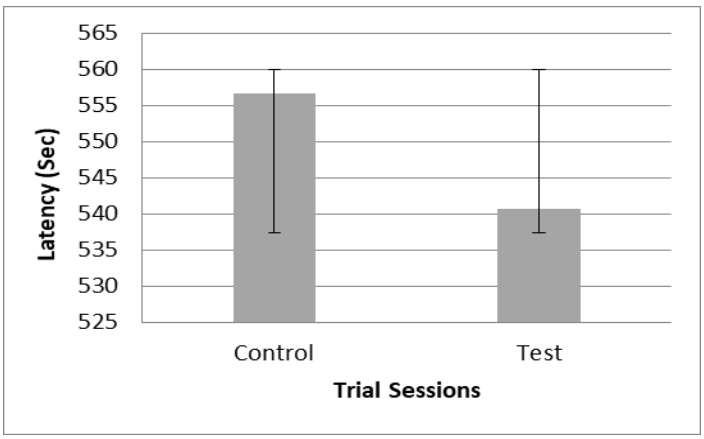
Cohort B

(Middle Age Mice)

Figure 25: Bar graph showing correct responses before administration of wild yam tincture

4.3.2 T-maze left-right discrimination task

Mice received ten trials daily for 16 days and the average latency for the group was determined. There were no significant differences in latency between the control mice and test mice before administration of wild yam tincture [**556.6 ± 9.2 sec (c) Vs 540.6 ± 4.2 sec (t), t = -1.5782, p=0.1250**]. A graphical representation of the results is shown in Figure 26.



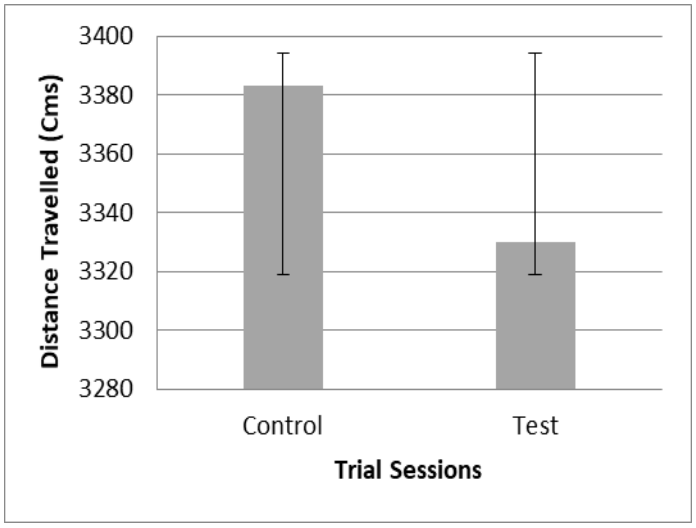
Cohort B

(Middle Age Mice)

Figure 26: Bar graph showing latency before administration of wild yam tincture

4.3.3 T-maze left-right discrimination task

Mice received ten trials daily for 16 days and the average total distance travelled by the group was determined. There were no significant differences in the distance travelled between the control mice and test mice before administration of wild yam tincture [**3367 ± 8.4 cm (c) Vs. 3375 ± 9.6 cm (t),t= 0.8963, p=0.3877**]. A graphical representation of the results is shown in Figure 27.



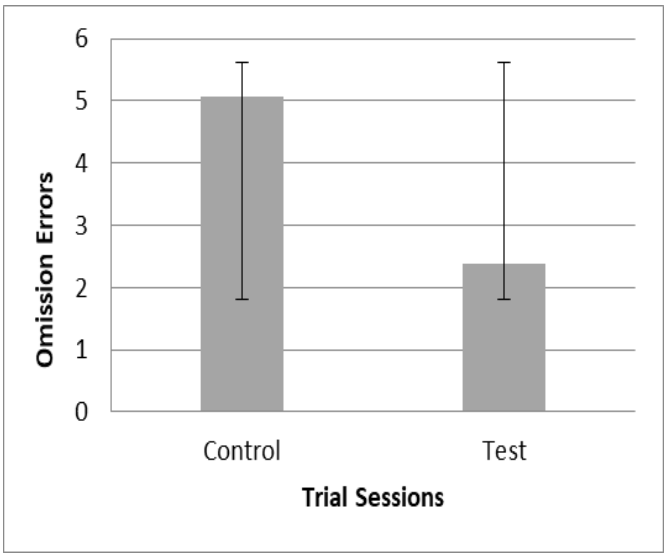
Cohort B

(Middle Age Mice)

Figure 27: Bar graph showing distance travelled before administration of wild yam tincture

4.3.4 T-maze left-right discrimination task

Mice received ten trials daily for 16 days and the average number of omission errors by the group was determined. There were significant differences in the number of omission errors between the control mice and test mice before administration of wild yam tincture [**2.4 ± 0.5 (c) Vs. 5.1 ± 0.7 (t), t= 3.31, p= 0.0025**]. A graphical representation of the results is shown in Figure 28.



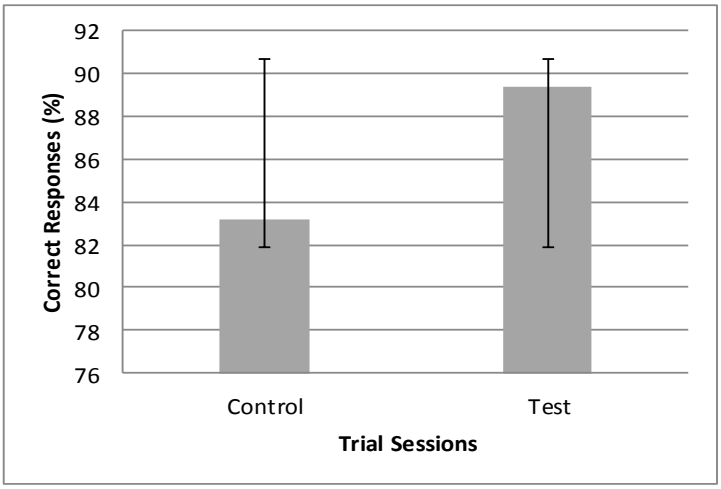
Cohort B

(Middle Age Mice)

Figure 28: Bar graph showing omission errors before administration of wild yam tincture

4.4.1 T-maze left-right discrimination task

Mice received ten trials daily for 7 days and the average percentage of correct responses for the group was determined. There were no significant differences in the percentage of correct responses between the control mice and test mice after administration of wild yam tincture [**83.2 ± 2.4 % (c) Vs. 89.4 ± 2.1 % (t), t= 1.94, p=0.0757**]. A graphical representation of the results is shown in Figure 29.



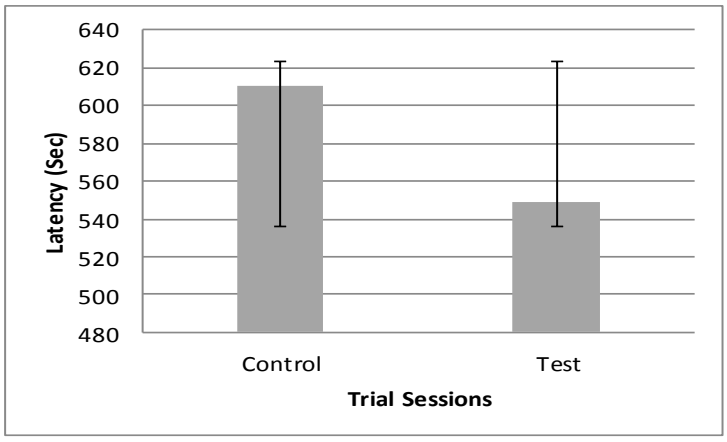
Cohort A

(Young Mature Adult)

Figure 29: Effect of wild yam tincture on correct response

4.4.2 T-maze left-right discrimination task

Mice received ten trials daily for 7 days and the average latency for the group was determined. There were significant differences in latency between the control mice and test mice after administration of wild yam tincture [**610.6 ± 3.6 sec (c) Vs. 549 ± 17.8 sec (t) , t=-7.1197, p<0.0001**]. A graphical representation of the results is shown in Figure 30.



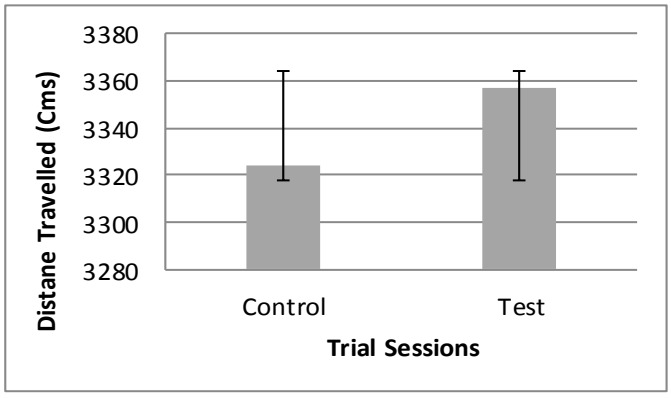
Cohort A

(Young Mature Adult)

Figure 30: Effect of wild yam tincture on latency

4.4.3 T-maze left-right discrimination task

Mice received ten trials daily for 7 days and the average total distance travelled for the group was determined. There were no significant differences in the distance travelled between the control mice and test mice after administration of wild yam tincture [**3324.3 ± 13.9 cm (c) Vs. 3357 ± 23.8 cm (t), t=1.20, p=0.2563**]. A graphical representation of the results is shown in figure 31.



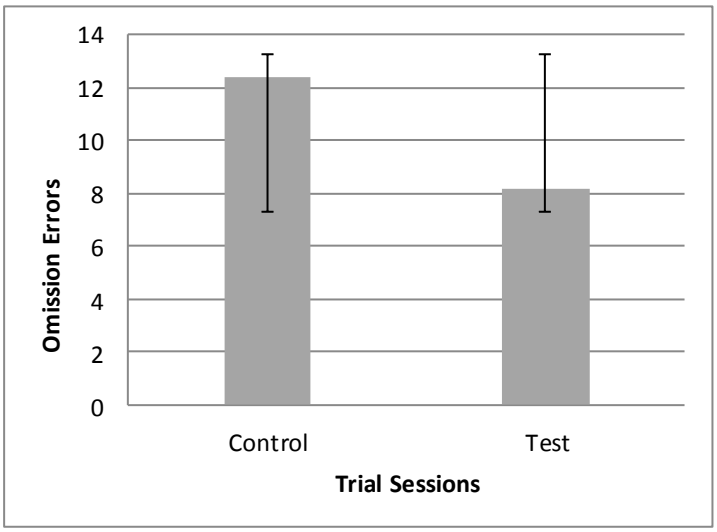
Cohort A

(Young Mature Adult)

Figure 31: Effect of wild yam tincture on distance travelled

4.4.4 T-maze left-right discrimination task

Mice received ten trials daily for 7 days and the average number of omission errors travelled for the group was determined. There were significant differences in the number of omission errors between the control mice and test mice after administration of wild yam tincture [**12.4 ± 1.34 (c) Vs. 8.2 ± 1.4 (t), t=2.2, p=0.0136**]. A graphical representation of the results is shown in Figure 32.



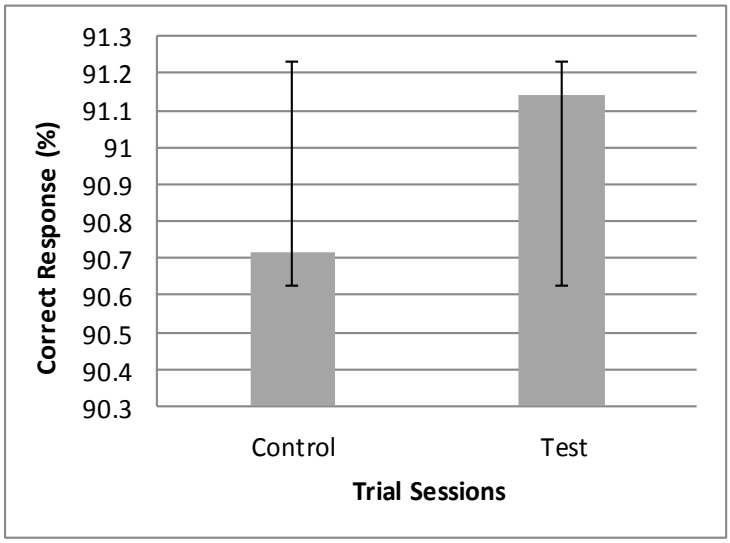
Cohort A

(Young Mature Adult)

Figure 32: Effect of wild yam tincture on omission errors

4.5.1 T-maze left-right discrimination task

Mice received ten trials daily for 7 days and the average percentage of correct responses for the group was determined. There were no significant differences in the percentage of correct responses between the control mice and test mice after administration of wild yam tincture [**90.7 ± 1.60% (c) Vs. 91.1 ± 2.15% (t), t=0.1603, p=0.8753**]. A graphical representation of the results is shown in Figure 33.



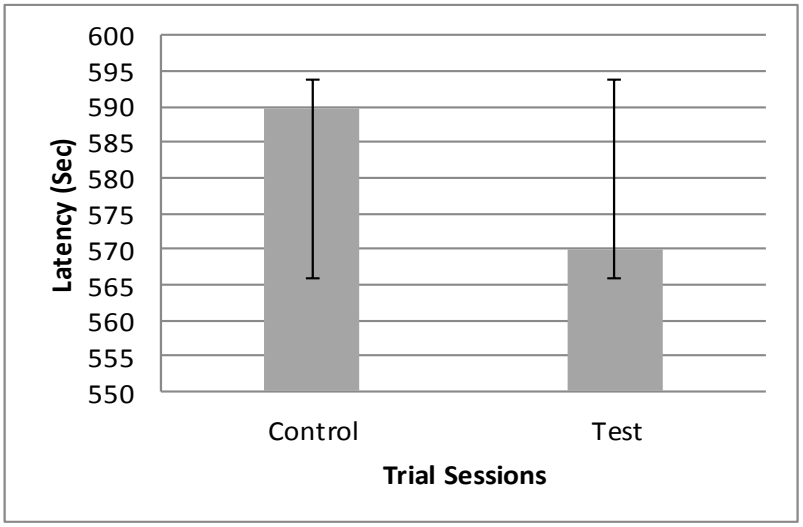
Cohort B

(Middle Age Mice)

Figure 33: Effect of wild yam tincture on correct responses

4.5.2 T-maze left-right discrimination task

Mice received ten trials daily for 7 days and the average latency for the group was determined. There were no significant differences in latency between the control mice and test mice after administration of wild yam tincture [**589.7 ± 2.5 sec (c) Vs. 569.9 ± 9.7 sec (t), t = -1.9828, p=0.0708**]. A graphical representation of the results is shown in Figure 34.



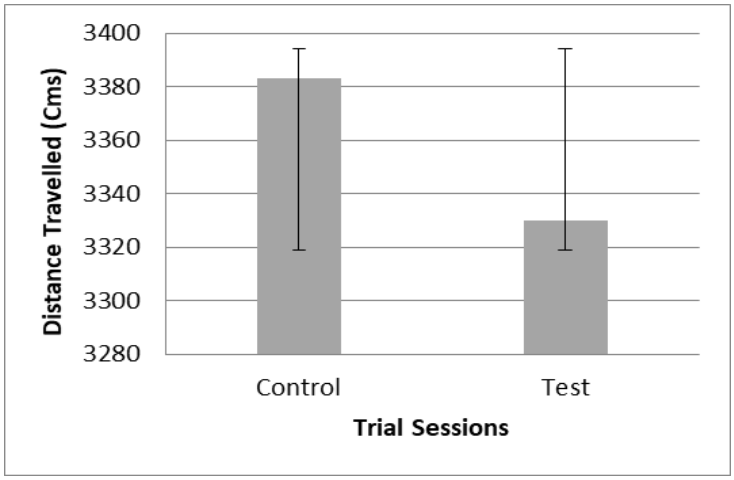
Cohort B

(Middle Age Mice)

Figure 34: Effect of wild yam tincture on latency

4.5.3 T-maze left-right discrimination task

Mice received ten trials daily for 7 days and the average total distance travelled by the group was determined. There were significant differences in distance travelled between the control mice and test mice after administration of wild yam tincture [**3383 ± 4.7 cm (c) Vs. 3330 ± 19.5 cm (t),t= -2.6460, p=0.0128**]. A graphical representation of the results is shown in Figure 35.



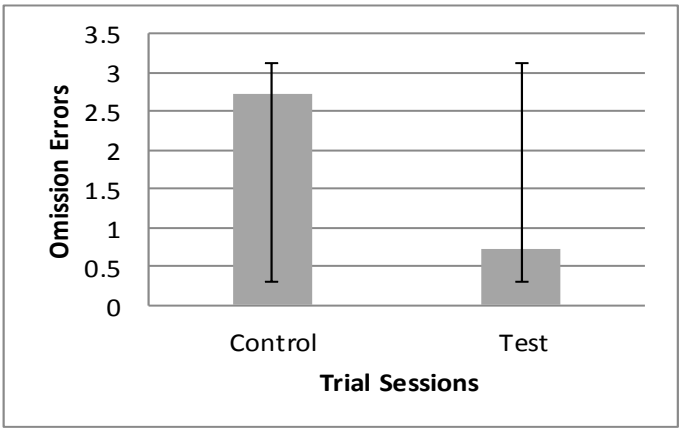
Cohort B

(Middle Age Mice)

Figure 35: Effect of wild yam tincture on distance travelled

4.5.4 T-maze left-right discrimination task

Mice received ten trials daily for 7 days and the average number of omission errors for the group was determined. There were significant differences in the number of omission errors between the control mice and test mice after administration of wild yam tincture [**0.7± 0.30 (c) Vs. 2.7 ± 0.70 (t), t=2.7111, p=0.0189**]. A graphical representation of the results is shown in Figure 36.



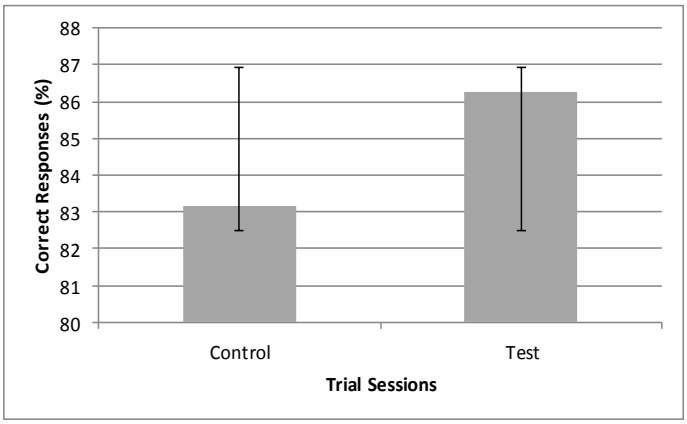
Cohort B

(Middle Age Mice)

Figure 36: Effect of wild yam on tincture on omission error

4.6.1 T-maze left-right discrimination task (Reversal)

Mice received ten trials daily for seven days and the average percentage of correct responses for the group was determined. There were no significant differences in the percentage of correct responses between the control mice and test mice after administration of wild yam tincture [**82.9 ± 2.5% (c) Vs. 86.6 ± 2.4% (t) , t=1.0674, p=0.3068**]. A graphical representation of the results is shown in Figure 37.



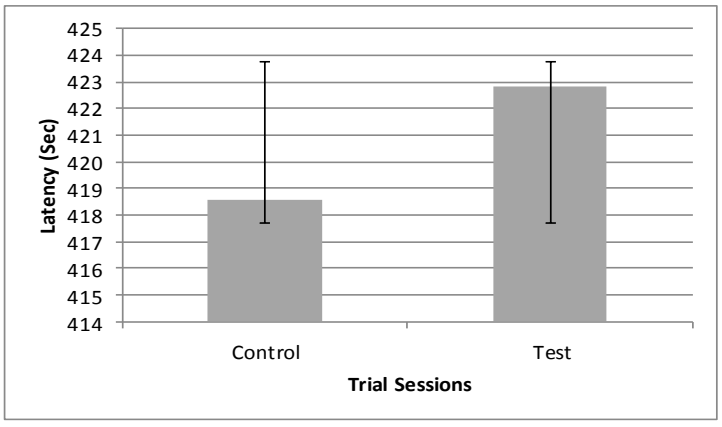
Cohort B

(Middle Age Mice)

Figure 37: Effect of wild yam tincture on correct responses

4.6.2 T-maze left-right discrimination task (Reversal)

Mice received ten trials daily for 7 days and the average latency for the group was determined. There were no significant differences in latency between the control mice and test mice after administration of wild yam tincture [**418.6 ± 6.6 sec (c) Vs. 422.9 ± 7.5 sec (t), t=0.4297, p=0.6751**]. A graphical representation of the results is shown in Figure 38.



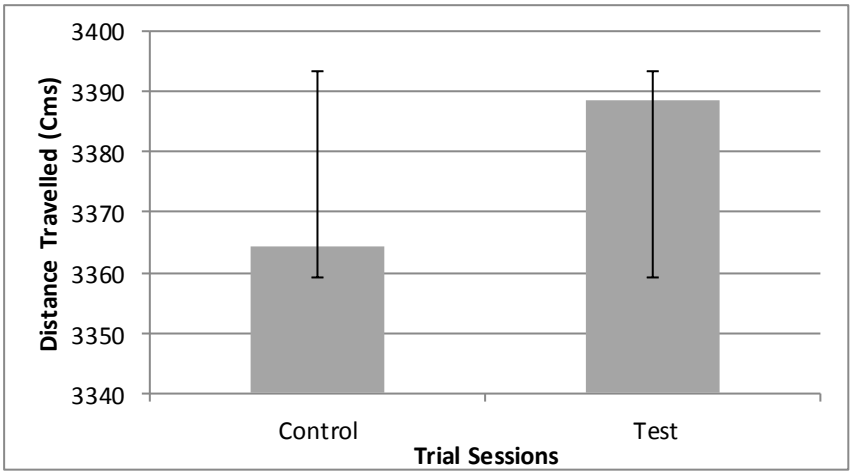
Cohort B

(Middle Age Mice)

Figure 38: Effect of wild yam tincture on latency

4.6.3 T-maze left-right discrimination task (Reversal)

Mice received ten trials daily for 7 days and the average distance travelled for the group was determined. There were no significant differences in the distance travelled between the control mice and test mice after administration of wild yam tincture [**3364.3 ± 17.4 cm (c) Vs. 3388.6 ± 5.1 cm (t), t=1.3370, p=0.2060**]. A graphical representation of the results is shown in Figure 39.



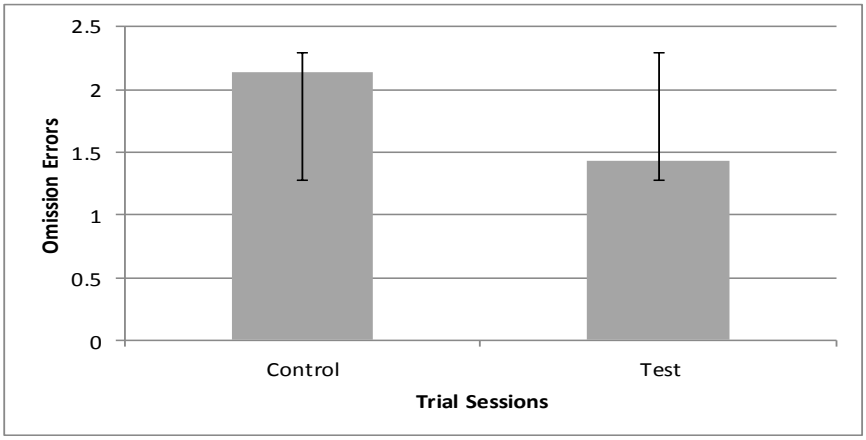
Cohort B

(Middle Age Mice)

Figure 39: Effect of wild yam tincture on distance travelled

4.6.4 T-maze left-right discrimination task (Reversal)

Mice received ten trials daily for 7 days and the average number of omission errors for the group was determined. There were no significant differences in omission errors between the control mice and test mice after administration of wild yam tincture [**2.14 ± 0.46 (c) Vs. 1.43 ± 0.53 (t) , t= -1.0206, p= 0.3276**]. A graphical representation of the results is shown in Figure 40.



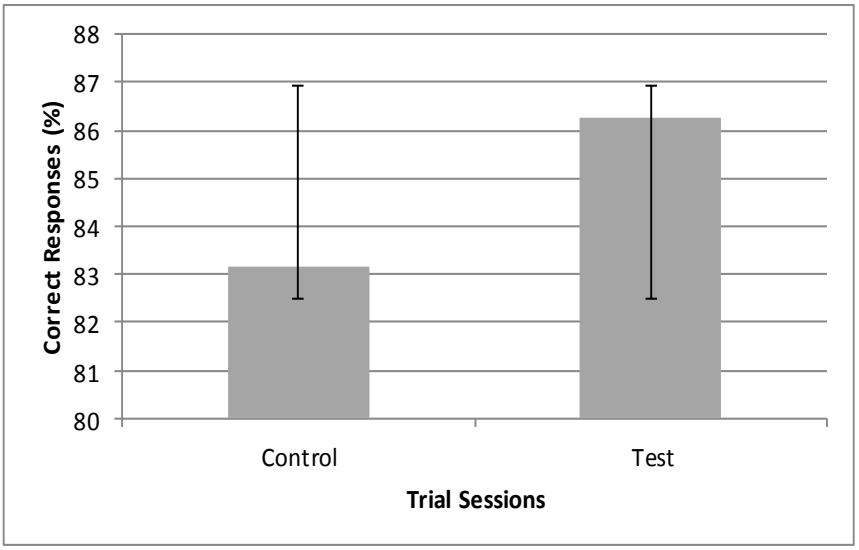
Cohort B

(Middle Age Mice)

Figure 40: Effect of wild yam tincture on omission errors

4.7.1 T-maze left-right discrimination task (Reversal)

Mice received ten trials daily for 7 days and the percentage of correct responses for the group was determined. There were no significant differences in the percentage of correct responses between the control mice and test mice after administration of wild yam tincture [**83.14 ± 2.5% (c) Vs. 86.3 ± 2.4 % (t), t=1.0674, p=0.3068**]. A graphical representation of the results is shown in Figure 41.



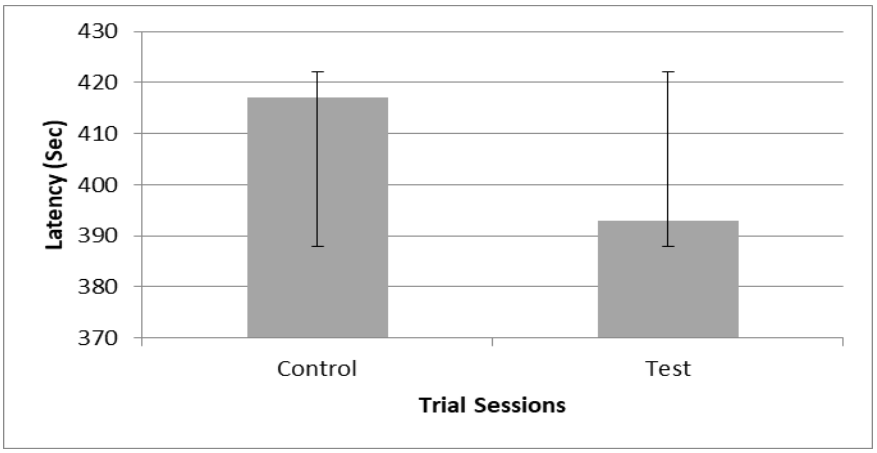
Cohort C

(Old Age Mice)

Figure 41: Effect of wild yam tincture on correct responses

4.7.2 T-maze left-right discrimination task (Reversal)

Mice received ten trials daily for 7 days and the average latency for the group was determined. There were no significant differences in latency between the control mice and test mice after administration of wild yam tincture [**417.14 ± 9.4 sec (c) Vs. 392.9 sec ± 10 sec (t),t= -1.7668, p=0.0585**]. A graphical representation of the results is shown in Figure 42.



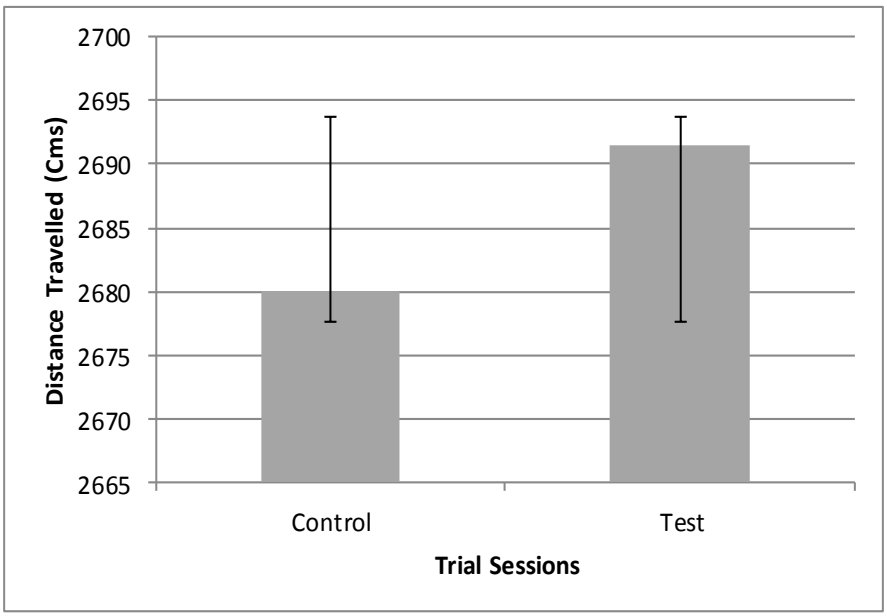
Cohort C

(Old Age Mice)

Figure 42: Effect of wild yam tincture on latency

4.7.3 T-maze left-right discrimination task (Reversal)

Mice received ten trials daily for 7 days and the average total distance travelled for the group was determined. There were no significant differences in the distance travelled between the control mice and test mice after administration of wild yam tincture [**2680 ± 21.7 cm (c) Vs. 2691 ± 10.3 cm (t) , t= 0.4753, p=0.6431**]. A graphical representation of the results is shown in Figure 43.



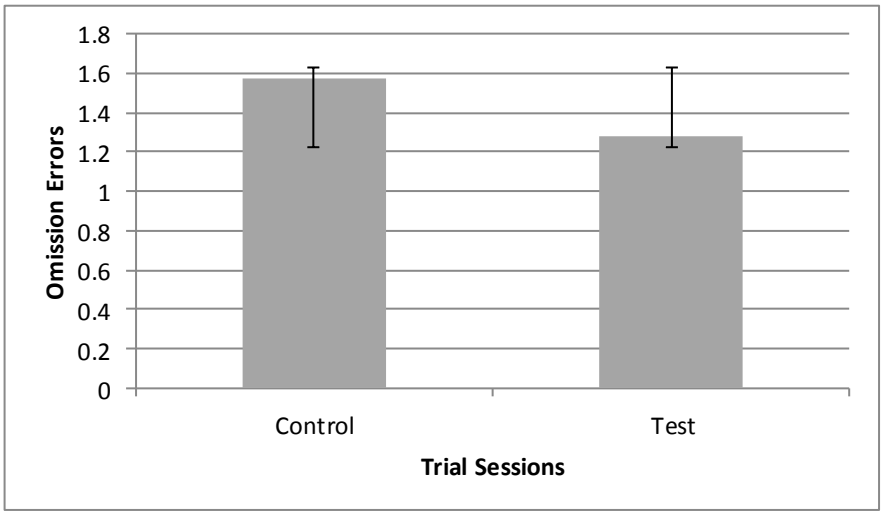
Cohort C

(Old Age Mice)

Figure 43: Effect of wild yam tincture on distance travelled

4.7.4 T-maze left-right discrimination task (Reversal)

Mice received ten trials daily for 7 days and the average number of omission errors for the group was determined. There were no significant differences in omission errors between the control mice and test mice after administration of wild yam tincture [**1.6 ± 0.8 (c) Vs. 1.3 ± 0.3 (t) , t= -0.3430, p=0.7375**]. A graphical representation of the results is shown in Figure 44.



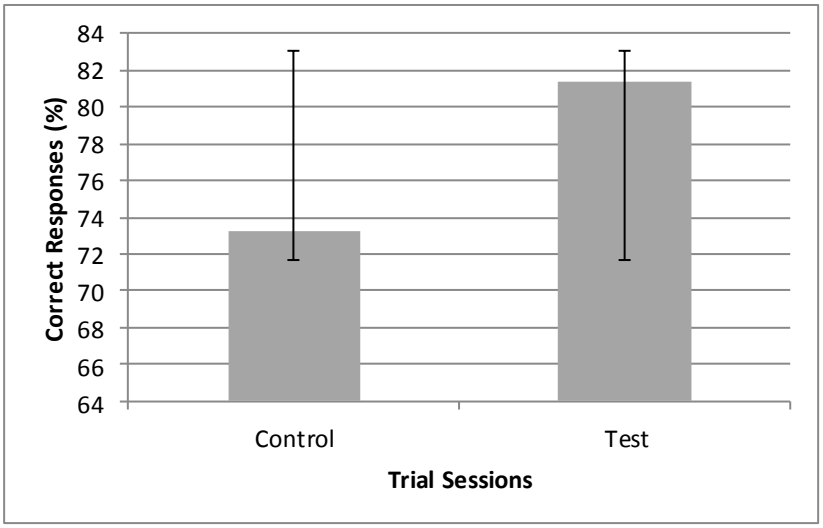
Cohort C

(Old Age Mice)

Figure 44: Effect of wild yam tincture on omission errors

4.8.1 T-maze forced alternation task

Mice received ten trials daily for 10 days and each trial consisted of both a forced and free choice run. A group average of the percentage of correct responses was determined. There were significant differences in latency between the control mice and test mice after administration of wild yam tincture [**73.3 ± 1.7% (c) Vs. 81.4 ± 1.7 % (t), t=3.2871 , p=0.0041**]. A graphical representation of the results is shown in Figure 45.



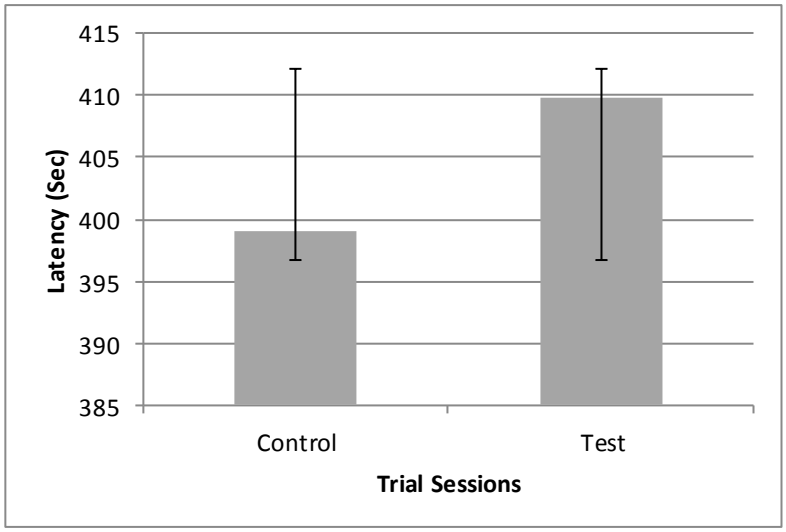
Cohort B

(Middle Age Mice)

Figure 45: Effect of wild yam tincture on correct responses

4.8.2 T-maze forced alternation task

Mice received ten trials daily for 10 days and each trial consisted of a forced and free choice run. A group average of the latency was determined. There were no significant differences in latency between the control mice and test mice after administration of wild yam tincture [**399 ± 5.8 sec (c) Vs. 409 ± 7.3 sec (t), t=1.1685 p= 0.2578**] . A graphical representation of the results is shown in Figure 46.



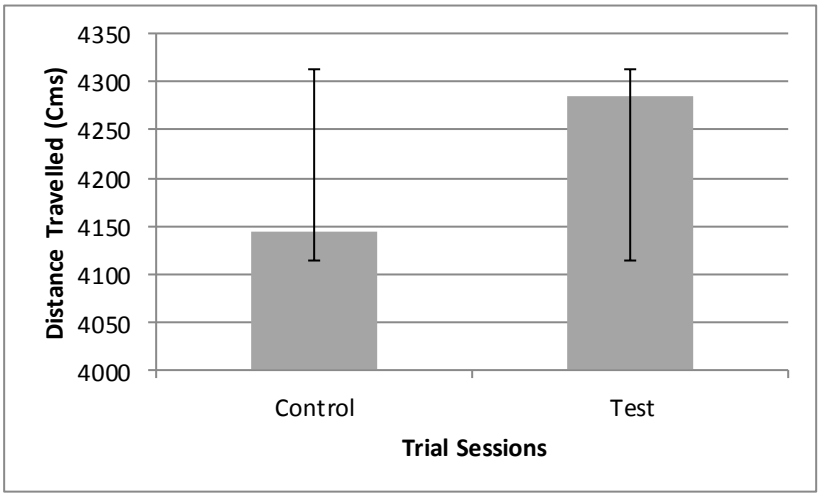
Cohort B

(Middle Age Mice)

Figure 46: Effect of wild yam tincture on latency

4.8.3 T-maze forced alternation task

Mice received ten trials daily for 10 days and each trial consisted of a forced and free choice run. A group average of the total distance travelled was determined. There were significant differences in the distance travelled between the control mice and test mice after administration of wild yam tincture [**4144 ± 37.5 cm (c) Vs. 4285 ± 13.4 cm (t), t=3.5411 , p= 0.0023**] . A graphical representation of the results is shown in Figure 47.



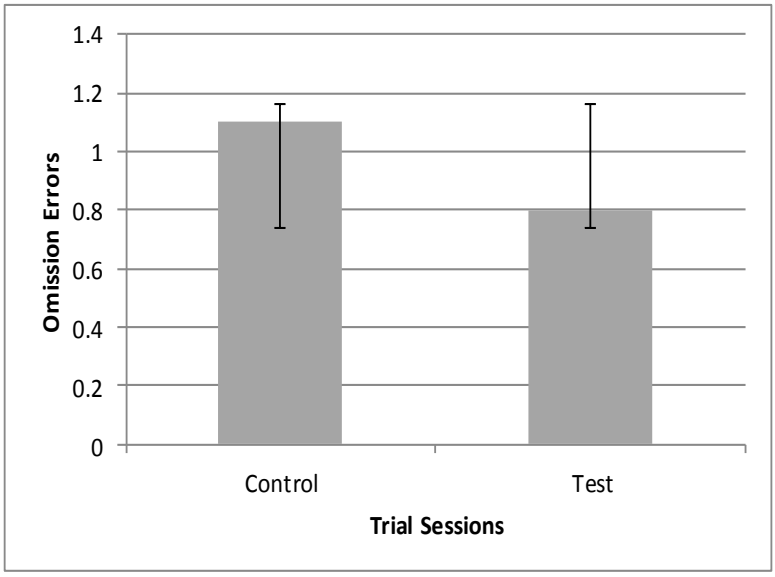
Cohort B

(Middle Age Mice)

Figure 47: Effect of wild yam tincture on distance travelled

4.8.4 T-maze forced alternation task

Mice received ten trials daily for 10 days and each trial consisted of a forced and free choice run. A group average of the omission errors was determined. There were no significant differences in omission errors between the control mice and test mice after administration of wild yam tincture [**1.1 ± 0.41 (c) Vs. 0.8 ± 0.33 (t), t=0.5750 , p= 0.5724**] . A graphical representation of the results is shown in Figure 48.



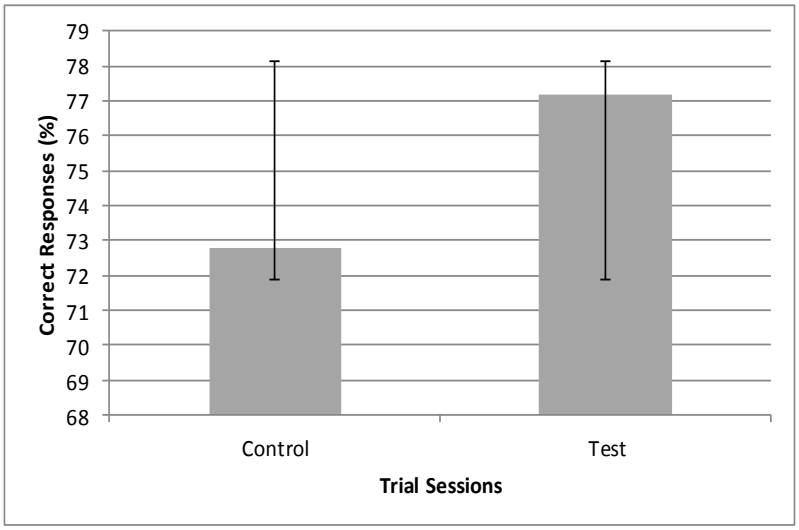
Cohort B

(Middle Age Mice)

Figure 48: Effect of wild yam tincture on omission errors

4.9.1 T-maze forced alternation task

Mice received ten trials daily for 10 days and each trial consisted of a forced and free choice run. A group average of the percentage of correct responses was determined. There were significant differences in the percentage of correct responses between the control mice and test mice after administration of wild yam tincture [**72.8 ± 1.3% (c) Vs. 77.2 ± 1.7% (t) , t= 2.0635, p= 0.0538**] . A graphical representation of the results is shown in Figure 49.



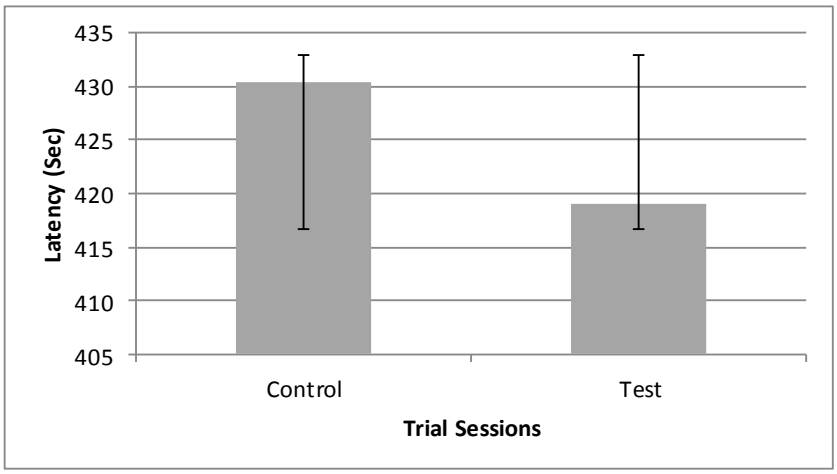
Cohort C

(Old Age Mice)

Figure 49: Effect of wild yam tincture on correct response

4.9.2 T-maze forced alternation task

Mice received ten trials daily for 10 days and each trial consisted of a forced and free choice run. A group average of the latency was determined. There were no significant differences in latency between the control mice and test mice after administration of wild yam tincture [**430.5 ± 9.05 sec (c) Vs. 419 ± 9.9 sec (t), t= 0.8555, p= 0.4035**] .A graphical representation of the results is shown in Figure 50.



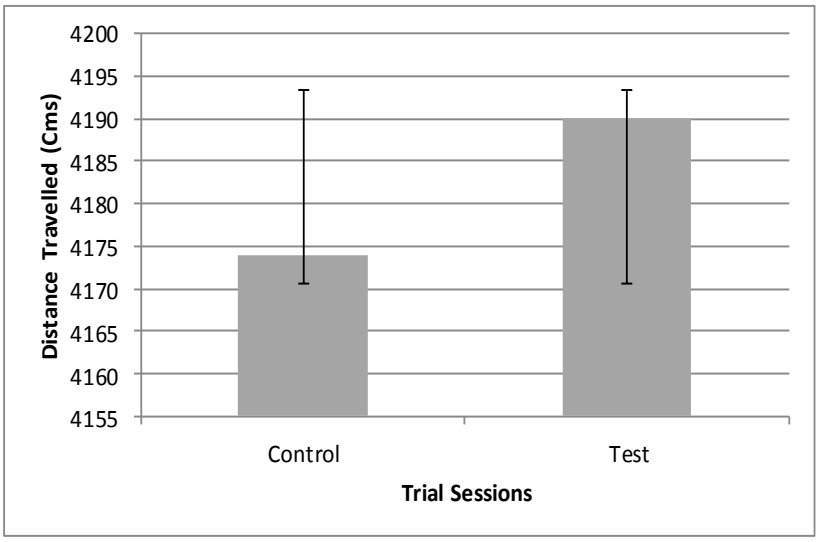
Cohort C

(Old Age Mice)

Figure 50: Effect of wild yam tincture on latency

4.9.3 T-maze forced alternation task

Mice received ten trials daily for 10 days and each trial consisted of a forced and free choice run. A group average of the total distance travelled was determined. There were no significant differences in the distance travelled between the control mice and test mice after administration of wild yam tincture [**4174 ± 50.4 cm (c) Vs. 4190 ± 30.4 cm (t), t= 0.2719, p = 0.7888**] . A graphical representation of the results is shown in Figure 51.



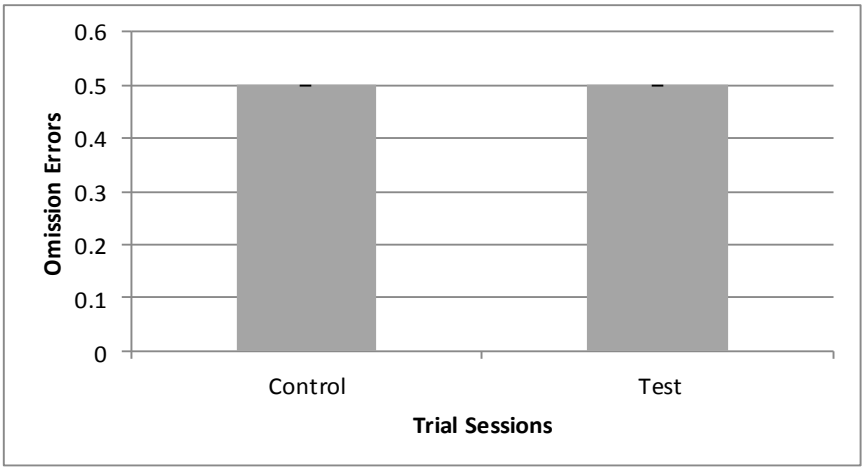
Cohort C

(Old Age Mice)

Figure 51: Effect of wild yam tincture on distance travelled

4.9.4 T-maze forced alternation task

Mice received ten trials daily for 10 days and each trial consisted of a forced and free choice run. A group average of the number of omission errors was determined. There were no significant differences in omission errors between the control mice and test mice after administration of wild yam tincture [**0.5 ± 0.17 (c) Vs. 0.5 ± 0.22 (t), t= 0.0000 p = 1.0000**] . A graphical representation of the results is shown in Figure 52.



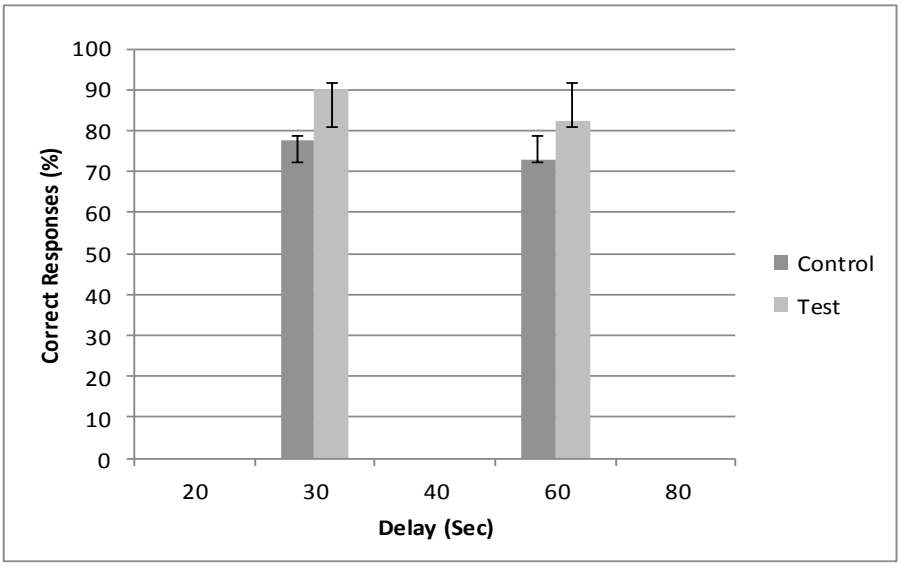
Cohort C

(Old Age Mice)

Figure 52: Effect of wild yam tincture on omission errors

5.1.1 T-maze delayed alternation task

Mice received ten trials daily for 2 days. Delays of 30 and 60 seconds were inserted between the trials and the percentage of correct responses was determined. There were significant differences in the percentage of correct response with delays of 30 seconds [**77.8 ± 3.2% (c) Vs. 90 ± 1.1% (t), t=3.6556, p = 0.0106]** and 60 seconds [**73 ± 1.8% (c) Vs. 82.3 ± 0.75% (t) , t= 4.6864 p= 0.0034**] between the control mice and test mice after administration of wild yam tincture. A graphical representation of the results is shown in Figure 53.



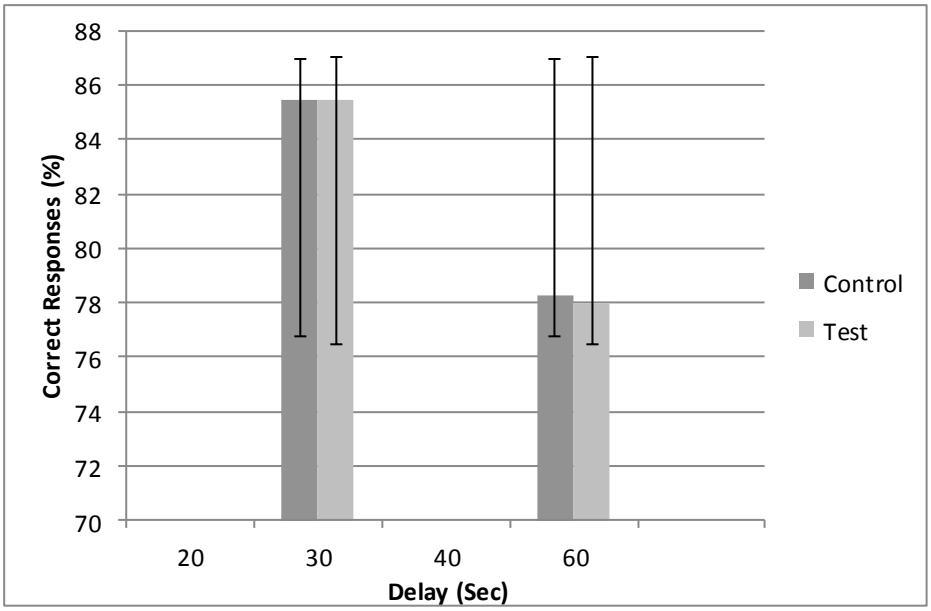
Cohort B

(Middle Age Mice)

Figure 53: Effect of wild yam tincture on correct responses with delays of 30 and 60 sec

5.1.2 T-maze delayed alternation task

Mice received ten trials daily for 2 days. Delays of 30 and 60 seconds were inserted between the trials and the percentage of correct responses was determined. There were no significant differences in the percentage of correct response with delays of 30 seconds [**85.5 ± 2.6% (c) Vs. 85.5 ± 1.8% (t) , t=0.00 , p = 1.00]** and 60 seconds [**78.3 ± 3.3% (c) Vs. 78 ± 3.2% (t) , t= 0.0543 , p = 0.9585**] between the control mice and test mice after administration of wild yam tincture. A graphical representation of the results is shown in Figure 54.



Cohort C

(Old Age Mice)

Figure 54: Effect of wild yam tincture on correct responses with delays of 30 and 60 sec

CHAPTER FIVE: DISCUSSION

The most important property of the brain is its ability for storage and retrieval of information. The brain has the normal and sometimes abnormal ability to forget stored information. Alternation behavior in rodents depends on their ability to remember the maze arms previously visited in order to respond with novelty especially when a reward is presented. This behavior is said to depend on the spatial working memory capability of the animals (Deacon and Rawlins, 2006). The animal's response to turn right or left in a maze changes constantly from trial to trial. Rodents generate a cognitive map of their surroundings during their navigations (Ciancia, 1991).

The goal arm's choice by the animal is a reflection of the rodents memory of which arm was previously entered. However, this choice can also be influenced by sensory, attentional and motivational factors. When rodents alternate their choice of a goal arm, it reflects their motivation to explore their environment as well as the function of their short term memory. (Shoji H et al.,2012). T-maze left-right discrimination task and forced alternation task are tests extensively used to assess cognitive functions in rodents. When executing a particular task the brain is said to have 'processors' that are associated with a memory system for a particular cognitive task (Dale et al., 1997). Research studies using maze apparatus have been useful in investigating the effects of medicinal substances and plant extracts on learning and memory in both humans and rodents. Studies indicate that the aging brain and cognitive decline are significantly influenced by the interaction between oxidative stress and inflammation. (Sergio et al., 2016).

In our study three cohorts of mice were subjected to memory tests/tasks in order to assess short term and long term memory, learned behavioral flexibility as well as retention of short term memory before and after administration of wild yam tincture. To my knowledge this is the first time that the effect of wild yam tincture on memory and learning in different age cohorts of male mice has been investigated

In T-maze tasks, the rodents performance and strategy may be influenced by spatial and non-spatial cues located within the maze or outside the maze (Shoji, H., et al. 2012). Other factors such as odour trail may influence location of the bait during trial sessions and hence influence learning and memory process.

Performance during the first trials are potentially devoid of the odour trail strategy. Alternation tasks in rodents are flexible and can be used to demonstrate cognitive enhancing properties of a substance.

Before the wild yam tincture was administered to the experimental animals the results suggest that no significant differences were found between the test and control mice in the T – maze task. These results are expected because both test and control groups were subjected to similar pre-training and training sessions before undertaking the memory tasks and hence their performance is comparable (figure 21 – 27). The test mice however showed significantly fewer omission errors than the control (**p= 0.0025**) (figure 28). This observation is unexpected, however consideration of the correct responses (**p= 0.4932**) and omission errors suggest comparable performance between the test and control groups.

In the left-right discrimination task after the wild yam tincture was administered, the young, mature adult and middle age test mice showed a shorter latency (**p<0.0001**) and made few omission errors (**p=0.0136**) than the control mice (figure 30 and 32). These results indicate a greater spatial learning ability and improved memory by the test animals. The test mice also showed a higher percentage of correct responses (**p=0.0757**) than the control mice (figure 29). Consideration of a high percentage of correct responses, short latency and significantly few omission errors indicate greater learning ability and improved performance in the T – maze. These findings indicate that wild yam tincture enhances long term memory (reference memory) in the young mature adult and middle age mice. It is important to note that the results shown in figures 29 – 32 represent the performance of both the young mature adult (Cohort A) and those young mature mice that had attained the middle age (A*) by the time the memory test was carried out as shown in Table 1 during the experimental period.

In the above task the old age mice showed significant differences in the distance travelled (**p=0.0128**) and made fewer omission errors (**p=0.0189**) than the control mice (figure 35 and 36). The test mice also showed a shorter latency (**p=0.0708**) than the control mice but the difference was not statistically significant. A consideration of the total distance travelled by the mouse in the maze, omission errors and a short latency indicates improved spatial learning ability and memory by the old mice. The above results represent the performance of the middle age mice (Cohort B) that had attained old age (B*) by the time the memory test was carried out as shown in Table 1 during the experimental period

In the reversal learning task which evaluates behavioral flexibility the middle age and old age mice showed a high percentage of correct responses, short latency, long distance travelled and less omission errors in comparison with the control group. These differences however were not statistically significant but they may be attributed to factors such as habituation level, learning strategy, locomotor activity or impulsive tendency towards the goal arm (Shoji, H., et al. 2012). These findings indicate that wild yam tincture has little or no effect on behavioral flexibility.

In the T – maze forced alternation task which evaluated short term memory, the middle age test mice showed a significant difference in correct responses (**p=0.0041**) and distance travelled (**p=0.0023**) than the control mice (figure 45 and 47). These findings suggest enhanced learning and motivation towards the goal arm by the test animals. The above findings suggest that wild yam tincture has a positive effect on short term memory in middle age mice. These results are supported by previous studies on rodents fed on fruits and vegetable extracts (Joseph et al., 2005).

In the above task, the old age test mice showed a higher percentage of correct responses (**p=0.0538**) than the control mice (figure 49). These results indicate enhanced learning ability and memory in old mice.

In the T- maze delayed alternation task, retention of memory was assessed with delay times of 30 and 60 seconds intervals. The test and control mice underwent the above test and the correct response parameter was measured.

In the delayed alternation task, the middle age test mice showed a significantly higher percentage of correct responses (**p=0.0034**) than the control mice at a delay time of both 30 and 60 seconds (figure 53). These results indicate enhanced memory retention and suggest that wild yam tincture has a positive effect on retention of memory in middle age mice. In this task the old age test mice and control did not have any significant differences with respect to percentage of correct response at delay times of thirty and sixty seconds. These findings indicate that wild yam tincture has no positive effect on memory retention in old mice. At the end of the experiment some of the signs of aging observed in mice were loss of body weight, hair, agility and death of some of the animals.

The results from our experiment with 0.34mg/kg of wild yam given to mice, suggest a positive effect on memory and learning in the test animals. The findings from our study indicate that wild yam tincture enhances long term memory in young mature, middle and old age mice. The

tincture enhances short term memory in middle and old age mice as well as memory retention in middle age mice These findings are supported by previous animal studies which indicate that Mexican wild yam *(D. Villosa)* supplements provide benefits for brain function in rats and mice.

The free radical theory of aging is one of the most common theory which indicates that increased generation of free radicals is the major cause of cellular damage. These free radical mediated damages are prevalent during aging and are associated with cognitive deficits and neurodegenerative diseases such as Alzheimer's disease (Schoniech, 1999; Schipper 2004).

Wild yam products are commonly available as dietary supplements taken for the anti-oxidant and anti-inflammatory properties attributed mainly to their diosgenin and polyphenolic content. Other constituents in wild yam include flavonoids, vitamins and minerals which show considerable anti-oxidant activities. Diosgenin is a major constituent of wild yam occurring as a steroidal saponin and is found abundantly in legumes and yams (Dioscorea Sp.). It is a precursor of various synthetic steroidal drugs extensively used in the pharmaceutical industry (Djerassi, 1992).

Several scientific studies have demonstrated the positive effects of diosgenin in memory and learning in rodents. A study by Alireza et al., (2007) showed that polyphenolic enriched supplements enhanced spatial memory in aged male rats. Research studies by Furthwine (2012) demonstrated that dioscorea improves memory function in neurodegenerative diseases by increased formation of nerve growth factors (NGF). Increased formation of NGF causes regeneration of nerve cells. These studies further showed that the anti-inflammatory effects in dioscorea are due to inhibition of histamine, serotonin and prostaglandins that mediate inflammatory processes. Diosgenin has been shown to attenuate inflammatory process in relevant animal models (Yamada et al.,2009;Tada et al.,2009) demonstrated anti-aging properties of diosgenin in relation to hormonal effects *in vivo*. A study by Chiu et al., (2011) showed that diosgenin improved cognitive functions and reduced oxidative damage in old mice. The memory enhancing effect of diosgenin maybe mediated by endogenous anti-oxidant enzymes Chiu et al., (2011).

Polyphenols and flavonoids present in wild yam have anti-oxidant activities and studies indicate that these compounds decrease the incidence of free radical induced lipid peroxidation in the central nervous system (CNS) of aged rats Balu et al., (2005). Epidemiological studies and

various scientific reports have shown that long term dietary supplementation of antioxidant rich foods improved cognitive performance in aged rats.

The dose level of wild yam tincture used in our study (0.5mls/mouse/day) was relatively low compared to dosages used in other studies (e.g. 6 – 12 mls /day). A higher dose level of the tincture would probably have yielded more significant results. However the test animals showed a high level of performance in most of the memory tasks. This study suggests that wild yam tincture enhances learning and memory in mice.

The findings of our current study is supported by other research which has demonstrated the efficacy of nutraceuticals as potential therapeutic supplements for cognitive deficits and neurodegenerative diseases. These natural antioxidants are associated with many biological activities which include; scavenging of free radicals, reduction of pro inflammatory cytokines and modulation of pro survival signaling pathways. Conventional drugs and pharmaceutical products operate through a single mechanism while nutraceuticals have multiple modes of action to mitigate oxidative stress and to promote neuronal growth and survival. Nutraceuticals therefore provide an opportunity for the development of novel therapies for cognitive impairments and disorders (Natalie et al., 2010). Phytochemicals found in foods and plant extracts are progressively gaining popularity over conventional synthetic drugs because they act via multiple molecular targets that synergize to efficiently prevent or treat ailments (Raju and Rao, 2009). Phytochemicals are considered safe with minimal or no toxic side effects and have relatively better bioavailability.

In our study no tests were carried out to assess safety of the wild yam tincture. However toxicology studies using relevant experimental models have established that therapeutic doses of diosgenin were safe and failed to cause systemic toxicity (Qin et al., 2009). More studies are recommended for further toxicological effects of diosgenin in common foods and supplements.

The current study had several limitations which include; inability to control precisely the external cues that determine the learning process and clarity required on what would constitute a sufficient amount of extra maze cue(s), performance may be influenced by many factors and animal characteristics such as species (strain), nutritional status, infection and stress, Content of active ingredients in the wild yam can range significantly due to the growth and quality of processing conditions. Possible sources of error include; inadequate habituation, intertrial handling of the animals, environmental cues (e.g. noise, external cues etc.), emotional and health

status of the animals. An automated T-maze apparatus has been developed and that would minimize occurrence of human error. However this apparatus is not available in Kenya yet.

5.1 CONCLUSION:

This study has demonstrated that wild yam tincture enhances spatial learning and memory in male mice and hence has potential benefits in alleviating memory deficits in the aging human brain. The findings of this study are also in-keeping with previously documented research studies in rodents. The results of this study recommend that further studies be undertaken to explore the benefits of wild yam in learning and memory in human volunteers considering the fact that wild yam products are widely used in traditional and modern therapy.

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