NEUROPROTECTIVE MECHANISMS OF GARLIC (*Allium sativum*) IN MODULATION OF HIPPOCAMPUS FUNCTION IN MODEL OF TYPE II DIABETIC WISTAR RATS

SEMUYABA IBRAHIM
BVM (MAK)
MSc.PHY/0001/142/DU

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE IN PHYSIOLOGY OF KAMPALA INTERNATIONAL UNIVERSITY

MAY, 2017

DECLARATION

I, the under signed declare that this work is an original research work.

Semuyaba Ibrahim
Signed.....
Date.....29/05/2017.....

This work has been prepared under the guidance of my supervisors

Dr.Niurka Figuredo (MBCHB, Msc)
Department of Physiology
Faculty of Biomedical Sciences
Kampala International University
Western Campus, Bushenyi.

Signed .....
Date.....30/05/2017.....

Mr.Abass Safiriyu (Bsc, Msc)
Department of Physiology
Faculty of Biomedical Sciences
Kampala International University
Western Campus, Bushenyi.

Signed .....
Date.....29/05/2017.....

ACKNOWLEDGMENT

To the almighty Allah be the glory for giving me life and keeping me healthy throughout the course of the study.

My sincere appreciation to my supervisors, Dr.Niurka Figuredo and Mr.Abass Safiriyu for their guidance, commitment and time while carrying out this research.

Also to the management of Kampala International University, to whom am very grateful for the opportunity offered to study this Master of Science degree under the Scholarship program.

Iam thankful to all staff of Department of Physiology, Faculty of Biomedical Sciences, Kampala International University ,Western campus, Bushenyi especially Mr.Okpanachi Alfred (HOD) for their encouragement and support.

Special thanks to Mr.Kiiza Ronald technician at Pharmacology laboratory Kampala International University, Mr. Bukenya Andrew laboratory technician Department of Biochemistry, Makerere University School of Health Sciences Mulago for their guidance during my laboratory work and analysis of my samples.

To all my family members and friends, am indeed lucky to have you in my life .I appreciate all your unconditional support.

TABLE OF CONTENTS

DECLARATION i
ACKNOWLEDGMENT ii
LIST OF ABBREVIATIONS AND ACRONYMS viii
ABSTRACT viiii
CHAPTER ONE: INTRODUCTION 1
1.1 Background 1
1.2 Problem statement 4
1.3 Purpose of the study 4
1.4 Objectives of the study 4
1.5 Research Hypotheses 5
1.6 Scope of study 5
1.7 Justification of the study 5
1.8 Conceptual frame work 6
1.8.1 Description of conceptual framework 6
CHAPTER TWO: LITERATURE REVIEW 7
2.1 Effect of diabetes mellitus and garlic on memory. 7
2.1.1 Diabetes mellitus 7
2.1.2 Pathophysiology of type 2 diabetes mellitus 7
2.1.3 Induction of type 2 diabetes mellitus in Wistar rats 8
2.1.4 The Hippocampus 9
2.1.5 Diabetic memory impairment 10
2.1.6 Garlic (A.sativum) its active compounda and medicinal properties 11
2.1.7 Bioactive properties of garlic 11
2.1.8 Medicinal properties of garlic 11
2.2 Effect of diabetes mellitus on hippocampus Na^+/K^+ ATPase 13
2.3 Effect of diabetes mellitus on hippocampus Ca^{2+} ATPase 14
2.4 Effect of diabetes mellitus on hippocampus glutamine synthetase activity 15
2.5 Effect of metformin on diabetes mellitus and memory 15
CHAPTER THREE:METHODOLOGY 16
3.1 Study design 16

3.2 Ethanol extraction of garlic 17
3.3 Induction of hyperglycaemia in Wistar rats 17
3.4 Confirmation of hyperglycaemia 17
3.5 Hippocampus dependant memory assessment 17
3.6 Collection of hippocampus samples and procesing 19
3.7 Biochemical analysis 19
3.7.1 Measurement of Na^+ /K^+ ATPase activity 19
3.7.2 Measurement of Ca^{2+} - ATPase activity 20
3.7.3 Measurement of glutamine synthetase activity 20
3.8 Statistical analysis 20
3.9 Limitations and delimitations 21
3.10 Ethical consideration 21
CHAPTER FOUR: PRESENTATION AND INTERPRETATION OF RESULTS 22
4.0 Effect of ethanol garlic (A.sativum) extract on blood glucose levels 22
4.1 Effect of ethanol extract of garlic on object location memory 23
4.2 Effect of ethanol garlic extract on activity of hippocampus Na^+ /K^+ ATPase 25
4.3 Effect of ethanol garlic extract on hippocampus calcium ATPase activity 27
4.4 Effect of ethanol garlic extract on hippocampus glutamine synthetase activity 29
CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMEDATIONS 31
5.1 Discussion 34
5.2 Conclusion 34
5.3 Recommendation 34
REFERENCES 35
APPENDICES 53

LIST OF FIGURES

Figure 1: Showing conceptual frame work.....6

Figure 2: Showing location of the hippocampus in the human brain.....10

Figure 3: Showing garlic plant and bulbs.....11

Figure 4: Showing scheme of study design.....16

Figure 5: Showing overhead view of object location memory box arena.....18

Figure 6: Effect of ethanol garlic (A. sativum) extract treatment on exploration time to familiar and novel object locations in different treatment groups.....22

Figure 7: Effect of treatment with ethanol garlic (A. sativum) extract on the novelty index among different treatment groups.....24

Figure 8: Shows effect of ethanol extract of garlic (A. sativum) on activity of hippocampus sodium- potassium ATPase in the different treatment groups.....26

Figure 9: Shows the effect of ethanol extract of garlic (A. sativum) on hippocampus calcium ATPase activity in different treatment groups.....28

Figure 10: Shows effect of ethanol extract of garlic (A. sativum) on hippocampus glutamine synthetase activity in different treatment groups.....30

LIST OF TABLES

Table 1: The effects of ethanol garlic (A.sativum) extract on blood glucose level.....22

Table 2: Effect of ethanol garlic (A. sativum) extract treatment on exploration time to familiar and novel object locations in the different treatment groups.....53

Table 3: Effect of treatment with ethanol garlic (A. sativum) extract on the novelty index in different groups.....53

Table 4: Effect of ethanol extract of garlic (A. sativum) on activity of hippocampus sodium-potassium ATPase in different treatment groups.....54

Table 5: The effect of ethanol extract of garlic (A,sativum) on hippocampus calcium ATPase activity in different treatment groups.....54

Table 6: The effect of ethanol extract of garlic (A.sativum) on hippocampus glutamine synthetase activity in different treatment groups.....54

LIST OF ABBREVIATIONS AND ACRONYMS

AMPA: Amino3-hydroxy-5-methylisoxazole-4-propionate acid

CNS: Central Nervous System

DM:Diabetes mellitus

GLU:Glutamate

GS: Glutamine synthetase

I.P: Intraperitoneal injection

IP_3: Inistol triphosphate

KIU: Kampala International University, Western Campus, Bushenyi.

NA: Nicotinamide.

NMDA: N-methyl-D-aspartate receptor

OLM: Object location memory

STZ:Streptozotocin

T2DM: Type II diabetes mellitus

ABSTRACT

Hippocampus functions have been reported to be affected by diabetes mellitus. Studies suggest that within diabetic rats, garlic (A.sativum) improves different forms of memory that depend on the hippocampus. However, the neuroprotective mechanisms of garlic (A.sativum) extract possibly mediated by the Na^+/K^+ ATPase, Ca^{2+}ATPase and glutamine synthetase (GS) in the hippocampus of type II diabetic Wistar rats has not been fully demonstrated. Therefore, the present study determined the effect of ethanol extract of garlic (A.sativum) on object location memory (OLM) and on hippocampus Na^+/K^+ATPase, Ca^{2+} ATPase and GS activities.

Thirty male Wistar rats divided into five groups, six rats each were used. Normal control rats in group A received 1ml of normal saline, normal rats in group B received 1000mg/kg of garlic extract, diabetic control rats in group C received 1ml of normal saline, diabetic rats in group D received 1000mg/kg of garlic extract and diabetic rats in group E received 50mg/kg of metformin. All treatments were administered orally for 21 days. Induction of hyperglycemia was achieved by a single intraperitoneal injection of 60mg/kg STZ followed by 120mg/kg nicotinamide while extraction of garlic (A.sativum) was done by cold maceration method. OLM was tested before induction of diabetes mellitus type II and at the end of the experiment in all the groups. After the test, the rats were sacrificed, the brain removed, the hippocampi were carefully excised and then homogenized. Homogenate was analyzed for Na^+/K^+ATPase, Ca^{2+} ATPase and GS activities.

Results obtained showed that, there was improvement in OLM. There was also a significant increase ($p < 0.05$) in hippocampus Na^+/K^+ ATPase, Ca^{2+} ATPase and GS activities.

In conclusion, it can be suggested that, the neuroprotective mechanisms of garlic (A.sativum) in modulating hippocampus function could be mediated through increasing the activities of hippocampus Na^+/K^+ ATPase, Ca^{2+} ATPase and glutamine synthetase.

CHAPTER ONE
INTRODUCTION

1.1 Background to the study

The brain consists of different structures of which are known to be specific for a different type of learning and memory. As a key brain area, the hippocampus has been shown to be responsible for spatial learning, spatial memory and object location memory (Squire *et al.*, 2004).

Memories of a spatial nature are responsible for recording information about one's environment and its spatial orientation (Squire *et al.*, 2004) . The hippocampus stores and processes information so that it can be retrieved later. It also provides animals with a spatial map of their environment(Dong *et al.*, 2012). The learning process involves alteration of neuronal membrane electrochemical gradient leading to activation of hippocampus neurons. The maintance of neuronal membrane electrochemical gradient or ionic equilibrium is done by the Na^{+}/K^{+} ATPase, Mg $^{2+}$ATPase and Ca $^{2+}$ ATPase (Rani and Panneerselvam, 2001). Memories occur at the synapses due to the release of neurotransmitters because of a previous neural activity caused by changes in ionic equilibrium. The synaptic release of glutamate which is a major excitatory neurotransmitter in the central nervous system activates post-synaptic N-methyl-D-aspartate receptor leading to memory formation (Banerjee *et al.*, 2010)

Diabetes mellitus (DM) type II is associated with cognitive dysfunction(Mc Crimmon *et al.*, (2012) and is characterized by chronic hyperglycaemia together with oxidative stress, which severely affects hippocampus dependant learning and memory (Amin *et al.*, 2013). Hyperglycaemia and oxidative stress causes damage to the hippocampus by altering its neuronal membrane, structure, neuronal signal input there by affecting memory signalling pathways (Hegazy and Ali, 2011). Also, there may be accumulation of neurotransmitter at the synapse which will be neurotoxic (Khakpai et al., 2012a). This leads to detrimental effects on learning and memory as observed in streptozotocin (STZ) and Zucker rat (genetic models of type II diabetes mellitus) with impaired performance in Morris water maze spatial test (Stepankova *et al.*, 2004).

The brain undergoes different alterations during diabetes which can either be morphological or functional in nature. It has been suggested that during ischemia both glial cells and neurons exhibit changes which are significant especially in experimentally induced diabetes (Li *et al.*, 1998). In diabetes there is decreased utilization of glucose by the brain (Pari, 2004) providing a possible pathway for increase in vulnerability to progression of the disease. During chronic elevated blood glucose conditions, there is an imbalance in oxidative state of CNS leading to ROS which damage the brain by peroxidation. Increased oxidative stress during diabetes, makes the tissues in the brain more prone to damage (Nazaroğlu and Dincel, 2009). Oxidative stress which causes an increase in generation of ROS leads to increased cell lipid peroxidation is suggested to have a major role in diabetes mellitus development. Indeed one of the cellular characteristics in diabetes of chronic nature is lipid peroxidation. (Sarkhail *et al.*,2007). It has been shown that increase in lipid degradation is vital for progression of experimentally induced hyperglycaemia by causing changes in the fluidity gradient of the transbilayer which hampers the functions of receptors and membrane enzymes (Dmitriev , 2010)

Proteins undergo oxidation which results into their modifications and this has been shown to be irreversible, deleterious leading to protein degradation, clearance and inactivation. (Grune *et al.*,2003). Oxidative modifications appears if oxidative stress is increased beyond the antioxidant defence system which then leads to damage (Forrester and Stamler, 2007). The reaction of the body to oxidative stress is important inorder to understand the defence mechanisms which the cells produce. (Macario and de Macario, 2005). Disorders of normal oxidative metabolism, or damages due to oxidative stress, have also been proven to be key players in a broad spectrum of diseases, from neurodegenerative disorders, such diabetes (Dalle-Donne *et al.*, 2003). The likely hood of enzyme modification by oxidation is used for selectively for regulation of enzyme degradation. As reported (Ortega and Roche, 1999) these effects of the metabolite would be responsible for the enzyme reaction to different responses such as in deficiencies due to nutrition causes.

Glutamine synthetase is an enzyme important in reducing intracellular concentration of glutamate and in ammonia assimilation. Glutamate accumulation initiates pathway of neuronal death known as excitotoxicity (Dienel and Hertz, 2005). The accumulation of glutamate in the extracellular fluid causes a decrease in glutamine synthetase which may lead to seizures (Perez *et al.,* 2012). Bui *et al.*, (2009) reported that when there is inhibition of glutamine synthetase, neurotransmission in the retina is affected. Loss of GS is associated with several diseases of animals in which there is severe neuronal loss, such as in canine glaucoma (Chen *et al.,* 2008) and equine recurrent uveitis (Hauck *et al.,* 2007)

The use of herbs as an alternative treatment of DM especially in poor countries has been utilized more frequently in treating patients with diabetes. In fact Ryan *et al.,* (2001) reported that 30% of diabetic patients use complementary medicine. World Health Organization indicated that more than 80% of the people in the world trust use of herbal medicines (Duraipandiyan *et al.*, 2006) and in developing countries, 3.5 billion are estimated to use medicinal plants (Jamison *et al.,* 2006)

Garlic (A. sativum) one of the herbs, has been shown to have numerous medicinal properties. It has been suggested to reduce blood pressure in hypertensive (Ried *et al,* 2013), has anti-angiogenic effects (Herman-Antosiewicz *et al.,* 2007), prevents apoptosis and toxicity to neurons by A-beta (Borek, 2001). Poses antidiabetic (Sher *et al.,* 2012) and anti-oxidative activity (Rahman *et al.,* 2012) in peripheral tissues, has ameliorative effects on memory impairment (Gebreyohannes *et al.,* 2013). Garlic (A.sativum) has been shown to improve learning and memory (Jalal *et al.,* 2011; Mukherjee and Banerjee, (2013); Jeong *et al.,* 2013, Sarkaki *et al.,* (2013) Tasnim *et al.,* (2015) also reported that A. sativum improves memory and attention in healthy human volunteers

According to available literature, no study has evaluated the effect of ethanolic extract of garlic (A.sativum) on hippocampus Na^{+}/K^{+} ATPase, Ca^{2+} ATPase and glutamine synthetase activities and linked it with object location memory in type II diabetic rats. This study sought to explore the possible neuroprotective mechanisms of garlic (A.sativum) involved in modulation of hippocampus function in type II diabetic rats.

1.2 Problem statement

High blood glucose levels and oxidative stress are characteristic of diabetes mellitus which leads to complex hippocampus cellular and structural changes (Bélanger *et al.*,2004a). Hippocampal damage results in alterations in Na^+/K^+ ATPase and Ca^{2+} ATPase activity. This alterations in Na^+/K^+-ATPase, Ca^{2+} ATPase activities and inhibition of glutamine synthetase leading to glutamate neurotoxicity, results in episodic spatial learning, spatial memory deficit and memory impairment(Khakpai *et al.*, 2012b).

Garlic has been shown to improve learning and memory in, fructose induced type II diabetes mellitus rats (Jalal *et al.*, 2011), amensic mice (Mukherje *et al.*, 2013). Also, fresh, cooked, raw garlic extract improved short term but not long term memory in diabetic rats (Sarkaki *et al.*, 2013). However, the effects of garlic (A.sativum) on hippocampus Na^+/K^+ ATPase, Ca^{2+} ATPase and glutamine synthetase activites in type II diabetic Wistar rats have not been fully elucidated. This study aimed at exploring the possible neuroprotective mechanisms through which garlic (A.sativum) extract on hippocampus function in type II diabetic Wistar rats.

1.3 Purpose of the study

To determine the neuroprotective mechanisms of ethanol extract of garlic (A.sativum) involved in modulation of hippocampus function in model of type II diabetic Wistar rats.

1.4 Objectives of the study

1.4.1 To determine the effect of ethanol extract of garlic (A.sativum) on object location memory in model of type II diabetic Wistar rats

1.4.2 To determine the effect of ethanol extract of garlic (A.sativum) on activity of hippocampus sodium- potassium ATPase activity in model of type II diabetic Wistar rats.

1.4.3 To determine the effect of ethanol extract of garlic (A.sativum) on hippocampus calcium ATPase activity in model of type II diabetic Wistar rats.

1.4.4 To determine the effect of ethanol extract of garlic (A.sativum) on hippocampus glutamine synthetase activity in model of type II diabetic Wistar rats.

1.5 Research hypotheses

1.5.1 Ethanol extract of garlic (A.sativum) treatment to model of type II diabetic Wistar rats does not have effect on object location memory.

1.5.2 Ethanol extract of garlic (A.sativum) treatment to model of type II diabetic Wistar rats does not have effect on hippocampus Na^+/K^+ ATPase activity.

1.5.3 Ethanol extract of garlic (A.sativum) treatment to model of type II diabetic Wistar rats does not have effect on hippocampus Ca^{2+} ATPase activity.

1.5.4 Ethanol extract of garlic (A.sativum) treatment to model of type II diabetic Wistar rats does not have effect on hippocampus glutamine synthetase activity.

1.6 Scope of study

The study involved a laboratory based model of type II diabetic Wistar rats obtained by injection of streptozotocin followed by nicotinamide. The diabetic rats confirmed hyperglycemic with blood glucose concentration of more than 250mg/dl were used for the study, to examine the underlying neuroprotective mechanism of garlic (A.sativum) by determining its effect on hippocampus Na^+/K^+ ATPase, Ca^{2+} ATPase and glutamine synthetase activities. The study was carried with in a period of five weeks.

1.7 Justification of the study

Diabetes mellitus is a metabolic disease that causes serious neuronal impairment and cognitive deficits and considered as one of the causes of progressive neurodegeneration (Sacai *et al.*, 2014) Diabetes has been shown to affect Na^+/K^+ ATPase, Mg^{2+} ATPase, Ca^{2+} ATPase and neurotransmitter synthesis or release in several brain regions (Tsakiris *et al.*, 2001). In rats induced with type II diabetes mellitus there is reduction in transmitter release from glutamatergic neuron (Khakpai *et al.*, 2012a). Garlic (A.sativum) is used due to its antidiabetic, antioxidant properties and ameliorative effects on memory. However the underlying neuroprotective mechanism of garlic (A. sativum) mediated by Na^+/K^+ ATPase, Ca^{2+}ATPase and glutamine synthetase activities has not been demonstrated. This generated knowledge will provide a basis for further research in humans on the use of garlic (A.sativum) in management of various disorders characterised with memory impairment and also in developing effective therapies in the management of memory impairment. Principles important in improving hippocampus functions by enhancing neuronal excitability, neurotransmitter release and prevention of neurotoxicity eventually strengthening memory were explained.

1.8 Conceptual frame work

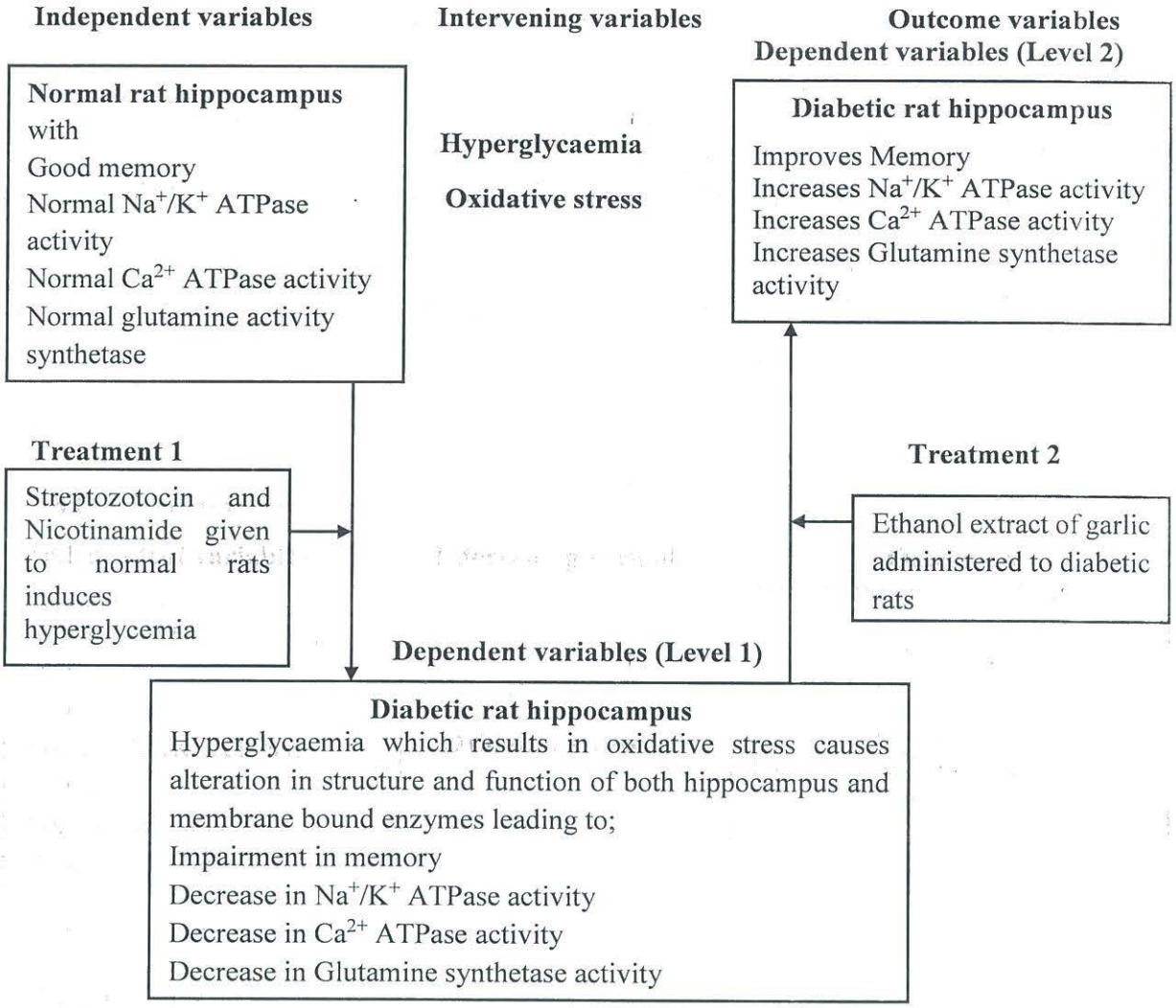


Figure 1. Showing Conceptual frame work based on literature (Kumar *et al.*, 2012)

1.8.1 Description of conceptual framework

Streptozotocin – NA induces hyperglycaemia which leads to increased production of reactive oxygen species (ROS) in the hippocampus. ROS attack membrane lipids, proteins resulting in increased lipid peroxidation, protein oxidation and protein nitration. ROS causes alteration in structure and function of the hippocampus, membrane bound enzymes leading to reduction in activities of hippocampus Na^+/K^+ ATPase, Ca^{2+} ATPase, glutamine synthetase leading to memory impairement. It is not known whether ethanol extract of garlic will have an effect on activities of hippocampus Na^+/K^+ ATPase, Ca^{2+}ATPase and glutamine synthetase.

CHAPTER TWO
LITERATURE REVIEW

2.1 Effect of diabetes mellitus and garlic (A. sativum) on memory

2.1.1 Diabetes mellitus

Is a group of metabolic disorders characterized by a chronic hyperglycaemic condition resulting from insufficient action of insulin. The main pathophysiological feature of type 2 diabetes, which represents majority of diabetic cases, arises due to impaired insulin secretion and increased insulin resistance. The impairment of pancreatic cell function notably shows progression over time. Type 2 diabetes is caused by a combination of genetic factors related to impaired insulin secretion and insulin resistance and environmental factors such as obesity, overeating, lack of exercise, and stress, as well as aging. Buchanan et al.,(2002). Diabetes mellitus, as an international public health concern, affects 5% of people worldwide Palsamy and Subramanian, (2010) and accounts for about 10% of total health care expenditure in many countries Pari and Saravanan, (2007). In human history, the 21st century has the most diabetogenic environment (Atkins and Zimmer, 2010). It has been estimated that 439 million people worldwide will have diabetes by 2030 (Chen *et al.*, 2012) and more than 100% increase in its incidence is expected between 2000 and 2030. Diabetes is one of the five leading causes of death in the world and about six deaths per minute are attributable to diabetes complications. In adults, type 2 diabetes is more frequent than type 1 and is mostly characterized by peripheral insulin resistance (Chatzigeorgiou *et al.,* 2009) and inadequate functional mass of β-cells (Chen *et al.*, 2005).

2.1.2 Pathophysiology of type 2 diabetes mellitus

Individuals with NIDDM have detectable levels of circulating insulin, unlike patients with IDDM and the pathophysiology of type 2. On the basis of oral glucose tolerance testing the essential elements of NIDDM can be divided into four distinct groups: i) Those with normal glucose tolerance, ii) Chemical diabetes (called impaired glucose tolerance), iii) Diabetes with minimal fasting hyperglycemia (fasting plasma glucose less than 140 mg/dl), iv) Diabetes mellitus in association with overt fasting hyperglycemia (fasting plasma glucose greater than 140 mg/dl). The individuals with impaired glucose tolerance have hyperglycemia inspite of having highest levels of plasma insulin, indicating that they are resistant to the action of insulin. In the progression from impaired glucose tolerance to diabetes mellitus, the level of insulin declines indicating that patients with NIDDM have decreased insulin secretion.

Insulin resistance and insulin deficiency are common in the average NIDDM patients. Insulin resistance is the primary cause of NIDDM, however some researcher contend that insulin deficiency is the primary cause because a moderate degree of insulin resistance is not sufficient to cause NIDDM (Raju and Raju, 2010). Most patients with the common form of NIDDM have both defects. Recent evidence has demonstrated a role for a member of the nuclear hormone receptor super family of proteins in the etiology of type 2 diabetes (Raju and Raju, 2010). Relatively new classes of drugs used to increase the sensitivity of the body to insulin are the thiazolidinedione drugs. These compounds bind to and alter the function of the peroxisome proliferators-activated receptor g (PPARg). PPARg is also a transcription factor and when activated, binds to another transcription factor known as the retinoid x receptor (RXR). When these two proteins are complexed a specific set of genes becomes activated. PPARg is a key regulator of adipocyte differentiation; it can induce the differentiation of fibroblasts or other undifferentiated cells into mature fat cells. PPARg is also involved in the synthesis of biologically active compounds from vascular endothelial cells and immune cells. (Raju and Raju, 2010).

2.1.3 Induction of type 2 diabetes mellitus in Wistar rats

Streptozotocin (STZ), [2-deoxy-2-(3-methyl-3-nitrosurea) 1-D-glucopyranose], is an antibiotic produced by Streptomyces achromogenes and a nitrosourea analogue in which N-methyl-N nitrosourea is linked to the carbon-2 of a hexose. STZ has selective toxic effects on β-cells because of high affinity for β-cell membrane, low capacity of β-cells to scavenge free radicals and low NAD^+/DNA ratio in islets. STZ accumulates in pancreatic β-cells by Glut2. Other organs like kidney, liver, and intestine are also damaged by STZ (Lenzen, 2008) . In β-cells, STZ acts by impairing glucose oxidation, decreases insulin synthesis and secretion (Szkudelski, 2001) and disrupts glucose transport and glucokinase activity (Rees and Alcolado, 2005)

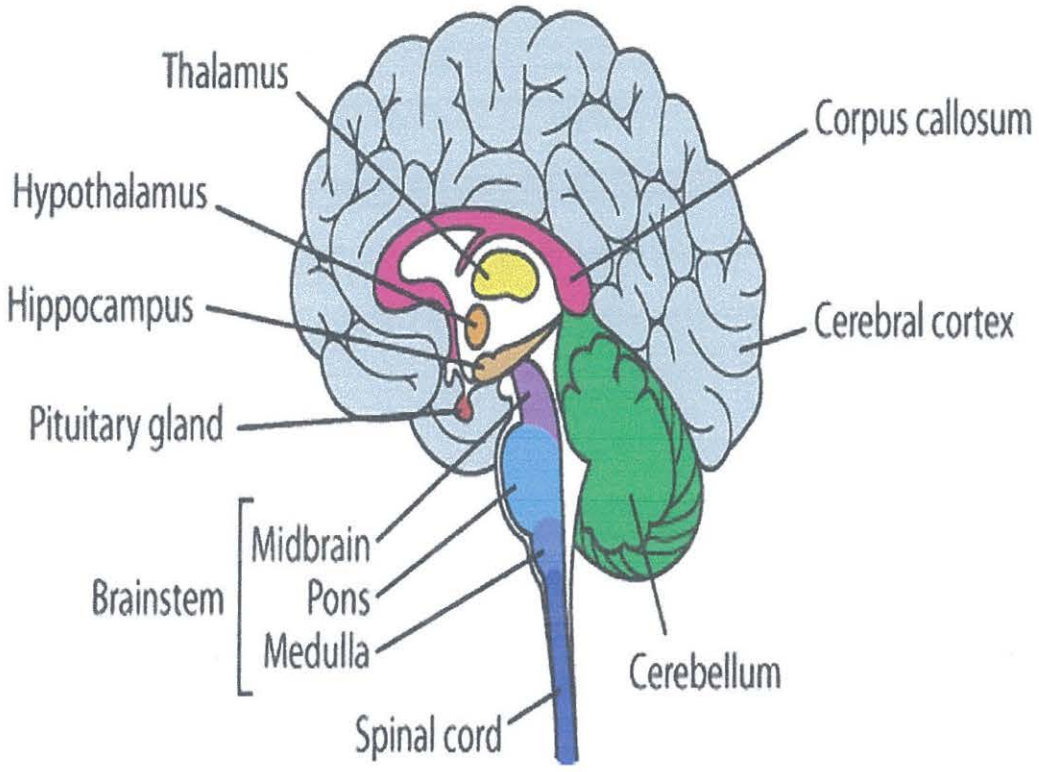
Nicotinamide (NA), pyridine-3-carboxamide, is a vitamin B3 (niacin) derivate with antioxidant capacity which reduces cytotoxic actions of STZ (Szkudelski, 2012). NA protects β-cell against STZ by several mechanisms. NA is a scavenger of oxygen-free radicals (Pociot *et al.,* 1993) and NO, inhibits both PARP with IC 50 value of 210 ± 2.9 μM , cytokine-induced MHC class II expression and provides NAD^+ (Szkudelski, 2001). NA also enhances β-cell regeneration and islet cell growth and inhibits apoptosis (Pandya *et al.*, 2010). In addition, NA may act as a methyl group acceptor, which reduces DNA methylation (Bennett and Pegg,

1981). NA is a cytoprotective agent that inhibits apoptosis through prevention of both externalization of phosphatidylserine and DNA degradation (Maiese *et al.*, 2009). The STZ-NA model of type 2 diabetes has been reported to be a good model for studies of diabetic complications and has been used in studies focused on diabetes complications Szkudelski, (2012) including diabetic nephropathy Palsamy and Subramanian, (2011) and neuropathy Sheela *et al.*, (2013) and cardiovascular complications of diabetes L'abbate *et al.*, (2007). The STZ-NA rat model of type 2 diabetes is based on protective effects of NA against β-cytotoxic effects of STZ. This model was first introduced by Masiello and Bergamini, (1977) using 10-week-old male Wistar rats. The STZ-NA model of type 2 diabetes has stable moderate hyperglycemia which does not require exogenous insulin to survive, reduction of β-cells (–40%) and reduced pancreatic insulin stores by 60%, glucose intolerance mainly due to impaired insulin secretion , presence (although impaired) glucose-stimulated insulin secretion and responsiveness to sulfonylureas (Szkudelski, 2012)

2.1.4 The hippocampus

The hippocampus is a major component of the brains of humans and other mammals. It belongs to the limbic system and plays important roles in long-term memory and spatial navigation. Like the cerebral cortex, with which it is closely associated, it is a paired structure, with mirror-image halves in the left and right sides of the brain. In humans and other primates, the hippocampus is located inside the medial temporal lobe, beneath the cortical surface. In rodents, the hippocampus has been studied extensively as part of the brain system responsible for spatial memory and navigation. Many neurons in the rat and mouse hippocampus respond as place cells: that is, they fire bursts of action potentials when the animal passes through a specific part of its environment. Hippocampal place cells interact extensively with head direction cells, whose activity acts as an inertial compass, and with grid cells in the neighbouring entorhinal cortex (Squire *et al.*, 2004).

Spatial learning and memory are functions of the hippocampus (Moser *et al.*, 1993). Evidence indicates that the hippocampus is necessary for acquisition and retrieval of spatial information as well as for consolidation and storage during the Morris water maze task (Morris, 1984)



Source: https://www.google.com/search?q=parts+of+the+brain

Figure 2. Showing location of hippocampus in human brain

2.1.5 Diabetic memory impairment

Spatial learning impairments have been reported in diabetes mellitus as one of its complications. Impairment of learning and memory have been shown to occur in STZ-diabetic rat with a decline in performance tested in the Morris water maze (Baydas *et al.*, 2003), passive avoidance test (Popovic *et al.*, 2001). This memory impairment seen in diabetic rats is due to hyperglycemia which induces oxidative stress in the hippocampus (Lee *et al.*, 2002) , resulting in apoptosis of hippocampus synapses and neurons (Zhang *et al.*, 2013). Hippocampus oxidative stress is associated with increased level of lipid peroxidation products (Mushtaq *et al.*, 2014) and diminution of activity levels of endogenous antioxidant enzymes (Moghaddam *et al.*, 2014). Diabetes mellitus has been reported to specifically impair the memory performance in experimental animals with powerful involvement of hippocampus and cerebral cortex. This finding may show impairment of acquisition and or consolidation of memory (Flood *et al.*, 1990).

2.1.6 Garlic (A. sativum), its active compounds and medicinal properties

Garlic plant Garlic bulbs/cloves

Source: *https://www.google.com/#q=garlic+plant*

Figure 3. Showing garlic plant and bulb

2.1.7 Bioactive compounds in garlic

Garlic its intact form contains allicin as the active ingredient which forms eight percent of the total formed thiosulfinates (McRae, 2005). Its action depends on the way the extract is prepared. Cutting, crushing or chewing of garlic bulbs leads to release of allinase (Stoll and Seebeck, 1949a, b). This herbal plant contains more than 33 compouds found to contain sulphur. These include diallyl dithiosulfinate (Tariq *et al.*, 1988), diallyl trisulfide, S-allylcysteine and diallyl disulphide (Wang and Ng, 1999)

2.1.8 Medicinal properties of garlic (A. sativum)

Garlic (A. sativum) is used as a food and for medicinal purposes (Haider *et al.*, 2008). Studies have shown that garlic extract lowers blood glucose levels, scavenges oxidants, increases superoxide dismutase, catalase, glutathione peroxidase, glutathione levels, inhibits lipid peroxidation and inflammatory prostaglandins (Borek, 2001). Garlic extract reduces cholesterol synthesis. Inhibition of cholesterol, LDL oxidation, and platelet aggregation by garlic inhibits arterial plaque formation, which is important in diabetes (Borek, 2001).

Fungal and bacterial diseases can be cured by garlic since it shows antifungal, antiviral and anti bacterial effects (Kim *et al.*, 2011). Garlic in its crude form is proven in treatment of gram-positive and gram-negative bacteria. Tsao and Hsu, (2003) and Zhou, (2003) showed that

freshly prepared garlic and its powdered form is effective against, Serratia marcescens, Klebsiella, Staphylococcus, Sarcina lutea, Salmonella, Streptococcus, Proteus, Escherichia coli and Bacillus. Al-Wailisaloom et al., (2007) reported that juice freshly prepared from garlic significantly inhibited activity of common microbial pathogens in humans. (Groppo *et al.,* (2007), Barki and Douglas (2005) made observation indicating that periodontitis is treatable using extract of garlic by inhibiting growth of pathogens in the mouth. Garlic extract is effective against forty fungi that cause fungal infections. Eight species out of fifteen of the zoopathogenic fungi genera treated with garlic showed retarded growth (Ledezma and Apitz-Castro, 2006). Yamada and Azuma, (1977) demonstrated the effectiveness of allicin as an anticandida agent. Aqueous garlic extract has been shown to inhibit and kill different types to Cryptococcus species (Fromtling and Bulmer , 1978). Adetumbi and Lau, (1986) reported the effect of aqueous garlic extract in inhibiting fungus growth of dimorphic nature. Lemar *et al.,* (2002) observed a reduction in growth of Candida albicans by inhibiting lipid, nucleic acid and protein synthesis when treated with aqueous extract of garlic. Josling (2001) reported that supplementation with garlic in146 individuals prevented infection with virus that causes common cold.

Observations indicate the use of onions and garlic prevented hypercholesterolemia due to feeding a high-fat diet. Sainani *et al.,* (1979) reported that in persons consuming garlic and onion had their serum levels of ß- lipoproteins, cholesterol, phospholipids and triglyceride were lower. Sumioka *et al.,*(2006) reported that fermented garlic extract of Monascus decreases hyperlipidemia indicating the potential to prevent arteriosclerosis. Consumption of garlic in amounts of 400 mg and allicin in 1mg two times in a day reduced significantly triglyceride, LDL-cholesterol, total cholesterol (Kojuri and Vosoughi , 2007) .

Aqel and Gharaibah , (1991) reported the hypertensive effect of garlic extract is due to smooth muscle relaxation. Andrianova and Fomchenkov , (2002) suggested that treatment with 600 mg/day caused a reduction in blood pressure. Recent studies suggest possible effects of garlic extracts in prevention of diseases associated with arteries. Abdullah et al, (1988) indicated that 139% to 159% tumor cells were destroyed by contents in raw extract of garlic. Aged extract of garlic prevents formation of tumours by suppressing endothelial tube formation, proliferation and mortality of cells (Matsuura *et al.,* 2006) . Extracts of garlic have been used as pesticides. Amonkar and Reeves, (1970) studied the effect of oil made from garlic and extracted from crude methanol is effective against Culex peus mosquito larvae in its 3rd stage.

Extracts of onion and garlic have been shown to have highly ameliorative effect on memory impairment (Nishimura *et al.,* 2006). Feeding aged garlic extract prevented deterioration of hippocampal based memory tasks in 7-month-old Tg2576 mice model, suggesting that aged garlic extract has a potential for preventing Alzheimer's disease progress (Chauhan , 2007) . On the other hand, studies suggest the possibility that aged garlic extract prevents physiological aging and age-related memory disorders in human (Moriguchi *et al* ., 1996). Fresh and aged garlic extracts improved short-term but not long-term memory in diabetic male rats assessed by passive avoidance method probably by inhibiting production and reducing scavenging free radicals in brain (Sarkaki *et al.,* 2013). Also, Iranian shallot or garlic extracts appear to improve spatial learning and memory impairments assessed by Morris water maze test in fructose-fed rats (Jalal *et al.,* 2011).

2.2 Effect of diabetes mellitus on hippocampus Na^+/K^+ ATPase

Brain oxidative metabolism is very active, mostly required to maintain cellular Na^+/K^+ gradients for keeping nerve impulse propagation, neurotransmitter release and cation homeostasis. Na^+/K^+-ATPase is a membrane bound enzyme involved in maintaining the Na^+ and K^+ gradient across the cell membrane. It is ubiquitously expressed in neurons (Pietrini *et al.,*1992). Hippocampal Na^+/K^+ ATPase has been linked to memory function in rodents (Heo *et al.,* 2012).It has been reported that a decrease in this enzyme activity or expression directly impairs neurotransmitter signaling with deleterious consequences on memory and anxiety behaviour (Palladino *et al.,* 2003). It has also been proposed that alterations in Na^+/K^+ ATPase activity may represent an important neurotoxic mechanism for neurons (Lees, 1991). It has also been shown The increase in activity of Na^+/K^+ ATPase may be due to alteration in permeability of the cell membrane in rat brain (Rajendra *et al.,* 2013). This is important because ionic imbalance across the cell membrane contributes to accumulation of intracellular potassium and extracellular sodium ions which is detrimental to the cell (Kimelberg, 2004). Intra axonal accumulation of sodium ions at the node leads to paranodal swelling due to increased inactivation of sodium channels (Li *et al.,* 2002). In addition, it has been suggested that increasing the activity of Na^+/K^+ ATPase allows constant exchange of Na^+ and K^+ ions across the neuronal membrane so as to maintain electrochemical gradient. This may prevent swelling and enlargement of neurons which leads to neuronal death a consequence of diabetes mellitus (Lee *et al.*, 2002).

Maintaining the electrochemical gradient in cells by Na^+/K^+ ATPase is energy-dependent. The decreased hippocampus Na^+/K^+ ATPase activity in diabetic rats is associated with depletion of ATP within the cell mainly due to decreased glucose utilisation (Lee *et al.*, 2002). Increased hippocampus Na^+/K^+ ATPase activity may prevent impairment of glucose transport as Na^+ is co-transported with glucose (Lee *et al.*, 2002). This facilitates the exchange of Na^+ and K^+ ions via the sodium pump against glutamine going into the neuron to be converted into glutamate in the cytoplasm under the glutamate-glutamine cycle (Ogundele and Madukwe, 2012).

2.3 Effect of diabetes mellitus on hippocampus Ca^{2+} ATPase

Diabetes has been shown to affect neurotransmitter synthesis or release in several brain regions (Welsh and Wecker, 1991) and it can be argued that the impaired ability of streptozotocin-treated rats to generate synaptic potentiation in the hippocampus is related to alteration at the presynaptic level, which possibly involves a reduction of transmitter release from glutamatergic neurons (Palladino *et al.*, 2003). The expression of Ca^{2+} ATPase pump has been reported in rodents and human hippocampus (Zacharias and DeMarco, 1997). Ca^{2+} ATPase regulates Ca^{2+} pump activity which acts as a second messenger in the control of cellular processes that plays a central role in mediating neurosecretion and inhibition of Ca^{2+} ATPase activity can in turn increase intracellular concentration of Ca^{2+} and alter the signal transduction pathways and cellular fluidity and eventually results in cell death (Aubier and Viires, 1998). Diabetes has also been reported to suppress activity of Ca^{2+} ATPase in rat whole brain (Kumar *et al.*, 2012). A decrease in Ca^{2+} ATPase in diabetic brain could be due to excessive non-enzymatic glycation of the enzyme itself or of calmodulin (Kamboj *et al.*, 2009)

In addition, increasing the activity of Ca^{2+}ATPase may prevent overload of intracellular Ca^{2+} influxes which induces neuronal death (Furukawa and Mattson, 1998). It has been proposed that inhibition of Ca^{2+}ATPase activity leads to increase in intracellular concentration of Ca^{2+} (Pekiner and Nebioğlu, 2005). This will alter cellular fluidity, signal transduction pathways, and eventually results in cell death (Aubier and Viires, 1998). The increase in activity of hippocampus Ca^{2+}ATPase may be due to stabilizing intracellular Ca^{2+} concentrations by inactivation of nuclear factor-κB (NF-κB). NF-κB modifies glutamate receptor expression (Furukawa and Mattson, 1998). In support of this argument, Lin et al., (2012) indicated that one of the active compounds in garlic; thiacremonone prevents amyloidogenesis and neuroinflammation by inhibiting the activity of NF-κB.

2.4 Effect of diabetes mellitus on hippocampus glutamine synthetase activity

Glutamatergic neurotransmission is known to play an important part in the course of hippocampal modulation of learning and memory processes (Khakpai *et al.,* 2013). In the brain, the conversion of glutamate to glutamine by glutamine synthetase, that takes place within the astrocytes, represents a key mechanism in the regulation of excitatory neurotransmission(Szatkowski and Attwell, 1994). Several studies have indicated that the activity of glutamine synthetase in astrocytes is diminished in several brain disorders, including diabetes (Eid *et al.,* 2013). In the rat models, the activity of glutamine synthetase, which converts glutamate into glutamine in astrocytes, is affected in type 1 diabetes (Bhardwaj *et al.,* 1998). Glutamine synthetase an enzyme mainly located in astrocytes is important in controlling the intracellular concentration of glutamate by converting it into glutamine (Gras *et al.,* 2006) and also involved in ammonia assimilation (Hertz *et al.,* 1999). It has been suggested that inhibition of GS leads to a build up of glutamate in the extracelluar fluid which is neurotoxic (Dienel and Hertz, 2005). This inhibition has been suggested to result in production of nitric oxide which is also toxic to neurones (Parathath *et al.,* 2007). A decrease in the activity of GS has been reported in excitotoxicity mediated by kainic acid (Swamy *et al.,* 2009).

2.5 Effect of metformin on diabetes mellitus and memory

Metformin, a biguanide antihyperglycemic agent, is widely used in the management of type 2 diabetes mellitus. It lowers the blood glucose concentration without causing hypoglycemia (Scheen, 1996). Metformin exerts its antihyperglycemic actions by suppressing hepatic glucose output and increasing insulin-mediated glucose disposal, without weight gain (Kirpichnikov, 2002). It has also be shown to improves, lipid profile by reducing hypertriglyceridemia, lowering plasma fatty acids and LDL-cholesterol and raising HDL-cholesterol in some patients (Ihara *et al.*, 2000). Metformin improved learning and memory behaviors by attenuating brain mitochondrial dysfunction and decreased the brain oxidative stress levels in high fructose diet fed rats (Pintana *et al.*,2012). Metformin improves learning and memory in mice with diabetes, and can protect hippocampus neurons (Zhang *et al.,* 2012). Metformin improves cognitive dysfunction in rats (Li *et al.,* 2013). Metformin reduces insulin resistance and improved the animal's spatial cognition ability (Yuan *et al.,* 2014) (Yuan *et al.,* 2014). Metformin may improve learning and memory deficits by activating AMPK and Na^{+}-K^{+} ATPase system (Mo and Qian, 2014).

CHAPTER THREE
RESEARCH METHODOLOGY

3.1 Study design

The experiment was carried out at the Biomedical Sciences animal house facility in Kampala International University (KIU), Western Campus, Bushenyi. Thirty (30) male Wistar rats weighing 150 – 200g and 3 months of age were purchased from Mbarara University animal house. The rats were housed in clean cages in the animal house facility at KIU western campus, Bushenyi and acclimatized to the new surroundings for two weeks under room temperature and 30% humidity. Rat pellets purchased from Nuvita feeds (U) Ltd and water was provided throughout the experimental period with exception when fasting blood glucose levels were taken (Ozougwu *et al.*, 2013).

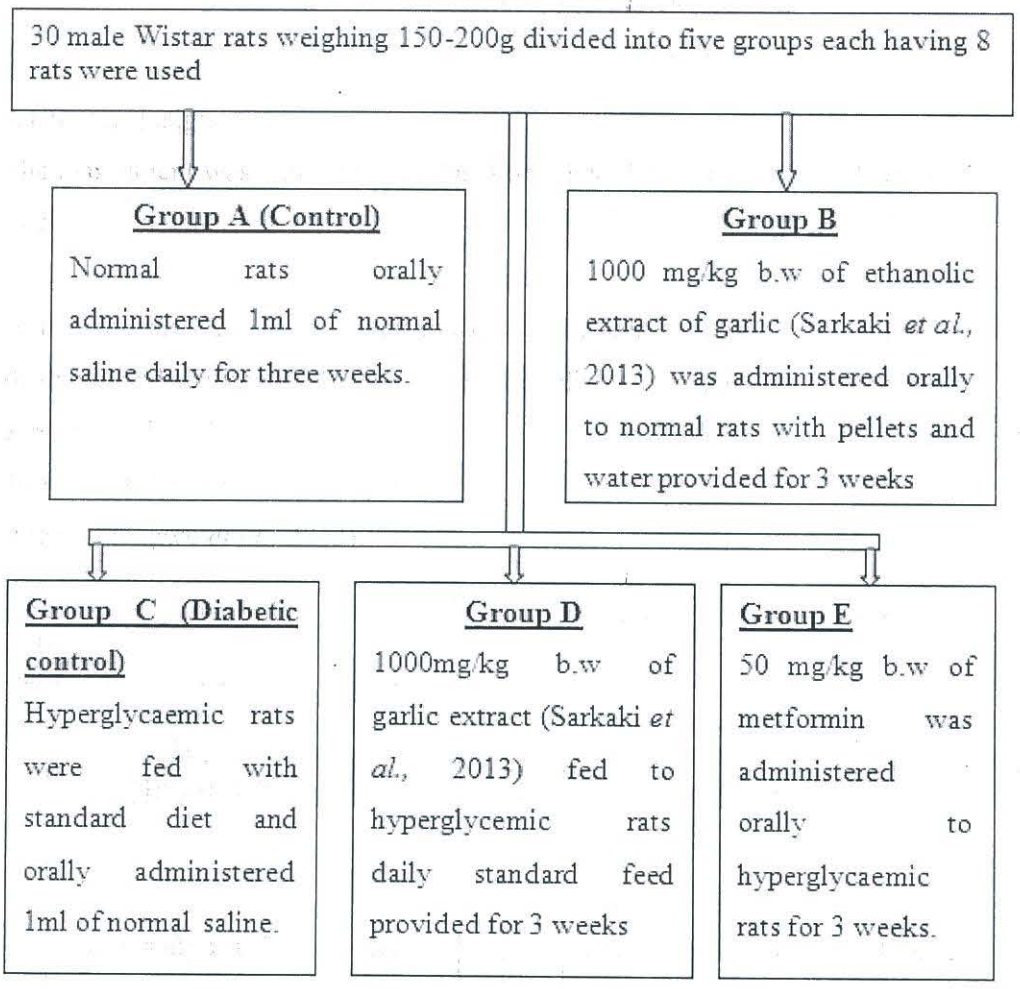


Figure 2. Showing scheme of study design

3.2 Ethanol extraction of garlic (A.sativum)

Extraction was done using cold maceration in the laboratory (Baluchnejadmojarad *et al.*, 2003) in the Department of Pharmacology, KIU Western Campus Bushenyi. Peeled garlic weighing 500 g was cut into small pieces and mixed in 70 ml of cold sterile 0.9% NaCl solution. The paste material was suspended in 80% ethanol for 48 hours in air tight glass jar using a rubber stopper, and the suspension was shaken periodically three times daily at an interval of 5 minutes. After 2 days, the suspension was filtered using Whitman filter paper to remove residue. Filtration was repeated 3 times and clear filtrate was obtained. The filtrate was concentrated using rotary evaporator at a bath temperature of 40°C. The extract concentrate obtained was then transferred to a cornical flask and further evaporated in oven drier at 50°C to obtain ultimately a gel like mass for the study (Baluchnejadmojarad *et al.*, 2003)

3.3 Induction of hyperglycemia in Wistar rats

Wistar rats were allowed to feed freely for five days. Sixty (60) mg/kg streptozotocin purchased from Sigma Aldrich was dissolved in 0.05ml of citrate buffer (pH 4.5) and120 mg/kg nicotinamide purchased from Zac's pharmacy in Bushenyi was dissolved in 0.5 ml of normal saline. Diabetes mellitus type II was induced in rats fasted overnight. It was done intraperitonealy with a single injection of 60 mg/kg STZ. Administration of 120 mg/kg of nicotinamide intraperitonealy was done 15 min after (Marudamuthu and Leelavinothan, 2008).

3.4 Confirmation of hyperglycemia

Elevated levels of blood glucose were confirmed after 3 days after induction by collecting blood samples from the rat tail vein through a small cut. Blood glucose levels were measured using one call glucometer after 3, 7, 14 and 21 days (Thulé *et al.*, 2006). Rats confirmed hyperglycemic with blood glucose concentration (≥250mg/dl) were used for the study (Marudamuthu and Leelavinothan, 2008).

3.5 Hippocampus dependant memory assessment

Assessment of hippocampus function was achieved using object location memory test (OLM). This test was performed, before induction of hyperglycemia and then 21 days after confirmation of diabetes mellitus type II (Grzęda and Wisniewska, 2008).

OLM apparatus: The experimental box was made of light wood and measured 40.65 cm x 640.65 cm x 630.5 cm. The saw dust was spread 2 inches on the floor and lighting using overhead bulb was used. The objects and arena was cleaned using 70% ethanol on each day of the experiment and to reduce olfactory cues fresh bedding was provided for each day. Object location memory was carried out for 3 days (Gerstein,*et al.*, 2013)

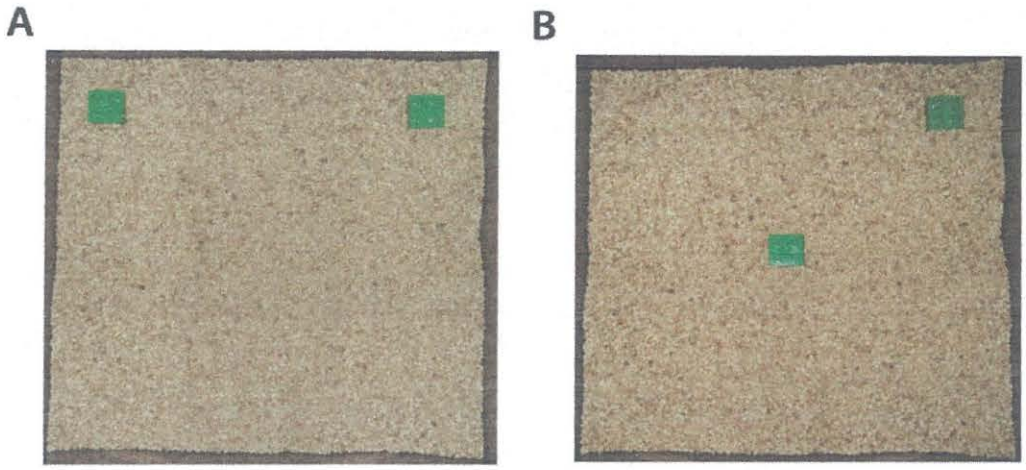


Figure 3. Shows overhead view of object location memory box arena. (A) Object locations on training day and (C) Object locations on testing day.

Experiment: On day one` (Habituation), the rats were allowed to explore the arena and behavioral room for two 5 minute exposures and no objects was placed in the arena during this day. The cage was cleaned to remove any feces and the rat was then returned after exploration. Habituation proceeded to another rat and repeated following the same arrangement after all rats had been habituated. Training day (Day 2), two identical objects were placed in the arena, measured 1.2561 X 2561 inches and the rats underwent training on the locations of the objects. The objects were then placed in the arena 2.5 cm from the wall of the box. The objects were located in corner A and B, 2.5 cm apart. The orientation of the box were in such a way that the side with cue marked X is east. Rats explored the two objects freely for a 10-minute trial. Any rats which failed to explore the objects on training day and had training trial of less than 5 seconds were not included for analysis. OLM testing occurred on day 3. Spatial memory was tested by measuring their preference for a novel location versus a familiar location. For each trial, one of the objects was placed in the center of the arena instead of its original location. The experiment was run similarly as during training, with 10-minute trials. All trials were videotaped and analyzed manually.

Object location memory was determined by measuring the object exploration time. Total amount of time (exploration time) spent exploring the novel and familiar objects was recorded for each rat and later analyzed. Exploration time was scored when the rat's head or nose was touching the object. Standing on, sitting on and sniffing the air above an object was also scored. The relative exploration time was recorded for each object and expressed as a novelty index: [(Time Spent (s) Investigating Object in Novel Location/Time Spent (s) Investigating Both Objects in Total) multiplied by 100%] (Gerstein,*et al.*, 2013)

3.6 Collection of hippocampus samples and processing

After the memory test, the rats were placed in a container with a lid containing a towel dipped in 99% diethyl ether for 2 minutes. The rats were then sacrificed, the brain removed, the hippocampi were carefully excised and then homogenized. Homogenate was then centrifuged to obtain a supernatant which was latter used for analyzing for Na^+/K^+ATPase, Ca^{2+} ATPase and GS activities.

3.7 Biochemical analysis

3.7.1 Measurement of Na^+ /K^+ ATPase activity

Thitry (30) hippocampus homogenates were analyzed for Na^+/K^+ ATPase according to the method of Tirri *et al.*, (1973). 0.1 grams of hippocampi was placed in a homogenizer and then Assay medium was added. The assay medium consisted of (in mM) 30 Tris-HCl buffer (pH 7.4), 50 NaCl, 6 $MgCl_2$, 5 KCl and 50 µg of protein in the absence of 1 mM ouabain, 0.1 EDTA, in a final volume of 350 µL. The reaction was started by the addition of ATP (adenosine triphosphate) to a final concentration of 3mM. After 30 min at 37° C, the reaction was then stopped by the addition of 50% (w/v) trichloroacetic acid (70 µL). Saturating substrate concentrations were used, and reaction was linear with protein and time. An appropriate control was included in the assays for non-enzymatic hydrolysis of ATP. The Pi (amount of inorganic phosphate) released was quantified calorimetrically, as previously described (Fiske and Subba Row, 1927), using 300KH_2PO_4 as reference standard. Specific Na^+, K^+-ATPase activity was calculated from the overall activity (in the absence of ouabain) and was computated as Pi/min/mg of protein in nmol.

3.7.2 Measurement of Ca^{2+} ATPase activity

The method of Desaiah and Ho (1979) was used to assay Ca^{2+} ATPase in 30 hippocampal homogenates. Pi (Inorganic phosphates) was estimated by the method of Fiske and Subba Row (1925). The assay medium had a final volume of 200 µL. It consisted of (in mM), 30 Tris-HCl, 100 µg of protein in the absence of 0.4 $CaCl_2$, buffer (pH 7.4), 3 $MgCl_2$and 0.1 EGTA. The reaction was started by the addition of ATP to a final volume of 3 mM. 60 min after at 37°C, the reaction was stopped by the addition of 50% (w/v), 70 µL of trichloroacetic acid. Substrate concentrations were used, the reaction was linear with time and concentration of protein. Controls included in the assays to assess non-enzymatic ATP hydrolysis. The Pi (concentration of inorganic phosphate) released was quantified colorimetrically, as previously described (Fiske and Subba Row, 1927), using KH_2PO_4 as a reference standard. The Ca^{2+} ATPase activity was determined by subtracting the activity measured from absence of Ca^{2+} (no added0.1 mM EGTA and Ca^{2+} and expressed as Pi/min/mg protein in nmol.

3.7.3 Measurement of Glutamine synthetase activity

The method of Rowe *et al.*, (1970) was used in the enzymatic assay of glutamine synthetase. In this method, 0.1 mL homogenates solubilized in 140 mM KCl was added to 0.1 mL of the reaction mixture in mM and incubated for 15 min (37°C). The reaction was stopped by 0.4 mL addition of a solution containing (in mM): 370 ferric chloride, 200 TCA and 670 HCl. The absorbance of the supernatant was measured at 530nm after centrifugation and standard quantities of ferric chloride reagent treated with c-glutamylhydroxamate were compared to the absorbance generated. Results were expressed as percentages of the control condition in mMol of gamma glutamyl hydroxamate / hr/mg protein.

3.8 Statistical analysis

Data obtained were expressed as mean ± SEM and were statistically analysed using one way analysis of variance (ANOVA). Tukey's multiple comparison post hoc tests to compare the level of significance between control and experimental groups were used. The values of $p < 0.05$ were considered significant.

3.9 Limitations and delimitations

I. Some of the rats didn't become hyperglycemic on induction and were not considered for the study.

II. Was unable to carry out insulin sensitivity or resistance test.

3.10 Ethical consideration

The study animals were handled according to "Guide for the care and the use of Laboratory Animals" published by the National Institute of Health, 2001. Animals were kept in clean cages, fed with water and rat pellets.

CHAPTER FOUR

PRESENTATION AND INTERPRETATION OF RESULTS

4.0 Effect of ethanol garlic (A. sativum) extract on blood glucose levels

The effect of ethanol garlic (A. sativum) extract on blood glucose levels in diabetic rats are shown in Table 1. The blood glucose levels for each week in diabetic rats were higher as compared to normal control rats. There was a significant decrease in blood glucose levels of diabetic rats treated with garlic (A. sativum) extract as compared with diabetic control rats (Table 1).

Table 1: The effects of ethanol garlic (A.sativum) extract on blood glucose levels

Fasting blood glucose levels (mg/dl)				
Groups	**Day 3**	**Day 7**	**Day 14**	**Day 21**
A. Normal	110 ± 0.64	108 ± 0.58	109 ± 0.39	108 ± 0.38
B. Diabetic control rats	323 ± 19.34	344 ± 12.42	449 ± 7.41	468 ± 6.07
C. Diabetic rats + 1000 mg/kg of ethanol garlic extract	330 ± 14.63	297 ± 21.36	143 ± 0.99	137 ± 0.41*

Results are expressed as Mean ± SEM (n=8). The data was analysed using ANOVA followed by Tukey's post hoc test. *P < 0.001 vs diabetic control rats.

4.1 Effect of ethanol extract of garlic (A. sativum) on object location memory

All rats in the different groups explored objects in familiar and novel locations equally. Exploration times to objects in familiar and novel locations were calculated. Results showed that, in the normal control rats exploration time to object in novel location (13.4 ± 0.50) was significantly ($p < 0.05$) higher than exploration time to object in familiar location (11.8 ± 0.03) (Figure 6) where as in diabetic control rats, exploration time to object in novel location (11 ± 0.32) was not significant ($p > 0.05$) as compared with exploration time of object in familiar location (10.9 ± 0.03). (Figure 6).

After three weeks of treatment of diabetic rats with ethanol garlic (A.sativum) extract at a dose of 1000mg/kg, the exploration time of object in novel location (14.8 ± 2.24) was significantly higher ($p < 0.05$) than exploration time of object in familiar location (12.3 ± 0.11). (Figure 6). In addition, treatment of diabetic rats with metformin at a dose of 50mg/kg , increased significantly ($p < 0.05$) the exploration time of object in novel location (29 ± 1.22) as compared to exploration time of object in familiar location (14.94 ± 0.01).(Figure 6).

The ratio between explorations of objects in the new (novel) location versus familiar location, the novelty index, of normal rats treated with garlic (59 ± 0.19) was significantly increased ($p < 0.05$) as compared to normal control rats (55 ± 0.11). The novelty index in the diabetic control group (49 ± 0.22) was significantly reduced ($p < 0.05$) as compared with that of normal rats (55 ± 0.11). (Figure 7).

However, when the diabetic rats were treated with garlic (A.sativum), there was significant difference ($p > 0.05$) in the novelty index (61.4 ± 0.20) as compared to the novelty index (49 ± 0.22) of the diabetic control rats. In addition, the diabetic rats treated with metformin at a dose of 50mg/kg for three weeks showed a significant increase ($p < 0.05$) in the novelty index (69 ± 0.05) as compared to that of diabetic control rats (49 ± 0.22). (Figure 7).

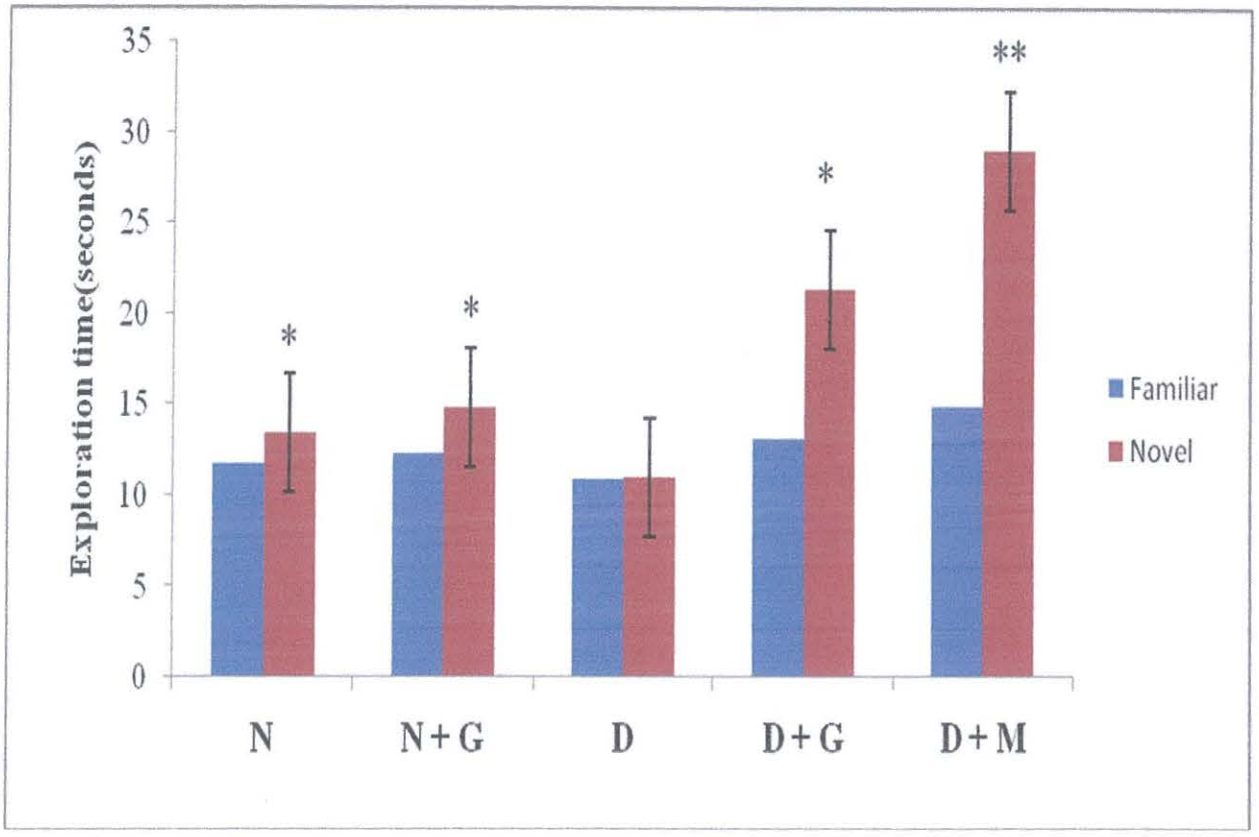


Figure 4. Effect of ethanol garlic (A.sativum) extract treatment on exploration time to familiar and novel object locations in different groups

Each bar represents a mean of six samples. * $P < 0.05$; **$P < 0.01$ vs respective groups.

Key:

N= Normal + saline.

N+G= Normal rats that received 1000mg/kg of garlic (A.sativum) extract.

D = Diabetic control rats + saline.

D+G= Diabetic rats that received 1000mg/kg of garlic (A.sativum) extract.

D+M=Diabetic rats that received 50mg/kg of metformin.

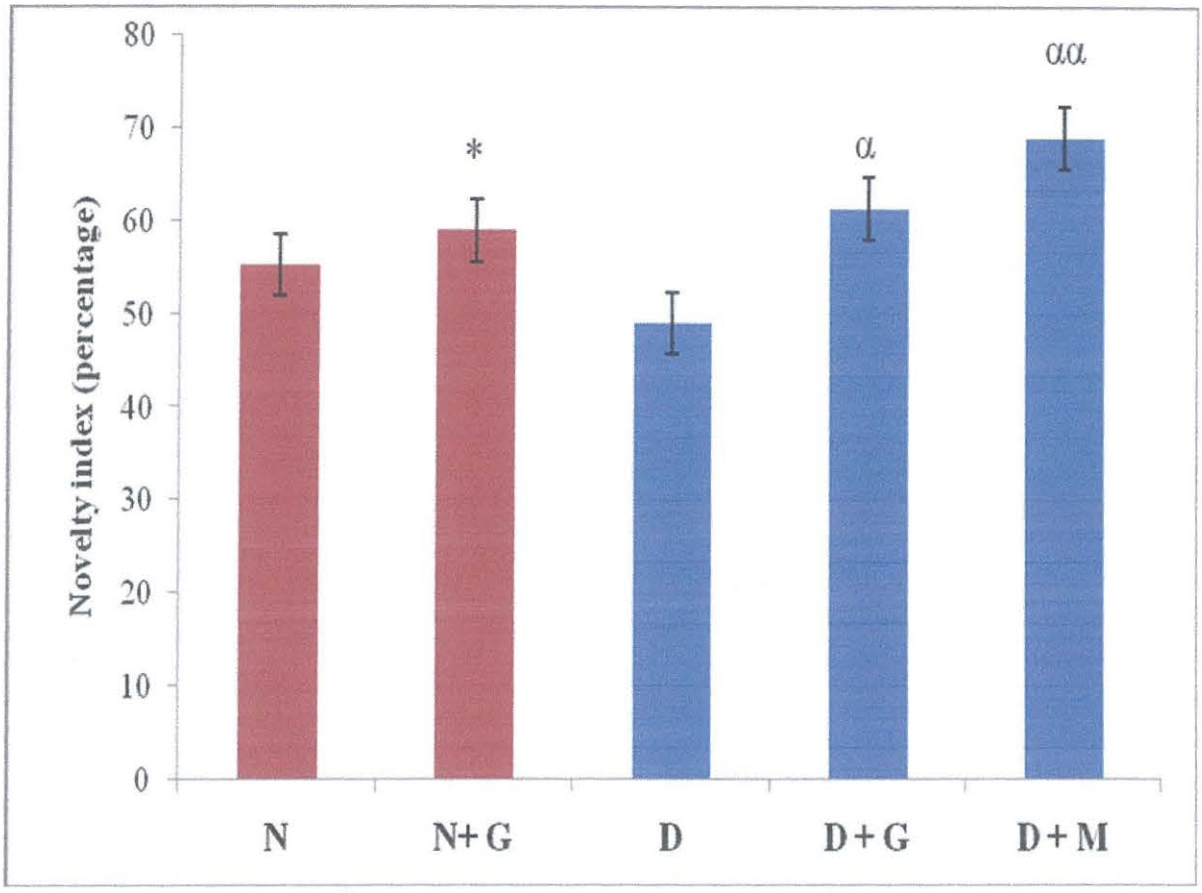


Figure 7. Effect of treatment with ethanol garlic (A. sativum) extract on novelty index among different treatment groups.

Each bar represents a mean of six samples. * $P < 0.05$ vs normal control group ,$^{\alpha}$ $P < 0.05$ vs diabetic control group, $^{\alpha\alpha}P < 0.01$ vs diabetic control group.

Key:

N= Normal + saline.

N+G = Normal rats that received 1000mg/kg of garlic (A. sativum) extract.

D = Diabetic control rats + saline.

D+G = Diabetic rats that received 1000mg/kg of garlic (A. sativum) extract.

D+M = Diabetic rats that received 50mg/kg of metformin.

4.2 Effect of ethanol extract of garlic (A. sativum) on activity of hippocampus sodium-potassium ATPase.

Results obtained revealed that hippocampus Na^+/K^+ ATPase activity was significantly higher ($p < 0.05$) in normal rats (0.53 ± 0.18) treated with garlic (A. sativum) at a dose of 1000mg/kg when compared to normal control rats (0.43 ± 0.11) that received normal saline (Figure 8).

In the diabetic control rats, the hippocampus Na^+/K^+ ATPase activity (0.338 ± 0.02) was significantly decreased ($p > 0.05$) when compared with normal control rats that received normal saline (0.43 ± 0.01). (Figure 8).

Administration of garlic (A, sativum) to diabetic rats at a dose of 1000mg/kg resulted in a significant increase ($p < 0.05$) in hippocampus Na^+/K^+ ATPase activity (0.68 ± 0.01) compared to diabetic control rats (0.338 ± 0.02). (Figure 8).

Following treatment with metformin to diabetic rats, hippocampus Na^+/K^+ ATPase activity (0.862 ± 0.02) was significantly increased ($p < 0.05$) as compared to diabetic control rats (0.338 ± 0.02). (Figure 8)

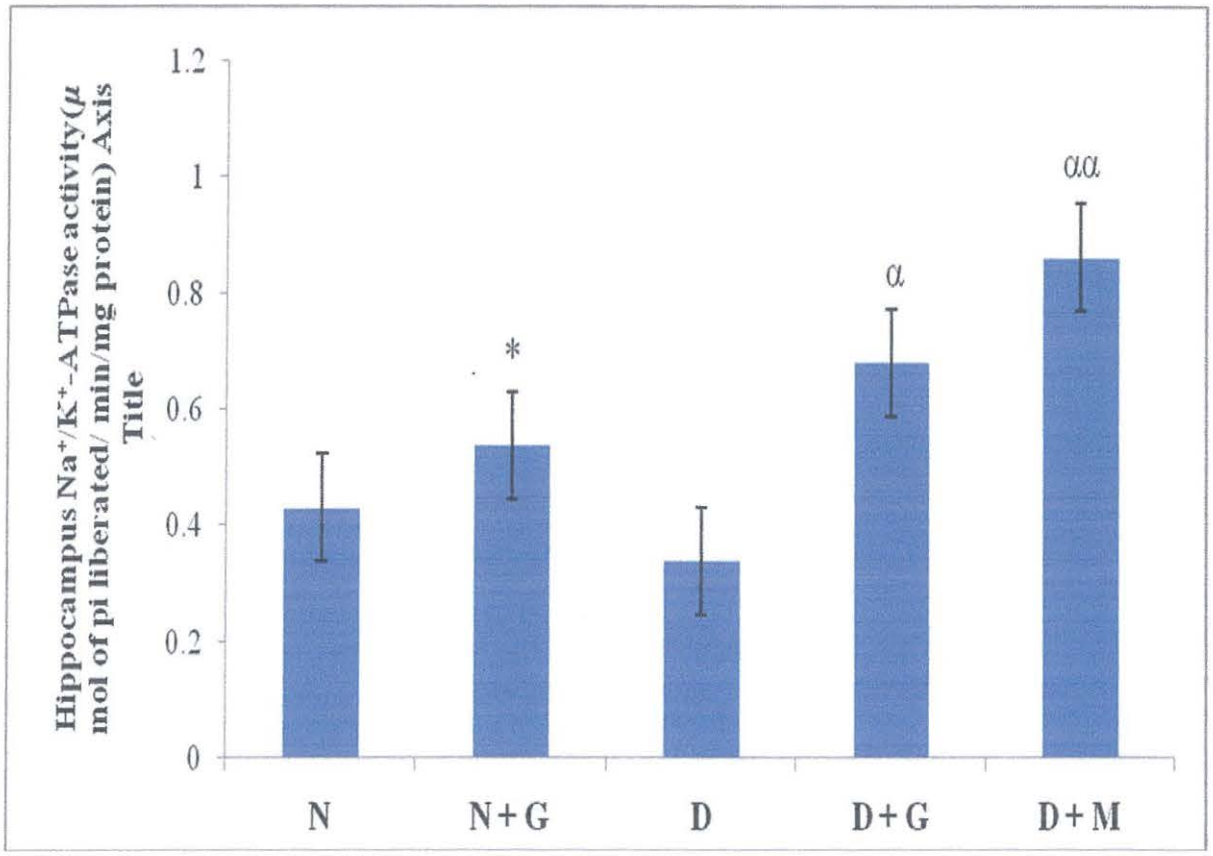


Figure 8. Shows effect of ethanol extract of garlic (A. sativum) on activity of hippocampus sodium- potassium ATPase in the different groups.

Each bar represents a mean of six samples. * $P < 0.05$ vs normal control group , $^{\alpha}$ $P < 0.05$ vs diabetic control group, $^{\alpha\alpha}$ $P < 0.001$ vs diabetic control group.

Key:

N= Normal + saline.

N+G= Normal rats that received 1000mg/kg of garlic (A. sativum) extract,

D = Diabetic control rats + saline.

D+G= Diabetic rats that received 1000mg/kg of garlic (A. sativum) extract.

D+M=Diabetic rats that received 50mg/kg of metformin.

4.3 Effect of ethanol extract of garlic (A. sativum) on hippocampus calcium ATPase activity

Ca^{2+}ATPase activity in the hippocampus of normal rats treated with garlic (A.sativum) at a dose of 1000mg/kg (0.78 ± 0.25) was significantly higher ($p < 0.05$) when compared to normal control rats (0.56 ± 0.18).(Figure 9).

However, hippocampus Ca^{2+}ATPase activity was significantly decreased ($p > 0.05$) in diabetic control rats (0.438 ± 0.019) when compared with normal control rats that received normal saline (0.56 ± 0.18). (Figure 9).

Following treatment of garlic (A. sativum) to diabetic rats at a dose of 1000mg/kg, hippocampus Ca^{2+}ATPase activity (1.22 ± 0.037) was significantly increased ($p < 0.05$) compared to diabetic control rats (0.438 ± 0.019).(Figure 9).

In addition, administration of diabetic rats with metformin significantly increased ($p < 0.05$) the hippocampus Ca^{2+}ATPase activity (1.612 ± 0.033) as compared to diabetic control rats (0.338 ± 0.02). (Figure 9)

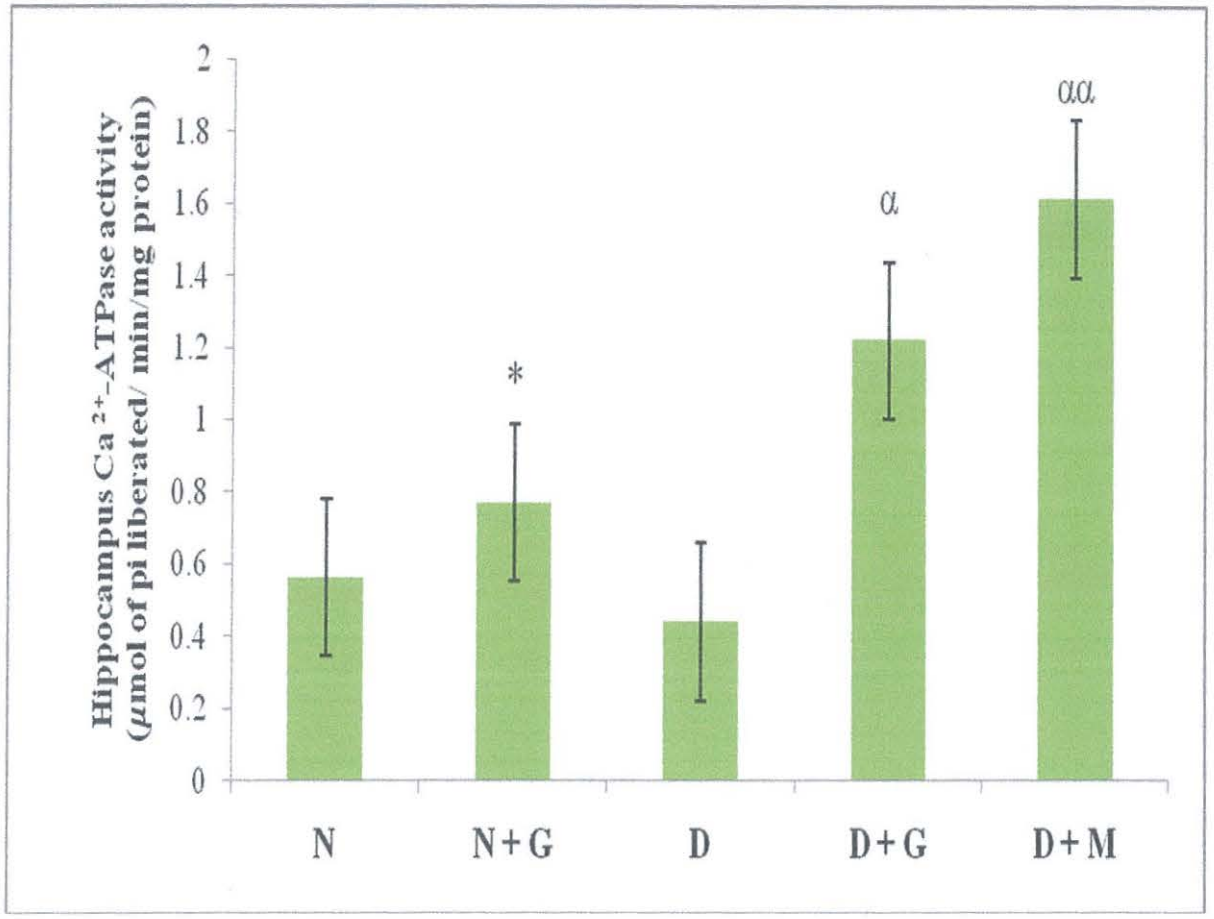


Figure 9. Shows the effect of ethanol extract of garlic (A. sativum) on hippocampus calcium ATPase activity in different groups.

Each bar represents a mean of six samples. * $P < 0.05$ vs normal control group ,$^{\alpha}$ $P < 0.05$ vs diabetic control group, $^{\alpha\alpha}$ $P < 0.001$ vs diabetic control group.

Key:

N= Normal + saline.

N+G = Normal rats that received 1000mg/kg of garlic (A.sativum) extract.

D = Diabetic control rats + saline.

D+G= Diabetic rats that received 1000mg/kg of garlic (A.sativum) extract.

D+M = Diabetic rats that received 50mg/kg of metformin.

4.4 Effect of ethanol extract of garlic (A. sativum) on hippocampus glutamine synthetase activity.

Results showed that hippocampus glutamine synthetase activity was significantly increased ($P < 0.05$) in normal rats treated with garlic (A.sativum) at a dose of 1000mg/kg (0.516 ± 0.005) as compared to normal control rats (0.478 ± 0.01). (Figure 10).

On the other hand, the diabetic control rats recorded a significantly decreased ($P > 0.05$) hippocampus glutamine synthetase activity (0.32 ± 0.001) when compared to normal control rats (0.478 ± 0.01). (Figure 10).

Treatment of diabetic rats with garlic (A.sativum) at a dose of 1000mg/kg increased significantly ($P < 0.05$) the hippocampus glutamine synthetase activity (0.77 ± 0.003) compared to diabetic control rats (0.32 ± 0.001). (Figure 10).

Administration of metformin at 50mg/kg to diabetic rats resulted in a significantly increased ($P < 0.05$) hippocampus glutamine synthetase activity (0.952 ± 0.007) as compared to corresponding diabetic control rats (0.32 ± 0.001). (Figure 10)

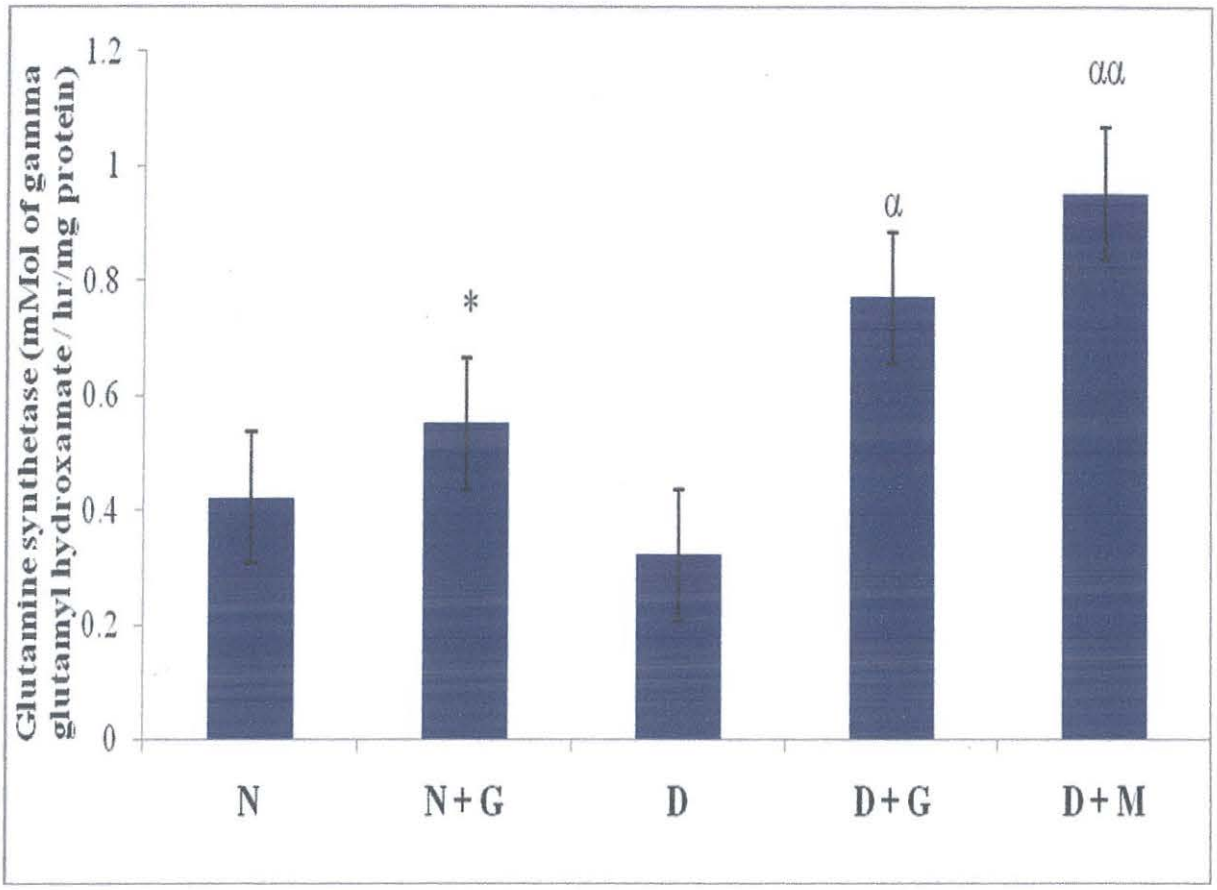


Figure 10. Shows effect of ethanol extract of garlic (A.sativum) on hippocampus glutamine synthetase activity in different groups.

Each bar represents a mean of six samples.
* $P < 0.05$ vs normal control group, $^{\alpha}$ $P < 0.05$ vs diabetic control group, $^{\alpha\alpha}$ $P < 0.001$ vs diabetic control group.

Key:

N= Normal + saline.

N+G= Normal rats that received 1000mg/kg of garlic (A.sativum) extract.

D = Diabetic control rats + saline.

D+G = Diabetic rats that received 1000mg/kg of garlic (A. sativum) extract.

D+M = Diabetic rats that received 50mg/kg of metformin.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

The purpose of the present study was to determine whether hippocampus Na^+/K^+ ATPase, Ca^{2+} ATPase and glutamine synthetase activities are involved in memory improvement in model of type II diabetic Wistar rats treated with garlic extract. Results showed that the exploration time of objects in novel and familiar locations in the diabetic control rats were equal indicating impairment in memory. This memory impairment seen in diabetic rats has been suggested to be associated with increased production of free reactive oxygen and nitrogen species in the hippocampus (Lee *et al.*, 2002). This result is consistent with studies (Chen *et al.*, 2013; Pierre *et al.*, 2012). However, when the diabetic Wistar rats were treated with garlic (A.sativum) extract, there was an increased preference for novelty and consequently the rats explored the object in novel location more than the one in familiar location indicative of improved memory. This result is in agreement with studies in which, Sarkaki *et al.*, (2013) suggested that fresh garlic but not cooked garlic improved short-term but not long term memory in diabetic rats. Nishimura *et al.*, (2006) suggested amelioration of memory impairment in diabetic rats treated with onion and garlic extracts.

Na^+/K^+ ATPase is important in regulating, cell volume, the active transport of K^+ into the cell with the exit of Na^+, transmembrane fluxes of Ca^{2+} and neurotransmitters release (Albers *et al.*, 2012). Results obtained revealed that ethanol garlic (A.sativum) extract increased significantly the activity of hippocampus Na^+/K^+ ATPase in diabetic rats. This indicates that garlic (A.sativum) extract increases functioning of hippocampus Na^+/K^+ ATPase in the diabetic rat hippocampus. However, hippocampus Na^+/K^+ ATPase activity was significantly reduced in diabetic rats. This is line with previously reported studies (Kumar *et al.*, 2012; Giribabu *et al.*, 2014). It has been suggested that neurotoxicity may be induced in neurons due to alterations in activity of Na^+/K^+ ATPase (Gras *et al.*, 2006). Since maintaining the activity of sodium-potassium ATPase is crucial for normal functioning of the hippocampus, reduced activity is related to selective neuronal damage in the rat and human brain (Leong and Leung, 1991). It has also been demonstrated that reduction in activity of Na^+/K^+ ATPase leads to cellular death by glutamate release (Lees, 1991).

Hippocampus neuronal death is associated with oxidative stress which leads to a reduction in activity levels of endogenous antioxidant enzymes (Moghaddam *et al.*, 2014). It has been suggested that garlic has antioxidant activity Rahman et al., (2012) and this would have accounted for the increase in hippocampus Na^+/K^+ ATPase activity.

Ca^{2+}ATPase maintain intracellular calcium levels and calcium ionic gradients across the cell membrane. The influx of calcium into the nerve terminal upon stimulation is essential for neuronal excitability and neurotransmitter release. Results from this study showed that Ca^{2+} ATPase activity was significantly decreased in the hippocampus of diabetic control group as compared to normal rats. This is in agreement with studies (Kumar *et al.,* 2012; Giribabu *et al.*, 2014). This could be due to increase in formation of lipid peroxides (Baquer *et al.*, 2009) or overload of intracellular calcium levels (Dog˘ru *et al.*, 2005). It has been suggested that increase in intracellular Ca^{2+} concentration occurs due to Ca^{2+} ATPase inhibition and leads to alteration in signaling pathways (Aubier and Viires, 1998). Administration of ethanol extract of garlic (A. sativum) to diabetic Wistar rats showed a significant increase in Ca^{2+}ATPase activity in the hippocampus. This finding suggests that the garlic (A.sativum) extract may have prevented inhibition of hippocampus Ca^{2+} ATPase activity and restored normal functioning of the enzyme. It has been suggested that the activity of this enzyme is related to the surrounding structural and lipid environment of the synaptosomal membrane (Hidalgo , 1987). Indeed, the decrease in activity of Ca^{2+}ATPase may be due to alterations in membrane phospholipids, which is related closely to the micro environment around the enzyme and this influences metabolism of intracellular calcium (Kuwahara *et al.*, 1997). The enhanced production of reactive oxygen species in diabetes cause increased degradation of lipids which inhibits the functioning of Ca^{2+}ATPase (Dmitriev and Titov, 2010) due to increased levels of lipid peroxidation products (Mushtaq *et al.*,2014). The reduction in lipid peroxidation by aged garlic extract (Dillon *et al.*, 2003) may have lead to increase in activity of hippocampus Ca^{2+} ATPase.

Neurotransmitter glutamate is released from glutamatergic neuronal vesicles through a calcium-dependent mechanism. Glutamine synthetase (GS) is an enzyme important in controlling the intracellular concentration of glutamate by converting it into glutamine (Blandini, 1996). Glutamine synthetase helps to maintain the concentration of ammonia within normal limits because excessive amounts of ammonia ions are toxic to the brain (Hertz *et al.,* 1999). Results

from this study indicated that there was a significant decrease in hippocampus glutamine synthetase activity in diabetic rats probably suggesting inhibition of extracellular clearance or uptake of glutamate. The decreased activity could be due to down regulation of the enzyme, increased clearance of glutamine synthetase and modulation of its activity by nitric oxide (Barger *et al.*, 2007). GS is suggested to be vulnerable to increased protein oxidation and nitration which inhibits its activity (Gorg *et al.*, 2006; Swamy *et al.*, 2009). Treatment with ethanol garlic (A. sativum) extract increased significantly the activity of hippocampus glutamine synthetase. This may have been due to reduction in formation of protein peroxides and products of protein nitration. It has been demonstrated that glutamine synthetase catalyses the conversion of glutamate to glutamine, a non toxic form(Gras *et al.*, 2006). This prevents excessive accumulation of glutamate in the synaptic cleft there by enabling transportation of glutamate in the extracellular space as glutamine (Blandini, 1996).

5.2 CONCLUSIONS

5.2.1 There was improvement in objection location memory in diabetic rats treated with ethanol garlic (A.sativum) extract.

5.2.1 There was a significant increase in hippocampus Na^+/K^+ ATPase activity in diabetic rats treated with ethanol garlic (A.sativum) extract.

5.2.2 Ca^{2+}ATPase activity in the hippocampus of diabetic rats treated with ethanol garlic (A. sativum) extract was significantly increased.

5.2.3 Hippocampus glutamine synthetase activity was significantly increased in diabetic rats treated with ethanol garlic (A.sativum) extract.

Based on the results of the present study showing that ethanol garlic (A. sativum) extract improves object location memory in type II diabetic Wistar rats, it is feasible to suggest that the neuroprotective mechanisms by which this medicinal herb modulates hippocampus function is by increasing the activities of hippocampus Na^+/K^+ ATPase, Ca^{2+} ATPase and glutamine synthetase.

5.3 RECOMMENDATIONS

5.3.1 More studies are required to determine which receptors on the postsynaptic membrane does garlic activate and the molecular pathways activated to bring about improvement in learning and memory in diabetic rats.

5.3.2 Electrophysiological studies on Na^+/K^+ ATPase after treatment with ethanol garlic (A. sativum) extract in diabetic rats are required.

5.3.3 Molecular mechanisms which regulate hippocampus Ca^{2+}ATPase activity on treatment with ethanol garlic (A. sativum) extract in diabetic rats should be investigated.

5.3.4 Determination of glutamate levels and glutaminase activity in hippocampus of diabetic rats treated with ethanol garlic (A. sativum) extract should be carried out.

REFERENCES

Abdullah TH, Kandil O and Elakdi A, C. J. (1988). Garlic revisited: therapeutic for the major diseases of our times. *Journal Natational Medical Association*, *80*, 439–45.

Adetumbi MA, L. B. (1986). Inhibition of in vitr ogermination and spherulation of Coccidioides immitis by Allium sativum. *Current Microbiology*, *13*, 73–6.

Albers RW, S. G. (2012). Membrane transport. Basic Neurochemistry*: Principles of Molecular, Cellular and Medical Neurobiology, 8th edn.*

Almeida, A., Delgado-Esteban, M., Bolaños, J. P and Medina, J. M. (2002). Oxygen and glucose deprivation induces mitochondrial dysfunction and oxidative stress in neurones but not in astrocytes in primary culture. *Journal of Neurochemistry*, *81*(2), 207--217. https://doi.org/10.1046/j.1471-4159.2002.00827.x

Alnaqeeb, M. A., Thomsonb, M., Bordiab, T., and Ali, M. (1996). Toxicology Letters Histopathological effects of garlic on liver and lung of rats. *Journal of Science*, *85*, 157–164.

Amin, S. N., Sc, M., Younan, S. M., and Youssef, M. F. (2011). Effect of Diabetes Mellitus on Rat Cognitive Functions and Related Hippocampal Synaptic Plasticity Markers, *F1000Res. 79*(2), 213–227 doi: 10.12688/f1000research.2-151.v1.

Amonkar SV and Reeves EL. (1970). Mosquito control with active principle of garlic, Allium sativum. *Journal of Econ Entomology*, *63*, 1172–5.

Andrianova IV, Fomchenkov IV and Orekhov AN . (2002). Hypotensive effect of long-acting garlic tablets allicor (a double-blind placebo-controlled trail). *Ter Arkh*, *74*, 76–88.

Aqel MB, Gharaibah MN and Salhab AS. (1991). Direct relaxant effects of garlic juice on smooth and cardiac muscles. *Journal of Ethnopharmacology*, *33*, 13–9.

Atkins RC and Zimmet P. (2010). Diabetic kidney disease: act now or pay later. *Acta Physiolology. Hung*, *97*, 52–56.

Aubier, M., and Viires, N. (1998). Calcium ATPase and respiratory muscle function. *European Respiratory Journal*, *11*(3), 758–766. https://doi.org/10.1183/09031936.98.11030758

Baluchnejadmojarad, T., Roghani, M., Homayounfar, H., and Hosseini, M. (2003). Beneficial effect of aqueous garlic extract on the vascular reactivity of streptozotocin-diabetic rats. *Journal of Ethnopharmacology*, *85*(1), 139–144. https://doi.org/10.1016/S0378-8741(02)00372-0

Banerjee A and Schepmann D, W. B. (2010). Synthesis and NMDA receptor affinity of fluorinated dioxadrol analogues. *Journal of Biorganic and Medicinal Chemistry*, *18*, 483–494.

Baquer, N. Z., Taha, A., Kumar, P., McLean, P., Cowsik, S. M., Kale R. K and Sharma, D. (2009). A metabolic and functional overview of brain aging linked to neurological disorders. *Journal of Biogerontology*, *10*(4), 377–413. https://doi.org/10.1007/s10522-009-9226-2

Barger, S. W., Goodwin, M. E., Porter, M. M., and Beggs, M. L. (2007). Glutamate release from activated microglia requires the oxidative burst and lipid peroxidation. *Journal of Neurochemistry*, *101*(5), 1205–1213. https://doi.org/10.1111/j.1471-4159.2007.04487.x

Barki IM, and Douglas CWI (2005). Inhibitory effect of garlic extract on oral bacterial. *Archieves of Oral Biology*, *50*, 645–51.

Baydas, G., Nedzvetskii, V. S., Nerush, P. A., Kirichenko, S. V., and Yoldas, T. (2003). Altered expression of NCAM in hippocampus and cortex may underlie memory and learning deficits in rats with streptozotocin-induced diabetes mellitus. *Journal of Life Sciences*, *73*(15), 1907–1916. https://doi.org/10.1016/S0024-3205(03)00561-7

Bélanger, A., Lavoie, N., Trudeau, F., Massicotte, G., and Gagnon, S. (2004). Preserved LTP and water maze learning in hyperglycaemic-hyperinsulinemic ZDF rats. *Journal of Physiology and Behavior*, *83*(3), 483–494. https://doi.org/10.1016/j.physbeh.2004.08.031

Bennett RA, and Pegg AE. (1981). Alkylation of DNA in rat tissues following administration of streptozotocin. *Cancer Research*, *41*, 2786–2790.

Bhardwaj, S.K., and Sharma. P, K.(1998). Alterations in free radical scavenger system profile of type I diabetic rat brain. *Journal of Molecular and Chemical Neuropathology*, *35*, 187–202.

Blandini F, Porter RH and Greenamyre J T.. (1996). Glutamate and Parkinson's disease. *Journal of Molecular Neurobiology*, *12*(1), 73–94.

Borek, C. (2001). Antioxidant health effects of aged garlic extract. *The Journal of Nutrition*, *131*(3s), 1010S–5S. https://doi.org/10.1080/00984100290071243

Buchanan TA, Xiang AH, Peters RK, et al. (2002). Preservation of pancreatic beta-cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high-risk hispanic women. *Journal of Diabetes*, *51*, 2796–2803.

Bui, B. V., Hu, R. G., Acosta, M. L., Donaldson, P., Vingrys, A. J., and Kalloniatis, M. (2009). Glutamate metabolic pathways and retinal function. *Journal of Neurochemistry*, *111*(2), 589–599. https://doi.org/10.1111/j.1471-4159.2009.06354.x

Chatzigeorgiou A, Halapas A, and Kalafatakis K, K. E. (2009). The use of animal models in the study of diabetes mellitus. *In Vivo*, *23*, 245–258.

Chauhan NB, and Sandoval J. (2007). Amelioration of early cognitive deficits by aged garlic extract in Alzheimer's transgenic mice. *Journal of Phytotherepeutic Research*, *21*, 629–640.

Chen, C. T., Alyahya, K., Gionfriddo, J. R., Dubielzig, R. R., and Madl, J. E. (2008). Loss of glutamine synthetase immunoreactivity from the retina in canine primary glaucoma. *Journal of Veterinary Ophthalmology*, *11*(3), 150–157. https://doi.org/10.1111/j.1463-5224.2008.00581.x

Chen D, and Wang MW. (2005). Development and application of rodent models for type 2 diabetes. *Journal of Diabetes, Obesity and Metabolism*, *7*, 307–317.

Chen L, Magliano DJ, and Zimmet PZ. (2012). The worldwide epidemiology of type 2 diabetes mellitus–present and future perspectives. *Nat. Rev. Endocrinology*, *8*, 228–236.

Dalle-Donne, I., Giustarini, D., Colombo, R., Rossi, R., and Milzani, A. (2003). Protein carbonylation in human diseases. *Trends in Molecular. Medicine*, *9*, 169–176.

Desaiah D and Ho IK (1979).Effect of acute and continuous morphine administration on catecholamine-sensitive adenosine triphosphatase in mouse brain. *J Pharmacol Exp Ther 208*: 80-85

Dienel, G. A., and Hertz, L. (2005). Astrocytic contributions to bioenergetics of cerebral ischemia. *Glia*, *50*(4), 362–388. https://doi.org/10.1002/glia.20157

Dillon SA, Burmi RS, Lowe GM, B. D. and R. K. (2003). Antioxidant properties of aged garlic extract: an in vitro study incorporating human low density lipoprotein. *Journal of Life Sci*, *72*, 83–94.

Dmitriev LF, and Titov. V. (2010). Lipid peroxidation in relation to ageing and the role of endogenous aldehydes in diabetes and other age-related diseases. *Ageing Research Reviews*, *9*, 200–210.

Dog˘ru, P. B., Daş, E. N., and Nebiog˘lu, S. (2005). Diabetes-induced decrease in rat brain microsomal Ca^{2+}-ATPase activity. *Journal of Cell Biochemistry and. Function.*, *23*(4), 239–243.

Dong Z, Gong B, Li H, Bai Y, Wu X, H. Y. et al. (2012). Mechanisms of hippocampal long- term depression are required for memory enhancement by novelty exploration. *Journal of Neurosci*, *32*(11980–90).

Duraipandiyan, V., Ayyanar, M., and Ignacimuthu, S. (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complementary and Alternative Medicine*, *6*, 35. https://doi.org/10.1186/1472-6882-6-35

Eid, T., Tu, N., Lee, T. S. W., and Lai, J. C. K. (2013). Regulation of astrocyte glutamine synthetase in epilepsy. *Neurochemistry International*. https://doi.org/10.1016/j.neuint.2013.06.008

Fiske, C.H. and Subba Row, Y (1925). The colorimetric determination of phosphorous. *Journal Biology Chem; 66*: 375-400.

Flood, J.F., Mooradian, A.D., and Morley, J.E., 1990. Characteristics of learning and memory in streptozocin-induced diabetic mice. Diabetes 39, 1391–1400

Forrester, M. T., and Stamler, J. S. (2007). A classification scheme for redox-based modifications of proteins. *J. Respiration. Cell Molecular Biology, 36*, 136–137.

Fritz, D.J. and Hamrick, M.E (1966) enzymatic analysis of (rat heart) adenosine triphosphatase. *enzymol Acta Bio Cat; 9*: 57-64.

Fromtling RA and Bulmer GS. (1978). In vitro effect of aqueous extract of garlic (Allium sativum) on the growth and viability of Cryptococcus neoformans. *Mycologia, 70*, 397–404.

Furukawa, K., and Mattson, M. P. (1998). The transcription factor NF-KB mediates increases in calcium currents and decreases in NMDA- and AMPA/kainate-induced currents induced by tumor necrosis factor-a in hippocampal neurons. *Journal of Neurochemistry, 70*(5), 1876–1886.

Gebreyohannes, G., & Gebreyohannes, M. (2013). Medicinal values of garlic : A review *International. Journal of Medical Science. 5*(9), 401–408. https://doi.org/10.5897/IJMMS2013.0960

Gerstein, H., Hullinger, R., Lindstrom, M. J., and Burger, C. (2013). A Behavioral Paradigm to Evaluate Hippocampal Performance in Aged Rodents for Pharmacological and Genetic Target Validation. *PLoS ONE, 8*(5). https://doi.org/10.1371/journal.pone.0062360

Giribabu, N., Srinivasarao, N., Swapna Rekha, S., Muniandy, S., and Salleh, N. (2014). Centella asiatica Attenuates Diabetes Induced Hippocampal Changes in Experimental Diabetic Rats. *Evidence-Based Complementary and Alternative Medicine, 2014*, 1–10. https://doi.org/10.1155/2014/592062

Gorg B, Bidmon HJ, Keitel V, and Foster N, G. R. (2006). Inflammatory cytokines induce protein tyrosine nitration in rat astrocytes. *Archives of Biochemistry and Biophysics, 449*(104–114).

Gras, G., Porcheray, F., Samah, B., and Leone, C. (2006). The glutamate-glutamine cycle as an inducible, protective face of macrophage activation. *Journal of Leukocyte Biology*, *80*(5), 1067–1075. https://doi.org/10.1189/jlb.0306153

Groppo FC, Ramacciato JC, Motta RH, and Ferraresi PM, S. A. (2007). Antimicrobial activity of garlic against oral streptococci. *International Journal of Dental Hygeine*, *2*, 109–15.

Grune, T., Merker, K., Sandig, G., and Davies, K. J. A. (2003). Selective degradation of oxidatively modified protein substrates by the proteasome. *Biochemistry and Biophysiological Research. Commun*, *305*, 709–718.

Grzęda E and Wiśniewska RJ. (2008) Differentiations of the effect of NMDA on the spatial learning of rats with 4 and 12 week diabetes mellitus.*Acta Neurobiol Exp (Wars); 68*(3): 398–406.

Gupta, R., Singh, M., and Sharma, A. (2003). Neuroprotective effect of antioxidants on ischaemia and reperfusion-induced cerebral injury. *Journal of Pharmacological Research*, *48*(2), 209–215. https://doi.org/10.1016/S1043-6618(03)00102-6

Haider, S., Naz, N., Khaliq, S., Perveen, T., and Haleem, D. J. (2008). Repeated administration of fresh garlic increases memory retention in rats. *Journal of Medicinal Food*, *11*(4), 675–9. https://doi.org/10.1089/jmf.2006.0229

Hauck, S. M., Schoeffmann, S., Amann, B., Stangassinger, M., Gerhards, H., Ueffing, M., and Deeg, C. A. (2007). Retinal Mueller glial cells trigger the hallmark inflammatory process in autoimmune uveitis. *Journal of Proteome Research*, *6*(6), 2121–2131. https://doi.org/10.1021/pr060668y

Hegazy, H. G., and Ali, E. H. A. (2011). Modulation of monoamines and amino-acids neurotransmitters in cerebral cortex and hippocampus of female senile rats by ginger and lipoic acid. *African journal of pharmacy and pharmacology*, *5*(August), 1080–1085. https://doi.org/10.5897/AJPP11.299

Heo, S., Csaszar, E., Jung, G., Beuk, T., Höger, H., and Lubec, G. (2012). Hippocampal levels and activity of the sodium/potassium transporting ATPase subunit alpha-3 (AT1A3) are

paralleling memory training in the multiple T-Maze in the C57BL/6J mouse. *Neurochemistry International*, *61*(5), 702–712. https://doi.org/10.1016/j.neuint.2012.07.006

Herman-Antosiewicz, A., Powolny, A. A., and Singh, S. V. (2007). Molecular targets of cancer chemoprevention by garlic-derived organosulfides. *Acta Pharmacologica Sinica*. https://doi.org/10.1111/j.1745-7254.2007.00682.x

Hertz, L., Dringen, R., Schousboe, A., and Robinson, S. R. (1999). Astrocytes: Glutamate producers for neurons. *Journal of Neuroscience Research*. https://doi.org/10.1002/(SICI)1097-4547(19990815)57:4<417::AID-JNR1>3.0.CO;2-N

Hidalgo .C. (1987). Lipid-protein interactions and the function of the Ca 2+ ATPase of sarcoplasmic reticulum. *Critical Reviews in Biochemistry*, *21*, 319–347.

Iciek, M., Kwiecien, I., and Wlodek, L. (2009). Biological Properties ofGarlic andGarlic-Derived Organosulfur Compounds. *Journal of Environmental and Molecular Mutagenesis*, *50* (February), 247–265. https://doi.org/10.1002/em

Ihara, Y., Yamada, Y., Toyokuni, S., Miyawaki, K., Ban, N., Adachi, T. and Seino, Y. (2000). Antioxidant alpha-tocopherol ameliorates glycemic control of GK rats, a model of type 2 diabetes. *FEBS Letters*, *473*(1), 24–6. https://doi.org/S0014-5793(00)01489-7 [pii]

J.L. Ortega and D. Roche, C. S.-G. (1999). Oxidative turnover of soybean root glutamine synthetase. In vitro and in vivo studies. *Journal of Plant Physiology*, *119*, 1483–1495.

Jalal, R., Bagheri, S. M., and Moghimi, A. (2011). The Effect of Iranian Shallot or Garlic Aqueous Extracts on Learning , Memory and Serum Biochemical Variables in Fructose-fed Wistar Rats. *Iranian journal of basic medical sciences*, *14*(3), 284–289.

Jamison, D., Breman, J., Measham, A., Alleyne, G., Claeson, M., Evans, D. and Musgrove, P. (2006). Disease Control Priorities in Developing Countries (2nd Edition): Default Book Series. *World Bank Group/E Library*, 1400. Retrieved from http://elibrary.worldbank.org/doi/abs/10.1596/978-0-8213-6179-5

Jeong, J. H., Jeong, H. R., Jo, Y. N., Kim, H. J., Shin, J. H., and Heo, H. J. (2013). Ameliorating effects of aged garlic extracts against Aβ-induced neurotoxicity and cognitive impairment. *BMC Complementary and Alternative Medicine*, *13*, 268. https://doi.org/10.1186/1472-6882-13-268

Josling P. (2001). Preventing the common cold with a garlic supplement: a double-blind, placebo-controlled survey. *Journal of Advancd Therapy*, *18*, 189–93.

Kamboj, S. S., Chopra, K., and Sandhir, R. (2009). Hyperglycemia-induced alterations in synaptosomal membrane fluidity and activity of membrane bound enzymes: beneficial effect of N-acetylcysteine supplementation. *Journal of Neuroscience*, *162*(2), 349–358. https://doi.org/10.1016/j.neuroscience.2009.05.002

Khakpai, F., Zarrindast, M. R., Nasehi, M., Haeri-rohani, A., Eidi, A., Universi-, T., and Azad, I. (2013). Mini review : The role of glutamatergic pathway between septum and hippocampus in the memory formation. *Journal of Behav brain research*, 41–51.

Khakpai F, Nasehi M, Haeri-Rohani A, Eidi A and Zarrindast MR (2012a). Scopolamine induced memory impairment possible involvement of NMDA receptor mechanisms of dorsal hippocampus and/or septum. *Journal of Behavoir Brain Research; 231*:1-10.

Khakpai F, Nasehi M, Haeri-Rohani A, Eidi A and Zarrindast MR(2012b). Septo-hippocamposeptal loop and memory formation. *Journal of Basic Clinical Neuroscience ;3*(5):1-4.

Kim, K., Lee, S. G., Kegelman, T. P., Su, Z. Z., Das, S. K., Dash, R. and Fisher, P. B. (2011). Role of Excitatory Amino Acid Transporter-2 (EAAT2) and glutamate in neurodegeneration: Opportunities for developing novel therapeutics. *Journal of Cellular Physiology*. https://doi.org/10.1002/jcp.22609

Kimelberg, H. K. (2004). Water homeostasis in the brain: Basic concepts. *Journal of Neuroscience*. https://doi.org/10.1016/j.neuroscience.2004.07.033

Kirpichnikov D, McFarlane SI and Sowers JR. (2002). Metformin: An update. *Annual Internal Medicine*, *137*, 25–33.

Kojuri J, Vosoughi AR, and Akrami A. M. (2007). Effects of Anethum graveolensand garlic on lipidprofile in hyperlipidemic patients. *Lipids Health Dis*ease *journal 1186*(1476–512).

Kumar, P., Kale, R. K., and Baquer, N. Z. (2012). Antihyperglycemic and protective effects of Trigonella foenum graecum seed powder on biochemical alterations in alloxan diabetic rats. *European Review for Medical and Pharmacological Sciences*, *16*(SUPPL. 3), 18–27.

Kuwahara, Y., Yanagishita, T., Konno, N., and Katagiri, T. (1997). Changes in microsomal membrane phospholipids and fatty acids and in activities of membrane-bound enzyme in diabetic rat heart. *Journal of Basic Research in Cardiology*, *92*(4), 214–22. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9342428

L'abbate A, Neglia D, Vecoli C, Novelli M, Ottaviano V, Baldi S, Barsacchi R, Paolicchi A, Masiello P, Drummond GS, and Mcclung JA, A. N. (2007). Beneficial effect of heme oxygenase-1 expression on myocardial ischemia-reperfusion involves an increase in adiponectin in mildly diabetic rats. *Am. J. Physiol. Heart Circ. Physiol*, *293*, H3532–H3541.

Ledezma E and Apitz-Castro. R. (2006). Ajoene the main active component of garlic (Allium sativum): a new antifungal agent. *Rev Iberoam Micol*, *23*, 75–80.

Lee, A. L., Ogle, W. O., and Sapolsky, R. M. (2002). Stress and depression: possible links to neuron death in the hippocampus. *Bipolar Disorders*, *4*(2), 117–128. https://doi.org/10.1034/j.1399-5618.2002.01144.x

Lee, K.-T., Chen, Y.-H., Lin, Ching-I Chiu, Wan-Chun Liao, H., and Lin, S.-H. (2013). Consumption of Oriental Plums Improved the Cognitive Performance and Modulated the Cerebral Neurodegeneration-Related Protein Expressions in Rats with Nicotinamide/Streptozotocin-Induced Diabetes. *Food and Nutrition Sciences journal*, *4*, 1145–1154.

Lees, G. J. (1991). Inhibition of sodium-potassium-ATPase: A potentially ubiquitous mechanism contributing to central nervous system neuropathology. *Brain Research Reviews*, *16*(3), 283–300. https://doi.org/10.1016/0165-0173(91)90011-V

Lemar KM, Turner MP, and Lloyd. D. (2002). Garlic (Allium sativum) as an anti-candida agent: a comparison of the efficacy of fresh garlic and freeze dried extracts. *Journal of Applied Microbiology, 93*, 398–405.

Leong, S. F., and Leung, T. K. C. (1991). Diabetes induced by streptozotocin causes reduced Na-K ATPase in the brain. *Journal of Neurochemical Research, 16*(10), 1161–1165. https://doi.org/10.1007/BF00966596

Li, X. H., Xin, X., Wang, Y., Wu, J. Z., Jin, Z. D., Ma, L. N., and Jin, M. W. (2013). Pentamethylquercetin protects against diabetes-related cognitive deficits in diabetic goto-kakizaki rats. *Journal of Alzheimer's Disease, 34*(3), 755–767. https://doi.org/10.3233/JAD-122017

Li, Z.-G., Zhang, W., Grunberger, G., and Sima, A. A. . (2002). Hippocampal neuronal apoptosis in type 1 diabetes. *Journal of Brain Research, 946*(2), 221–231. https://doi.org/10.1016/S0006-8993(02)02887-1

Li PA, Gisselsson J, Keuker J, Vo gel ML, and Kuschinsk . S. (1998). Hyperglycemia-exaggerated ischemic brain damage following 30 min of middle cer ebral artery occlusion is not due to capillary obstruction. *Journal of Brain Research, 804*, 36–44.

Macario, A. J. L., de Macario, E. C., S. chaperones. (2005). cellular stress, and disease. *N. Engl. J. Med, 353*, 1489–1501.

Maiese K, Chong ZZ, Hou J, S. Y. (2009). The vitamin nicotinamide: translating nutrition into clinical care. *Molecules, 14*, 3446–3485.

Masiello P, and Bergamini . E. (1977). Nicotinamide and streptozotocin diabetes in the rat. Factors influencing the effectiveness of the protection. *Journal of Experientia, 33*, 1246–1247.

Matsuura N, Miyamae Y , Y amane K, Nagao Y , Hamada Y , Kawaguchi N, Katsuki T , Hirata K, and Suni S, I. H. (2006). Aged garlic extract inhibits angiogenesis and proliferation of colorectal carcinoma cells. *Journal of Nutrition, 136*, 8425–65.

McCrimmon, R. J., Ryan, C. M., and Frier, B. M. (2012). Diabetes and cognitive dysfunction. *Lancet*, *379*(9833), 2291–2299. https://doi.org/10.1016/S0140-6736(12)60360-2

Marudamuthu, A.S and P. Leelavinothan, (2008). Effect of pterostilbene on lipids and lipid profiles in Streptozotocin-Nicotinamide induced type 2 rats. *Journal of Applied Biomedicine, 6*: 31-37.

McCrimmon, R. J., Ryan, C. M., and Frier, B. M. (2012). Diabetes and cognitive dysfunction. *Lancet*, *379*(9833), 2291–2299. https://doi.org/10.1016/S0140-6736(12)60360-2

McRae MP (2005). A review os studies of garlic (Allium sativum) on serum lipid levels and blood pressure and after 2004: does the amount of allicin released from garlic powder tablets play a role. *Journal of chiropratic medicine*; *4*:82-90

Mo W and Qian GF. (2014). Metformin for high fat diet induced insulin resistance the effect of space cognition, learning and memory and cerebral energy metabolism. *Chinese Journal of gerontology; 34*: 2813-2815

Moghaddam, H. K., Baluchnejadmojarad, T., Roghani, M., Khaksari, M., Norouzi, P., Ahooie, M., and Mahboobi, F. (2014). Berberine ameliorate oxidative stress and astrogliosis in the hippocampus of STZ-induced diabetic rats. *Journal of Molecular Neurobiology*, *49*(2), 820–826. https://doi.org/10.1007/s12035-013-8559-7

Moriguchi, T., Saito, H., and Nishiyama, N. (1996). Aged garlic extract prolongs longevity and improves spatial memory deficit in senescence-accelerated mouse. *Biological & Pharmaceutical Bulletin*, *19*(2), 305–307. https://doi.org/10.1248/bpb.19.305

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, *11*(1), 47–60. https://doi.org/10.1016/0165-0270(84)90007-4

Moser, E. I., Moser, M. B., and Andersen, P. (1993). Spatial learning impairment parallels the magnitude of dorsal hippocampal lesions, but is hardly present following ventral lesions. *The Journal of Neuroscience*, *13*(9), 3916–3925.

Mukherjee, D., and Banerjee, S. (2013). Learning and memory promoting effects of crude garlic extract. *Indian Journal of Experimental Biology*, *51*(12), 1094–1100.

Mushtaq, N., Schmatz, R., Pereira, L. B., Ahmad, M., Stefanello, N., Vieira, J. M. and Schetinger, M. R. (2014). Rosmarinic acid prevents lipid peroxidation and increase in acetylcholinesterase activity in brain of streptozotocin-induced diabetic rats. *Journal of Cell Biochemistry and Function*, *32*(3), 287–293. https://doi.org/10.1002/cbf.3014

Nazaroğlu NK, and Dincel AS, A. N. (2009). The effects of sulfonylurea glyburide on superoxide dismutase, catalase, and glutathione peroxidase activities in the brain tissue of streptozotocin induced diabetic rat. *Journal of Diabetes and Its Complications*, *23*, 209–213.

Nishimura, H., Higuchi, O., Tateshita, K., Tomobe, K., Okuma, Y., and Nomura, Y. (2006). Antioxidative activity and ameliorative effects of memory impairment of sulfur-containing compounds in Allium species. *BioFactors*, *26*(2), 135–146. https://doi.org/10.1002/biof.5520260204

Ogundele, O.M and Madukwe, J. (2012). Blockade of Na^+ /K^+ ATPase Pump Amplifies Toxicity of Cyanide in Oxygen Deprived Neurons. *Journal of Medicine and Medical Sciences*, *3*(11), 703–709. Retrieved from http://www.interesjournals.org/JMMS %5Cr

Ozougwu, J., Obimba, K., Belonwu, C., and Unakalamba, C. (2013). The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. *Journal of Physiology and Pathophysiology*, *4*(4), 46–57. https://doi.org/10.5897/JPAP2013.0001

P.Kumar., Kale, R. K., Mclean, P and Baquer, N. Z.. (2012). Antidiabetic and Neuroprotective Effects of Trigonella Foenum-graecum Seed Powder in Diabetic Rat Brain. *Prague Medical Report*, *113*(1), 33–43. https://doi.org/PMR2012A0004 [pii]

Palladino, M. J., Bower, J. E., Kreber, R., and Ganetzky, B. (2003). Neural dysfunction and neurodegeneration in Drosophila Na^+/K^+ ATPase alpha subunit mutants. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *23*(4), 1276–1286. https://doi.org/23/4/1276 [pii]

Palsamy P, and Subramanian S. (2010). Ameliorative potential of resveratrol on proinflammatory cytokines, hyperglycemia mediated oxidative stress, and pancreatic beta-cell dysfunction in streptozotocin-nicotinamide-induced diabetic rats. *Journal of Cellular Physiology*, *224*, 423–432.

Pandya KG, and Patel MR, L.-C. C. (2010). Comparative study of the binding characteristics to and inhibitory potencies towards PARP and in vivo antidiabetogenic potencies of taurine, 3-aminobenzamide and nicotinamide. *Journal of Biomedical. Science*, *17 Suppl 1*, S16.

Parathath, S. R., Gravanis, I., and Tsirka, S. E. (2007). Nitric oxide synthase isoforms undertake unique roles during excitotoxicity. *Stroke*, *38*(6), 1938–1945. https://doi.org/10.1161/STROKEAHA.106.478826

Pari L and Latha . M. (2004). Effect of Scopariadulcis (Sweet Broom weed) plant extract on plasma antioxidants in streptozotocininduced experimental diabetes in male albino Wistar rats. *Pharmazie*, *59*, 557–560.

Pari L and Saravanan. R. (2007). Beneficial effect of succinic acid monoethyl ester on erythrocyte membrane bound enzymes and antioxidant status in streptozotocin-nicotinamide induced type 2 diabetes. *Chem. Biological Interaction*, *169*, 15–24.

Pekiner, B. D., Evcimen, N. D., and Nebioğlu, S. (2005). Diabetes-induced decrease in rat brain microsomal Ca^{2+}-ATPase activity. *Journal of Cell Biochemistry and Function*, *23*(4), 239–243. https://doi.org/10.1002/cbf.1144

Perez, E. L., Lauritzen, F., Wang, Y., Lee, T. W., Kang, D., Zaveri, H. P. and Eid, T. (2012). Neurobiology of Disease Evidence for astrocytes as a potential source of the glutamate excess in temporal lobe epilepsy. *Neurobiology of Disease*, *47*(3), 331–337. https://doi.org/10.1016/j.nbd.2012.05.010

Pierre, W., Jeugo, A., Gildas, H., Ulrich, M. C., Modeste, W., Benoît, N. T., and Albert, K. (2012). Hypoglycemic and hypolipidemic effects of Bersama engleriana leaves in nicotinamide / streptozotocin-induced type 2 diabetic rats. *BMC Complementary and Alternative Medicine*, 2–7.

Pietrini, G., Matteoli, M., Banker, G., and Caplan, M. J. (1992). Isoforms of the Na,K-ATPase are present in both axons and dendrites of hippocampal neurons in culture. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(18), 8414–8418. https://doi.org/10.1073/pnas.89.18.8414

Pintana, H., Apaijai, N., Pratchayasakul, W., Chattipakorn, N., and Chattipakorn, S. C. (2012). Effects of metformin on learning and memory behaviors and brain mitochondrial functions in high fat diet induced insulin resistant rats. *Journal of Life Sciences*, *91*(11–12), 409–414. https://doi.org/10.1016/j.lfs.2012.08.017

Pociot F, Reimers JI, and Andersen. H. (1993). Nicotinamide biological actions and therapeutic potential in diabetes prevention. *Diabetologia*, *36*, 574–576.

Popovic, M., Biessels, G., Isaacson, R. L., and Hendrik, W. (2001). Learning and memory in streptozotocin-induced diabetic rats in a novel spatial / object discrimination task. *Behavior brain research*, *122*, 201–207.

Rahman, M. M., Fazlic, V., and Saad, N. W. (2012). Antioxidant properties of raw garlic (Allium sativum) extract. *International Food Research Journal*, *19*(2), 589–591.

Rajendra Wudayagiri Gunduluru Swathi and Cherukupalle Bhubaneswar. (2013).) Modulation of Na^{+}/K^{+}, Mg^{2+} and Ca^{2+} atpase activity in different regions of rat brain during rotenone induced parkinson's disease and protective role of bacopa monnieri. *Evidence-Based Complementary and Alternative Medicine*, *4*(1).

Raju SM and Raju B (2010). Illustrated medical biochemistry. 2nd Edition. Jaypee Brothers Medical Publishers ltd, New Delhi, India. 645pp

Rani P.J.A. and Panneerselvam, C. (2001). The role of L-Carnitine in the activities of membrane-bound enzymes in the brain of aged rats. *Journal of Anti-Aging Medicine*, *4*, 147–155.

Rees DA and Alcolado. J. (2005). Animal models of diabetes mellitus. *Diabetes and Medicine*, *22*, 359–370.

Ried, K., Frank, O. R., and Stocks, N. P. (2013). Aged garlic extract reduces blood pressure in hypertensives: a dose-response trial. *European Journal of Clinical Nutrition*, *67*(1), 64–70. https://doi.org/10.1038/ejcn.2012.178

Rowe Bruce, W., Ronzio, R. A., Wellner, V. P., and Meister, A. (1970). Glutamine synthetase (sheep brain). *Journal of Methods in Enzymology*, *17*(PART A), 900–910. https://doi.org/10.1016/0076-6879(71)17304-1

Ryan, E. , Pick, M. E., and Marceau, C. (2001). Use of alternative medicines in diabetes mellitus. *Diabetic Medicine : A Journal of the British Diabetic Association*, *18*, 242–245. https://doi.org/10.1046/j.1464-5491.2001.00450.x

Sainani GS, Desai DB, Gorhe NH, Natu SM, and Pise DV, S. P. (1979). Effect of dietary garlic and onion on serum lipid profile in jain community. *Indian Journal Medical Research*, *66*, 776–80.

Sarkaki, A., Valipour Chehardacheric, S., Farbood, Y., Mansouri, S. M. T., Naghizadeh, B., and Basirian, E. (2013). Effects of fresh, aged and cooked garlic extracts on short- and long-term memory in diabetic rats. *Avicenna Journal of Phytomedicine*, *3*(1), 45–55.

Sarkhail P, Rahmanipour S, Fadyevatan S, Mohammadir ad A, Dehghan G, Amin G, and Shafiee A, A. M. (2007). Antidiabetic effect of Phlomisanisodonta: effects on hepatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes. *Pharmacologica L Research*, *56*, 261–266.

Scheen, A. . (1996). Clinical pharmacokinetics of metformin. *Clinical Pharmacokinetics*, *30*, 359–371.

Sharma, G., and Harikumar, S. L. (2015). Diabetes Associated Memory Impairment : Perspective on Management Strategies, *4*(June), 62–72.

Sheela N, Jose MA, and Sathyamurthy D, K. B. (2013). Effect of silymarin on streptozotocin-nicotinamide-induced type 2 diabetic nephropathy in rats. *Iran Journal of. Kidney Disease*, *7*, 117–123.

Sher, A., Fakhar-Ul-Mahmood, M., Shah, S. N. H., Bukhsh, S., and Murtaza, G. (2012). Effect of garlic extract on blood glucose level and lipid profile in normal and alloxan diabetic rabbits. *Advances in Clinical and Experimental Medicine*, *21*(6), 705–711.

Sima F., Z. G. Li, W. Zhang, and G. Grunberger (2002). "Hippocampal neuronal apoptosis in type 1 diabetes," Brain Research, vol.946, no.2, pp.221–231.

Squire, L. R., Stark, C. E. L., and Clark, R. E. (2004). The m edial t emporal lobe *Annual review in neuroscience*, 279–306. https://doi.org/10.1146/annurev.neuro.27.070203.144130

Stepankova, K., Fenton, A. A., Pastalkova, E., Kalina, M., and Bohbot, V. D. (2004). Object – location memory impairment in patients with thermal lesions to the right or left hippocampus. https://doi.org/10.1016/j.neuropsychologia.2004.01.002

Stoll A and Seebeck E. (1949b.). Über die spezifitat der allinase und die synthesen mehrerer dem Alliin verwandter verbindungen. *Helv Chim Acta*, *32*, 866–77.

Sumioka I, Hayama M, Shimokawa Y , Shiraishi S, T. okunaga A. (2006). Lipid-lowering effect of monascus garlic fermented extract (MGFE) in hyperlipidemia subjects. *Hiroshima Journal of Medical Science*, *55*, 59–64.

Swamy, M., Sirajudeen, K. N., and Chandran, G. (2009). Nitric oxide (NO), citrulline-NO cycle enzymes, glutamine synthetase, and oxidative status in kainic acid-mediated excitotoxicity in rat brain. *Drug Chem Toxicol*, *32*(4), 326–331. https://doi.org/10.1080/01480540903130641

Szatkowski, M. and Attwell, D. (1994). Triggering and execution of neuronal death in brain ischaemia: Two phases of glutamate release by different mechanisms. *Trends Neurosci.*, *17*(9), 359–365.

Szkudelski .T, (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Journal of Physiological. Research*, *50*, 537–546.

Szkudelski.T . (2012). Streptozotocin-nicotinamide-induced diabetes in the rat. Characteristics of the experimental model. *Exp. Biol. Med. (Maywood)*, *237*, 481–490.

Szkudelski, L. (2008). The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*, *51*, 216–226.

Tarriq HA,Kandil O,Elkadi A and Carter J. (1998).Garlic revisted: therapeutic for major diseases of our times. *Journal of National Medical Association*; *80*:439-445.

Tasnim, S., Haque, P. S., Bari, S., Hossain, M., Mohd, S., Islam, A. and Sayeed, B. (2015). Allium sativum L . Improves Visual Memory and Attention in Healthy Human Volunteers, Evidence-Based Complementary and Alternative Medicine *2.10.18*. https://doi.org/10.1155/2015/103416

Thulé, P. M., Campbell, A. G., Kleinhenz, D. J., Olson, D. E., Boutwell, J. J., Sutliff, R. L., and Hart, C. M. (2006). Hepatic insulin gene therapy prevents deterioration of vascular function and improves adipocytokine profile in STZ-diabetic rats. *American Journal of Physiology. Endocrinology and Metabolism*, *290*(1), E114–E122. https://doi.org/10.1152/ajpendo.00134.2005

Tsakiris, S., Koromilas, C., and Schulpisb, K. H. (2001). R educed M g^{2+} A T Pase A ctivity in the Hypoglycemic Adult Rat Brain. *Journal Neurophysiology*, 912–914.

Tirri, R., Lagrspetz, K.Y.H and Kohomen, J (1973) Temperature dependence of the ATPase activity in brain homogenates during the postnatal development of rat. *Journal of Comp Biochemistry and Physiology* .44: 473.

Tsao SM, Hsu CC, andYin. M. (2003). Garlic extract and two diallyl sulphides inhibits methicillin-resistant Staphylococcus aur eusinfection in BALB/cA mice. *Journal of Antimicrobiol Chemotherepay*, *52*, 974–80.

Vague, P., Coste, T. C., Jannot, M. F., Raccah, D., and Tsimaratos, M. (2004). C-peptide, Na^{+}, K^{+}--ATPase, and Diabetes. *Experimental Diabesity Research*, *5*(1), 37–50. https://doi.org/10.1080/15438600490424514

Wang HX and Ng TB (1999). Natural products with hypoglycemic, hypotensive, hypocholesterolemic, antiatherosclerotic and antithrombic activities. Journal of life Sciences; 65:2663-2677

Welsh, B., and Wecker, L. (1991). Effects of Streptozotocin-Induced Diabetes on Acetylcholine Metabolism in Rat Brain. *Neurochemical Research*, *16*(4), 453–460.

Yamada Y and Azuma. K. (1977). Evaluation of the in vitro antifungal activity of allicin. *Antimicrob Agents Chemother*, *11*, 743–9.

Yuan X, Chen YB, Gan DN, and Cheng YF, X. J. (2014). Metformin improves learning and memory of rats induced by high-fat diet. *Military Medical Sciences*, *38*, 17–21.

Zacharias D. A., S. J. DeMarco, and E. E. S. (1997). "mRNA expression of the four isoforms of the human plasma membrane Ca 2+-ATPase in the human hippocampus,." *Molecular Brain Research*, *45*(1), 173–176.

Zhang, X., Xu, L., He, D., and Ling, S. (2013). Endoplasmic reticulum stress-mediated hippocampal neuron apoptosis involved in diabetic cognitive impairment. *BioMed Research International*, *2013*. https://doi.org/10.1155/2013/924327

Zhang WH, Liu Z, and Shi FG, C. Z. (2012). Huanglian wendan tang in mice with type 2 diabetes and the impact on the learning and memory and the form of hippocampal nerve cells. *Lishizhen Medicine and Materia Medica Research*, *23*, 948–949.

Zhou. W. (2003). Acute lymphangitis treated by moxibustion with garlic in 118 cases. *J Tradit Clin Med 2003*, *23*, 198.

APPENDICES

APPENDIX 1:Tables

Table-2: Effect of ethanol garlic extract (A.sativum) on exploration time to familiar and novel object locations in different treatment groups.

Groups		Exploration time (Seconds)	
		Familiar location	Novel location
A	Normal control	11.8 ± 0.03	13.4 ± 0.50*
B	Normal rats + Garlic at 1000mg/kg	12.3 ± 0.11	14.8 ± 2.24*
C	Diabetic control	10.9 ± 0.03	11 ± 0.32
D	Diabetic rats + Garlic at 1000mg/kg	13.2 ± 0.05	21.4 ± 1.86*
E	Diabetic rats + Metformin at 50mg/kg	14.94 ± 0.01	29 ± 1.22**

Results are expressed as Mean ± SEM (n=6). The data was analysed using ANOVA followed by Tukey's post hoc test. *P < 0.05, **P < 0.01 vs respective groups.

Table-3: Effect of ethanol garlic extract on the novelty index in different groups.

Groups		Novelty index
A	Normal control	55 ± 0.11
B	Normal rats + Garlic at 1000mg/kg	59 ± 0.19*
C	Diabetic control	49 ± 0.22
D	Diabetic rats + Garlic at 1000mg/kg	61.4 ± 0.20 $^{\alpha}$
E	Diabetic rats + Metformin at 50mg/kg	69 ± 0.05 $^{\alpha\alpha}$

Results are expressed as Mean ± SEM (n=6). The data was analysed using ANOVA followed by Tukey's post hoc test.* P < 0.05 vs normal control, $^{\alpha}$ P < 0.05; $^{\alpha\alpha}$ P < 0.01 vs diabetic control group.

Table 4: Effect of ethanol garlic (A.sativum) extract on activity of hippocampus sodium-potassium ATPase in different groups.

Groups		Hippocampus Na^+/K^+-ATPase activity(μmol of pi liberated / min/mg protein)
A	Normal control	0.43 ± 0.01
B	Normal rats + Garlic at 1000mg/kg	0.538 ± 0.02*
C	Diabetic control	0.338 ± 0.02
D	Diabetic rats + Garlic at 1000mg/kg	$0.68 \pm 0.01^{\alpha}$
E	Diabetic rats + Metformin at 50mg/kg	$0.862 \pm 0.02^{\alpha\alpha}$

Results are expressed as Mean ± SEM (n=6). The data was analysed using ANOVA followed by Tukey's post hoc test.* $P < 0.05$ vs normal control, $^{\alpha}P < 0.05$; $^{\alpha\alpha}P < 0.001$ vs diabetic control group.

Table 5: Effect of ethanol garlic (A.sativum) extract on hippocampus calcium ATPase activity in different treatment groups

Groups		Hippocampus Ca^{2+}-ATPase activity(μmol of pi liberated/ min/mg protein)
A	Normal control	0.562 ± 0.02
B	Normal rats + Garlic at 1000mg/kg	0.77 ± 0.03*
C	Diabetic control	0.438 ± 0.019
D	Diabetic rats + Garlic at 1000mg/kg	$1.22 \pm 0.037^{\alpha}$
E	Diabetic rats + Metformin at 50mg/kg	$1.612 \pm 0.033^{\alpha\alpha}$

Results are expressed as Mean ± SEM (n=6). The data was analysed using ANOVA followed by Tukey's post hoc test.* $P < 0.05$ vs normal control, $^{\alpha}P < 0.05$; $^{\alpha\alpha}P < 0.001$ vs diabetic control group.

Table 6: Effect of ethanol garlic (A.sativum) extract of on hippocampus glutamine synthetase activity in different treatment groups

Groups		Glutamine synthetase (mMol of gamma glutamyl hydroxamate / hr/mg protein)
A	Normal control	0.478 ± 0.01
B	Normal rats + Garlic at 1000mg/kg	0.516 ± 0.005*
C	Diabetic control	0.32 ± 0.001
D	Diabetic rats + Garlic at 1000mg/kg	$0.77 \pm 0.003^{\alpha}$
E	Diabetic rats + Metformin at 50mg/kg	$0.952 \pm 0.007^{\alpha\alpha}$

Results are expressed as Mean ± SEM (n=6). The data was analysed using ANOVA followed by Tukey's post hoc test.* $P < 0.05$ vs normal control, $^{\alpha}P < 0.05$; $^{\alpha\alpha}P < 0.001$ vs diabetic control group.

APPENDIX 2: Letter of approval to carryout research