EFFECTS OF TRANS-CINNAMALDEHYDE ON HIPPOCAMPAL HISTOMORPHOLOGY AND FUNCTIONS IN HIGH-FAT DIET AND STREPTOZOTOCIN TREATED RATS

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CERTIFICATION

This is to certify that this Thesis, submitted by OLORUNNADO, Samson Ehindero, has been read and approved as meeting the requirements of the Department of Anatomy, University of Ilorin, Ilorin, Nigeria, for the Award of the Doctor of Philosophy (Ph.D.) degree in Anatomy.

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DEDICATION

This Thesis is dedicated to God Almighty for His dependable promises.

DECLARATION

I, Samson Ehindero OLORUNNADO, a Ph.D. student in the Department of Anatomy, University of Ilorin, Ilorin, hereby declare that this Thesis entitled "Effects of Trans-Cinnamaldehyde on Hippocampal Histomorphology and Functions in High-Fat Diet and Streptozotocin Treated Rats", submitted by me is based on my actual and original work. Any materials obtained from other sources or work done by any other persons or institutions have been duly acknowledged. In addition, the research has been approved by the University of Ilorin Ethical Review Committee.

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LIST OF ABBREVIATIONS

AD	Alzheimer’s disease
ADI	Alzheimer’s disease International
APP	Amyloid precursor protein (APP).
DLB	Dementia with Lewy bodies
HFD	High-fat diets
IR	Insulin resistance
MIBG	Meta-iodobenzylguanidine
ND	Normal diet
PD	Parkinson’s disease dementia
PET	Positron emission tomography
PS-1	Presenilin-1
PS-2	Presenilin-2
STZ	Streptozotocin
SPEC	Single-photon emission computed tomography
T2DM	Type two diabetes mellitus
TCA	Trans- cinnamaldehyde
WHO	World Health Organization

ABSTRACT

The incidence of insulin-resistance is on the increase globally. Earlier reports linked impaired insulin signaling and glucose intolerance to cognitive decline, suggesting that improving insulin signaling could enhance neuronal survival. Trans-cinnamaldehyde (TCA) is an active component of cinnamon and it has many pharmacological importance. However, the effects of TCA on insulin resistance-induced cognitive deficit is unclear. This study therefore aimed at evaluating the effects of trans-cinnamaldehyde on hippocampal histomorphology and functions in insulin-resistant rats. The objectives of this study were to: (i) evaluate the effects of TCA on blood glucose levels and assess insulin resistance in Wistar rats; (ii) assess behavioural changes in insulin-resistant Wistar rats after TCA intervention; (iii) investigate the histoarchitectural changes in the hippocampus of insulin-resistant Wistar rats treated with TCA; (iv) investigate the effects of TCA on inflammatory markers in the hippocampus of Wistar rats; (v) and investigate the effects of TCA intervention on the hippocampal NeuN expression.

Sixty-four adult female Wistar rats were divided into eight groups with the following treatments: (I) normal control; (II) high-fat diet (HFD) and streptozotocin (STZ) 30 mg/kg bw (insulin-resistant control); (III) oral TCA alone at 60 mg/kg bw; (IV) normal diet, HFD, STZ (30 mg/kg bw) and oral TCA at 60 mg/kg bw; (V) HFD, STZ (30 mg/kg bw) and oral TCA 60 mg/kg bw; (VI) oral TCA alone at 40 mg/kg bw; (VII) normal diet, HFD, STZ (30 mg/kg bw) and oral TCA at 40 mg/kg bw; (VIII) HFD, STZ (30 mg/kg bw) and oral TCA at 40 mg/kg bw. Generally, HFD was administered for eight weeks, followed by low dose STZ intraperitoneally; and oral TCA administration for four weeks. Fasting blood glucose was determined using glucometer, while insulin resistance was determined using homeostasis model assessment of insulin resistance (HOMA-IR). Morris Water Maze test was conducted for cognitive function. The Wistar rats were sacrificed and the expression of nuclear factor kappa B (NF-κB), tumour necrosis factor alpha (TNF-α), histoarchitectural changes and NeuN expression were evaluated using biochemical, histological and immunohistochemical techniques. Data were analysed using one-way analysis of variance, with Tukey's *post hoc* test. A significant difference was defined as $p < 0.05$.

The findings of the study were that:

i. oral TCA significantly reduced ($p < 0.05$) blood glucose (119±4.9 mg/dl) and insulin resistance (22.13± 3 mg/dl) when compared with the untreated rats (217±10 mg/dl) and (41.7±2 mg/dl) respectively;
ii. TCA administration to insulin-resistant rats significantly reduced ($p < 0.05$) escape latency (26.67±1.4 s) when compared with HFD + STZ (38.17±1.3 s) in Morris water maze test;
iii. TCA administration to insulin-resistant rats histologically reduced pyknosis, astrogliosis, and neurodegenerative changes in the hippocampus when compared with untreated (insulin-resistant) rats;
iv. TCA significantly reduced TNF-α, NF-κB when compared with untreated rats; and
v. hippocampal cyto-architecture was improved in TCA-treated rats as indicated by enhanced NeuN expression.

The study concluded that TCA protected the hippocampus from insulin-resistance-induced neuronal degeneration via anti-inflammatory mechanism. The study recommended that trans-cinnamaldehyde be explored as a therapy for insulin-resistance-induced cognitive impairment.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Insulin resistance is a state of reduced responsiveness of target tissues to normal circulating levels of insulin. It is also a state of impairment in the blood glucose- lowering effect of circulating or injected insulin which is the central feature of type 2 diabetes mellitus (T2DM) and metabolic syndrome (Feldman, 2012; Czech, 2017).

Insulin resistance, hyperglycemia and hyper-insulinemia are the most common features of type two diabetes mellitus (T2DM) (Taylor, 2012). The incidence of diabetes mellitus is on steady increase globally. The global prevalence of diabetes in 2019 was 9.3% (463 million people). This figure is projected to rise as high as 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045 (Thuy *et al.,* 2020). Significant healthcare challenge has resulted because of the financial burden due to the treatment of diabetes for many countries of the world (Taylor, 2012). Insulin resistance occurs due to the decreased in insulin sensitivity of liver, muscle and fat cells to insulin (Leahy *et al.,* 2014). Decreased insulin sensitivity ultimately leads to reduction in uptake of circulating blood glucose for glycogenesis which subsequently results into chronic hyperglycemia as one of the pathological hallmarks of insulin resistance (Leahy *et al.,* 2014).

Neurodegenerative disease is a progressive impairment in the functionality of the brain usually resulting from loss or death of neurons traceable to multiple causes including insulin resistance, and it disproportionally affects the elderly and worsens with age (Despa, 2019). The causative factor for neurodegeneration is attributable to specific or general neuronal impairment (McKusick, 2007; Despa, 2019). The resulting neurodegenerative changes lead to a compromise in some specific neuronal functions such as hearing, vision, memory or general

brain function. Most neurodegenerative disorders are commonly associated with some age-related symptoms such as obesity, insulin resistance and diabetes (Reddy, 2017; Madhusudhanan *et al.,* 2020).

There are both basic and clinical evidences that supports the fact that incidence of neurodegenerative disorders is more common among patients with insulin resistance than the general population; impaired neuropsychological functions has been reported among the diabetics (Teixeira *et al.,*2020). Several findings reveal that hyperglycemic patients have a higher prevalence of global cognitive impairment and greater cognitive decline (Elham *et al*., 2016; Lindsay *et al*., 2016) when compared to normoglycemic population. Insulin resistance and diabetes have therefore been implicated as a risk factor for dementia (Jayaraj *et al.,* 2020). Deficit in hippocampal related function, including memory formation has been reported in insulin resistant state (Bonds *et al.,* 2020).

Alzheimer's disease (AD) which is the most common form of dementia among the elderly population was first diagnosed by German psychiatrist and neuropathologist, Alois Alzheimer in 1906, it is a form of dementia associated with gradual loss of cognitive and memory abilities (Lynn *et al.,* 2010; Bhumsoo, 2015; Gohar *et al.,* 2015; Sedighi *et al.,* 2019). The hippocampus has been shown to be one of the first structures in the brain that is affected by the disease.

AD at cellular level is generally characterized by a progressive loss of pyramidal cells in the entorhinal cortex and the hippocampus which are responsible for maintenance of higher cognitive functions, in its early-stage AD is characterized by loss of synaptic functions that interrupts connections between neural circuits, ultimately resulting in gradual loss of memory (Kashyap *et al.,* 2019). Recently, AD was declared as the sixth major cause of death in the

world, individuals affected with AD suffer a progressive decline of cognitive abilities and memory functions until they are unable to perform routine functions (Bryan *et al.,* 2014).

From clinical perspective, AD can be classified into two subtypes which are; the late-onset (sporadic AD) which constitute about 95% of AD cases and occurs in patients aged 65 years or older. Secondly, the early-onset (familial AD) which constitute about 5% of AD cases, and occurs in people within their thirties, forties, or fifties (Vo *et al*., 2019). The disease pathology is caused by mutation in three known genes which are presenilin-1 (PS-1), presenilin-2 (PS-2) and amyloid precursor protein (APP). Although PS-1 mutations account for most of the familial AD, there are still some unknown mutations outside these three genes (Dorszewska *et al.*, 2016).

Presently, treatment for AD is limited to the administration of cholinesterase inhibitors (donepezil, rivastigmine, galantamine) and memantine an N-methyl-D-aspartate receptor antagonist (Jennifer *et al*., 2016). Some of the recently synthesized drugs affect multiple AD pathophysiological pathways and can act as inhibitors of monoamine oxidases (MAO-A, MAO-B), modulators of amyloid-beta binding alcohol dehydrogenase and antioxidants, inhibitors of cholinesterases (AChE, BuChE), modulators of mitochondrial permeability transition pores (Jennifer *et al*., 2016).

1.2 Global impact of dementia

The common cause of dementia is AD, as of 2015, around 46.8 million people worldwide suffered from dementia (ADI, 2015). This number by 2050 is expected to reach 131.5 million except if there is medical intervention. In terms of gender prevalence, AD is more common among women partly because of longer life expectancy among women when compared to men (Luy, 2014). AD has pose serious economic burden globally, with studies indicating that AD management cost 818 billion USD in 2016 (Wimo *et al*., 2017).

Sub-Saharan African countries are experiencing rapid transitions with increased life expectancy, therefore the burden of age-related conditions such as diabetes and dementia is on the increase (Alain, 2014). As a result of the increasing aging population in sub-Saharan Africa, number of people with dementia is expected to rise above 7.6 million by 2050 (ADI, 2018).

Trans-cinnamaldehyde

Over some decades, there has been a geometrical increase in efforts concerning finding treatment for dementia. Phytochemicals derivatives has been used to combat various pathological conditions (Saeideh *et al.*, 2018).

Cinnamon is a derivative of a Greek word meaning sweet wood, it comes from the inner bark of tropical evergreen cinnamon trees (Ballal, 2008). *Cinnamon* is one of the most commonly used flavouring agents in the beverage and food industry globally, it is also well recognized for its medicinal properties one of the major essential oil is trans-cinnamaldehyde. Cinnamon extracts have been used in traditional Ayurvedic medicine for ailments such as diarrhoea, arthritis and menstrual irregularities (Bandara, 2014).

Experimental evidences indicate that trans-cinnamaldehyde possesses antimicrobial activity, antioxidant, cholesterol-lowering, antineoplastic, antibacterial and antifungal properties (Saeideh *et al*., 2018).TCA has been reported to inhibit the production of NO and IL-1β and expression of iNOS and COX-2 by suppressing activation of NF-κB in LPS-stimulated microglia as a model of activated microglia (Yan *et al.*, 2017).

1.3 Justification of the study

The intake of high-fat diets (HFD) containing mainly saturated fats continue rise over the past decades leading to increased incidence of insulin resistance, diabetes and other metabolic syndromes (Wolfgang, 2019). This increase intake of HFD has directly or indirectly resulted to increase in dementia (Alain, 2014). In Sub-Saharan Africa, it is projected that the number of people with dementia will rise to 7.6 million by 2050 (ADI, 2018). Despite this alarming figure, no effective treatment methods have been found. It is because of these facts that this study explored the therapeutic potentials and the mechanisms of trans- cinnamaldehyde on the hippocampal histomorphology and functions in insulin-resistance

1.4 Aim of the Study

The aim of this research was to explore the therapeutic potentials and the mechanisms of trans- cinnamaldehyde on the hippocampal histomorphology and functions in insulin-resistant rats.

1.5 Specific objectives of the Study

The specific objectives of the study were to:

i. assess behavioural changes in insulin-resistant Wistar rats after TCA intervention;
ii. investigate the histoarchitectural changes in the hippocampus of insulin-resistant Wistar rats treated with TCA;
iii. evaluate the effects of TCA on blood glucose levels and insulin resistance in Wistar rats;
iv. determine the degree of insulin resistance;
v. evaluate the effects of TCA on blood glucose level;
vi. investigate the effects of TCA on inflammatory markers in the hippocampus of Wistar rats; and

vii. evaluate the therapeutic potentials of TCA on insulin- resistant rats using immunohistochemical techniques.

1.6 Research hypothesis

The study hypothesizes that trans- cinnamaldehyde confers therapeutic benefits on neurons of the hippocampus following the administration of high fat diet and streptozotocin.

1.7 Significance of the Study

This study would determine the therapeutic potentials of trans-cinnamaldehyde (TCA) following HFD/STZ induced insulin resistance in adult Wistar rats. It would also give clarity to the mechanism of the therapeutic potential of TCA on insulin- resistance induced cognitive decline in Wistar rats.

CHAPTER TWO

LITERATURE REVIEW

2.1 The role of insulin-resistance in the pathophysiology of neurodegenerative diseases

Insulin resistance (IR) is the reduced sensitivity of target organs to insulin commonly associated with metabolic defects and hyperinsulinemia (Shulman, 2018). Proper insulin signaling is crucial to maintaining glucose homeostasis, there is impairment of insulin signaling at multiple levels in insulin resistant state, leading to imbalance between glucose uptake and its production in peripheral tissues (Feldman, 2012). In the pathogenesis of AD, IR has been implicated, because of the similarity in some molecular and biochemical features between both types of diabetes and AD, AD is commonly described as "type 3 diabetes" (Craft, 2017; Hemachandra *et al.,* 2017), The association between diabetes and AD may partly be due to the systemic mitochondrial dysfunction that is common to these pathologies (Craft, 2017).

Brain insulin signaling plays vital roles in the regulation of food intake, body weight, learning and memory (Zabolotny *et al*., 2016). Dysregulation of insulin signaling pathway is associated with cognitive decline and AD (Hölscher, 2019; Sami *et al*, 2019). Deficits in hippocampal function has been attributed to peripheral insulin resistance and hyperlipidemia induced by a high-calorie diet (Biessels & Reagan, 2015; Matteo *et al.,* 2019). Several findings linked impaired insulin function and glucose metabolism to the risk of developing AD-type neurodegeneration (Madhusudhanan *et al.,* 2020; Yanan *et al.,* 2020).

Perturbation in insulin signaling pathway is associated with decreased cognitive ability and development of dementia. Neurons are metabolically active insulin-dependent tissues, while in insulin resistant state the ability to respond properly to the neurotrophic properties of insulin is lost, resulting in neuronal injury, neuronal dysfunction, AD and related diseases

(Feldman, 2012). Long term IR in the peripheral tissues promoted IR in the brain by suppressing the uptake of insulin and accelerating the accumulation of Aβ in the brain (Sima *et al.,* 2018), it has been proposed that neurons can develop hyperinsulinemia-induced insulin resistance (Feldman, 2012). Chronic hyperinsulinemia may lead to a compromise in blood-brain barrier and subsequently abrogate insulin activity. Prolong neuronal exposure to hyperinsulinemic environment has been reported to result in irreversible cognitive dysfunction and neuronal degeneration (Karvani *et al.,* 2019).

HFD contribute prominently to insulin resistance and cognitive impairment, the effect of such diets on the brain are poorly understood but insulin resistance may play an important role by acting on brain energy metabolism and neuroprotective mechanisms (Vishal *et al,* 2017).

Types of Dementia

Vascular dementia

The term vascular dementia substantially means "disease with a cognitive impairment resulting from cerebrovascular disease and ischemic or hemorrhagic brain injury

Vascular dementia is the second leading cause of dementia (Dichgans and Leys, 2017). Vascular cognitive impairment can result from widespread small vessel dysfunction, which adversely affects cerebral perfusion, cerebrovascular reactivity, and blood brain barrier and white matter integrity (Smith and Beaudin, 2018; Frantellizzi *et al.,* 2020). Although vascular cognitive impairment and its associated pathophysiology are most commonly attributed to hypertension, the degree to which peripheral and brain insulin resistance contributes to the underlying pathology requires further investigation, particularly given the multifactorial role of insulin in vascular function. Insulin acts as a vasoactive hormone that modulates both cerebral and peripheral blood flow (Hughes and Craft, 2016) binding to receptors on endothelial cells, where it increases nitric oxide that acts to dilate blood vessels. However,

insulin can alternatively constrict blood vessels by stimulating production of endothelin-1 via the MAPK pathway (Muniyappa and Yavuz, 2013). Through these effects, insulin acts as vasodilator when at normal concentrations and a vasoconstrictor at high concentrations. As a result, insulin resistance-associated chronic hyperinsulinaemia promotes vasoconstriction resulting in higher blood pressure and reduced cerebral perfusion, a pattern that might be observed years before the onset of cognitive symptoms characteristic for vascular cognitive impairment (Muniyappa and Yavuz, 2013).

Lewy Bodies Dementia

Unlike Alzheimer's disease, memory impairment is not necessarily a prominent early feature in Lewy bodies dementia, but this will usually appear with progression of the disease. Instead, deficits in attention, executive function, and visuospatial ability are often prominent early symptoms.

There are three core features for diagnosis. Two of these core features should be present for a diagnosis of probable dementia with Lewy bodies, while one core feature should be present for a diagnosis of possible dementia with Lewy bodies. The three core features are (i) fluctuating cognition with pronounced variation in attention and alertness, (ii) recurrent visual hallucinations (iii) spontaneous features of parkinsonism.

There are also three suggestive features for diagnosis. In the presence of one core feature, the additional finding of a suggestive feature justifies a diagnosis of probable dementia with Lewy bodies. In the absence of any core features, the presence of a suggestive feature justifies a diagnosis of possible dementia with Lewy bodies. The three suggestive features are (i) a REM sleep behavior disorder, (ii) severe neuroleptic sensitivity, and (iii) low dopamine transporter uptake in the basal ganglia demonstrated by SPECT or PET imaging.

Supportive features are often present, but are not sufficient for diagnosis. These include repeated falls and syncope; transient, unexplained loss of consciousness; severe autonomic dysfunction (eg, orthostatic hypotension, urinary incontinence), nonvisual hallucinations, systematized delusions, depression, relative preservation of medial temporal lobe structures on CT or MRI scans, generalized low uptake on SPECT/PET perfusion scans with low occipital activity, abnormally low uptake on MIBG myocardial scintigraphy, and prominent slow wave activity on EEG with temporal lobe transient sharp waves.

These diagnostic criteria are considered in light of other confounding clinical conditions. Thus, a diagnosis of dementia with Lewy bodies is less likely in the presence of clinical or imaging evidence of cerebrovascular disease, in the presence of other clinical conditions that might explain the clinical findings, and if parkinsonism appears only as a late complication in a severely demented patient (Robert, 2007).

The most specific immunohistochemical method for the detection of Lewy bodies employs antibodies to alpha-synuclein. Anti-ubiquitin antibodies will also detect Lewy bodies, but this technique also highlights Alzheimer-type neurofibrillary changes, and thus is less useful in cases with co-existent Alzheimer pathology.

Dementia with Lewy bodies (DLB) is one of the most common causes of dementia after Alzheimer disease (AD) and vascular dementia. DLB often presents a diagnostic challenge given this clinical heterogeneity and overlap with other neurodegenerative diseases. Further, it was initially often overlooked pathologically because of the difficulty in identifying cortical Lewy bodies with routine histochemical stains.

Lewy body dementia encompasses both dementia with Lewy bodies and Parkinson’s disease dementia (Chouliaras *et al*., 2020).

Dementia with Lewy bodies (DLB) and Parkinson's disease dementia (PDD) are classified together under the umbrella term Lewy Body Dementia (LBD). LBD is the second most common type of neurodegenerative dementia in older people, responsible for around 10% of all cases (Kane *et al.,* 2018; Vann and O'Brien, 2014). PDD and DLB are closely aligned. The essential difference lies in the order of clinical symptom onset. In PDD the motor features associated with PD precede the memory problems associated with dementia by at least one, and usually several, years. In DLB, however, memory and motor problems start within one year of each other (Aarsland *et al.,* 2017). Hence, the two conditions may be viewed as a continuum under the term LBD, combining PDD and DLB, rather than two separate conditions (Walker *et al.,* 2015).

DLB shares features with both Alzheimer's (AD) and Parkinson's disease (PD). As well as well-described differences in clinical features, compared to AD people with DLB have a higher risk of falling, lower quality of life, greater caregiver burden and a higher mortality rate (Mueller *et al*., 2019; Price *et al.,* 2017). DLB primarily affects people over 65, more commonly men. Suspected cases are diagnosed on the basis of neuropsychiatric assessment, neuroimaging, and biomarker assays (Walker *et al.,* 2015).

The consensus criteria for diagnosis of DLB involve presence of dementia syndrome along with two core clinical features or one core clinical feature and an indicative biomarker (Walker *et al.,* 2015).

Core clinical features of DLB are visual hallucinations, parkinsonism, fluctuating cognitive function and REM sleep behaviour disorder. Indicative biomarkers are reduced basal ganglia dopamine uptake (single-photon emission computed tomography (SPECT)/positron emission tomography (PET), low cardiac Iodine-123 meta-iodobenzylguanidine (MIBG) and confirmation of REM sleep disorder without atonia on polysomnography. Case series of

postmortem studies have shown that pathology consistent with a diagnosis of LBD is the second most common finding after AD pathology (Beach *et al.,* 2008).

Pathophysiology of LBD

The major pathological hallmark of LBD is the intraneuronal deposition of α-synuclein, including in the form of Lewy bodies, protein aggregates which also contain ubiquitin. As the disease progresses, Lewy bodies manifest throughout the brainstem, limbic areas and neocortex (McKeith *et al.,* 2017; Montine *et al.,* 2012). Apart from the Lewy bodies themselves, α-synuclein also accumulates in axons, forming amyloid fibrils of distinct morphology (Jellinger, 2018). These Lewy neurites are characteristically abundant in the amygdala, hippocampus and striatum and may emerge early in disease progression.

Mixed Dementia

Mixed dementia is the coexistence of Alzheimer's disease and cerebrovascular disease (CVD) in the same demented patient (Nilton, 2017).

2.2 Common models of dementia

Different animal models are used in dementia research. They mimic the various pathological stages involved in the condition thereby providing insight into how dementia is initiated and propagated (Gotz and Gotz, 2009). Dementia is a complex condition attributable to several etiological factors, impacting a multitude of biological pathways. Developing a research model that truly encapsulates this complexity has, thus, been a scientific challenge. Even so, many models have been developed to gain insights into various aspects of dementia. Animal models of dementia include both transgenic animals as well as natural, non-transgenic models. Together, these animal models can be used to simulate dementia pathology to understand disease progression. Several *in vitro* models have also been developed to

understand the more complex tissue-specific pathologies associated with dementia (Li *et al.*, 2016).

2.2.1 Animal models

Rodents are the most widely used animal for study of dementia because of their biological similarity to humans, low cost, ease of manipulation, well characterized behaviours, short life-cycles, and existing tools to induce mutations.

Additionally, researchers can have total control of the environment for rodents, increasing the ability to limit extraneous variables in experiments. The investigation of AD mechanisms in these models range from very precise mutations to study specific pathological features to combined mutations to generate more comprehensive models of AD pathology (Arnold *et al*., 2019).

2.2.2 Transgenic Animal Models

Transgenic mice have served as a genetic tool to study the effects of several genes implicated in etiology of AD. The most widely-used and notable mouse models express the human (hAPP). The levels and spatial expression of the hAPP protein are driven by varying the promoters used in mice. Commonly used promoters are platelet-derived growth factor B-chain PDGF-B (e.g., J20), thymocyte differentiation antigen 1 Thy-1 (e.g., 5xFAD), and prion protein (PrP) genes (Arnold *et al*., 2019).

Other mice models include Tau transgenic mice that express the human tau protein using various promoters, APP-tau double transgenic mice, triple transgenic mice that express APP, PSEN11, and Tau, and five transgenic mice that express 5 familial AD mutant genes. These mice exhibit the expected, hallmark neurological symptoms associated with AD such as

cognitive deficits, motor deficits, and memory loss, albeit at varying levels (Arnold *et al*., 2019).

2.2.3 In vitro models

In vitro models facilitate a more direct and in-depth examination of dementia related pathologies on a cellular and molecular level. These include tissue models such as brain slices and cultured brain tissue, as well as cell models such as AD-derived induced pluripotent stem cells and neuroblastoma cells, and molecular simulation models such as antibubble biomachinery developed to study the impact of inflammation on AD.

Summarily, experimental models of dementia have greatly enhanced our understanding of the disease process in dementia and promoted the development of novel therapies. While transgenic animal models enable researchers to explore the genetic etiology of AD, and have led to the establishment of more complete models of AD which may be used for genetic manipulation and the identification of novel therapies, non-transgenic animal models recapitulate the natural process of AD including its occurrence, evolution and prognosis, which has shown a high application value in earlier studies. By contrast, in vitro models isolate specific molecular pathways from others in AD, permitting therapeutic screening in a rapid and direct way. Among them, transgenic models are the most popularly used in AD research (Arnold *et al*., 2019).

Insulin

Insulin plays a central role in the regulation of human metabolism. The hormone is a 51-residue anabolic protein that is secreted by the β-cells in the Islets of Langerhans. Containing two chains (A and B) connected by disulfide bonds, the mature hormone is the post-translational product of a single-chain precursor, designated proinsulin (Michael *et al*., 2014).

Insulin enhances glucose uptake by increasing the number of transporters in the plasma membrane of target cells. This was first demonstrated in adipocytes and subsequently in skeletal and cardiac muscle. Insulin stimulation of such cells mobilizes transporters from intracellular compartments to the plasma membrane to facilitate glucose transport. Translocation of receptors to the plasma membrane has been demonstrated to occur within 30 seconds of insulin stimulation; as the stimulus dissipates the decrease in the number of plasma membrane receptors declines coincident with a decline in glucose transport. The impaired ability of insulin, on binding and activation of the IR, to signal Glut4 translocation from intracellular stores contributes to postprandial hyperglycemia in Type 2 DM. Animal studies have also demonstrated that insulin resistance is associated with a decreased translocation of glucose transporters to the plasma membrane in muscle cells. In fact, decreased insulin levels in animal models of DM have been shown not only to decrease transporter translocation, but also to attenuate expression of Glut4 in muscle cells. Thus, it appears that insulin provides both a short-term signal to increase glucose-transporter translocation and a long-term signal to maintain a basal level of expression of such transporters in target cells. The combination of acute and basal actions provides a common mechanism in Type 1 DM (characterized by low or vanishing endogenous insulin levels) or Type 2 DM (characterized by insulin resistance) could cause pathologically high plasma glucose levels: loss of regulation and expression of transmembrane glucose transporters. Glut2, expressed on surface of β-cells, contributes to the regulation of insulin secretion. Accordingly, a β-cell specific IR knock-out (KO) model indicated that insulin likely positively regulates its own secretion from the β-cell (Michael *et al*., 2014)

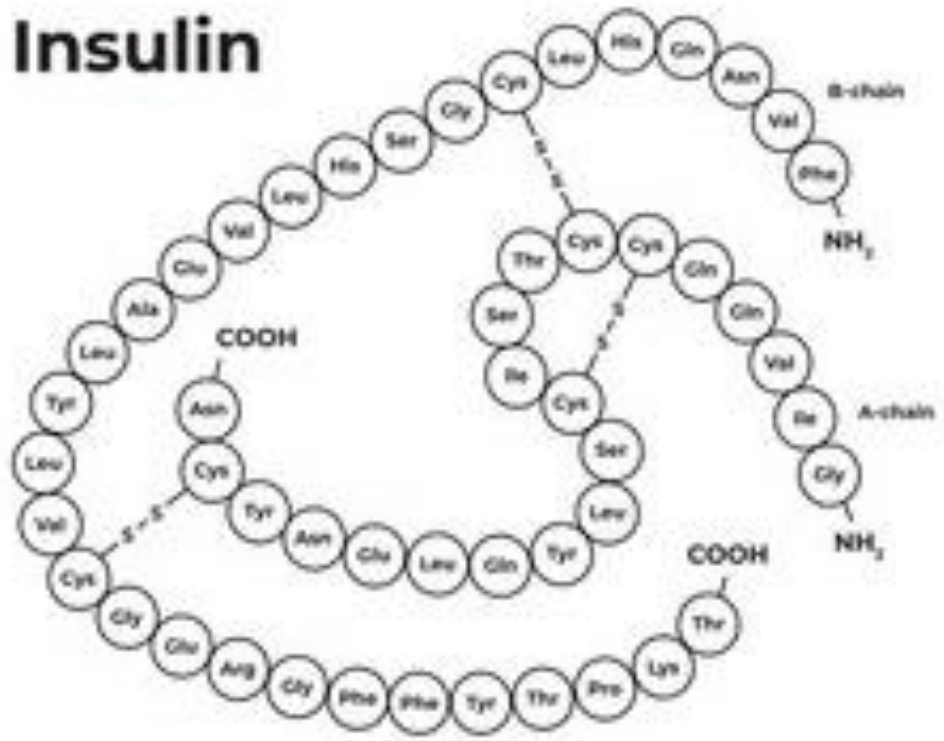


Figure 2.1: Structure of insulin (Michael *et al*., 2014)

2.3 The pathological link between insulin resistance and Dementia

Insulin resistance is a state of decreased responsiveness of target tissues to insulin, and a major feature of type 2 diabetes, glucose intolerance, hypertension, dyslipidemia, and cardiovascular disease. Some large population studies support an association between type 2 diabetes caused by insulin resistance and dementia (Chatterjee *et al.,* 2016; Gudala *et al.,* 2013). In several recent studies that involved both human and animal models, insulin resistance was suggested to have negative effects on cognition, particularly learning and memory (Willette *et al.,* 2015). Experimental observations are identifying that markers of metabolic dysregulation are also present in AD, the most remarkable being insulin resistance (De Felice, 2013; Boles *et al.,* 2017). However, the molecular mechanisms underlying this crosstalk are still elusive, as well as how central and peripheral insulin signaling operate in AD (Biessels and Despa, 2018).

There is strong epidemiological connection between diabetes, obesity, and dementia, the key intersection among the three diseases is insulin resistance, which has been classically described to occur in peripheral tissues in diabetes and obesity and has recently been shown to develop in Alzheimer's disease (AD) brains (Lais *et al.,* 2018).

2.3.1 Mechanisms of Insulin resistance-induced dementia

There are several possible mechanisms to explain the role of insulin resistance in the development of dementia. First, cerebrovascular disease, a consequence of insulin resistance, can induce the development and progression of vascular dementia (VD) and AD by causing multifocal ischemic lesions, and has been shown to predict the development or progression of cognitive decline in several clinical studies (Love *et al.,* 2016). Secondly, the alteration of brain insulin signaling may be due to insulin resistance associated with induction of cognitive impairments and neurodegeneration (Folch *et al.,* 2019). Thirdly, insulin resistance-related

syndromes, such as diabetes and dyslipidemia, are well-known risk factors for dementia. In a recent meta-analysis that included 19 community-based studies, type 2 diabetes patients had dementia 1.6 times more often than patients without type 2 diabetes (Kivimaki *et al.,* 2019). In another meta-analysis of 17 studies, high plasma cholesterol in mid-life was associated with a 2.14-fold increased risk of AD dementia but not in late life (Anstey *et al.,* 2017). Individuals with insulin resistance, T2DM, hyperlipidemia, obesity, or other metabolic disease may have increased risk for the development of AD and similar conditions, such as vascular dementia. This association may in part be due to the systemic mitochondrial dysfunction that is common to these pathologies. Accumulating evidence suggests that mitochondrial dysfunction is a significant feature of AD and may play a fundamental role in its pathogenesis. In fact, aging itself presents a unique challenge due to inherent mitochondrial dysfunction and prevalence of chronic metabolic disease (Bryan, 2017).

Subtle cognitive changes that can accompany early stages of insulin resistance due to aging, type 2 diabetes, and other factors may eventually develop into clinically significant cognitive impairment, including dementia

Dementia develops as a result of a complex interplay of clinical and biological factors and is beset by multiple underlying pathological features. People with type 2 diabetes represent an important risk group for cognitive impairment and dementia caused by both Alzheimer's disease, dementia and vascular brain injury. For example, a recent meta-analysis found that type 2 diabetes was associated with a 60% increase in risk for all-cause dementia (Gudala *et al*., 2013) and a population-based longitudinal study found a 16% increased risk for dementia even among those in which type 2 diabetes onset was recent (Haroon *et al*., 2015).

2.3.2 The pathological link between type II diabetes and dementia

Diabetes mellitus a predominant global epidemic has a strong link with the incidence of neurodegenerative disorders. Hyperglycemic condition has strong correlation with cognitive decline, AD and neurodegeneration in general. The mechanisms through which diabetes and hyperglycemia mediates neurodegenerative conditions are largely unknown (Barbagallo & Dominguez, 2014). Although it has been more than a decade since the idea of diabetes mellitus as a causal disorder of many neuronal diseases originated, this link has been less explored (Yanan *et al*., 2020). This oversight is likely due to inadequate methodologies and lack of appropriate testable models. In AD, irreversible neurodegeneration causes severe damage to the brain tissue and a reduction in size of the brain (Bernardes *et al.,* 2017).

Evidences from epidemiological and biological studies support a strong link between T2DM and Alzheimer's disease, patients with diabetes mellitus have a higher incidence of cognitive decline and they have increased risk of developing all types of dementia. Cognitive decline in persons with diabetes mainly affect the areas of psychomotor efficiency, attention, learning and memory, mental flexibility and speed, and executive function. The strong epidemiological association has suggested the existence of a physiopathological link (Gohar *et al.,* 2015). The determinants of the accelerated cognitive decline in T2DM, however, are less clear. Cortical and subcortical structural atrophy has been reported in both cases of T2DM and AD. Insulin and insulin resistance has been reported as the possible links between diabetes and AD. Imbalance in brain insulin signaling pathway may contribute to the histopathological, biochemical and molecular abnormalities in AD. Hyperglycemia on its own is a risk factor for dementia and cognitive dysfunction (Barbagallo and Dominguez, 2014; Gohar *et al.,* 2015).

The progressive degenerative and irreversible neurological disorder induced by AD is largely characterized by the formation of neurofibrillary tangles, amyloid β (Aβ) plaques, loss of neurons and synapses and amyloidal angiopathy (Sisi *et al.,* 2017). Studies reveal that obesity in middle age increases the risk for AD and there is a strong correlation between AD and glucose metabolism disorder (Wangb, 2014).

Neurodegenerative diseases are generally characterized by cellular accumulation of misfolded proteins, ROS production due to mitochondrial dysfunction, and disruption of the autophagy machinery in neuronal cells (Madhusudhanan *et al.*, 2020).

Recent progress in AD research has demonstrated that there are several other external factors widely causing the emergence of AD pathologies, such as obesity, diabetes, brain injury, neurotoxicity, and infections (Pugazhenthi *et al.*, 2017).

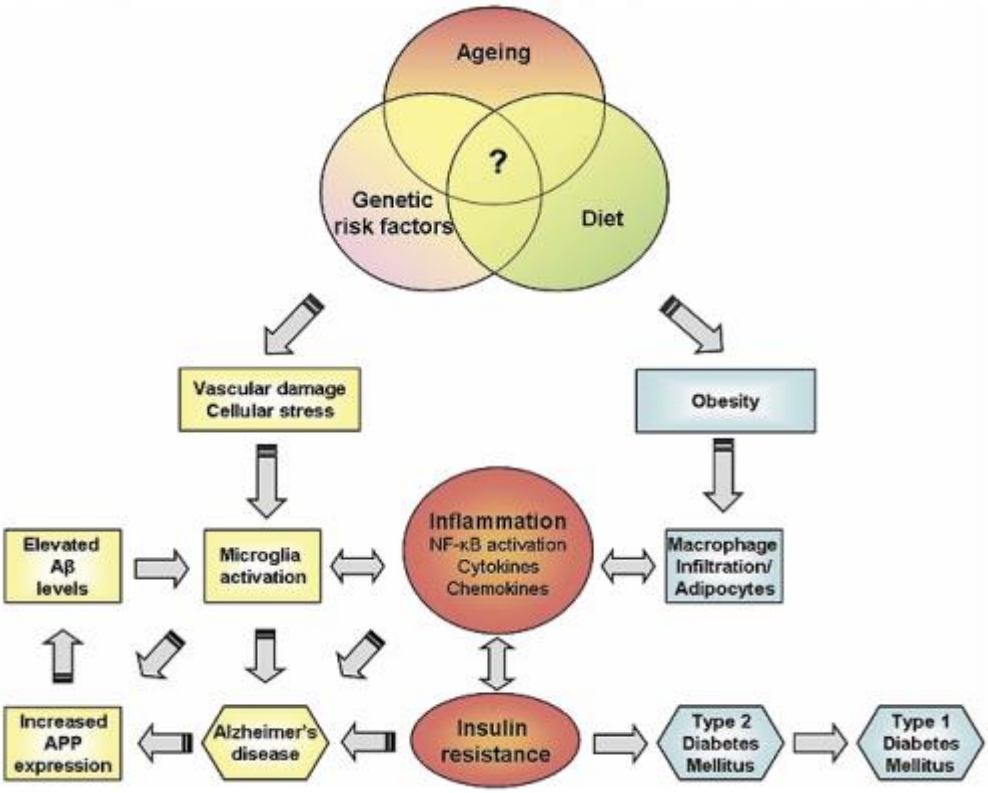


Figure 2.1: Illustration of the pathological pathways between AD and diabetes (Mihalea *et al.*, 2009).

Recent clinical studies have demonstrated a dramatic correlation between AD and metabolic diseases such as type 2 diabetes mellitus. Hence, AD is now recapturing the attention of neuroscientists as a possible complication of defective glucose metabolism (Bianchi & Manco, 2018). According to a recent report by International Diabetes Federation the number of patients with diabetes in the world has increased from 108 million in 1980 to 425 million in 2017, indicating that every 11th person in the world is diabetic (Bianchi & Manco, 2018). These numbers probably underestimate the actual number of patients with diabetes, since one out of two people remains undiagnosed in most developing countries. According to estimates by the World Health Organization, developing countries will contribute five times more than developed countries to the prevalence of diabetes and diabetes-related deaths by 2030 (Wild *et al.,* 2004). This could also be an indication of the alarming number of patients with AD in developing and underdeveloped countries, where lack of modern diagnostic techniques and new treatment strategies for AD are contributing to a major health crisis (Kalaria *et al.,* 2008).

2.3.3 Amylin in neurodegenerative disease

Amylin also known as Islet amyloid polypeptide (IAPP) is a hormone that is co-secreted by the pancreatic β cells along with insulin (Despa and DeCarli, 2013). It is secreted in minute quantities compared to insulin exerting similar physiological functions like insulin. The roles of Amylin in AD has recently become a point of interest (Mietlicki-Baase, 2016). The proteolytic processing of amylin is similar to that of Aβ (Akter *et al.,* 2016). Aggregates of amylin have been reportedly found in the pancreatic islets of T2DM patients (Mietlicki-Baase, 2016). Accumulation of amylin contributes to insulin resistance and oxidative stress responses observed in pancreatic islets cells (Lutz & Meyer, 2015). Amylin possesses the ability to cross the blood brain barrier and its receptors are distributed in some parts of the CNS similar to what is observable in the case of insulin and its receptors

(Mietlicki-Baase & Hayes, 2014). There is an accumulation of amylin in peripheral tissues of T2DM patients, leading to the hyperamylinic condition found in the brain of the diabetic (Jackson *et al.,* 2013). Hyperamylinemia subsequently give rise to brain injury culminating in AD symptoms (Lim *et al.,* 2013). The mechanism through which amylin mediates neurodegeneration is largely unknown, however studies from AD patients with type 2 diabetes and diabetic rats expressing human islet amyloid polypeptide reveals that deposition of amylin-Aβ in brain of AD patients leads to the activation pro inflammatory cytokines leading to neuronal degeneration (Verma *et al.,* 2016). Amylin with its associated analogs subsequently interact leading to the activation of different downstream molecules along the insulin signaling pathway (Nassar *et al*., 2018).

Parallel research exploiting the structural and biophysical similarities between amylin and beta-amyloid peptide has unearthed another fascinating finding that patients with AD significantly overexpress amylin receptors (Jhamandas *et al.,* 2011). It is already known that Aβ and amylin can bind to the same receptor, which indicates a probable amylin receptor-mediated Aβ action in patients with AD (Nassar *et al.,* 2018). In vitro studies have shown that blocking amylin receptors could mitigate the electrophysiological effects of Aβ and confer neuroprotection (Jhamandas *et al*., 2011). These studies provide the rationale for considering amylin receptors as a reliable novel therapeutic target for the treatment of AD.

2.3.4 Insulin signaling dysfunction as a factor in the formation of beta-amyloid and tau hyperphosphorylation

Insulin signaling is vital for several functions of the brain. Some of these include synaptogenesis, plasticity, neuroregeneration, learning, memory and repair (Tumminia *et al.,* 2018). Insulin also regulates APP metabolism in neurons (Tumminia *et al.,* 2018). Hence, an imbalance in insulin signaling can reflect on the metabolism and processing of APP, which eventually leads to the accumulation of Aβ in the cell—a major cause for neurodegeneration

in AD. As evidence for the potential role of insulin signaling in neurodegeneration in AD, significantly reduced expression of insulin receptor has been observed in the brains of patients with AD (Frazier *et al.,* 2019). Furthermore, hyperphosphorylation of the tau protein, one of the critical features of AD pathology is also increased due to impaired insulin signaling in the brain of patients with T2DM (Tumminia *et al.*, 2018).

2.3.5 Neuroinflammation and its deleterious effects to insulin signaling pathway

It is well known that neuroinflammatory pathways can cause deleterious effects on neuronal cells. In the hyperglycemic condition, neuroinflammatory pathways can be induced in numerous ways. First, increased mitochondrial activity creates a stressful environment within the cell, thus enhancing ROS production which leads to the activation of inflammatory pathways. One of the other key features of T2DM is the overproduction of proinflammatory cytokines such as TNF-α and IL-6, in part due to hyperactivation of microglia and astrocytes, the immune cells of the brain (Nasoohi *et al.,* 2018). Persisting inflammation and abnormal levels of circulating cytokines that may even breach the blood brain barrier can be observed in patients with T2DM (Nasoohi *et al.,* 2018). TNF-α promotes various stress-sensitive kinases which induce serine phosphorylation of IRS-1, an essential molecule in the insulin signaling cascade which is usually activated by phosphorylation at a tyrosine residue to propagate the insulin signal (Nasoohi *et al.,* 2018). Thus, increased cytokine levels in the brain can lead to defective insulin signaling, which is one of the mechanisms through which T2DM affects brain functions (Ferreira *et al.*, 2014). It is clear that T2DM-induced chronic inflammation has a significant impact on the brain and is one of the important causal mechanisms of many neurological disorders such as AD and multiple sclerosis (Van & Lacoste, 2018).

2.3.6 Cognitive decline in type II diabetes mellitus and insulin resistance

The CNS is one of the most important targets of insulin. Insulin receptors (IRs) are widely expressed in different parts of the brain, especially in the hippocampus. Insulin mediates metabolic homeostasis and regulates neurotrophic processes and synaptic plasticity of the brain (Calvo-Ochoa and Arias, 2015; Nguyen *et al*., 2018).

2.3.7 High-fat diet and Metabolic Disorders

High fat diet (HFD) also called Western diet is essentially rich in saturated fat and refined sugar, has been shown to increase cognitive decline with aging and Alzheimer's disease, and to affect cognitive functions that are hippocampal dependent, including reversal learning and memory processes (Tamashiro, 2015).

Previous investigations have established a clear association between high fat diet intake and metabolic disorders such as cardiovascular diseases, obesity and diabetes and large data of studies now suggests that diets high in fat can also have negative impact on behaviour, the brain and cognition (Heather *et al*., 2013; Tamashiro, 2015). Evidence in humans and animal models suggests that obesity, insulin resistance, diabetes and cardiovascular diseases are associated with an increased risk of Alzheimer's disease and other forms of cognitive impairment (Subbiah *et al.*, 2017).

Animal disease models of dementia are essential tools for studying the pathophysiology of the disease, thereby assisting in the development of potent therapeutic molecules within a very short time (Engel *et al.*, 2018).

2.4 Streptozotocin

Streptozotocin (STZ) is a glucosamine-nitrosourea compound derived from soil bacteria which was originally developed as an anticancer agent, it was found to induce diabetes in

experimental animals in 1963. Streptozotocin (STZ) is currently the most used diabetogenic agent in testing insulin and new antidiabetic drugs in animals (Nidal, 2015).

2.4.1 Physical Properties of STZ

In terms of solubility, it is very soluble in water, ketones and lower alcohols, but slightly soluble in polar organic solvents. Streptozotocin has a molecular formula of $C_8H_{15}N_3O_7$, molecular weight of 265 g/mol and the structure is composed of nitrosourea moiety with a methyl group attached at one end and a glucose molecule at the other end (Chinedum *et al*., 2013).

2.4.2 Chemical properties of STZ

It is a cytotoxic methyl nitrosourea moiety (N-methyl-N-nitrosourea) attached to the glucose (2-deoxyglucose) molecule.

It is a glucosamine derivative.

It is a toxic beta cell glucose analogue

It is a hydrophilic compound

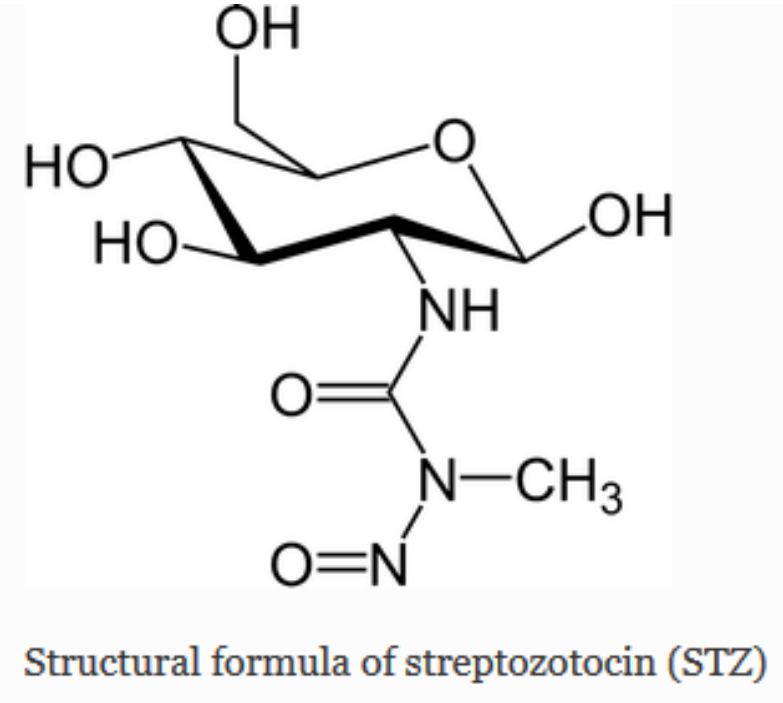
It is an alkylating agent

STZ is a toxic glucose (Glu) and N-acetyl glucosamine (GlcNAc) analogue that is accumulated preferentially in pancreatic β-cells via GLUT 2 transporter uptake (Ventura-Sobrevilla *et al.,* 2011).

It is relatively stable at pH 7.4 and 37°C at least for up to I hr.

It has a biological half-life of 5–15 minutes

When reconstituted into a solution, it can be stored at room temperature or refrigerator but must be used within 12 hrs if stored at room temperature and protected from sunlight (Sharma, 2010).



Structural formula of streptozotocin (STZ)

Figure 2.2: Chemical structure of streptozotocin

2.4.3 Pharmacodynamics of STZ

STZ has been used to create models of diabetes in experimental animals. It is selectively toxic toward pancreatic beta cells, as a result of its cellular uptake by the low-affinity glucose transporter 2 (GLUT2) protein located in pancreatic cell membranes. STZ is able to exhibit its cytotoxic property due to DNA alkylation which leads to cellular necrosis (Grieb, 2015).

2.5 Trans-cinnamaldehyde

Some of the essential oils of cinnamon includes trans-cinnamaldehyde, cinnamic acid, and cinnamate which has been reported to possess antioxidant, antimicrobial, anticancer, lipid-lowering, and cardiovascular-disease-lowering properties (Camacho *et al.,* 2015; Pasupuleti,

2014). The active compound of Cinnamon with hypoglycaemic activity is a contentious issue (Richard *et al.,* 2016).

The roles cinnamon in obesity and diabetic conditions have been reported (Wan-Nurdiyana, 2014). Cinnamon is reputed from both nutritional and pharmacological points of view and its beneficial health promoting properties is mainly attributed to the polyphenolic composition and the volatile essential oils coming from different parts of the plant (bark, leaves, flowers, or buds). The cinnamon bark essential oils such as cinnamate, cinnamic acid, cinnamic aldehyde, cinnamyl aldehyde eugenol and trans-cinnamaldehyde are the major components of the leaves (Saeideh *et al*., 2018).

Beneficial use of Trans-cinnamaldehyde

Flavoring agent: Trans-cinnamaldehyde is commonly used as a flavouring agent in chewing gum, ice cream, candy, and beverages (Gutiérrez *et al*., 2009) it is also used in some perfume so as to give them fruity or aromatic scents (Chang *et al.*,2001).

Agrichemical: TCA is widely used in agrochemical industries as fungicide because of its low toxicity, well known properties (Pedro *et al.,* 2013). It has also been reported to be an effective insecticide with potent ability to kill mosquito larva and ability to repel animals such as cats and dogs (Cheng *et al.,* 2004).

Antimicrobial agent: Cinnamaldehyde is widely used as antimicrobial agent (Gomes *et al.,* 2011; Balaguer *et al.,* 2013) with ability to inhibit oral microbial growth (Filoche *et al*., 2005), trans-cinnamaldehyde is also used as an antimicrobial additive in poultry feeds (Balaguer *et al.*, 2013)

Anticancer agent: The anticancer property of TCA *invitro* and in vivo has been documented, at high doses of TCA, proliferation, invasion, and tumor growth were inhibited in a murine A375 model (rats and mice) of human melanoma (Balaguer *et al.,* 2013)

2.5.1 Physical properties of trans-cinnamaldehyde

Trans-cinnamaldehyde is a clear yellow liquid with strong odour of cinnamon, the molecular formula is C_9H_8O and chemical formula is C_6H_5CH: CHCHO (Siti *et al.,* 2019).

2.5.2 Chemical properties of trans-cinnamaldehyde

The molecular formula of trans-cinnamaldehyde was determined in 1834 by French chemists Jean Baptiste André Dumas and Eugène Melchior Péligot (Balaguer *et al.,* 2013)

The natural product of *trans*-cinnamaldehyde molecule consists of a phenyl group attached to an unsaturated aldehyde with low solubility in water (Balaguer *et al.,* 2013).

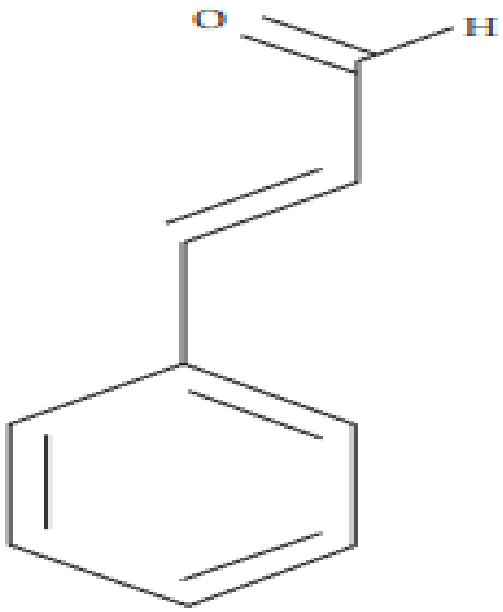


Figure 2.3: Chemical structure of trans-cinnamaldehyde (Xia *et al.,* 2019)

2.6 Insulin resistance, Type II Diabetes and Dementia in Africa

African governments seem not to recognize the catastrophic tendency of the diabetes epidemic, type 2 diabetes accounts for 70% - 90% of diabetes cases in Africa (Levitt, 2008; Mufunda *et al.* , 2006) it is more prevalent among the wealthy, incidence is higher in urban areas where people tend to be less physically active, eat diets rich in saturated fat and refined sugars and are more obese. Obesity is a key contributor to increased prevalence of diabetes mellitus in both urban and rural areas, but more so in the former (Hossain and Kawar, 2007; Sobngwi *et al.*, 2004). According to the World Health Organization (WHO) estimates of obesity in Africa, it was observed that more than one-third of the women are obese compared to one-fourth of the men (Hossain and Kawar, 2007).

Recent survey conducted on AD indicated dementia of the Alzheimer's type is a major cause of health concern among adult population (Robert *et al.,* 2012). Similar trend has been predicted in most African countries as a result of expected increase in life expectancy (Fratiglioni & Agüero-Torres, 2012). The prevalence of dementia in Ibadan, Nigeria was reported to be 2.29% and AD constituted 1.41% of the reported cases (Mbuyi, 2014). Similar study was conducted in Cotonou in Benin, a non-significantly higher prevalence (3.70%) was reported (Mbuyi, 2014). A relatively lower prevalence of 2.85% was also reported at a neurology clinic in Yaoundé in Cameroon (Callixte *et al.,* 2013). A cross sectional survey conducted in northern Nigeria, reported 2.79% cases among Hausa-Fulani aged 65 and above with AD constituting 66.67% of cases (Abdulkareem *et al.,* 2010).

The number of people with dementia expected to rise above 7.6 million by 2050 as a result of increasing aging population in sub-Saharan Africa (ADI, 2018). There is urgent need to study the possible risk factors and potential intervention so as to curtail the growing burden of the disease (ADI, 2018).

Sub-Saharan African (SSA) countries are experiencing rapid transitions with increased life expectancy. As a result the burden of age-related conditions such as neurodegenerative diseases might be increasing (Alain *et al.,* 2014). Population ageing is considered a global public health success, but also brings about new health challenges in the form of chronic diseases including cardiovascular diseases, cancers, as well as neurodegenerative disorders (Alain *et al*., 2014).

2.6.1 Risk factors for dementia

Dementia is an aging-related condition and a progressive neurodegenerative disorder clinically characterized by deterioration in memory, thinking, behavior, and the ability to perform everyday activities, it is one of the major causes of disability and dependency among older people worldwide. More so, dementia also constitute social and economic burden, not only on patients with dementia, but also on their caregivers, families, and society at large. Dementia results from a variety of diseases and injuries such as Alzheimer's disease (AD) or stroke that are primarily or secondarily associated with the brain. Among the types of dementia, AD is the most common, comprising up to 60–80% of dementia cases, and vascular dementia (VD) is the second most common, accounting for 10–20% of dementia cases (Podcasy *et al.,* 2016).

Dementia is a growing health problem with an expected number of 115 million cases worldwide in 2050 (WHO, 2021). The prevalence of dementia increases steeply with age from a prevalence of 2.6% in subjects aged 65–69 years and a prevalence of 43.1% in subjects aged 90 years and older (WHO, 2012). Insight in the risk factors for cognitive decline is essential in the search for preventive strategies for cognitive impairment and dementia. Former studies identified a range of potential risk factors including the APOE

(apolipoprotein E) ε4 allele, cardiovascular risk factors, depressive symptoms, inflammation markers and lifestyle factors (Cheng *et al.,* 2012; Smith *et al*., 2013; Baumgart *et al*., 2015).

There are some reported associated risk factors of AD in sub-Saharan Africa, such risk factors include the following;

2.6.1.1 Ageing

Age is the strongest known risk factor for dementia. Though it is possible to develop the condition earlier in life, at least 1 in 20 people with dementia developed it at age under 65, the chances of developing dementia rise significantly with ageing. Above the age of 65, a person's risk of developing Alzheimer's disease or vascular dementia doubles roughly every 5 years. It is estimated that dementia affects one in 14 people over 65 and one in six over 80. This may be due to factors associated with ageing, such as higher blood pressure, increased risk of cardiovascular diseases, changes to nerve cells, DNA and cell structure, loss of sex hormones after mid-life changes, the weakening of the body's natural repair systems and changes in the immune system (Alain *et al.,* 2014).

2.6.1.2 Gender and genetics

Women are more likely to develop Alzheimer's disease than men. The reasons for this are still unclear. It has been suggested that Alzheimer's disease in women is linked to a lack of the hormone oestrogen after the menopause. Studies have shown that women have a two- to eight-fold increased risk of dementia attributed partly to their longevity when compared to men, increasing age has been closely associated with the risk of dementia. Genes such as apolipoprotein E (APOE) gene has been reported to increase the risk of Alzheimer's disease among people in the U.S., however research in sub-Saharan Africa is inconclusive. Indianapolis-Ibadan project reveals that the allele was not associated with Alzheimer's disease in elderly Yoruba, where as another study reported that the allele was associated with

AD. A novel mutation in presenilin 1, was found to cause familial or early-onset Alzheimer's disease in South Africa.

For most dementias other than Alzheimer's disease, men and women have much the same risk. For vascular dementia, men are actually at slightly higher risk than women. This is because men are more prone to stroke and heart disease, which can cause vascular and mixed dementia (Alain *et al.,* 2014).

2.6.1.3 Ethnicity

There is some evidence that people from certain ethnic communities are at higher risk of dementia than others. For example, South Asian people (from countries such as India and Pakistan) seem to develop dementia – particularly vascular dementia – more often than white Europeans. South Asians are well known to be at a higher risk of stroke, heart disease and diabetes, and this is thought to explain the higher dementia risk. Similarly, people of African or African-Caribbean origin seem to develop dementia more often. They are known to be more prone to diabetes and stroke. All of these effects are probably down to a mix of differences in diet, smoking, exercise and genes (Alain *et al.,* 2014; WHO, 2018).

Scientists have known for some time that the genes we inherit from our parents can affect whether or not we will develop certain diseases. The role of genes in the development of dementia is not yet fully understood, but researchers have made important advances in recent years. More than 20 genes have been found that do not directly cause dementia but affect a person's risk of developing it. For example, inheriting certain versions (variants) of the gene apolipoprotein E (APOE) increases a person's risk of developing Alzheimer's disease. Having a close relative (parent or sibling) with Alzheimer's disease increases your own chances of developing the disease very slightly compared to someone with no family history. However, it does not mean that dementia is inevitable for you. It is also possible to inherit

genes that directly cause dementia, although these are much rarer than the risk genes like APOE. In affected families there is a very clear pattern of inheritance of dementia from one generation to the next. This pattern is seen in families with familial Alzheimer's disease (a very rare form of Alzheimer's which appears usually well before the age of 60) and genetic frontotemporal dementia. If a person has the faulty gene then each of their children has a 50 per cent chance of inheriting it and so developing the dementia (Alain *et al.*, 2014).

2.6.1.4 Medical conditions and diseases

Cardiovascular factors There is very strong evidence that conditions that damage the heart, arteries or blood circulation all significantly affect a person's chances of developing dementia. These are known as cardiovascular risk factors. Among the Yoruba ethnic group, a study reveals that elderly participants with high blood pressure had an increased risk of dementia, compared to people with normal blood reading, high cholesterol and peripheral arterial disease was also associated with dementia. .Having cardiovascular disease or type 2 diabetes increases a person's risk of developing dementia by up to two times. These cardiovascular conditions are most strongly linked to vascular dementia. This is because vascular dementia is caused by problems with blood supply to the brain. Recent research suggests that many people with dementia have mixed dementia, or they have Alzheimer's disease with some vascular damage in the brain (Hall *et al.,* 2006).

2.6.3.5 Depression and other Conditions

People who have had periods of depression – whether in mid-life or later life – also seem to have increased rates of dementia. Whether depression is a risk factor that in part causes dementia is not clear, and the answer probably differs with age. There is some evidence that depression in middle age does lead to a higher dementia risk in older age. In contrast,

depression in later life, ie when a person is in their 60s or older, may be an early symptom of dementia rather than a risk factor for it.

Other medical conditions that can increase a person's chances of developing dementia include Parkinson's disease, multiple sclerosis and HIV. Down's syndrome and other learning disabilities also increase a person's risk of dementia. A number of other conditions have been linked to dementia in some studies, but evidence on them is still emerging. These conditions include chronic kidney disease, hearing loss, anxiety and sleep apnoea (where breathing stops for a few seconds or minutes during sleep). There is also growing evidence that loneliness and social isolation may increase someone's risk of dementia (Alain *et al.,* 2014).

2.6.3.6 Head injuries

A severe blow to the head – especially being knocked out – increases the risk of later dementia such as Alzheimer's disease. About a fifth of professional boxers go on to develop a different form of dementia. This used to be known as dementia pugilistica but is now known as chronic traumatic encephalopathy. This is thought to be caused by protein deposits formed in the brain as a result of head injury. Recent evidence suggests that professional American footballers, who often have repeat mild head injuries, may also be at risk of chronic traumatic encephalopathy (ADI, 2018).

2.6.3.7 Early life and Education

A study in Central African shows a correlation between losing a parent in early childhood and dementia, the impact of environmental factors such as birthweight and nutrition on dementia has not been reported. Association between education and dementia in sub-Saharan Africa is inconclusive, some studies observed association between low education and dementia, the association appears to be greater in women than men (ADI, 2018)

2.6.3.8 Lifestyle, environment and behavior

The Yoruba Nigerians whose diets are predominantly grains, vegetables and fish low in calories and fats were found to have low cholesterol and low incidence of AD when compared to African Americans. The findings on the association between dementia and alcohol in African population are mixed. Exposure to neurotoxins such as heavy metals in the environment has been reported to be a common experience of people living in developing countries, which may pose a potential risk and trigger increased incidence of dementia. (ADI, 2018). Some SSA studies have identified weak social network as a risk factor for AD, African communal way of living has been reported to have protective effect by keeping the brain active (ADI, 2018).

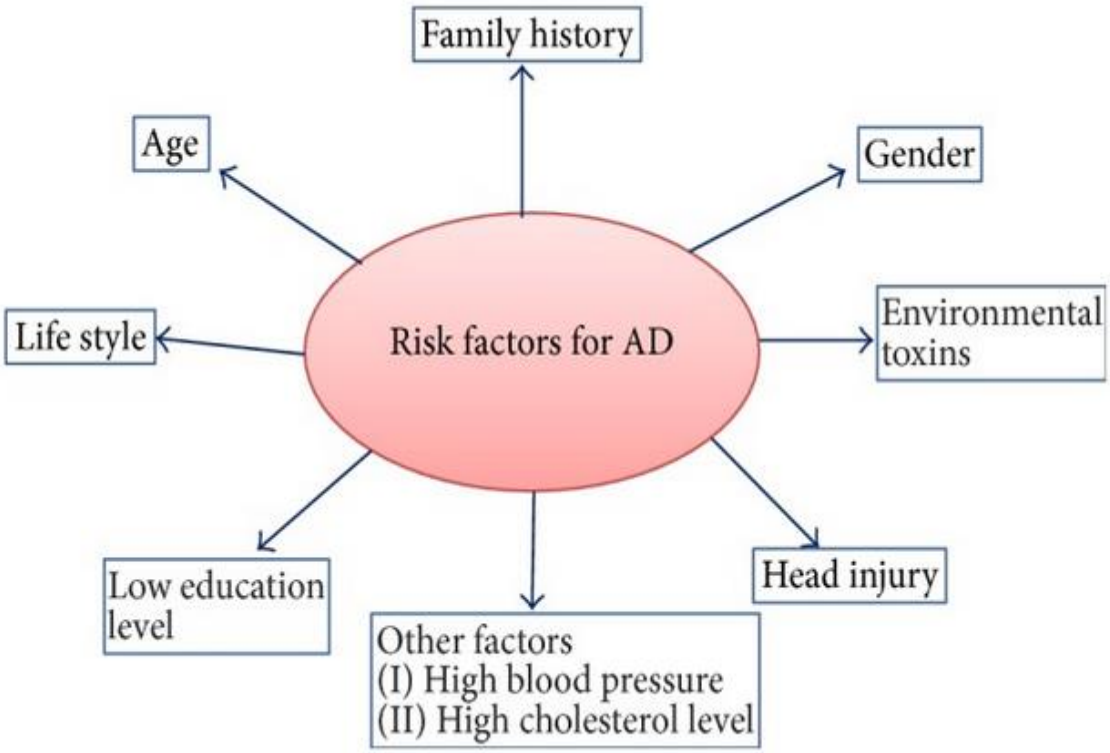


Figure 2.4: Risk factors for developing AD (Sandeep *et al.*, 2016)

2.7 Pathology and pathophysiology of dementia

The etiology of Alzheimer's disease (AD) as a neurodegenerative disorder is incompletely defined. The drugs currently available on provides momentary assistance with symptoms. Despite the ongoing extensive research on AD and the generation of several studies, the precise mechanism, disease course and cure still remain largely unknown. (Zamani *et al.*, 2019).

Marked atrophy, shrinkage of the gyri, broadened of sulci has been reported in the brain of AD patient, in most cases affecting every part of the cerebral cortex. However, the occipital lobe is often relatively spared. The cortical ribbon often become thinned with ventricular dilatation usually around the temporal horn as a result of the atrophy of the hippocampus and amygdala. There are series of ongoing studies on the pathology of AD using different animal models so as to obtain valuable information on the pathogenic mechanisms of AD. The pathology of AD can be divided into three; (a) positive lesions (b) negative lesions, and (c) inflammation and plasticity. Positive lesions are very common and readily detectable and they form the basis for diagnosis of AD. Negative lesions are difficult to evaluate, it involves neuronal and synaptic loss, their impact is directly related to cognitive deficit. The main pathological hallmarks of AD are the accumulation of senile plaques and neurofibrillary tangles, these features are also found in other neurodegenerative diseases and clinically normal individuals. Burnt out plaques and diffuse plaques are also found in the AD brains apart from senile plaques, burnt out plaques consist of an isolated dense amyloid plaque while diffuse plaques consist of poorly defined amyloid. The abnormal processing of the amyloid-β protein precursor along the amyloidogenic pathway has been reported to lead to the production of fragments, among which $A\beta_{42}$ peptide is the most toxic (Zamani *et al.*, 2019).

Alzheimer's disease (AD) concedes as progressive neurodegenerative disorder, the foremost cause of dementia in late adult life. Intracellular neurofibrillary tangles (NFTs) and extracellular amyloidal protein deposits as the senile plaques characterize it pathologically. Accumulations of Aβ are amyloid plaques in the brain parenchyma and in the cerebral blood vessels where it is known as congophilic angiopathy also known as cerebral amyloid angiopathy (CAA). NFTs formed the paired helical filaments with hyperphosphorylated tau proteins. These NFTs characterized by the neuronal and synaptic loss and some certain distinctive lesions. (Kumar and Singh, 2015).

Neuronal loss and/or pathology may be seen particularly in the hippocampus, amygdala, entorhinal cortex and the cortical association areas of the frontal, temporal and parietal cortices, but also with subcortical nuclei such as the serotonergic dorsal raphe, noradrenergic locus coeruleus, and the cholinergic basal nucleus. The deposition of tangles follows a defined pattern, starting from the trans-entorhinal cortex; consequently, the entorhinal cortex, the CA1 region of the hippocampus and then the cortical association areas, where frontal, parietal and temporal lobes are particularly affected. The extent and placement of tangle formation correlates well with the severity of dementia, much more so than numbers of amyloid plaques (Kumar and Singh, 2015).

One of the main pathological features of AD is the formation of senile plaques (SP), which is caused by amyloid beta (Aβ) deposition. Normally, Aβ are soluble small peptides, which are produced by the splitting of the precursor protein of amyloid (APP) by the action of α-secretase, β-secretase and γ-secretase. The imbalance between β-amyloid (Aβ) production and clearance leads to various types of toxic oligomeric, namely protofibrils, fibrils and plaques depending upon the extent of oligomerization. The reason of the formation of Aβ is still unclear, but the sequence, concentration and conditions of stability of Aβ are important

factors (Liu *et al.*, 2017). The pathophysiology of Alzheimer's disease is credited to a number of factors such as the cholinergic dysfunction, amyloid/tau toxicity and oxidative stress/mitochondrial dysfunctions (Mohamed and Shakeri, 2016)

Vascular risk factors (hypertension, hyperlipidemia, diabetes) and behavioral factors (obesity, physical inactivity) are associated with dementia (O'Donnell *et al*., 2010). Vascular risk factors may lead to cerebrovascular dysfunction through pathways mediated by β-amyloid and the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, a major source of vascular oxidative stress. Cerebrovascular dysfunction and BBB alterations may compromise the cerebral microenvironment and increase the vulnerability of regions critical for cognition to ischemic-hypoxic brain damage leading to neuronal dysfunction and cognitive deficits (Iadecola *et al.*, 2009).

2.7.1 Inflammation and dementia

Neuroinflammation is the presence of activated microglia and astrocytes leading to neuronal injury via the release of pro-inflammatory cytokines (Monte, 2017). Neuroinflammatory factors have been known to play significant role in the etiology of dementia such as AD (Mattson, 2004). Aβ peptide plays critical role in the neuroinflammation hypothesis of AD, Aβ accumulation results in increased levels of inflammatory molecules which are products of chronically activated glia resulting in neuronal damage which further induces glia activation leading to detrimental cycle of neuroinflammation and neurodegeneration (Griffin *et al*, 1998).Chronic inflammation amplifies the pathogenic processes through sustained NF-κB activation and increased pro-inflammatory cytokines, leading to insulin resistance or increased amyloid-β (Aβ) production and microglia activation and subsequently AD (Mihalea *et al.*, 2009).

2.8 Alzheimer's disease hypothesis

Several hypotheses have been formulated to explain the etiology of AD, Although αβ accumulation along with tau hyper-phosphorylation is the most proposed pathogenetic mechanism (Fuyuki, 2018) of a recent, mitochondrial cascade hypothesis has attracted much interest (Swerdlow, 2018). Amyloid cascade hypothesis proposes that, accumulation of amyloid beta induces the histologic, biochemical and clinical changes observable in AD patients (Hillen, 2019). Other hypotheses that are under debate includes: vascular hypothesis (Torre, 2010) tau hypothesis (Maccioni *et al.*, 2010) inflammatory hypothesis (Lamb, 2018) metal hypothesis (Tanzi, 2008) cell cycle hypothesis (Reddy, 2006) oxidative stress hypothesis (Christen, 2000) and cholesterol hypothesis (Gibson *et al.*, 2014)

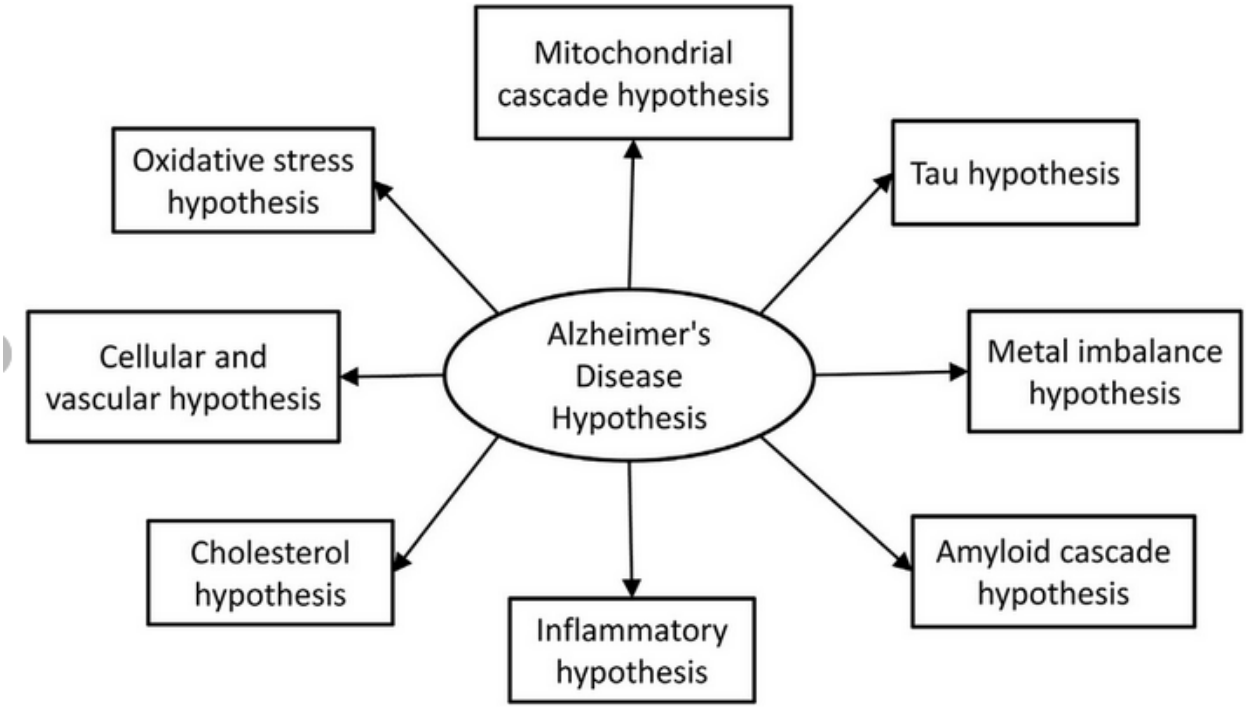


Figure 2.5: Alzheimer's disease hypothesis (Hroudová *et al.*, 2016)

AD and Type 2 Diabetes Mellitus (T2DM) are the two most common diseases among the aging population worldwide. Epidemiological studies reveal that people with T2DM stand a higher risk of developing AD. AD brains are reportedly less effective in glucose uptake thereby mimicking brain insulin resistance (Mudher, 2018).

2.9 Anatomy of the hippocampus

The hippocampus plays a major role in learning and memory, it is embedded deep into temporal lobe. It is a vulnerable structure that readily gets damaged by a variety of stimuli. Neurological and psychiatric disorders have been reported to readily affect it (Bahniwal *et al.,* 2017).

The hippocampus has three distinct zones: the dentate gyrus, the hippocampus proper, and the subiculum, the dentate gyrus and hippocampus proper form two C-shaped rings that interlock. The subiculum is thus a transition zone, linking the hippocampus proper with the dentate gyrus. (Bahniwal *et al*., 2017).

The Cornu Ammonis (CA) is a seahorse-like or ram's horn-like structure that describes the different layers of the hippocampus. There are four hippocampal subfields CA1, CA2, CA3, and CA4. CA3 and CA2 border the hilus of the dentate gyrus on either side. CA3 is the largest in the hippocampus and receives fibers from the dentate granule cells on their proximal dendrites (Daugherty *et al.,* 2016; Bahniwal *et al.,* 2017).

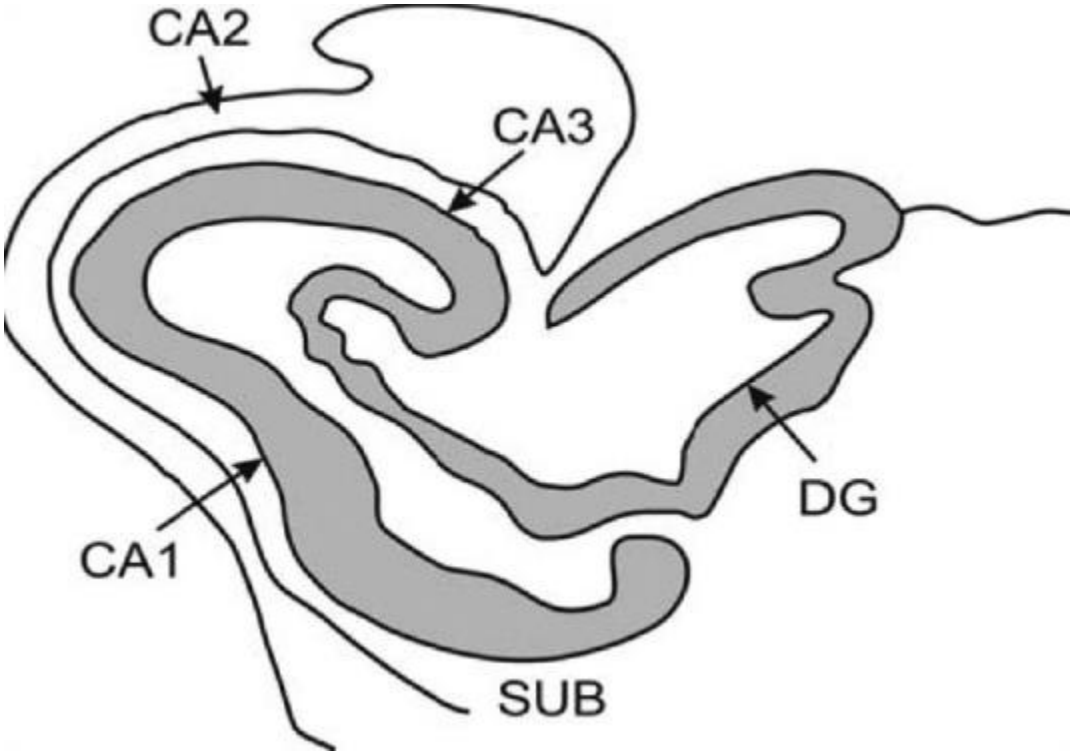


Figure 2.6: Structure of the hippocampus (Bahniwal *et al.*, 2017).

Embryology of the Hippocampus

The hippocampus originates in the isocortex as part of the fifth limbic lobe of the brain in the cerebral hemisphere's medial surface. It is also considered part of the olfactory cortex (Wang *et al.,* 2019). It is drawn to the temporal lobe by a strand of fibers called the fornix. Choroid fissure helps the choroid plexus invaginate into the lateral ventricle. The hippocampus itself is a mammalian innovation, while the isocortex as a whole is part of the phylogenetical ancient brain. The hippocampus is a deep structure hidden between the mesencephalon and the medial aspect of the temporal lobe. Three important changes are necessary for the complex shape and location of the hippocampus

i. Rotation of the lateral parts of the developing telencephalon dorsocaudally, then ventrally and rostrally, forming the parietal, occipital, and temporal lobes.

ii. The hippocampal sulcus then invaginates into the medial wall of the temporal lobe

iii. Finally, the hippocampal sulcus rotates along a longitudinal axis of the hippocampus, forming a complex structure that is present in the medial aspect of the temporal lobe.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Ethical approval

The approval for this research protocols was given by the University of Ilorin Ethical Review Committee (UERC) with approval number UERC/ASN/2018/1157

3.2 Animal acquisition and handling

Sixty-four (64) adult female Wistar rats were purchased from Ladoke Akintola University, Ogbomosho. These rats were acclimatized for fourteen days and accommodated in the animal House of the Faculty of Basic Medical Sciences, University of Ilorin, Nigeria. The rats had access to food and water *ad libitum* except at certain period of the experiment. Standard guidelines for animal handling as approved by University of Ilorin Ethical Review Committee (UERC) were followed.

3.3 Induction of insulin-resistance

To induce insulin- resistance, animals were fed with high-fat diet as previously described by Akinola *et al*., 2018 for eight weeks, and 30mg/kg STZ intraperitoneally (Zhang *et al.*,2008) at the end of 8 weeks. After the treatment, blood was withdrawn from the tail vein of the animals, and the blood glucose level was checked using a digital glucometer (Accu-Check, Roche, Belgium). Animals with fasting blood glucose concentrations not less than 200mg/mol were included in the study.

3.4 Animal grouping and administration of trans-cinnamaldehyde

The rats were randomly assigned into eight groups with the following treatment administered:

Group I (Normal control) received oral dose of olive oil throughout the experiment.

Group II (Insulin-resistant control) received HFD for eight weeks and 30 mg/kg of STZ (i.p)

Group III (TCA only 60mg/kg) received 60 mg/kg of TCA (oral) for four weeks (Haripriya and Vijayalakshmi, 2014).

Group IV (Insulin resistant-ND 60mg/kg) received HFD for eight weeks, 30 mg/kg of STZ (i.p) for four weeks, 60 mg/Kg of TCA for four weeks and normal diet after HFD administration was withdrawn.

Group V (Insulin- resistant-HFD 60mg/kg) received HFD for eight weeks, 30 mg/kg of STZ (i.p) for four weeks, and 60 mg/kg of TCA (oral) for four weeks.

Group VI (TCA only 40mg/kg) received 40 mg/kg of TCA (oral) for four weeks.

Group VII (Insulin-resistant-ND 40mg/kg) received HFD for eight weeks, 30 mg/kg of STZ (i.p) for four weeks, 40 mg/kg of TCA (oral) for four weeks and normal diet after HFD administration was withdrawn.

Group VIII (Insulin-resistant-HFD 40mg/kg) received HFD for eight weeks, 30 mg/kg of STZ (i.p) for four weeks, and 40 mg/kg of TCA (oral) for four weeks.

3.5 Measurement of Fasting Blood Glucose Levels

Blood was withdrawn from the tail vein of the animals, and the blood glucose level was checked using a digital glucometer (Accu-Check Roche, Belgium). Animals with fasting blood glucose concentrations of at least 200mg/mol were included in the study.

Measurement of Body Weight

Body weights of the rats were taken fortnightly using an electronic balance (SF-400, China).

3.6 Behavioural Tests for Cognitive Function

3.6.1 Y-Maze Test

Short-term spatial memory was assessed in rats using Y- maze apparatus. The Y-maze apparatus, made of wood, is shaped like a Y, with three identical arms labelled A, B and C with an angle of 120° between each pair of arms (Rasoolijazi *et al.,* 2013). Each of the arm is 40 cm long, 30 cm high, and 15 cm wide. Each animal was set out at the end of one arm and was then allowed to move freely inside the maze. When the base of the animal's tail was completely placed in the arm, each arm entrance was recorded visually (Nittaa, 2002).

3.6.2 Morris Water Maze

After the TCA treatment, the spatial learning and memory of rats were evaluated using Morris water maze. A circular pool of 150cm in diameter and 60cm high was filled with water (25± 2^{o}C) to a height of 40 cm. The pool was divided into four equal quadrants; North, South, East, and West. A transparent escape platform (10 cm in diameter) was hidden 2 cm below the surface of the water at a fixed location in one of the quadrants to ensure being invisible to the rats but high enough for the rats to stand on it. Rats were trained once in a day for three days. Following the training, milk was added to the water to render it opaque, rats were gently put into the water and were given 60 s to freely search for the platform. Rats that found the platform in allotted time were allowed to stay on the platform for another 15s, while those who failed to detect the platform in 60 s were guided to the destination and also allowed to stay for 15 s. The time required for reaching the platform (escape latency), was recorded and measured by a video tracking system.

3.7 Collection of Brain Samples

The following day after the behavioural studies, rats were sacrificed, hippocampus was then excised, weighted and then rinsed in 0.25 M sucrose 3 times for 5 mins each and placed in 30% sucrose in which they were stored at 4°C.

3.8 Biochemical Assays

Concentrations of tumor necrotic factor-alpha (TNF-α), nuclear factor kappa b(NFk-B) and insulin in the hippocampus were determined using rat ELISA kits (Diaclone, London, UK or (eBioscience, USA), according to the manufacturer's protocol.

3.8.1 Homogenate

After behavioural tests (Morris Water Maze and Y Maze), animals were anaesthetized with ketamine and sacrificed. The skull was opened up and the hippocampus was harvested, weighed, and then kept in ice before being transferred into the freezer at 20°C in a Phosphate Buffer Saline (PBS) of volume 4 times the brain weight before homogenization. The homogenates were centrifuged, the pellet was discarded and the supernatants were immediately separated in to various portions for ELISA assays.

3.8.2 Estimation of Brain Tumour Necrosis Factor-alpha (TNF-α)

Hippocampal TNF-α concentration was estimated using ELISA MAX^{TM} Deluxe kit (BioLegend, USA) according to the manufacturer's instructions. All the samples, reagents and standard solutions were kept in room temperature before use. Briefly, TNF-α enzyme immunoassay was done by adding 100 µL of hippocampal sample, standards and controls to each wells of an overnight (18hr, 4°C) mouse TNF-α capture antibody incubated 96 well plate. After which, plate was sealed with adhesive foil and incubated for 2 hours at room temperature (25°C) on a shaker (approx. 500 rpm). Then, 100 µL of biotinylated goat polyclonal anti-mouse TNF-α detection antibody and avidin-horseradish peroxidase (avidin-

HRP) solutions were added to each wells; plate was sealed and incubated for 1hr. 3min. at room temperature (25°C) on a shaker (approx. 500 rpm). Thereafter, 100 µL of the chromogenic substrate [3, 3′, 5, 5′- tetramethy lbenzidine (TMB) was added to each well and incubated in the dark for 15min at room temperature (25°C) before the addition of stop solution (100 µL) and the absorbance was read at 450nm within 15min using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional microplate reader equipped with Softmax Pro v 5.4 (SMP 5.4). Thereafter, a log-log logistic 4-parameter curve-fitting was used to determine the hippocampal concentrations of TNF- α in pg/mL.

3.8.3 Estimation of Hippocampal Nuclear Factor-kappa B (NF-kB)

The Nf-kB concentrations were estimated in the hippocampus using ELISA MAX™ Deluxe kit (BioLegend, USA) according to the manufacturer's instructions. All reagents, standard solutions and samples were brought to room temperature before use. Nf-kB enzyme immunoassay was carried out by adding 100µL of standards, control and hippocampal samples to each wells of an overnight (18hr, 4°C) mouse Nf-kB capture antibody incubated 96 well plate. After which, microplate was sealed with adhesive foil and incubated for 2hr room temperature (25°C) on a shaker (approx. 500 rpm). Then, biotinylated rat monoclonal anti-mouse Nf-kB detection antibody (100µL) and avidin-HRP (100µL) solutions were added to each wells, and plates were sealed and incubated for 1hr 3min at room temperature (25°C) on a shaker (approx. 500rpm). Thereafter, 100µL of the chromogenic substrate (TMB) was added to each well and incubated in the dark for 20min at room temperature (25°C). The absorbance was read at 450nm within 15min using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional microplate reader equipped with Softmax Pro v 5.4 (SMP 5.4), after the addition of 100µL of stop solution on a shaker to achieve homogenous solutions. A log-log logistic 4-parameter curve-fitting was used to determine the concentration of Nf-kB in the hippocampus in pg/mL.

3.8.4 Estimation of Hippocampal Insulin Concentration

Hippocampal insulin concentration was estimated using ELISA MAX™ Deluxe kit (BioLegend, USA) according to the manufacturer's instructions. All the samples, reagents and standard solutions were kept in room temperature before use. Briefly, insulin immunoassay was done by adding 100 µL of hippocampal sample, standards and controls to each wells of an overnight (18hr, 4°C) mouse insulin capture antibody incubated 96 well plate. After which, plate was sealed with adhesive foil and incubated for 2 hours at room temperature (25°C) on a shaker (approx. 500 rpm). Then, 100 µL of biotinylated goat polyclonal anti-mouse insulin detection antibody and avidin-horseradish peroxidase (avidin-HRP) solutions were added to each wells; plate was sealed and incubated for 1hr. 3min. at room temperature (25°C) on a shaker (approx. 500 rpm). Thereafter, 100 µL of the chromogenic substrate [3, 3′, 5, 5′- tetramethy lbenzidine (TMB) was added to each well and incubated in the dark for 15min at room temperature (25°C) before the addition of stop solution (100 µL) and the absorbance was read at 450nm within 15min using Spectramax M-5 (Molecular Devices, Sunnyvale, CA) multifunctional microplate reader equipped with Softmax Pro v 5.4 (SMP 5.4). Thereafter, a log-log logistic 4-parameter curve-fitting was used to determine the hippocampal concentrations of insulin in pg/mL.

Calculation of HOMA-IR

HOMA-IR was calculated using the formula = (glucose in mmol/L x insulin in mIU/mL)/22.5.

3.9 Histopathological Examinations

3.9.1 Preparation of Brain Tissues for Histology and Immunohistochemistry

Animals were anaesthetized with ether and perfused transcardially with sterile Phosphate Buffered Saline (PBS). Then, the rats were dissected, flushed with normal saline and perfused

with 10% buffered formaldehyde. Thereafter, their brains were harvested and fixed with 10% phosphate buffered formaldehyde. The brains were then subjected to the routine method for paraffin wax embedment to obtain paraffin wax embedded tissue blocks.

3.9.2 Staining techniques

3.9.2.1 Haematoxylin and Eosin

This technique was meant to reveal the normal histoarchitecture of the hippocampus. It was also used for stereological analysis of the cells. (Pearse, 1980).

3.9.2.2 Protocol for Haematoxylin and Eosin Staining of the Brain section

Slides containing paraffin sections were placed in a slide holder and deparaffinization and rehydration of sections were done in the following reagents respectively: 3 times for 3 mins in Xylene, 3 times for 3 mins 1:1 Xylene with 100% ethanol, 1 time for 3 mins in 95% ethanol, 1 time for 3 mins in 80% ethanol and then 1 time for 5 mins in deionized H_2O (excess water was blotted from the slide holder before taking them into haematoxylin). Subsequently, haematoxylin staining was done with the following procedure: 1 time for 3 mins haematoxylin, 1 time for 5 mins in tap water (to allow stain to develop), slides were dipped 12 times in (fast) acid ethanol to destain), rinsed 2 times for 1 mins of tap water, then rinsed 1 time for 2 mins in deionized water (and left overnight). Excess water was blotted from slide holder before going into eosin.

For eosin staining and dehydraytion, the following procedures were followed: slides were placed 1 time for 30 sec in eosin (up to 45 sec for older batch of eosin) then 3 times for 5 mins in 95% ethanol, followed by 3 times for 5 mins in 100% ethanol (blotted excess ethanol before going into xylene) and then 3 times for 15 mins in xylene. Following this, slides were cover slipped using distrene plasticizer in xylene (DPX) as mountant (one drop of DPX was

placed on the slide using a glass rod, taking care to leave no bubbles). Coverslips were angled to let them fall gently on the slide. Slides were then dried overnight in the hood.

3.9.2.3 Tissue staining with Nissl Stain

Procedure for Nissl stain in brain samples (Hippocampus, prefrontal cortex and striatum) Sections were taken to water, followed by staining in 1% of Cresyl fast violet for 30mins. After which it was then rinsed in water; differentiation was done by using 70% alcohol until the stained section appeared pale. Differentiation was controlled by observation through the microscope. Dehydration in absolute alcohol and then cleared in xylene. This was followed by mounting in DPX (Wolfgang, 2003). The sections were viewed under a light microscope.

3.9.2.4 Immunohistochemical study of the hippocampus

Immunohistochemistry of Beta-amyloid, GFAP, and NeuN

The immunohistochemistry method was used to quantify the level of beta-amyloid, GFAP and NeuN using the beta-amyloid, GFAP and NeuN kits (Santa Cruz, Germany) according to the manufacturer's instructions and modified method of Edelstein *et.al.*. (2014).

Briefly, brain tissue section hippocampus was subjected to the process of deparaffinization and hydration using xylene and graded alcohols (100, 90 and 80%) for 5 min, respectively. The slides were then washed twice with distilled water and incubated with peroxidase block for 5-10 min at room temperature (25°C). Thereafter, tissue sections were rinsed with distilled water, placed in citrate buffer tank and heated in a water bath for 3-5 min for antigen retrieval. Slides were washed with phosphate buffer saline (PBS) containing 0.02% Tween 20 thrice, before adding protein blocking solution for 5-10 min at room temperature (25°C). Tissue sections were incubated with primary antibody (1:300) for 20-30 min at room temperature (25°C). Slides were then washed with PBS 5-7 times and incubated with one-step horseradish peroxidase (HRP) polymer for 20-30 min at room temperature (25°C). Also,

tissue sections were rinsed 5-7 times with PBS containing 0.02% Tween 20 and 2-3 times with distilled water. Few drops of ready to use 3, 3′- diaminobenzidine (DAB) reagent was added on each tissue sections and allowed to incubate for 6-10 min at room temperature (25°C) before washing with PBS 5-7 times and then with distilled water. Then, slides were incubated with hematoxylin for 30-60 s, rinsed with distilled water and allowed to drain before mounting with appropriate mountant.

3.10 Photomicrography

Stained tissue sections were viewed under a light binocular microscope (Olympus, USA) and images were captured with an Amscope camera (MD 500).

3.11 Morphometric analysis

Morphometric analysis of the tissue was done using Image J software (NIH, USA) and plugins to analyse cell count of neuron on the photomicrographs.

3.12 Statistical Analysis

All quantitative data were analysed using GraphPad (Version 6) and SPSS (Version 20) All the biochemical parameter outcomes were analysed with One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. Significance was set at $p < 0.05$ (95% confidence interval). The results were represented in bar charts with error bars to show the mean and standard error of mean respectively.

CHAPTER FOUR

RESULTS

4.1 Body weight changes

Body weight changes in treated and control rats were monitored and the average weight of animals in each group were compared at the end of the treatment.

Results from figure 4.1 show that there was an increase in body weight among HFD fed animals. The weight increase was not significantly high partly because of STZ associated weight loss and the age of the rats.

Body weight

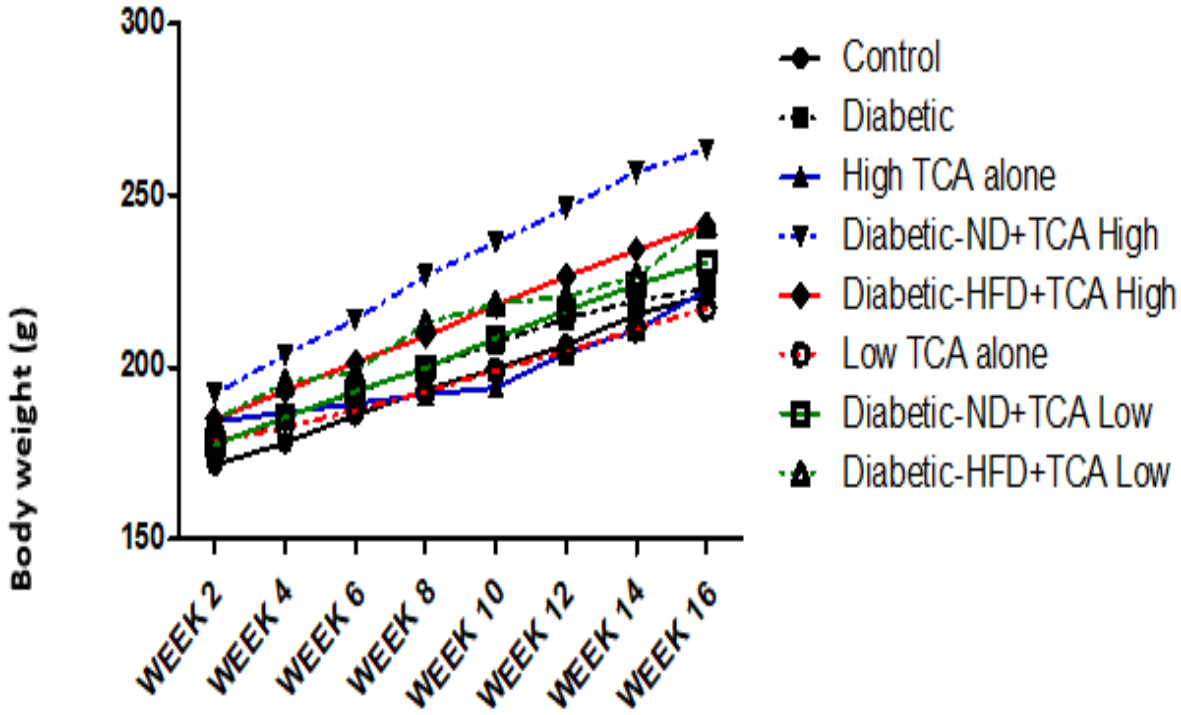


Figure 4.1: Showing body weight changes with high fat and normal diet feeding.

Values are expressed as mean ± SEM (n = 5 per group; * = statistical significance; $p < 0.05$; TCA=Trans-cinnamaldehyde; ND=Normal Diet; HFD=High Fat Diet)

HFD increases body weight among the treated groups

4.2 Fasting blood glucose level

Administration of HFD/STZ significantly increased the blood glucose level of the treated groups when compared to the control.

4.2.1 Blood glucose level after HFD and STZ administration in insulin-resistant rats

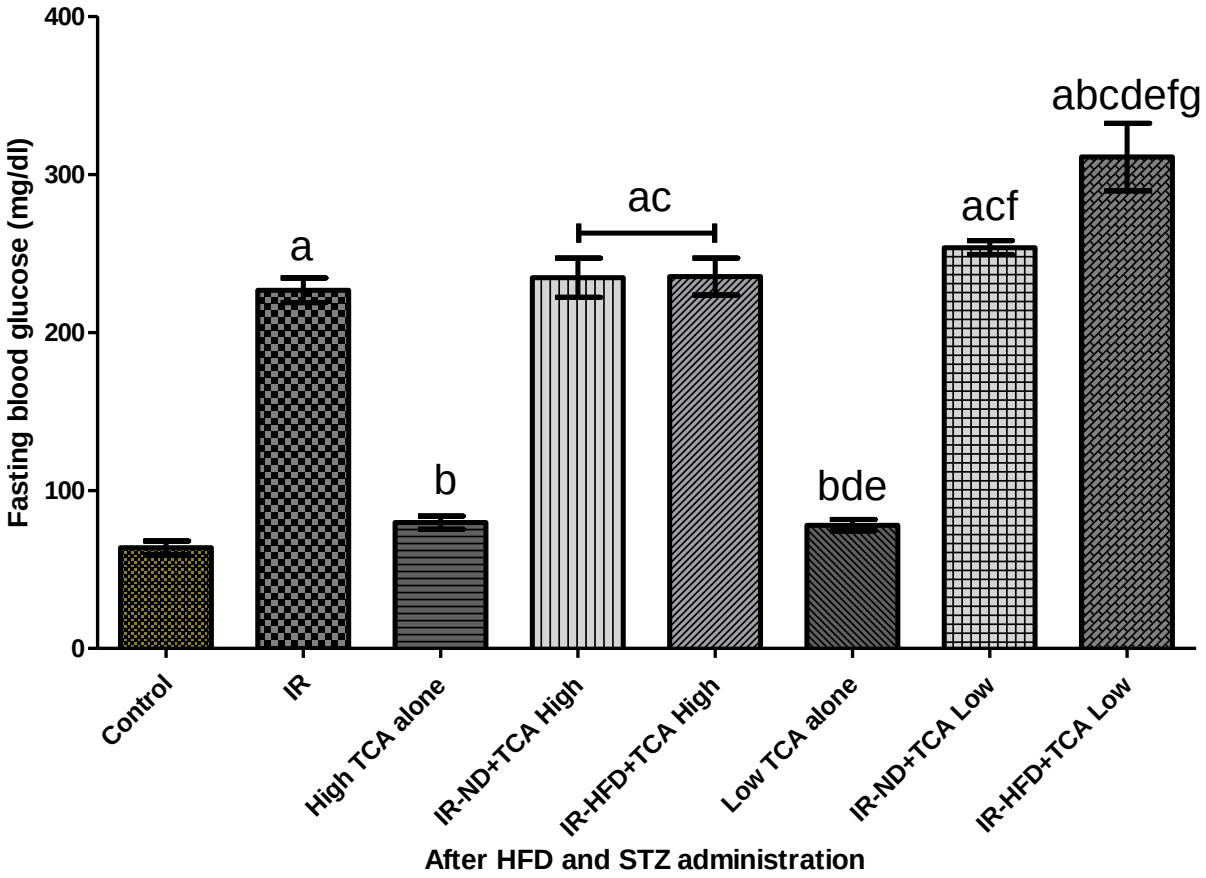


Figure 4.2: Fasting blood glucose level (After HFD and STZ)

Values are expressed as mean ± SEM. n = 5 per group; the superscript alphabets above the bars represents significance difference with other groups. [a] represent difference with control; [b] represent difference with insulin resistant control; [c] represent difference with high TCA; [d] represent difference with IR-ND+TCA high; [e] represent difference with IR-HFD+TCA high; [f] represent difference with low TCA alone. TCA=Trans-cinnamaldehyde;IR=insulin-resistant; ND=Normal Diet; HFD=High Fat Diet)

4.2.2 Blood glucose level after TCA treatment

TCA treatment significantly reduces blood glucose of the normal control and group ($p < 0.05$) (119±4.9 mg/dl) when compared to the untreated rats (217±10 mg/dl)

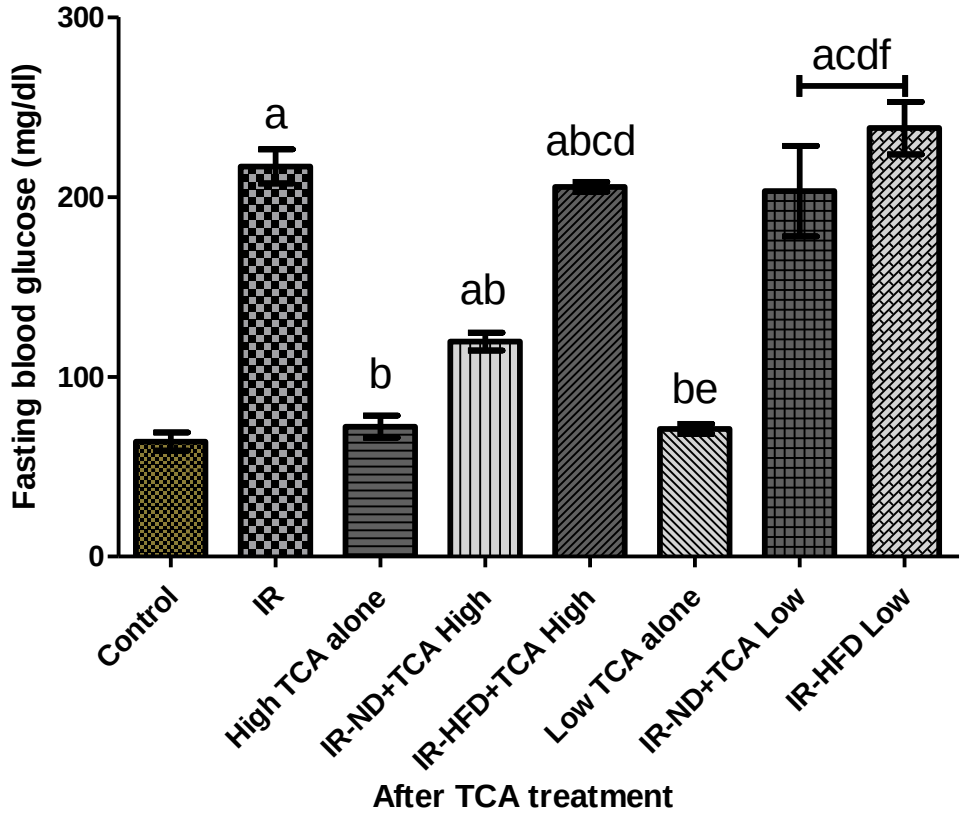


Figure 4.3: Blood glucose after TCA intervention

The superscript alphabets above the bars represents significance difference with other groups.[a] significantly different from control;[b] significantly different from IR control ;[c] significantly different from high TCA alone;[d] significantly different from IR-ND+TCA high ;[e] significantly different from IR-HFD+TCA high ;[f] significantly different from low TCA alone

Values are expressed as mean ± SEM (n = 5 per group; * = statistical significance; p < 0.05; TCA=Trans-cinnamaldehyde; ND=Normal Diet; HFD=High Fat Diet)

4.3 Behavioural Tests for Cognitive Functions

4. 3.1 Morris Water Maze

The IR groups show increased escape latency (38.17±1.302)**.** When compared to normal control (22.33±1.022) however TCA treatment at both high and low doses significantly reduce the escape latency when compared to insulin-resistant control group; IR-ND+TCA low (35.50±0.8466); High TCA alone (12.17±0.7032), IR-ND+TCA High (27.67±1.358), IR-HFD+TCA High (26.67±1.229), Low TCA alone (20.67±1.256) and IR-ND+TCA Low (22.83±1.600)

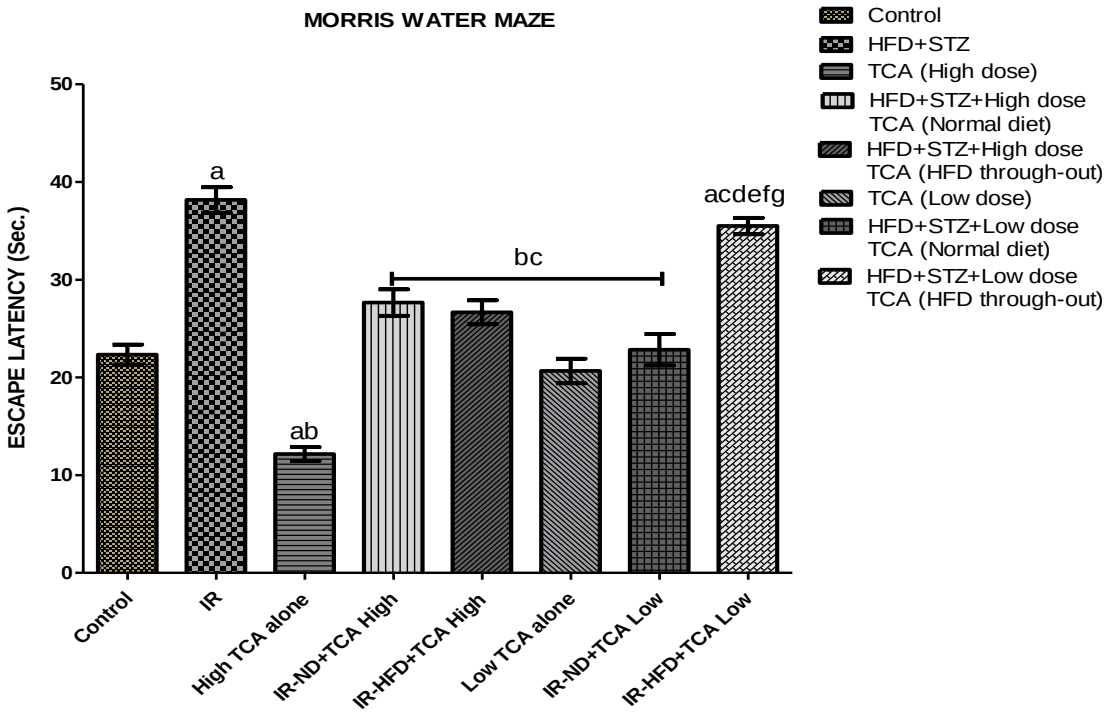


Figure 4.4: Rat escape latency using Morris water maze performance.

The superscript alphabets above the bars represents significance difference with other groups.

Values are mean ± SEM of data obtained. [a] significantly different from control; b significantly different from IR; [c] significantly different from High TCA alone ;[d] significantly different from IR-ND+TCA High; [e] significantly different from IR-HFD+TCA High;[f] significantly different from low TCA; [g] significantly different from IR-ND+TCA low $P < 0.05$.

Escape latency in Morris water maze performance for working memory.

4.3.2 Y-maze Test

Percentage of alternation**:** the mean latency for group Insulin- resistant group (39.67±1.453), IR-ND+TCA High (71.67±2.028), IR-ND+TCA Low (48.67±2.028) and IR-HFD+TCA Low (22.00±1.732) were significantly different from control (57.67±2.028), that of High TCA alone (57.67±1.453), IR-HFD+TCA High (61.00±1.732) and TCA alone Low (56.00±2.082) were significantly different from insulin-resistant group.

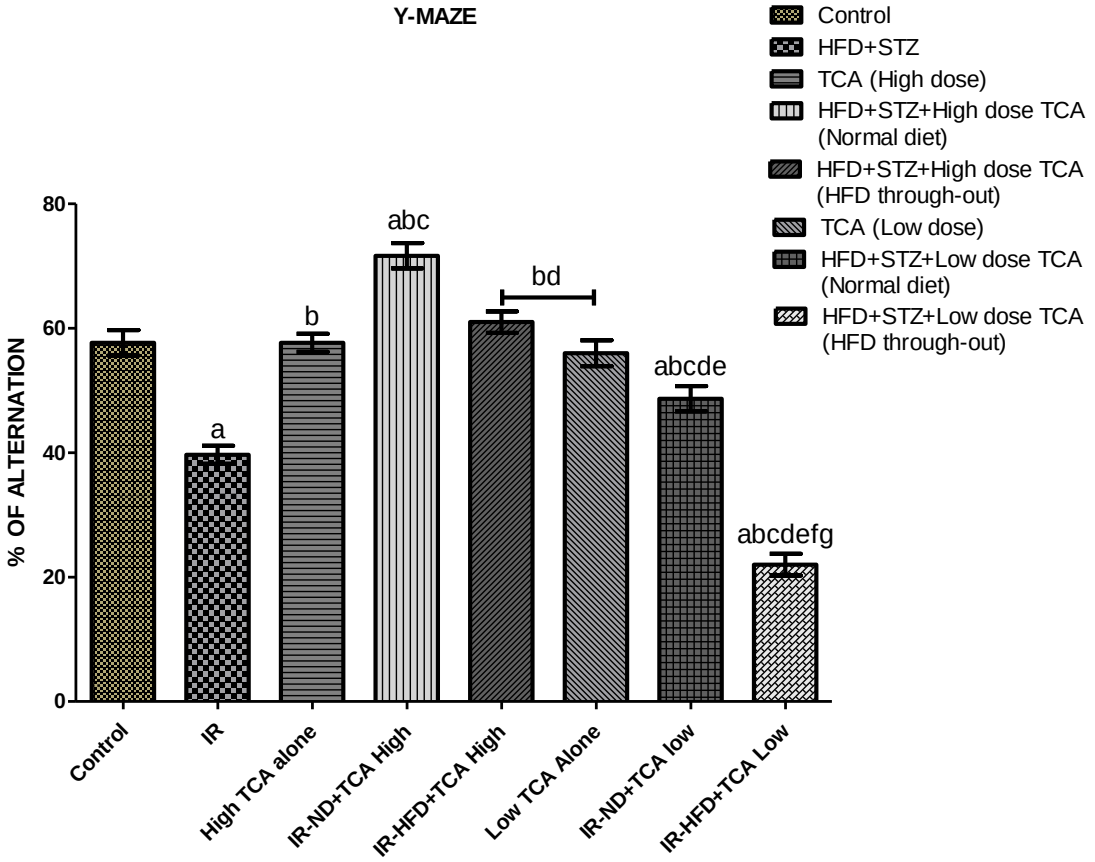


Figure 4.5: The percentage of alternation using Y-maze test performance.

The superscript alphabets above the bars represents significance difference with other groups.

Values are mean ± SEM of data obtained **[a]** significantly different from control;**[b]** significantly different from IR; **[c]** significantly different from High TCA alone;**[d]** significantly different from IR-ND+TCA High; **e**= significantly different from IR-HFD+TCA High;**[f]** significantly different from Low TCA alone; **[g]** significantly different from IR-ND+TCA Low $P < 0.05$.

4.4 Changes inflammatory markers

4.4.1 Changes in the level of tumor necrotic factor-α

TCA administration significantly suppresses neuroinflammation among the treated groups when compared to the insulin resistant control group.

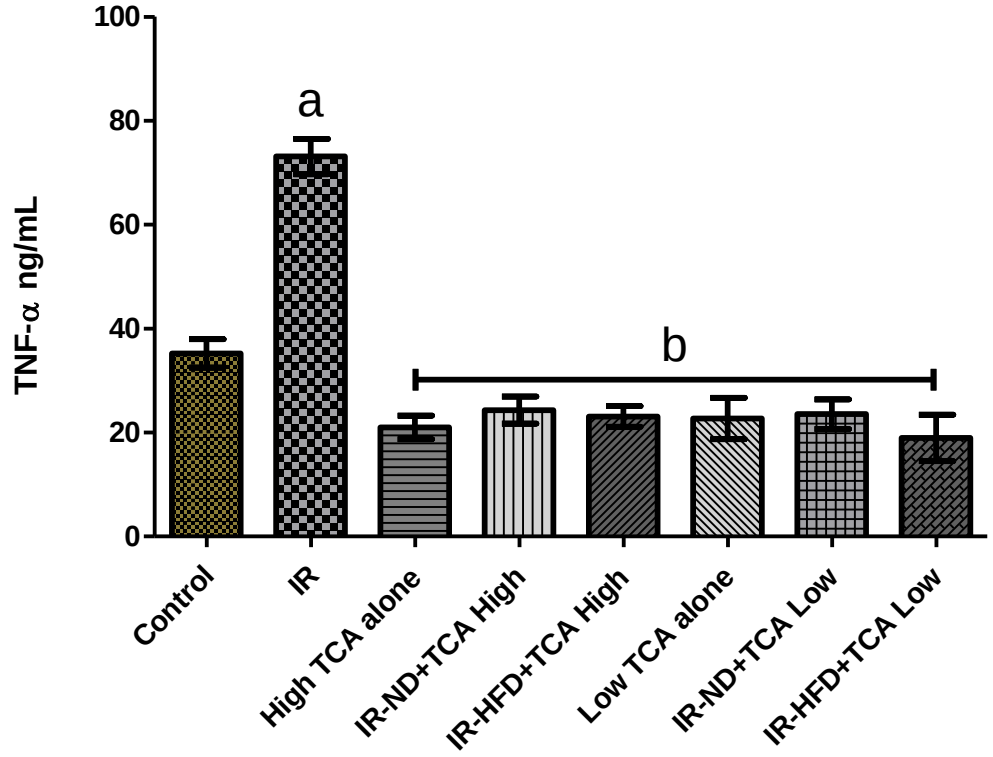


Figure 4.6: Hippocampal level of TNF-α

The superscript alphabets above the bars represents significance difference with other groups

[a] significantly different from control;[b] significantly different from IR

Values are expressed as mean ± SEM (n = 5 per group; * = statistical significance; $p < 0.05$; TCA=Trans-cinnamaldehyde; ND=Normal Diet; HFD=High Fat Diet; IR= Insulin resistant)

4.4.2 Changes in the level of nuclear factor kapa b

TCA treatment at high and low doses significantly reduced the activities of nuclear factor kappa b in a dose dependent manner.

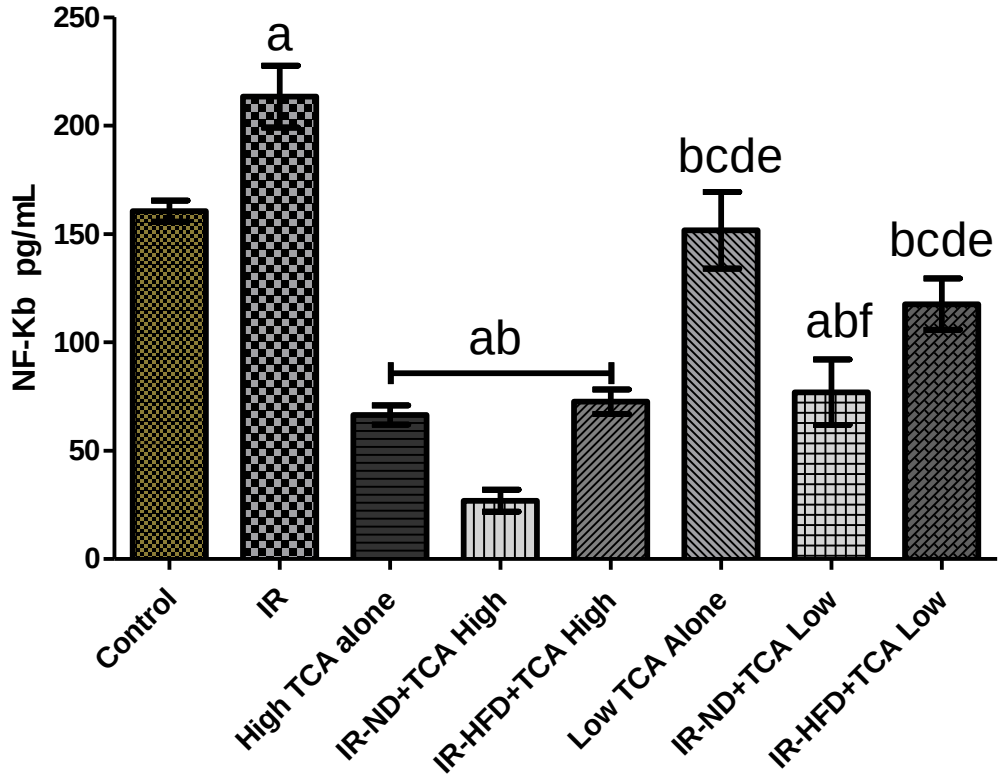


Figure 4.7: Hippocampal level of NF-Kb

The superscript alphabets above the bars represents significance difference with other groups

[a] significantly different from control group; [b] significantly different from IR group;

[c] significantly different from High TCA alone;[d] significantly different from IR-ND+TCA High;[e] significantly different from IR-HFD+TCA High ;[f] significantly different from Low TCA alone

Values are expressed as mean ± SEM (n = 5 per group; * = statistical significance; $p < 0.05$; TCA=Trans-cinnamaldehyde; ND=Normal Diet; HFD=High Fat Diet; IR=Insulin resistant)

4.5 Variation in serum insulin level

TCA intervention significantly ameliorates hyperinsulinemic condition among treated groups when compared to the insulin resistant control.

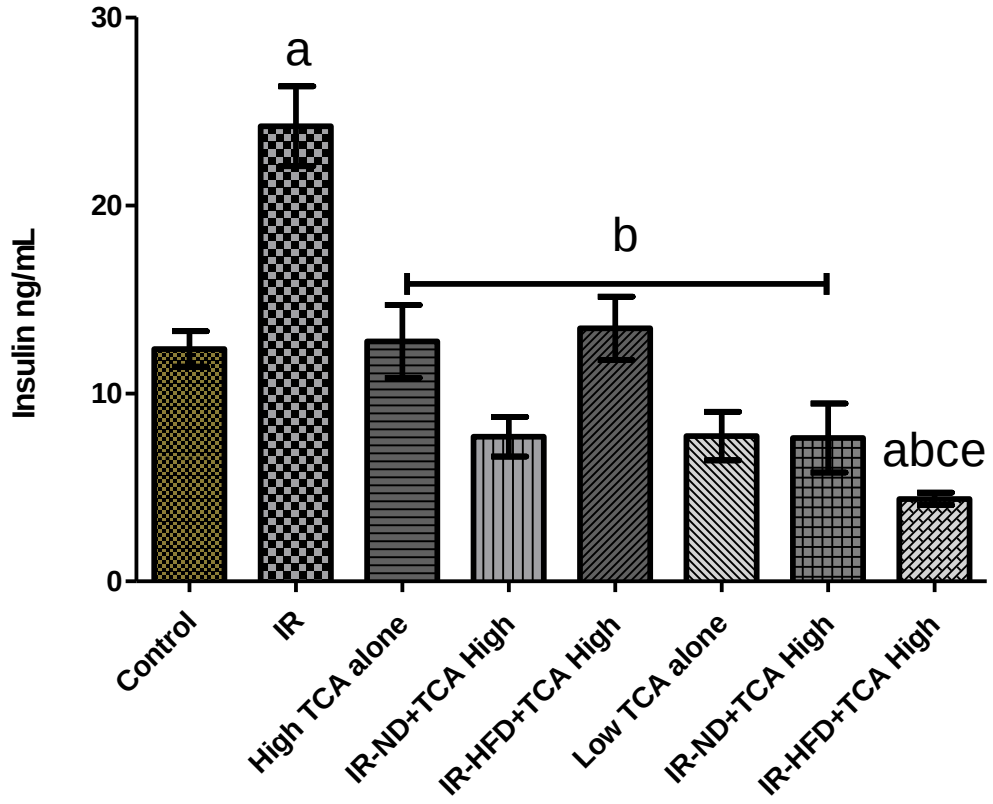


Figure 4.8: Serum insulin levels

The superscript alphabets above the bars represents significance difference with other groups

[a] significantly different from control;[b] significantly different from IR group;[c] significantly different from High TCA alone;[e] significantly different from IR-HFD high TCA

Values are expressed as mean ± SEM (n = 5 per group; $p < 0.05$; TCA=Trans-cinnamaldehyde; ND=Normal Diet; HFD=High Fat Diet; IR=insulin resistant)

4.6 Brain weight

4.6.1 Hippocampal weight

Mild hippocampal atrophy was observed among the insulin-resistant control group; however, TCA treatment restores the insulin- resistance induced hippocampal atrophy.

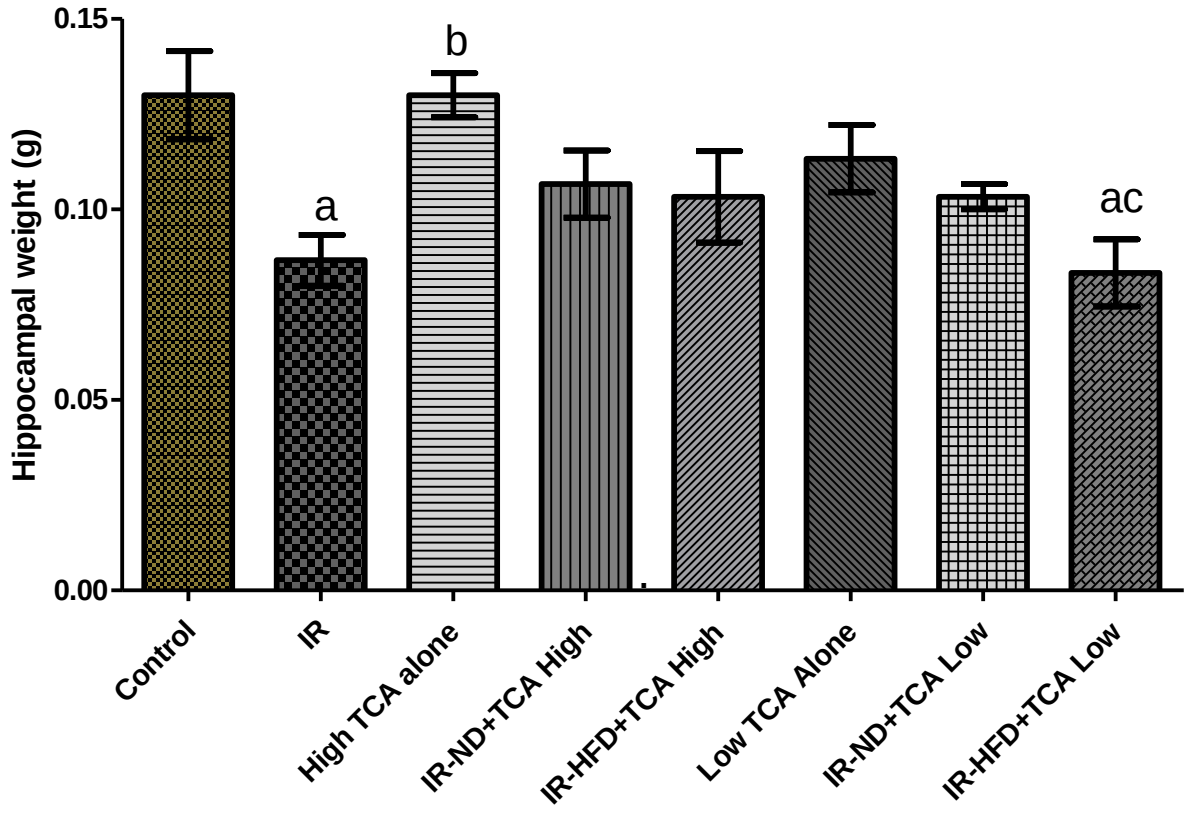


Figure 4.9: Hippocampal weight

The superscript alphabets above the bars represents significance difference with other groups

[a] significantly different from control;[b] significantly different from diabetic group;[c] significantly different from High TCA alone

Values are expressed as mean ± SEM (n = 5 per group; $p < 0.05$; TCA=Trans-cinnamaldehyde; ND=Normal Diet; HFD=High Fat Diet)

4.6.2 Relative organ weight (Brain/Hippocampus)

The percentage ratio of hippocampus to the entire brain was significantly reduced among the insulin-resistant control and the group that received high fat diet with low dose TCA

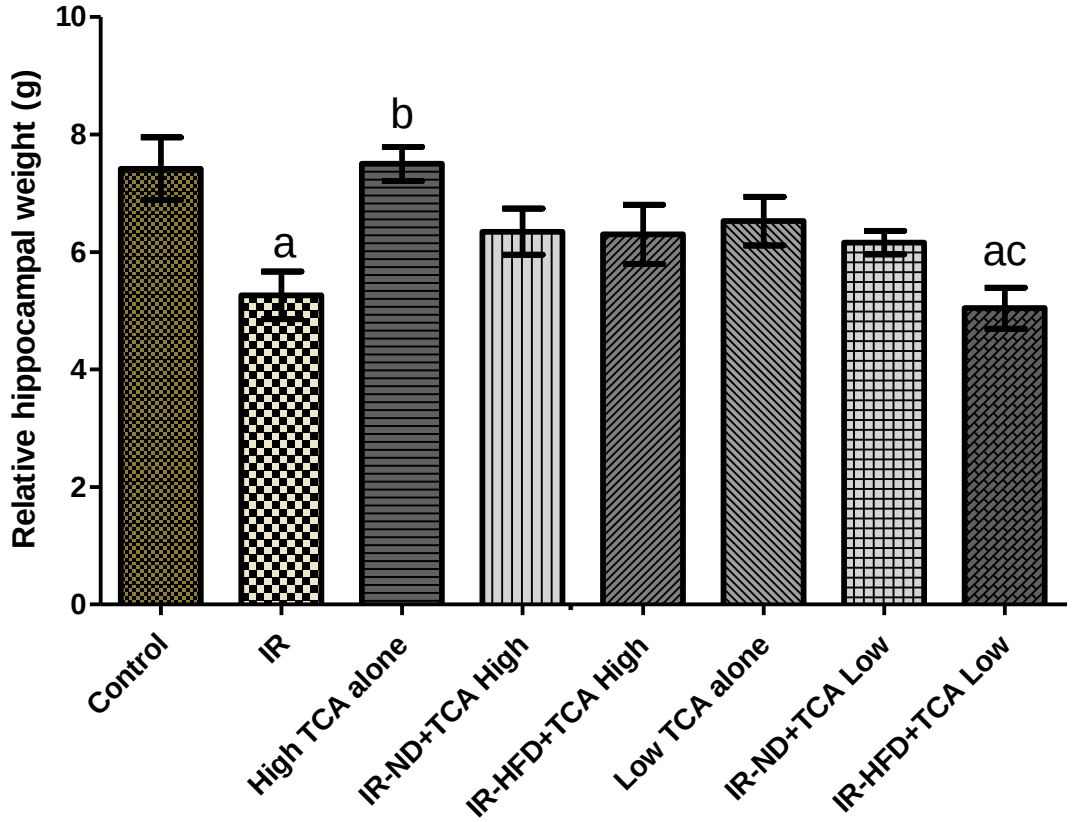


Figure 4.10: Relative organ weight (Brain/Hippocampus)

The superscript alphabets above the bars represents significance difference with other groups

[a] significantly different from control group;[b] significantly different from IR group;

[c] significantly different from High TCA alone

Values are expressed as mean ± SEM (n = 5 per group; $p < 0.05$; TCA=Trans-cinnamaldehyde; ND=Normal Diet; HFD=High Fat Diet; IR= Insulin resistant)

4.7 HOMA-IR

HOMA-IR was significantly increased among the insulin-resistant control group, however TCA intervention at high and low doses significantly reduced the trend.

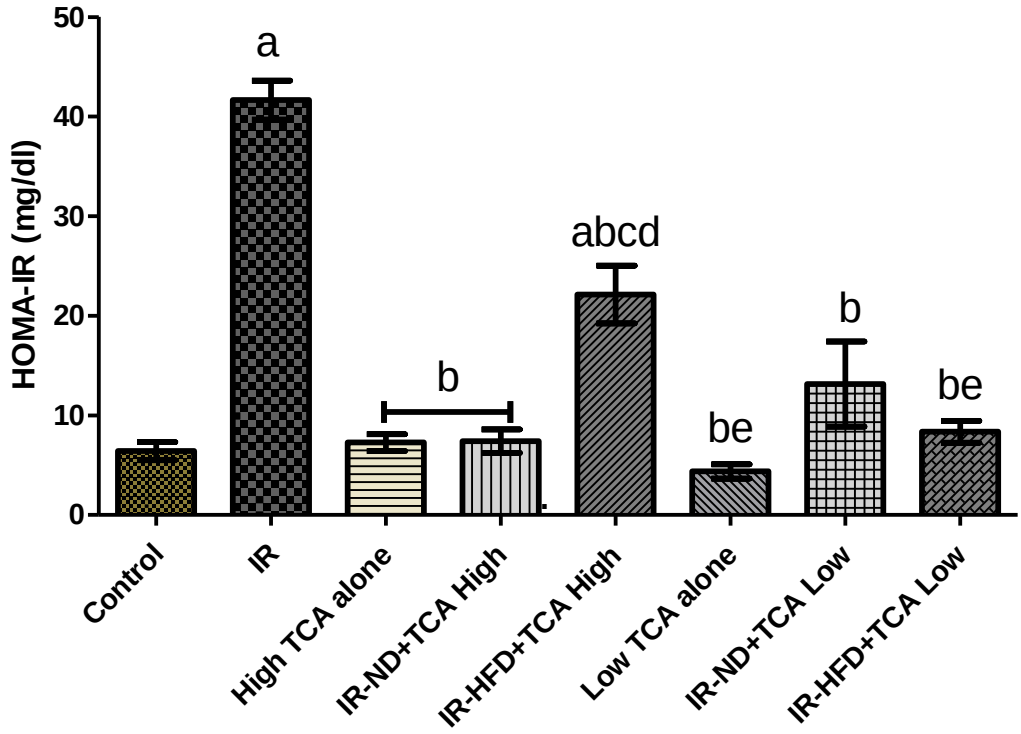


Figure 4.11: HOMA-IR

The superscript alphabets above the bars represents significance difference with other groups

[a] significantly different from control group;[b] significantly different from IR group;[c] significantly different from High TCA alone; [e] significantly different from Diabetic-HFD+TCA High

Values are expressed as mean ± SEM (n = 5 per group; $p < 0.05$; TCA=Trans-cinnamaldehyde; ND=Normal Diet; HFD=High Fat Diet

4.8 Histological observation of the hippocampus

4.8.1 Haematoxylin and Eosin stain observation

Representative micrographs of H & E staining showing the general cytoarchitecture of the Hippocampus in wistar rats. Normal histological features of the hippocampus were observable in the control and TCA treated groups. The histology of these two groups presents normal neuronal layers (yellow arrows) with well-organized cells. In contrast, the HFD/STZ treated groups was characterized by various degenerative changes with pyknotic nuclei (red arrows), however the cyto-protective property of TCA was evident in the HFD/STZ+ TCA groups with organized cellular layers an indication of restoration of neuronal cytoarchitecture.

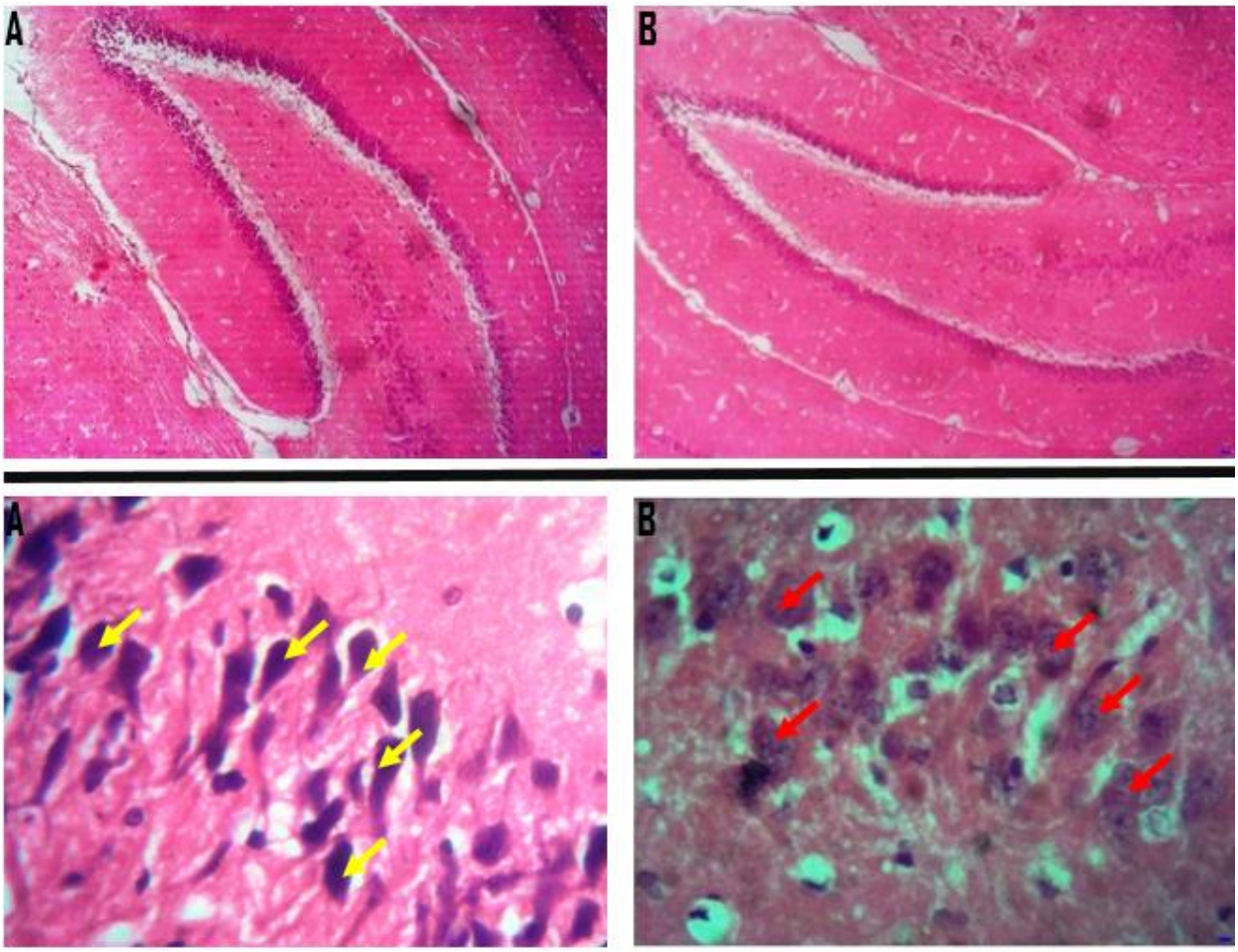


Figure 4.12a&b: **(control group), the tissue presents normal neuronal layers (yellow arrows) with well-organized cells. In contrast, the insulin resistant group (Fig 4.12 B) was characterized by various degenerative changes with pyknotic nuclei (red arrows), reduced layer of neuronal cell and neuronal vacuolation; H&E Scale bar: 450µm (A &B top) and 45 µm (A &B below)**

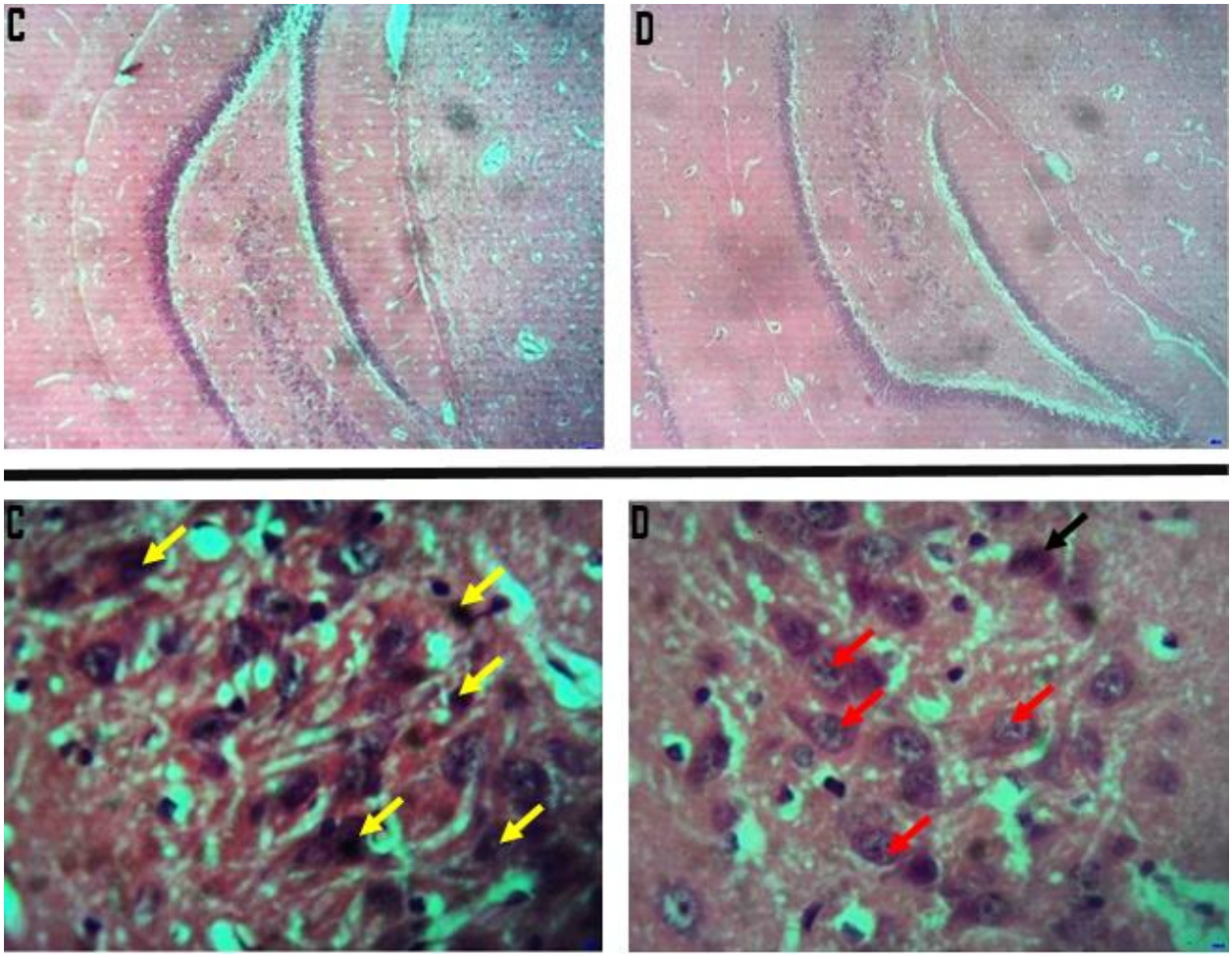


Figure 4.12 c&d: **Normal histological features of the hippocampus were observable in the high TCA alone treated group (Fig. 4.12C) with normal neuronal layers (yellow arrows) composed of well-organized cells. Treatment with high dose TCA shows some organized neuronal cell layer, an indication of the cytoprotective property of TCA. H&E; Scale bar: 450µm (C & D top) and 45 µm (C &D below)**

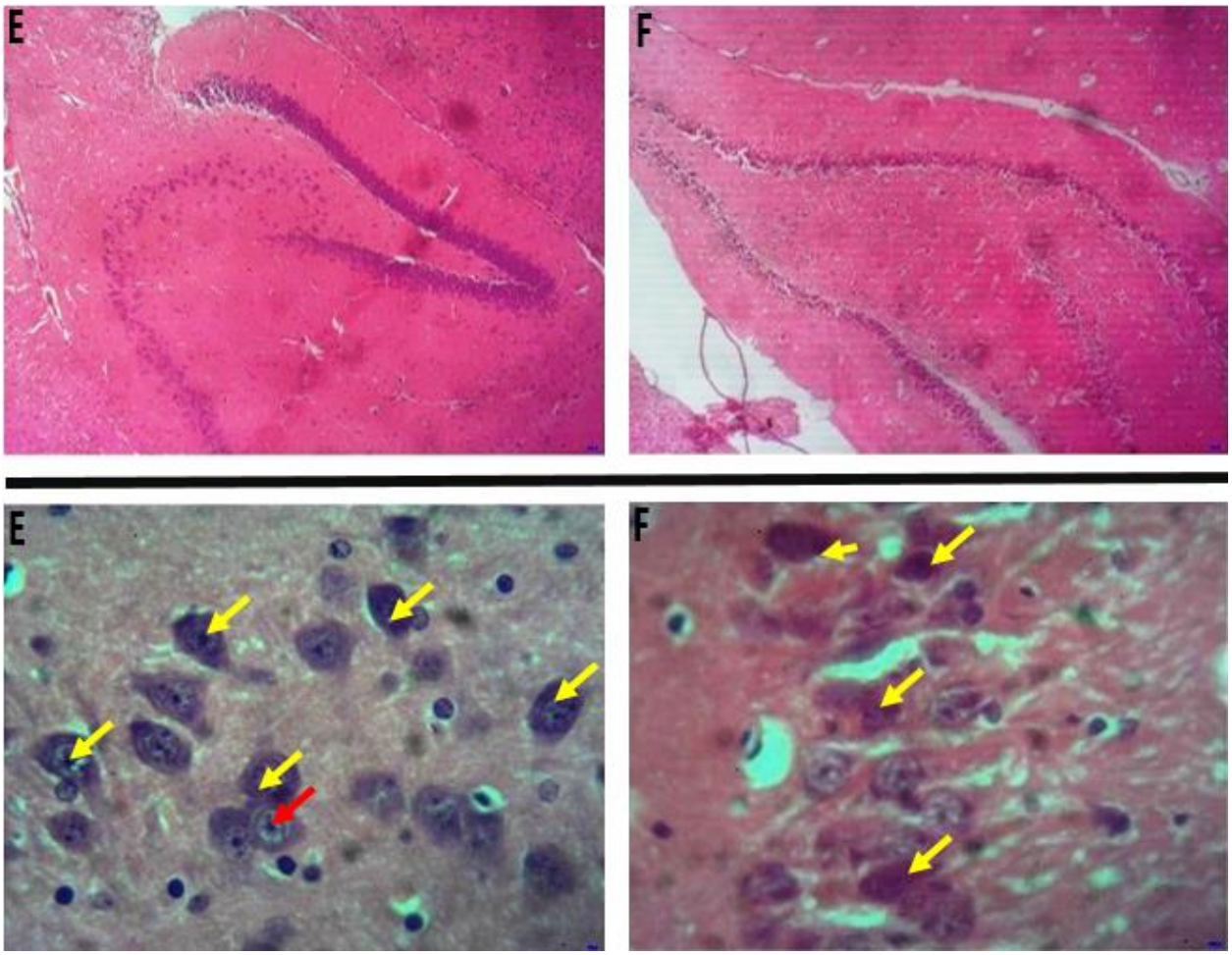


Figure 4.12e&f: **Treatment with high dose TCA with concurrent intake of HFD (4.12E) shows reduction in pyknotic cells and organized neuronal cell layer, an indication of the cytoprotective property of TCA, Normal histological features of the hippocampus were observable in the low dose TCA alone (Fig. 4.12F). The histology of this group presents normal neuronal layers (yellow arrows) with well-organized cells. H&E; Scale bar: 450µm (E &F top) and 45 µm (E &F below)**

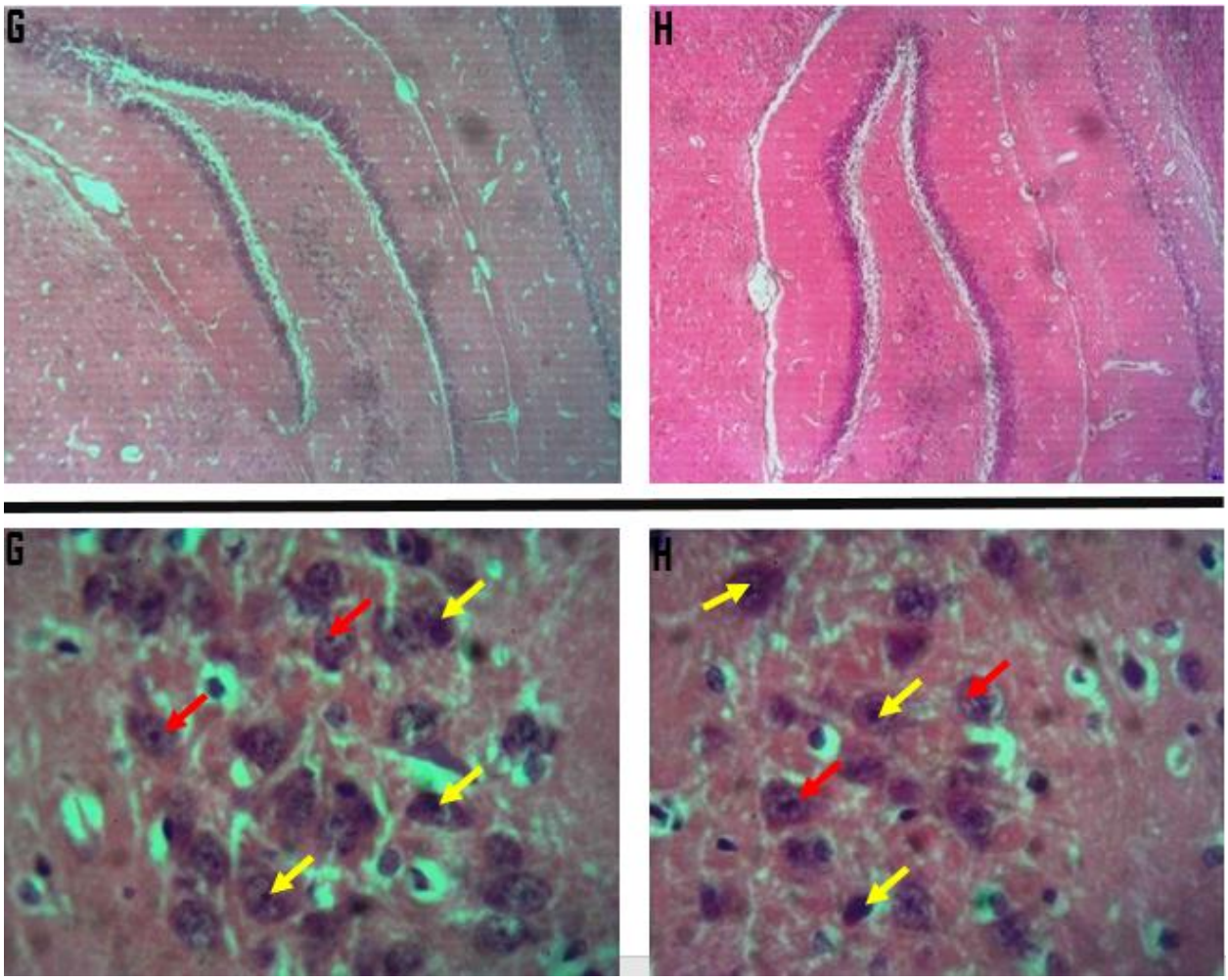


Figure 4.12g&h: **Hippocampus of insulin-resistant rats treated with low dose of TCA showing mild neuronal degeneration with reduced neuronal cell layer. H& E; Scale bar: 450µm (G &H top) and 45 µm (G &H below)**

Cresyl Fast Violet

Hippocampal sections stained with CFV, reveals a chromatogenic neuronal cell layers within CA3 region of the hippocampus in both the control and TCA treated groups. The pyramidal cell layer of the hippocampus exhibits a well stained intensity and are well arranged. HFD/STZ administration caused a marked distortion as seen in the hippocampal sections of rats in this group showing a highly chromatolysis of pyramidal cells (red arrows) within the CA3 region. Treatment of insulin resistant rats with TCA shows an improved chromatogenic properties of the pyramidal cells when compared with the insulin resistant control group.

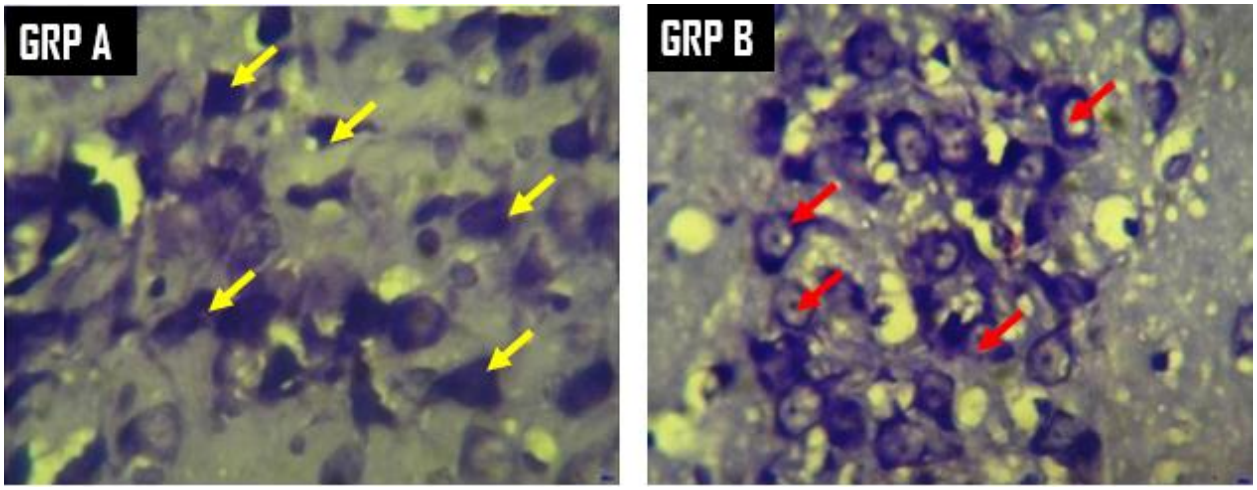


Figure 4.13a&b: **Hippocampal stained section showing highly chromatogenic (yellow arrows) cells in the control group (Fig. 4.13 A), the insulin resistant group (Fig. 4.13B) showing highly chromatolytic cells (red arrows). CFV; Scale bar: 45 µm**

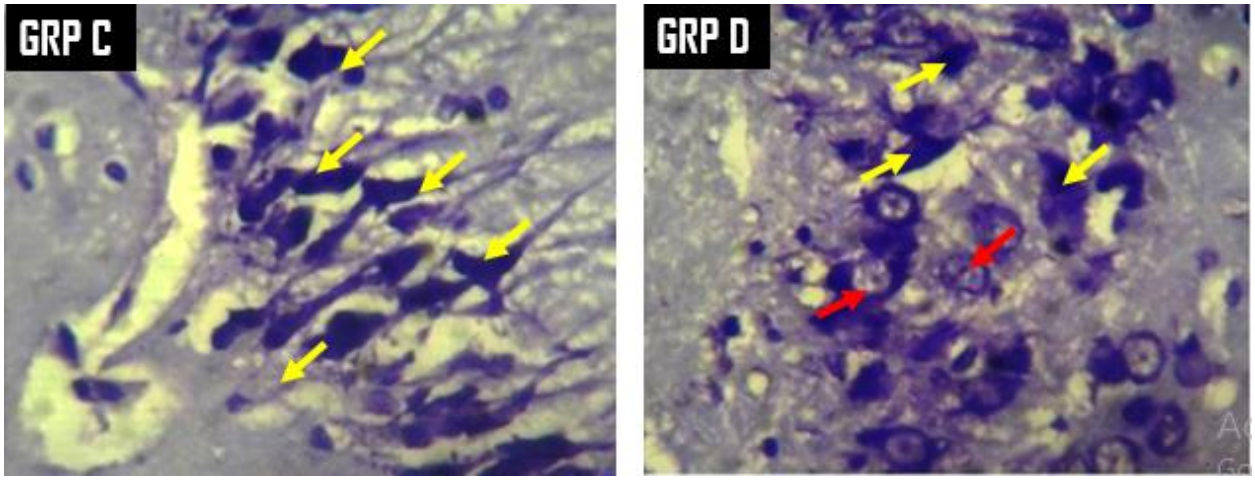
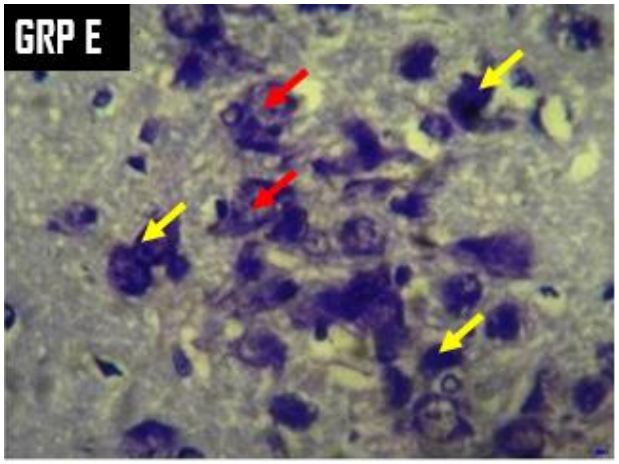


Figure 4.13c&d: **Administration of high dose TCA alone (Fig. 4.13C) showing normal neuronal cell layer, treatment with high dose TCA (Fig. 4.13D) following HFD/STZ administration shows an improved chromatogenic properties of the pyramidal cells and neuroglia within the CA3 region of the hippocampus. CFV; Scale bar: 45 µm**



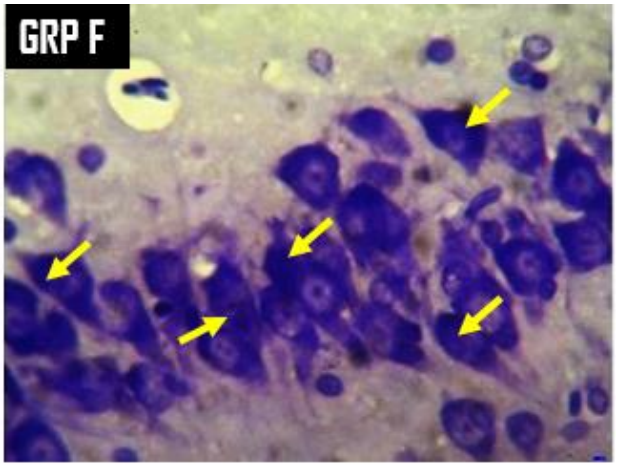
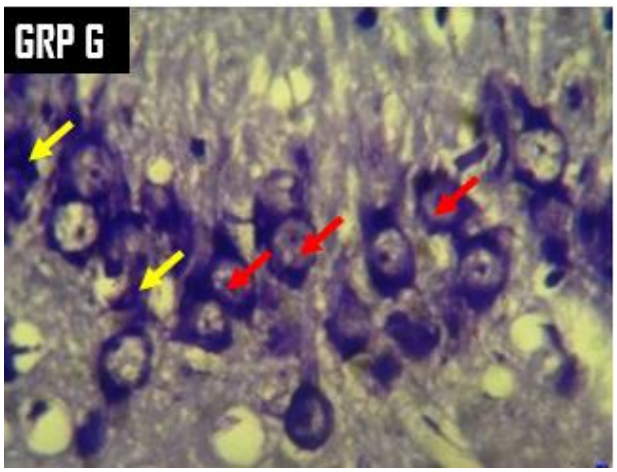


Figure 4.13e&f: **Treatment with high dose TCA with concurrent administration of HFD (Fig. 4.13E) shows restoration of the chromatolytic properties of the neuronal cells. (Fig. 4.13F) Administration of low dose TCA alone shows normal neuronal cell layer.CFV; Scale bar: 45 µm**



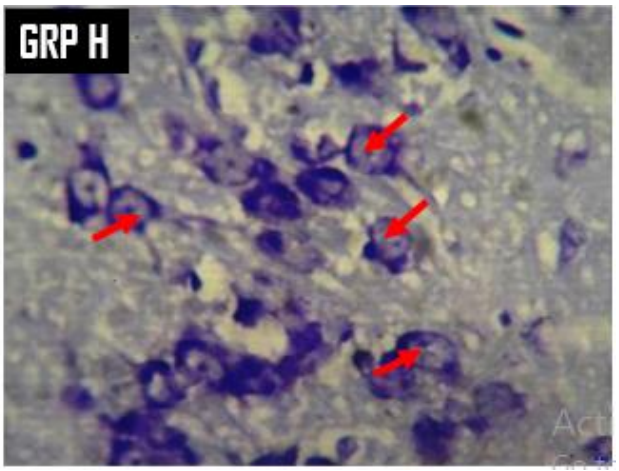


Figure 4.13g&h: **Treatment of insulin-resistant rats with low dose TCA shows persistent chromatolytic cells in the CA3 region of the hippocampus. CFV; Scale bar: 45 µm**

Immunohistochemical observation of the hippocampus

Figure 4.14: Amyloid expression

Control and TCA alone treated groups shows normal amyloid distribution, however insulin-resistant control groups shows multiple amyloid deposition, treatment with low and high doses of TCA reduces the amyloid burden.

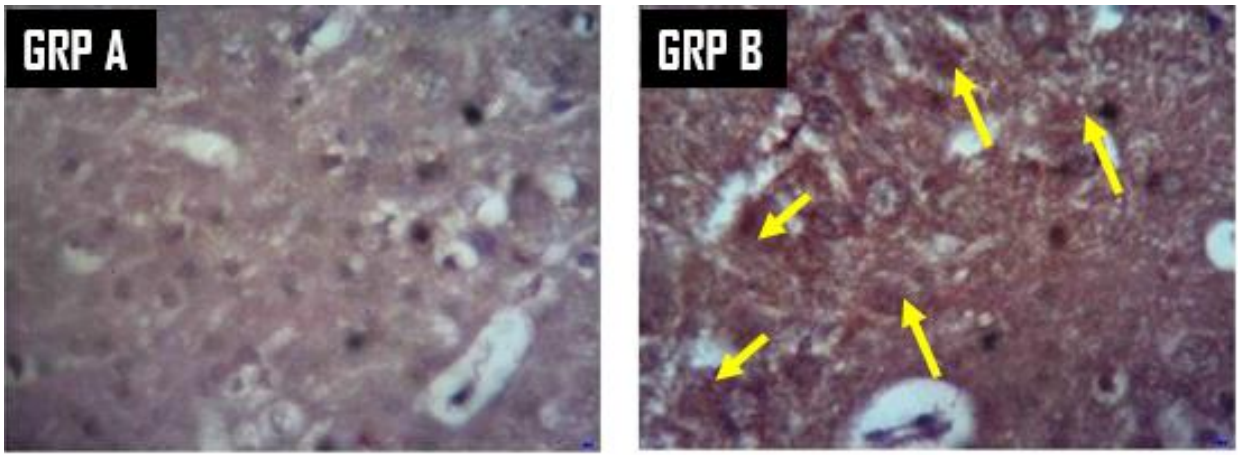


Figure 4.14a&b: **CA3 region of the hippocampus of normal control rats with moderate amyloid deposition (A), multiple deposition of amyloid plaques was observed in the insulin-resistant control group (B) (yellow arrows), Scale bar: 45 µm**

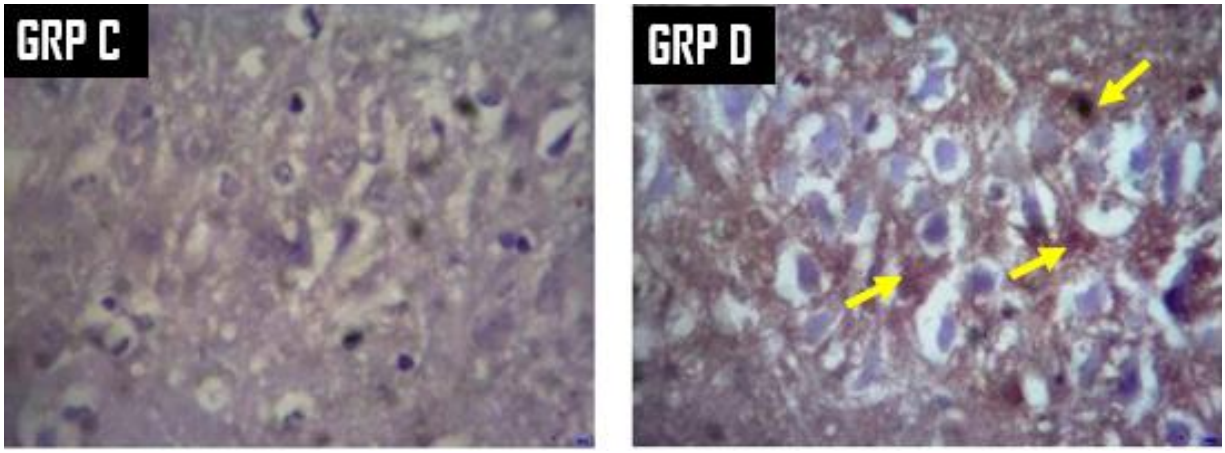


Figure 4.14c&d: **Showing reduced amyloid deposition in high TCA alone (C) and after high TCA intervention (D); Scale bar: 45 µm**

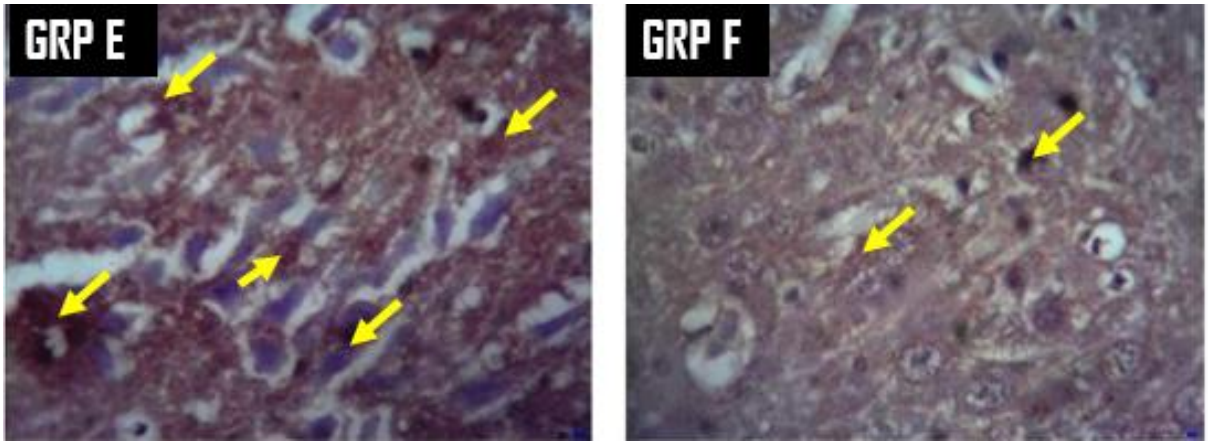


Figure 4.14e&f: **Showing relatively increased amyloid deposition after high TCA treatement with concurrent HFD intake (E) and after low TCA alone intervention (D); Scale bar: 45 µm**

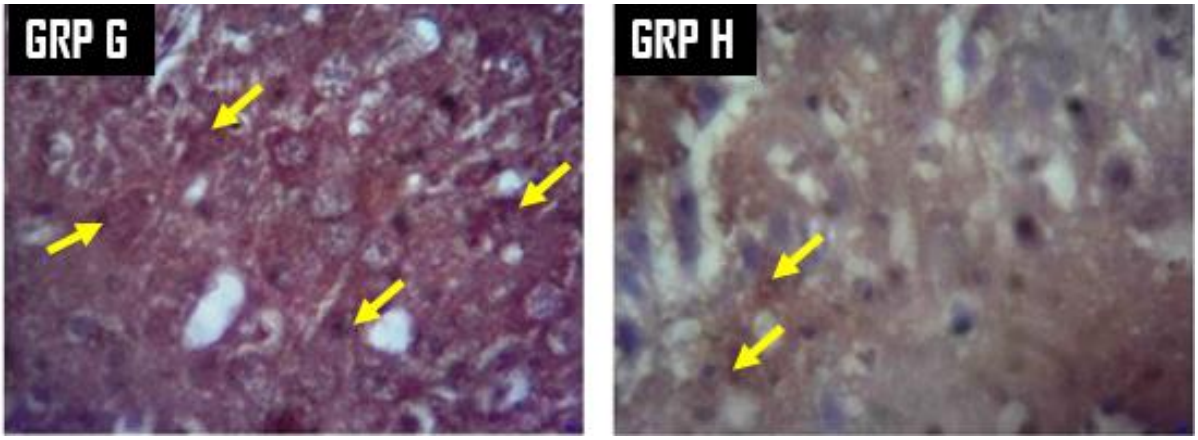


Figure 4.14g&h: **Showing relatively increased amyloid deposition after low dose of TCA treatement with normal diet (G) and concurrent HFD intake (H); Scale bar: 45 µm**

Glia Fibrillary Acidic Protein (GFAP)

Figure 4.15 Representative micrographs of immunohistochemical staining of the hippocampus of Wistar rats using GFAP

The normal control and TCA alone treated groups (A,C,&F)shows normal astrocyte distribution, the groups that received TCA intervention both at high and low doses (D,E,G&H) have similar astrocyte distribution with insulin resistant control group, the hippocampus of animals in these groups is similarly characterized by reactive astrocytes (red arrows), however in contrast to insulin-resistant group, the astrocyte morphology has similar appearance to the normal control group.

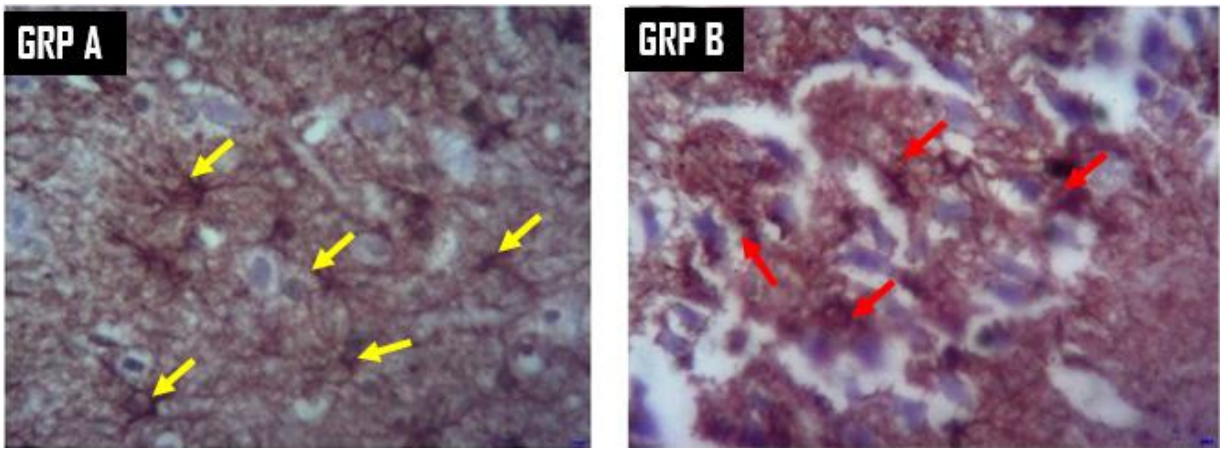


Figure 4.15a&b: **The normal control (A) is characterized by normal astrocytic expression with regular distribution, size and numerous processes which forms an array of network (yellow arrows), on the other hands astrocyte integrity in the hippocampus of rats in the insulin-resistant control group (B) was characterized by series of deleterious changes such as astrogliosis and increased astroglia size (red arrows). Scale bar: 45 µm**

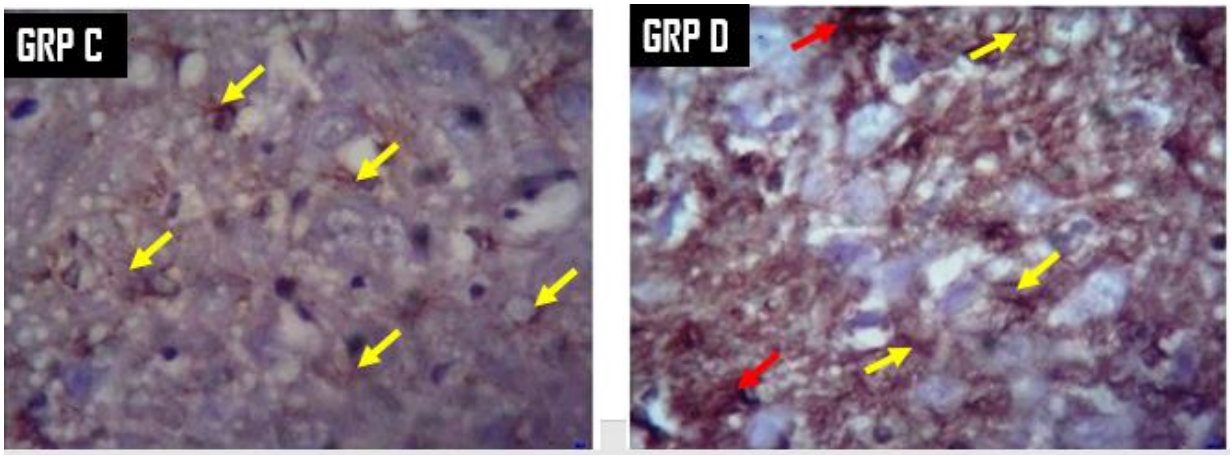


Figure 4.15c&d: **High TCA alone treated group (D) is characterized by normal astrocytic expression with regular distribution (yellow arrows), treatment with high dose TCA with normal diet feeding restores normal astrocytic morphology; Scale bar: 45 µm**

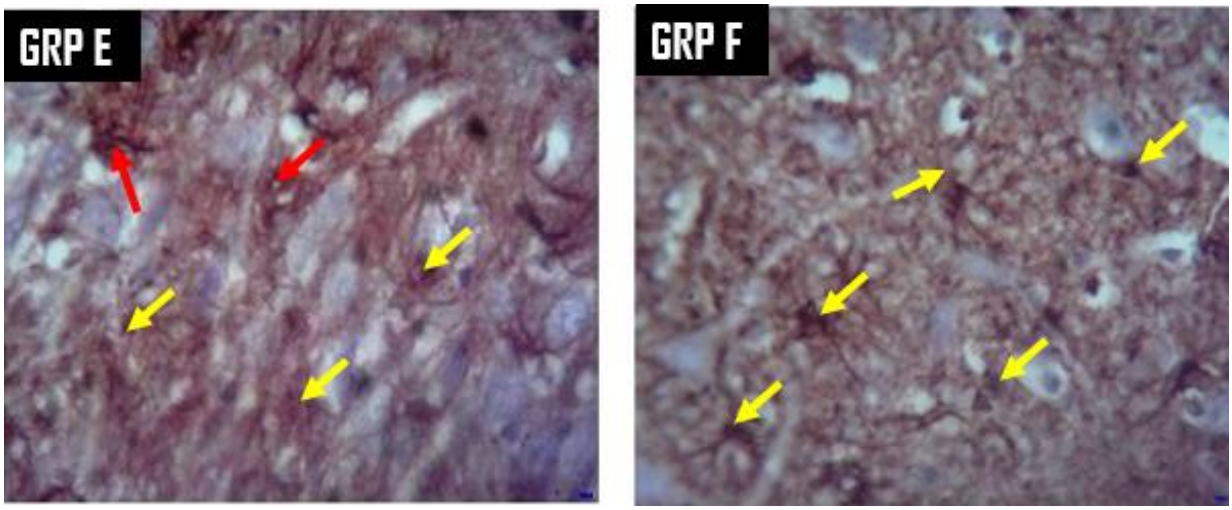


Figure 4.15e&f: **High TCA treated group (E) shows improved astrocytic morphology (yellow arrows) low TCA alone treated group (F) is characterized by normal astrocytic expression with regular distribution (yellow arrows); Scale bar: 45 µm**

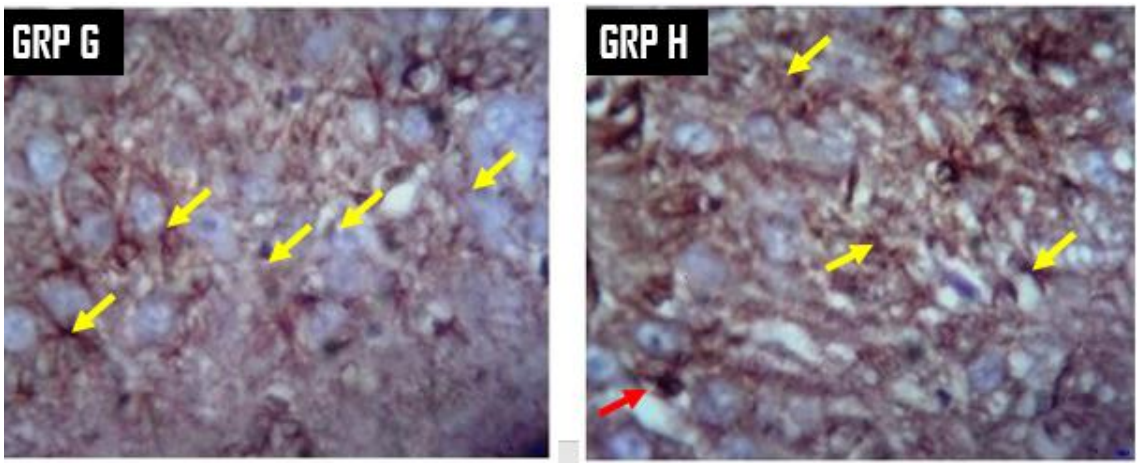


Figure 4.15g&h: **Low TCA alone treated group with normal diet (G) is characterized by normal astrocytic expression with regular distribution (yellow arrows), treatment with low dose TCA with concurrent high fat diet feeding restores normal astrocytic morphology; Scale bar: 45 µm**

NeuN immunostaining

Figure 4.16: The normal control and TCA treated groups were characterized by increased NeuN immunoreactivity (A,C,F) with normal cellular architectural layout, however in the insulin-resistant control group (B) there is decreased NeuN immunoreactivity and nuclear degeneration (yellow arrows) in neurons as a result of pathological changes, administration of TCA restores IR induced nuclear degeneration (D,E,G&H).

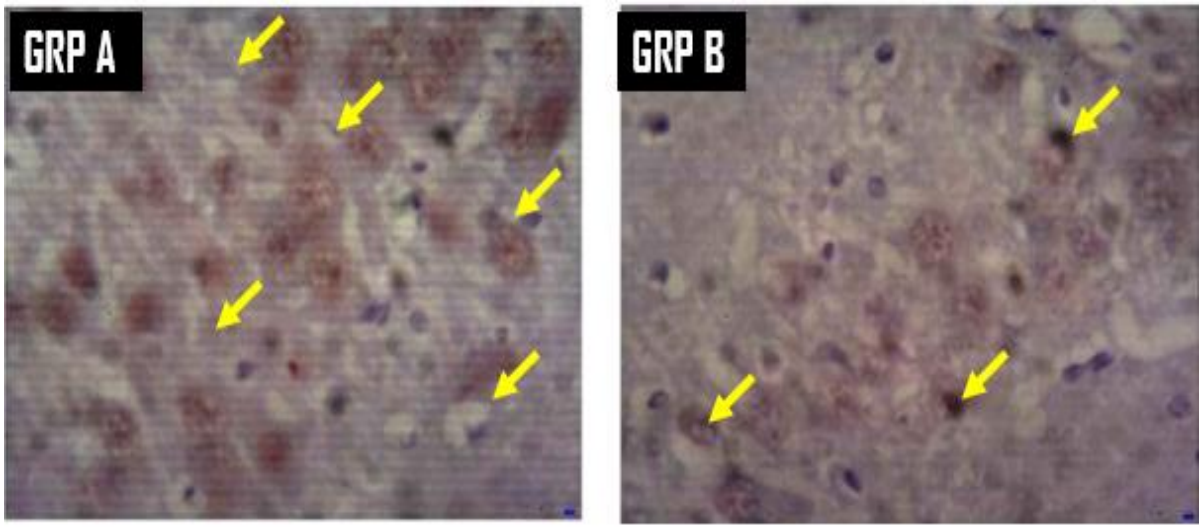


Figure 4.16a&b: The normal control and TCA treated groups were characterized by increased NeuN immunoreactivity (A), yellow arrows, with normal cellular architectural layout, however in the insulin-resistant control group (B) there is decreased NeuN immunoreactivity and nuclear degeneration (yellow arrows). NeuN; Scale bar: 45 µm

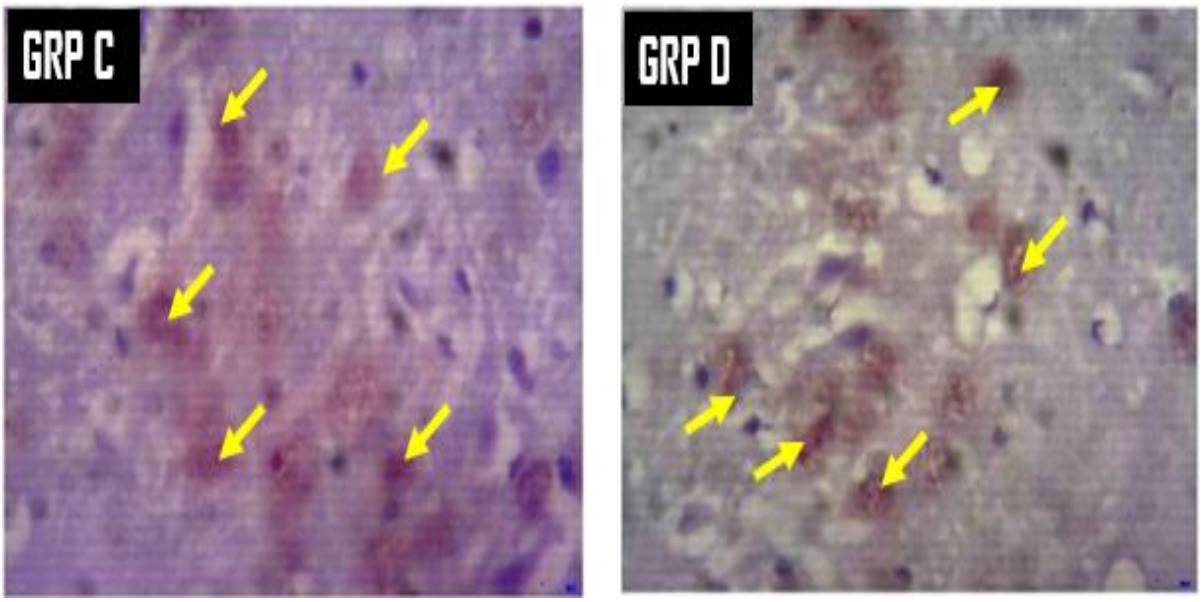


Figure 4.16c&d: **High TCA alone (C) and with normal NeuN immunoreactivity (yellow arrows), yellow arrows, treatment with high dose TCA alone with concurrent normal diet intake (D) shows reduced neurodegenerative changes NeuN; Scale bar: 45 µm**

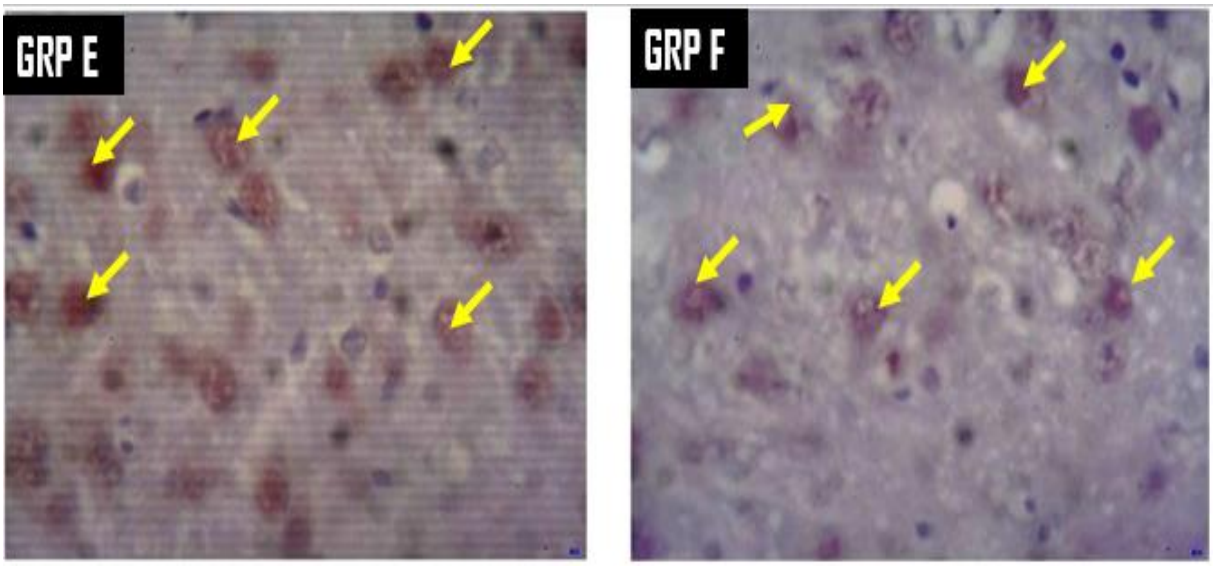


Figure 4.16e&f: **High TCA alone with HFD (E) and with normal NeuN immunoreactivity (yellow arrows), yellow arrows, treatment with high dose TCA alone with concurrent normal diet intake (D) shows reduced neurodegenerative changes NeuN; Scale bar: 45 µm**

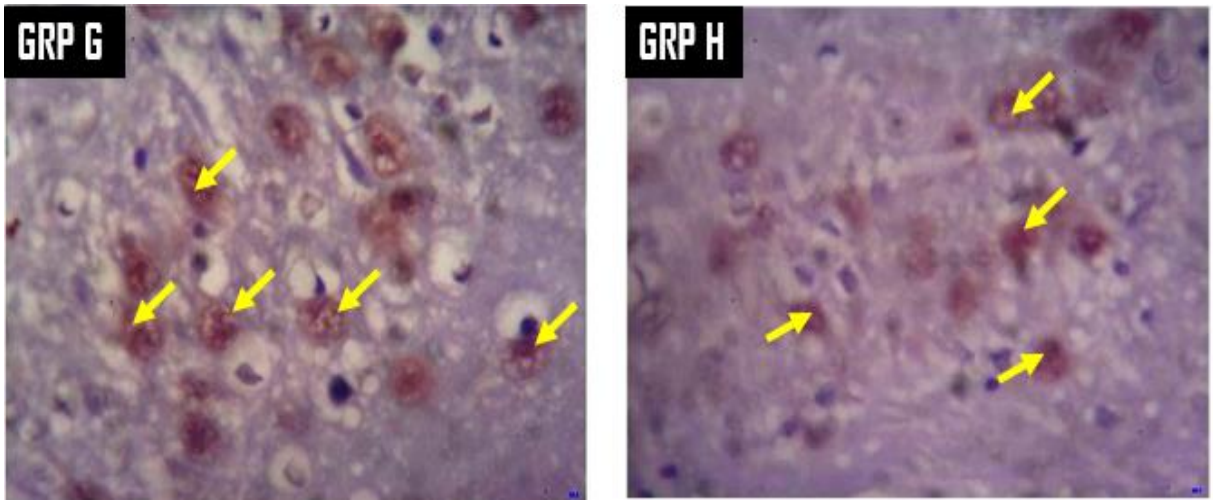


Figure 4.16g&h: **Low TCA alone with HFD (G) showing relatively normal NeuN immunoreactivity (yellow arrows), treatment with low dose TCA with concurrent normal diet intake (H) showing restoration of cellular histo -architectural layout. NeuN; Scale bar: 45 µm**

Stereological observation

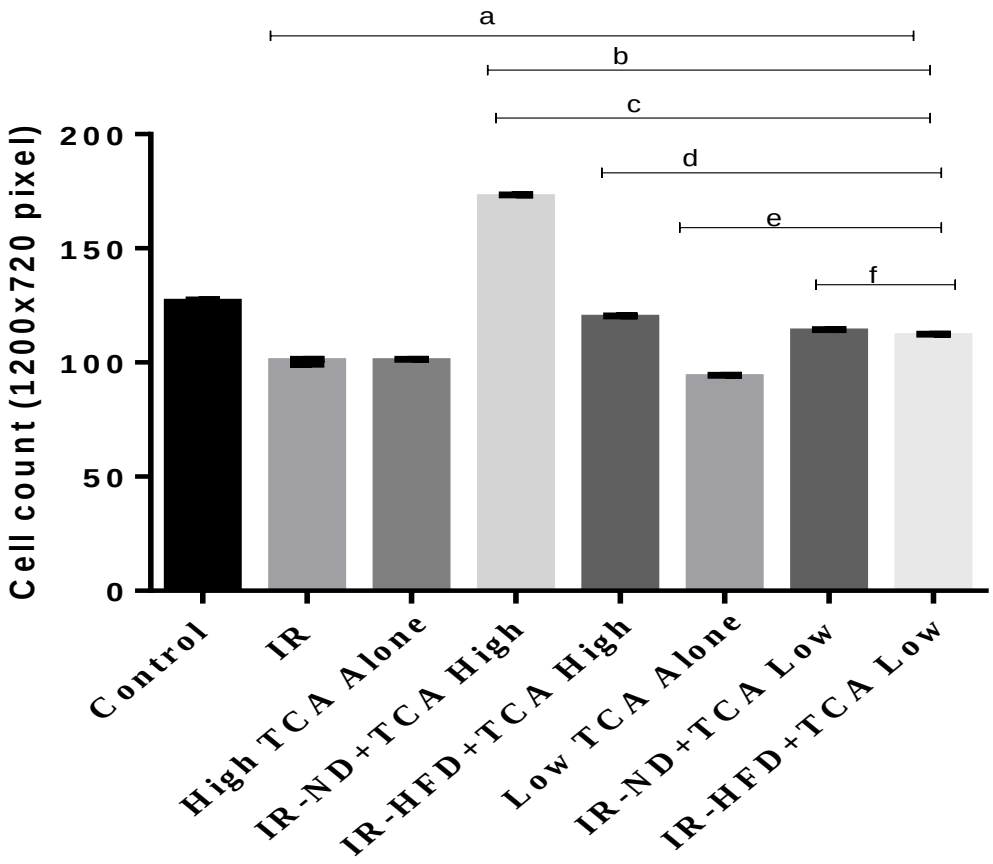


Figure 4.17: Neuronal count

A significant decrease was observed in the cell count between the control and IR treated groups.

The superscript alphabets above the bars represents significance difference with other groups

[a] significant different from Control ;[b] significant different from IR;[c] significant different from High TCA Alone;[d] significant different from IR-ND+TCA High;[e] significant different from IR-HFD+TCA High;[f] significant different from Low TCA Alone

CHAPTER FIVE

5. 1 Discussions

The mechanisms by which insulin resistance and diabetes alter brain functioning are not clearly understood, the most appropriate methods to diagnose and treat cognitive dysfunction associated with insulin-resistance is yet to be defined (Shaimaa *et al*., 2013). AD which is the most common form of dementia causes irreversible and degenerative neurological changes and it is always characterized by the formation of amyloid β (Aβ) plaques, neurofibrillary tangles, neuronal and synaptic loss (Sisi *et al.*, 2017). Epidemiological studies have demonstrated that obesity, insulin resistant and T2DM are risk factors for AD (Wang *et al*., 2014).

High-Fat Diet and low dose STZ has been suggested as a better way to initiate insulin resistance which is one of the characteristic features of type 2 diabetes. Researchers are now developing rat models that will mimic the natural history of insulin resistance by feeding the rats with high fat diet followed by low dose of STZ (Reed *et al.,* 2000; Srinivasan *et al.,* 2005). The coadministration of HFD and STZ has been widely used to create models of type I and type II diabetes mellitus through the induction of β cell death via alkylation of DNA (Szkudelski, 2001). High-dose STZ severely impairs insulin secretion mimicking type 1 diabetes, however, low-dose STZ has been used to induce a mild impairment of insulin secretion which mirrors features common in insulin-resistant state (Reed *et al,*. 2000; Srinivasan *et al.,* 2005).

HFD and STZ model provides a relative cheaper, easily accessible and practical for the testing of various compounds with potential therapeutic properties for the treatment of insulin resistance and type II diabetes. Low-dose injections of STZ possess the ability to induce a stepwise β cells destruction rather than the rapid destruction caused by a single injection of

high-dose STZ (Kannan *et al.,* 2004). High fat diet feeding combined with low dose STZ was prescribed for the creation of animal models insulin resistance (Vatandoust *et al.,* 2018).

In this present work, adult female Wistar rats were fed with HFD for eight weeks followed by low dose intraperitoneal injection of STZ, female adult Wistar rats were used because insulin resistance and T2DM tend to occur later in life and among women, contrary to vast majority of insulin resistance models that used young Wistar rats (Skovsø, 2014).

5.1 High fat diet and streptozotocin administration increases body weight in Wistar rats

The body weight (BW) of each rat was recorded fortnightly, findings from this study revealed marked increase in body weight (figure 4.1) among Wistar rats treated with HFD/STZ when compared to rats fed with normal diet. This corroborates previous studies which reported that HFD increases body weight but the combination of HFD/STZ leads to a slight decrease in body weight (Jinshan *et al.,* 2015; Magalhães *et al.,* 2019).

Multiple environmental factors such as excess food intake and lifestyle play critical roles in the onset of insulin resistance. The use of HFD and STZ to model IR is now common in recent years, the HFD and low dose STZ induces insulin resistance resulting in mild β-cells dysfunction without compromising insulin secretion completely. This model closely mimics the natural course of insulin resistance (Wang *et al.,* 2009; Shatwan *et al.,* 2013).

The slight decrease in body weight observed in this study may be due to insulin-resistance induced muscular atrophy and also the age of the rats used, one of the uniqueness of this work is that adult wistar rats were used because insulin resistance tends to occur later in life unlike bulk of previous findings that used younger rats, treatment with high dose TCA with concurrent replacement of high fat diet with normal diet led to a slight increase in body weight among the group treated with high dose TCA and normal diet (figure 4.1) showing

that adjustment of lifestyle or diet may play a synergistic role in therapeutic intervention, there was no significant increase in body weight among the group that were administered low dose TCA and the group that continued with high fat diet with high dose TCA.

Furthermore, there was observable decrease in body weight among untreated insulin resistant group, the weight gained among HFD/STZ treated groups was not significantly high until the end of the treatment. Previous studies also reported a reduction in the body weight of animals fed with HFD diet followed by different STZ doses (Jinshan *et al.,* 2015; Xiao-xuan *et al.,* 2018)

5.2 Trans- cinnamaldehyde intervention prevents hippocampal atrophy

The degree of hippocampal atrophy has been used clinically in the diagnosis and clinical trials of dementia (Susanne *et al.,* 2010). Mild cognitive impairment in patients has reportedly led to 10-15% hippocampal volume loss, in patients with early AD the volume loss is around 15-30% ,the loss in moderate AD can be as high as 50% (Dhikav *et al.*, 2011).

There was an observed decrease in the hippocampal weight in the HFD/STZ treated group when compared to the control and TCA treated groups, the reduction in hippocampal weight observed in this study may be due to the insulin resistance induced neuronal damage, the hippocampus has been reported to be one of the first structures in the brain that is affected by AD and metabolic syndrome (Sedighi *et al.,* 2019).

Hippocampal-brain ratio was also examined in this work, insulin resistance significantly reduced hippocampal-brain weight, however TCA intervention increases hippocampal-brain weight an indication of its cognitive enhancement ability. Insulin resistance is associated with hyperglycemia, the observed hyperglycemia may have detrimental effects on the overall weight of the hippocampus, hyperglycemia and neurodegeneration have been reported to be

associated with atrophy of structures relevant to aging and neurodegenerative processes such as the hippocampus (Cherbuin *et al.,* 2012; Monte, 2017). The decrease in hippocampal weight observed in this study may be responsible for poor memory performance observed in MWM and Y-Maze tests.

5.3 Trans-cinnamaldehyde enhanced cognitive functions in insulin-resistant rats

The results from this study (Fig. 4.5) show a significant decrease in the percentage alternation in insulin resistant group in Y-Maze test, however treatment with high dose TCA (60mg/kg) significantly increased the percentage alternation when compared to the control and TCA treated groups, administration of low dose TCA with subsequent withdrawal of HFD significantly reverses the memory impairment which further consolidates the fact that lifestyle adjustment may play a significant role in therapeutic intervention in dementia, this findings agree with previous reports that deficit in the hippocampal based memory performance occurs in diabetic individuals (Rajamani, 2014) previous findings had reported that western diet, rich in saturated fat and refined sugar accelerates cognitive decline with aging and Alzheimer's disease, affect cognitive functions that are hippocampal dependent including reversal learning and memory processes (Tamashiro, 2015).

Evaluating behavioural parameters in neurodegeneration in therapeutic targets represents an important means of measuring the effectiveness of treatment (Zheng-hui *et al.,* 2013). Diabetes associated cognitive decline also known as encephalopathy is becoming a source of concern (Mijnhout *et al.,* 2006). Diabetes-associated cognitive decline is a complication of the diabetic brain, it manifests as a progressive decline in cognitive function, the exact mechanisms of encephalopathy is not yet known, however impaired insulin signaling pathway which plays roles in the metabolism of Amyloid Protein (Aβ) and tau also plays an important role in Diabetes associated cognitive decline (Kroner, 2009).

Gradual decline of memory functions and cognitive abilities is a hallmark event in patients that are affected with AD until the disease renders them incompetent in the discharge of routine functions (Bryan *et al.,* 2014). Late-stage insulin resistance is associated with decreased dendritic complexity, hippocampal atrophy, impairment of synaptic plasticity and decline of hippocampal neurogenesis (Magariños, 2000; Suzanne, 2013). The above pathological features are associated with mild learning and memory impairment in early life and increased dementia risk or Alzheimer's disease in the elderly (Geert *et al.,* 2006).

From Morris water maze observation in this study, there was a significant increase in escape latency among HFD/STZ treated animals when compared to the other groups, escape latency was significantly reduced among the groups treated with high dose TCA and the group that received low dose TCA followed by HFD withdrawal, however in group treated with low dose TCA with continuous intake of HFD the observed escape latency was significantly high (figure 4.4) these results are in agreement with other studies that have also verified cognitive impairment in streptozotocin-induced diabetes mellitus (Kuhad *et al.,* 2009).

In the Morris water maze test, the insulin resistant groups showed a significant increase in escape latency (figure 4.4) when compared with normal control group showing a poorer learning performance due to HFD/STZ administration, decline in hippocampal based memory has been recorded in patients with insulin resistance (Yau *et al.,* 2010). Impaired learning behavior in Morris water maze task after HFD feeding had been reported (Heather *et al.,* 2013). Performance in the Morris Water Maze is correlated with the function of the hippocampus, it has also been associated with hippocampal NMDA receptor function from previous studies using NMDA receptor antagonists. MWM test has also been used to demonstrate that various HFD can accelerate cognitive decline found in insulin resistant state (Heather *et al.,* 2013). These results are in agreement with other studies that have also

verified cognitive impairment in streptozotocin-induced diabetes mellitus (Kuhad and Sethi , 2008; Schmatz *et al.,* 2009).

The impairment in hippocampal based tests observed in this work may be partly due to the hippocampal atrophy and hyperglycemia associated cognitive decline earlier reported in this study.

These findings indicate that treatment with TCA was able to prevent learning and memory impairment induced by insulin resistance.

5.4 Trans- cinnamaldehyde reduces blood glucose level in HFD/STZ induced insulin resistance in wistar rats

From this study, insulin resistance significantly increases the fasting blood glucose among the HFD/STZ treated groups before the onset of TCA treatment (Figure 4.2), hyperglycemia has been implicated in the etiology of Alzheimer's disease as reported from previous findings (Rojas *et al.,* 2018), previous studies had reported increased fasting blood glucose in type diabetes models (Jinshan *et al.*, 2015) More so, hyperglycemia has been implicated in mild to moderate cognitive dysfunction, decline in learning and memory, or even AD (Petrova *et al.,* 2010).

However, treatment with TCA at high and low doses significantly reduced the blood glucose (figure 4.3), Shatwan *et al*. (2013) reported that dietary intake of cinnamon rich food regulates lipid profile, adipose tissue hormones and blood glucose in type 2 diabetic rats, similar work observed the ability of cinnamon to normalize lipid abnormalities, weight changes and glucose metabolism (Anand *et al.,* 2010; Subash *et al.,* 2007).

Accumulating evidence established close association between insulin resistance, type 2 diabetes mellitus and age-dependent Alzheimer's disease, Alzheimer's disease has even been

classified as type 3 diabetes. Some pathophysiological features such as dysfunctional insulin signaling, hyperglycemia and oxidative stress link insulin resistance to Alzheimer's disease (Song *et al.,* 2012).

Hyperglycemia is a risk factor for cognitive dysfunction, AD and dementia in general, the mechanism of hyperglycemia mediated dementia is unknown (Barbagallo & Dominguez, 2014; Gohar *et al.,* 2015). The close relationship between insulin resistance, type II diabetes mellitus and AD has been linked to the sustained hyperglycemia on the nervous system.

The active compound in cinnamon that elicits hypoglycemic activity is a contentious issue (Richard *et al.,* 2016), however, this study has clearly shown that the active ingredient in cinnamon with hypoglycemic activity is TCA, the hypoglycemic activities of TCA was not apparent in normal rats that received TCA both at low and high doses which corroborates previous findings (Kannappan *et al.,* 2006). This study clearly indicates that TCA is one of the active ingredients in cinnamon with hypoglycemic activity.

Chronic exposure to hyperglycemia has been reported to deteriorate cognitive function (Overman *et al.,* 2017). Hyperglycemia-induced impairment of cognitive function is considered a brain complication of diabetes (Dolan *et al.,* 2018). This may serve as a link between T2DM and AD which further explained poor memory related performance observed in this work.

Hyperglycemia progressively increases amyloid beta aggregation, impairs neuronal integrity, neuroinflammation resulting in neurodegeneration (Shannon *et al.,* 2015; Gaspar *et al.,* 2016; Kim *et al.,* 2016;).

Hyperglycemia induces increased amyloid beta production by inhibiting APP degradation (Yang & Zhang, 2013). Most of the neuronal loss in AD occurs as a result hyperglycemia

mediated apoptosis, glucose plays critical roles in neuronal function and metabolism (Mousavi *et al.,* 2010).

5.5 Trans- cinnamaldehyde treatment significantly reduced HOMA-IR in insulin-resistant rats

Induction of insulin resistance significantly increased blood glucose and insulin levels as well as HOMA-IR in insulin resistant control group compared to the normal control and TCA treated groups. Groups treated with TCA showed a significant decrease in blood glucose, insulin and HOMA-IR when compared to insulin resistant control group. Similar findings by Jatla *et al.,* (2012) reported that TNF-α concentration increased together with increase in concentrations of insulin, and HOMA-IR.

The key pathogenetic mechanism of glucose metabolism disorders insulin resistance (IR), can be assessed using the Homeostasis Model Assessment of insulin resistance (HOMA-IR) (Dagmar *et al.,* 2019). Insulin resistance is known to cause disruption in energy metabolism, oxidative stress, impairment in mitochondrial function and DNA damage which ultimately leads to increased atrophy of the hippocampus resulting in cognitive impairment (Suzanne *et al.,* 2013). Homeostasis of blood glucose is maintained by the activities of different hormones such as insulin and glucagon as well as cytokines under normal conditions (Lee *et al.,* 2018). Diabetes and dementia have overlapping risk factors such as mitochondrial dysfunction, inflammation and oxidative stress (Lee *et al.,* 2018).

5.6 Trans- cinnamaldehyde suppresses neuroinflammation in insulin resistant treated Wistar rats

Cytokines plays an essential role in the coordination of immune responses in the body. Cytokine dysregulation is a key event in neuroinflammation, demyelination and neurodegeneration in the central nervous system. Activation of microglia can occur as a result of pathological states within the nervous system which may mediate glia cell injury through

the production of proinflammatory cytokines. Inflammation under physiological condition may be of beneficial effects such as clearance of pathogen and phagocytotic destruction of apoptotic cells, however, uncontrolled inflammation may result in the production of neurotoxic factors that can aid neurodegeneration. (Ramesh *et al.,* 2013). Inflammation is a common characteristic feature of many chronic diseases such as Alzheimer's disease and diabetes mellitus (Mihalea *et al.,* 2009).

This study shows that administration of HFD/STZ induces neuroinflammation in the hippocampus of Wistar rats evidenced by significant increase in the expression of TNF-α and NFkB (figures 4.5.1 and 4.5.2) which agrees with some studies that reported inflammation as a key pathogenic factor in the development of AD (Kenawy *et al.,* 2017). TNF-α has been implicated in the pathogenesis Alzheimer's disease (Mihalea *et al.,* 2009). The alteration in the production of TNF-α has been reported in the etiology of variety of metabolic disorders including insulin resistance (Jatla *et al.,* 2012).

From this studies, it was observed that HFD/STZ treatment increases the activity of tumour necrosis factor-alpha (TNF-α) and nuclear factor kappa b (NFk-B) (figures 4.5.1 and 4.5.2) however, TCA treatment reduces the expression of TNF-α and NFk-B showing that TCA has the ability to suppress neuroinflammation similar to previous findings (Chinjarernpan *et al.,* 2014).

Increase TNF-α has been reported in AD (Mihalea *et al.,* 2009). Tumor necrosis factor (TNF) predominantly confers protection against some tumors and infections, they as well assist in some reparative processes in the central nervous system. TNF-α is a subset of TNF proteins and a major player in the onset of TNF induced inflammation. Over expression of TNF-α in AD brains, and its elevated level in the cerebrospinal fluid and blood of AD patients has been reported. (Vivek *et al.,* 2012). The hippocampus is the part of the brain with the sole

responsibility of consolidating short and long-term memory, shown over the years to be prone to inflammation and injury in metabolic disease (William *et al.,* 2008). Dysregulation of TNF-α inhibits insulin transduction and glucose metabolism playing crucial role in the pathophysiology of insulin resistance (Jatla *et al.,* 2012).

In the progression of AD, nuclear factor-kappa β (NF-κβ) plays an active role in the, impairment in the NF-κβ signaling pathway initiates some pathological changes such as oxidative stress, neuroinflammation, microglia activation and apoptotic cell death, which may initiate neurodegenerative changes in normal neurons (Jha *et al.,* 2019). NF-κβ signaling pathway is involved in normal brain functioning, the pathway plays a critical role in maintaining synaptic plasticity and balance between learning and memory. Impairment in the pathways associated with NF-κβ signaling causes alteration in neuronal function (Jha *et al.,* 2019).

The increased activity of pro inflammatory cytokine observed in this study is partly due to the chronic activation of NF-κβ which has the ability to sustain microglia activation and cell death subsequently leading to neurodegenerative changes of AD-type.

The anti-inflammatory activities of TCA observed in this study may be as a result of its ability to inhibit inflammatory mediators and NF-κβ (Chinjarernpan *et al.,* 2014), from this study the expression of NF-κβ was significantly blocked by the administration of TCA (figure 4.7)

Franziska *et al.,* (2014) attributed the anti-inflammatory properties of cinnamon to its ability to block nuclear factor-κB activation in immune cells. Nuclear factor-κB (NF-κB) plays important roles, however aberrant NF-κB activation contributes to development of various inflammatory, therefore inhibiting NF-κB signaling pathway may hold a has potential

therapeutic intervention in inflammatory diseases (Hong, 2016). Constitutive and inducible activated NF-κB, has been reported to be related with various human diseases like AIDS, atherosclerosis, AD and asthma. Constitutively formed NF-κB is usually expressed in different regions of glutamatergic neurons of the hippocampus. Accumulation of Aβ can lead to the activation of NF-κB which can lead to the production of nitric oxide and subsequent neurotoxicity (Zamani *et al.,* 2019). Hyperglycemia, insulin resistance and hyperinsulinemia were reported in this work, this observed pathological changes may be as a result of the disturbances in the NF-κB, NF-κB plays a major role in the etiology of diabetes mellitus and AD, it is assumed that it is at the core of the relationship between both disorders (Mihalea *et al.,* 2009).

From this study, it was observed that TCA not only reduced the expression of pro-inflammatory cytokine TNF-α but also downregulated the nuclear transcription factor kappa-B (NF-κB) which further uphold its anti-inflammatory property. The above observations provide substantial evidence supporting the anti-inflammatory nature of TCA wherein it significantly normalized the TNF α activity which was significantly high after HFD/STZ administration in wistar rats.

5.7 Trans- cinnamaldehyde possesses anti hyperinsulinemic properties

In this study, HFD/STZ treated rats, characterized by hyperglycemia and hyperinsulinemia, produced marked impairment in cognitive function as revealed by the behavioural studies which was coupled with marked increases in amyloid deposition in the hippocampus.

Hyperinsulinemia, hyperglycemia and insulin resistance are all hallmark features in type 2 diabetes (Taylor, 2012). Altered glucose regulation has been reported to clinically impair learning and memory (Messier, 1996). The potential mechanisms altered glucose-mediated

impairment of learning and memory may be directly related to hyperinsulinemia and hyperglycemia (Brands *et al.,* 2004; Lobnig *et al.,* 2006).

Neurodegenerative disorders have been reported to be more prevalent in insulin resistant state and diabetics compared to incidence in general population, impairment in neuropsychological functioning in diabetic patients has been reported clinically (Coker, 2003). Hyperinsulinemia was observed in the HFD/STZ treated group, greater cognitive decline has been reported among diabetics (Gregg *et al.,* 2000) compared to normoglycemic individuals. The hyperglycemia and hyperinsulinemia observed in this study further strengthens the memory impairment observed in the Y-maze and MWM tests earlier reported in this study

Hyperglycemia has been linked to cognitive decline, Alzheimer's disease, dementia and neurodegeneration in general (Barbagallo & Dominguez, 2014). TCA treated groups show improvement in insulin and glucose level, similar reports had shown that cinnamon (of which TCA is an active component) plays an essential role in obesity and diabetic conditions (Verspohl *et al.,* 2005; Wan-Nurdiyana, 2014).

Insulin degrading enzyme is highly expressed in the brain and fosters amyloid beta (Aβ) clearance and intracellular degradation. It is also responsible for the clearance of insulin. Hyperinsulinemia may result in competitive inhibition of insulin degrading enzyme thus, preventing Aβ degradation, resulting in Aβ accumulation in the brain leading to AD-like pathology (Verdile, 2015).

Treatment with TCA shows improvement in insulin level by reducing hippocampal insulin (figure 7). Similar reports had shown that cinnamon (of which TCA is an active component) possesses anti hyperinsulinemic properties. (Bolin *et al.,* 2010).

5.8 Trans-cinnamaldehyde treatment prevents hippocampal tissue distortion potentially caused by HFD/STZ

Histopathological study is a common approach used to identify specific mechanism that mediate biochemical and behavioural changes.

Microscopical examination of hippocampus was observed by staining with Hematoxylin&Eosin and Cresyl fast violet.

It was shown from the result that the hippocampus of the normal control and the group treated with TCA (figure 4.12 a,c and f) had normal basic histological features of the hippocampus comparable to the normal control. The histological presentations of these groups were dominated by distinctly arranged pyramidal cell layers. The well-arranged hippocampal cellular layer and neuronal morphology in these groups suggest an appropriate interconnectivity within the hippocampus. Insulin resistant control group however, shows disorganization of neuronal cell layers, with degenerating and pyknotic cells. Such alteration in cellular morphology may be responsible to the behavioural deficits observed in Y-Maze and MWM tests, this observation is similar to what was earlier reported by Shaimaa *et al*., (2013).

The insulin resistant control group (figure 4.12 b) showed various histopathological changes ranging from neuronal degeneration with pyknotic nuclei, reduced layer of neuronal cell and morphology when compared with normal control group (figure 4.12 a) and the groups treated with TCA alone, treatment with TCA at a dose of 40 mg/kg (figure 4.12 g and h) did not prevent the neuronal degeneration. However, TCA treatment at a dose of 60 mg/kg (figure 4.12 d and e) shows a decrease in neuronal degeneration and normal histo-architecture comparable to the normal control group, normal layer of neuronal cell when compared with insulin resistant control group (figure 4.12 b).

This result shows that TCA was able to protect the histo-architectural morphology of the brain following neurotoxicity. The normal histological features were preserved in the normal control group and TCA treated groups contrast in the HFD/STZ treated groups, there were marked change in the CA3 region of hippocampus in the form of disorganization and cell loss.

5.9 Trans-cinnamaldehyde treatment reduces the expression of beta amyloid and plaques

There was an observed increased deposition of amyloid plaque and proteins in the hippocampus of HFD/STZ treated rats (plates 4 and 5), Alzheimer's disease is primarily characterized by the formation of amyloid β (Aβ) plaques, amyloidal angiopathy, neurofibrillary tangles, loss of neurons and synapses (Sisi *et al.*, 2017). However, treatment with TCA reduced amyloid plaque burden, this may be due to the anti-inflammatory and hypoglycemic activities of TCA earlier reported in this study, had shown that cinnamon have activities against neurological disorders, such as Parkinson's and Alzheimer's diseases (Pasupuleti *et al.*, 2014; Camacho *et al.*, 2015). The increased Aβ accumulation in the HFD/STZ may partly be due to increased levels of TNF-α earlier reported (Griffin *et al*., 1998).

AD-like pathological changes such as abnormal Aβ deposition and tau hyperphosphorylation were observed in patients with insulin resistance and type II diabetes (Judith *et al*., 2010) and diabetic animal models (Li and Zhang, 2007).

Brain insulin resistance and amyloidogenesis are central for hyperglycemia-induced impairment of cognitive function. Neuroinflammation, oxidative stress, and mitochondrial dysfunction are known to aggravate brain insulin resistance and amyloid beta accumulation in brain lesion. High levels of amyloid beta in brain can lead to neuronal structure deterioration

which can lead to poor cognitive performance observed in the neurobehavioral tests conducted in this study.

Treatment with TCA significantly ameliorated cognitive deficits, with significant decreases in amyloid burden and TNF-α levels via inhibition of NF-κB in brain as well as attenuation of hyperglycemia and hyperinsulinemia.

Cognitive dysfunction and impaired synaptic plasticity in both types of diabetes have been linked to hyperglycemia, insulin resistance and altered insulin signaling.

HFD/STZ administration (figure 4.14 b) resulted in increased beta-amyloid plaques (red arrows) whereas treatment with various doses of TCA resulted in reduction in the distribution of beta-amyloid plaques (figure 4.14 d,e,g and h).

5.10 Trans- cinnamaldehyde intervention reduces astrogliosis

In this study, GFAP was significantly increased in the untreated insulin-resistant group (figure 4.15 b) compared to the control and TCA alone groups (figure 4.15 a,c and f), which disagrees with findings by Shaimaa *et al*., (2013) that reported decrease in GFAP activity in insulin resistance model. The reaction of astrocytes had been reported to be the earliest response of the brain tissue to an altered glucose metabolism (Shaimaa *et al.*, 2013).

However, for the groups treated with HFD/STZ followed by TCA has a very similar appearance with that of control and TCA group. GFAP immunohistochemistry revealed that many of the neurons between astrocytes have normal morphology. These findings elucidate the neuroprotective properties of TCA in preventing astrogliosis in the hippocampus of rats induced by TCA administration.

Astrocytes constitute the most abundant class of neuroglia, they are widely distributed in mammalian nervous system where they serve wide range of adaptive functions (Clarke *et al.,* 2013). Astrocytes interact with neurons to provide structural, tropical and metabolic support, they are now emerging as key components in many aspects of brain development, function and disease (Clarke *et al.,* 2013).

Astrocytes have increasingly been implicated in most demyelinating diseases. Astrocytes are critical for the survival of neurons in the central nervous system (CNS) by playing a role in the energy metabolism, maintenance of the blood-brain barrier, vascular reactivity, regulation of extracellular glutamate levels and finally protection from reactive oxygen species. These cells react to the neuronal damage, resulting from physical or chemical insults, by over expression of the glial fibrillary acidic protein (GFAP), an intermediate cytoskeletal filament protein specific for astrocytes.

Presence of reactive astrocytes surrounding amyloid plaques, appeared hypertrophied with increased thickness of their cytoskeletal processes was demonstrated in the insulin-resistant control group (figure 4.15 b) compared to normal control, TCA treatment at high and low doses shows reduction in the thickness of these processes compared to the insulin-resistant control group. Reactive gliosis, including astrocyte and microglial activation detected by increased glial fibrillary acidic protein (GFAP) and microglial levels, are other important features of AD neuropathology (Leticia *et al.,* 2014), moreover, prominent astrogliosis was revealed in GFAP-immunohistochemically stained, these astrocytes play a vital role in degradation of amyloid plaques through the astrocytic processes which internalize and degrade Aβ deposits (Koistinaho *et al.,* 2004). However, they secrete inflammatory mediators that lead to neuronal injury (Johnstone and Gearing, 1999)

This result is in agreement with different studies that showed that HFD/STZ intoxication often leads to the activation of astrocytes and that treatment.

5. 11 Trans cinnamaldehyde treatment restores neuronal loss and increases cell count in HFD/STZ treated rats

NeuN is a neuron-specific nuclear protein expressed in the nucleus and cytoplasm of most neuronal cell types in vertebrate nervous systems. (Tippett *et al.,* 2007).

In this work, there was an observable decrease in NeuN immunoreactivities, studies have suggested that quantitative changes in NeuN immunoreactivity can be a determinant of neuronal loss in several pathologies including neurodegenerative diseases (Tippett *et al.,* 2007).

Neuronal degeneration and cognitive impairment are the most typical features of Alzheimer's disease (Song *et al.,* 2014) which can have direct impact on the ability of patient to recall or recognize new information processed in the hippocampus (Song *et al.,* 2014)

In the untreated diabetic group, there is the loss of NeuN immunoreactivity (an indication of neuronal loss), the administration of TCA at high and low doses restores the immunoreactivity, numerous studies have confirmed neurogenesis in hippocampal neurons (Dong *et al.,* 2003; Mu *et al*., 2011).

TCA treatment alleviated neuronal damage in the hippocampus of rats administered HFD/STZ this result indicates that TCA at 60 mg/kg/d can promote neuronal recovery and at the same time improve neurogenesis in the hippocampus. Changes in neurogenesis had been reported to alter some hippocampal dependent functions such learning and memory (Jason *et al.,* 2011). Neurogenesis and neuroplasticity in the hippocampus are sensitive to many pathogenic and treatment factors that are associated with metabolic diseases including

diabetes. Previous studies provide strong evidence that diabetes adversely affects the structural integrity of the hippocampus, which may contribute to diabetes induced cognitive impairment (Sommersa and Irwin, 2013).

The insulin resistant group shows a significant decrease in cell count (figure 4.17), this is significant because it may be responsible for the memory deficits earlier reported in this study and may provide one of the mechanisms underlying the cognitive decline associated with T2DM and AD, however, treatment with TCA significantly restored the cell count.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study revealed some of the morphometric, morphological and histological changes that occurred in the brain of insulin resistant rats following TCA treatment.

HFD/STZ produces AD-like pathological changes in the hippocampal regions of Wistar rats.

Morphometrically, there was an observed decrease in the hippocampal weight among the HFD/STZ treated group

HFD/STZ administration resulted in hyperglycemia, hyperinsulinemia, insulin resistance and chronic inflammation with accompanied neurobehavioral deficit in hippocampal dependent memory tests.

The hippocampus of Wistar rats exposed to HFD/STZ produces alteration in the normal histoarchitectural layout of the hippocampus characterized majorly by pyknotic cellsls and

Immunohistochemical findings reveal increased astrogliosis, accumulation of amyloid plaques and neurodegeneration among HFD/STZ treated group.

TCA administration confers therapeutic advantage to the hippocampus of wistar rats in a dose dependent manner.

TCA administration at higher and lower doses significantly restores memory performance, improves glucose and insulin level, and suppresses neuroinflammation among HFD/STZ treated rats.

TCA treatment restores normal histoarchitectural structures of the hippocampus among wistar rats challenged with HFD/STZ.

6.2 Contributions to knowledge

This study has further reiterated the fact that HFD/STZ treatment can lead to insulin resistance and other neurodegenerative changes.

Structural atrophy can be one of the mechanisms through which neurodegenerative changes occur.

Significant memory impairment is associated with HFD/STZ administration

Trans- cinnamaldehyde is the active ingredient in cinnamon with potent anti-hyperglycemic activities.

Trans- cinnamaldehyde possesses memory enhancing properties.

Trans- cinnamaldehyde suppresses neuroinflammation by blocking nuclear factor kappa b pathway.

Significant histomorphological alterations of the hippocampus may to a greater extent be responsible for the neurobehavioral and cognitive impairment associated with AD-like pathological changes.

6.3 Recommendation

With gradual shift in traditional diets to western diet, care must be taken in the intake of western diet

Increase in life expectancy is associated with age related diseases, attention is needed for the aged to avoid exponential outbreak of age-related neurodegenerative diseases.

Neuro-pharmacological industries should explore further the therapeutic potentials of Trans-cinnamaldehyde against neurodegenerative diseases.

6.4 Limitation of the study

Electron microscopy could not be carried out in this study to better demonstrate some observed changes at subcellular level.

6.5 Further study

The next phase of this study will employ the use of electron microscopy to investigate subcellular changes

In vitro studies would be used to evaluate the therapeutic potentials of TCA so as to further buttress findings from this study

Clinical trials would be necessary so as to validate the reported findings from this work

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