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Curcumin Ameliorate Cortical Histomorphological Deficits in Streptozocin and Western Diet Induced Neurodegeneration in Wistar Rats.

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ABSTRACT

We employed a rodent model to typify histomorphological changes connected to dementia-like symptoms and co-morbid insulin resistance; we examined cortical neuronal density and morphological changes; evaluated amyloid (Aβ) deposits and probed the association between phosphatidylinositol 3-kinase (PI3K), serine/threonine Protein kinase (AKT) and glycogen synthase kinase 3β (GSK3β) activity and oral curcumin regimen. 36 adult Wistar rats were randomized into six groups (n=6), control group rats received 1ml olive oil; the curcumin group received 200mg/kg BW curcumin; the diabetic model received HFD for 60 days, then three doses of 40mg/kg BW of streptozotocin (STZ); protective group were administered HFD, three doses of 40mg/kg BW of STZ and a concurrent treatment with 200mg/kg BW curcumin within 60 days; The preventive group rats received a pretreatment of 200mg/kg BW of curcumin, HFD for 60 days and three doses of 40mg/kg BW of STZ; the therapeutic group rats were administered HFD, three doses of 40mg/kg BW of STZ within 60 days, followed by 200mg/kg BW of curcumin for 21 days; Cortical sections were stained for microscopic investigations. ELISA was used for quantifying PI3K, AKT and GSK3β activity. Data was analyzed using one-way ANOVA and Turkey's *post hoc* test. $p<0.05$ was considered significant. Findings indicate that insulin resistance was associated with cortical cyto-architectural deficits while curcumin ameliorated observed deficits; furthermore, Oral curcumin reduced cortical Aβ deposits and corrected impaired PI3K, AKT and GSK-3β activities in the studied models. Curcumin showed it ameliorative potentials by up regulating PI3K and AKT activity and inhibiting GSK-3β.

Keywords: Diabetes Mellitus, Insulin resistance, Alzheimer's disease, Prefrontal cortex, Neurodegenerative disease, Curcumin.

INTRODUCTION

The Human brain is an organ of the central nervous system saddled with the responsibility of integration of received impulses and interpretations of same for proper coordination of activities. This very important organ with a mass of 1.4 kg which is approximately 2% of the total body weight is one of the most intricate of the human organs and consumes approximately 20% of the total energy produced in the body[1]. The main source of energy to the brain is glucose, hence glucose utilization and balance is critical to efficient brain function. Glucose regulation is achieved primarily by insulin which plays an important role in the regulation of energy balance and glucose homeostasis[1]. An imbalance in glucose homeostasis leads to metabolic disorders, the chief of which is Diabetes mellitus (DM). Diabetes mellitus is a disorder of insulin regulation of blood glucose levels, it constitutes an epidemic and is characterized by a sustained marked increase in blood glucose level (hyperglycemia) which could be insulin dependent, described as Type I diabetes mellitus (T1DM) or non-insulin dependent referred to as Type II diabetes mellitus (T2DM)[2]. T2DM is characterized by hyperglycemia and hyperinsulinemia due to inability of cells to react to insulin availability and signaling, this eventually leads to insulin resistance in the brain and other tissues, accumulation of defective proteins in brain regions, and ultimately, neuronal disorders[2].

The World Health Organization reported a global prevalence of about 422 million people with diabetes, of this figure; more than 95% of the people have T2DM. An estimated 1.5 million fatalities were recorded on account of diabetic complications in the year 2019[3]. Uloko *et al.,*[4], estimated the country-wide prevalence of DM to be 5.77% which translates to about 11.2 million Nigerians, this figure translates to one out of every 17 adult in Nigeria. This epidemic is further aggravated by the fact that T2DM which was previously believed to be adult-onset diabetes is fast becoming more common in young people, challenging the idea that it usually occurs exclusively in people older than 30 years of age[5,6].

Dementia is a brain illness associated with a loss of cerebral ability severe enough to truncate usual occupational functioning and/or typical social activities[7]. Dementia is characterized by severe compromise of memory and was believed to be solely due to amyloid-β accumulation; however a critical evaluation of the neuronal energy metabolism revealed an association between poor glucose homeostasis and neurodegenerative disorders via disruption in insulin signaling and insulin resistance, this ultimately places dementia in the core of other well established metabolic disorders and a complication of poorly managed T2DM[1,8]. These aforementioned points highlight the key regulatory role insulin play in energy balancing and maintenance of glucose utilization and storage. A significant tilt in the normal glucose homeostasis results in production of reactive oxygen species, mitochondria dysfunctions, oxidative stress, compromised protein synthesis leading to defective degradation of amyloid precursor hence the consequent accumulation of amyloid-β and the complementary abnormal phosphorylation of Tau protein. The later of these events lead to poor signal

transduction, neurotransmission is compromised and the neurons while unable to function efficiently degenerates gradually and eventually die off[1].

Protein changes, oxidative stress, inflammation, dysregulated immunology, poor neuronal-glial communication, and an increase in neurotoxic chemicals all contribute to neuronal death in Alzheimer's disease. Aβ pathology (Aβ plaques) and tau pathology (neurofibrillary tangles (NFTs)) are the two pathologies that constitute Alzheimer's disease[9]. There is mounting evidence that when tau proteins take on pathological forms, they impair neuronal function and cause neuronal death, implying that tau is a key facilitator of Aβ toxicity and, the resultant, AD pathology[10]. Tau proteins bind to tubulin to stabilize microtubules and vesicular transport in the normal state but Tau proteins that have been hyperphosphorylated and aggregated form neurofibrillary tangles, these tangles becomes insoluble and loses its affinity for microtubules when it is in hyperphosphorylated form, resulting in neurodegeneration[11,12].

Insulin resistance promotes age-related memory deficits and is a risk factor for Alzheimer's disease, according to numerous studies. The biochemical and cellular relationship between insulin resistance and Alzheimer's disease, however, remains ambiguous. In the same vein, impaired insulin function has been increasingly demonstrated in AD, suggesting that decreased brain insulin levels/action may constitute the link between both pathologies[2]. Several earlier researches have looked into the role of metabolic abnormalities in the etiology of Alzheimer's disease. Balanced glucose levels are necessary for energy production, neurogenesis, neuronal survival, and synaptic plasticity, all of which are important for learning and memory[2,7].

We hypothesize that insulin resistance will reduce neuronal sensitivity to insulin signaling; this will hereby result in hyperinsulinemia, and the impairment in insulin signaling plays a key role in AD pathogenesis, manifesting as inflammation of the brain tissue, oxidative stress, changes in amyloid beta (Aβ) levels, and finally neuronal cell death[13].

Medications that control insulin resistance have been found in human and experimental animal trials to diminish Aβ build-up in the brain as well as improve cognitive abilities[14]. As a result, therapeutic methods that focus on elucidating the relationship between insulin resistance and Alzheimer's disease may aid the development of future Alzheimer's medications[15].

One of such promising agent of therapeutic importance is Curcumin (CUR). It is an antioxidant and anti-inflammatory molecule in the root of turmeric, a relative of the popular ginger. Turmeric has been used for thousands of years as a medicinal preparation and as a preservative and coloring agent in foods[16]. Curcumin is the principal curcuminoid found in turmeric (Curcuma longa Linn.), a popular spice in Asian cuisine. It is widely consumed and generally believed to be beneficial for human health[16]. Curcumin extract from rhizomes of turmeric has been shown to contain compounds with anti-inflammatory and antidiabetic properties and direct free radical scavenging properties at high concentrations (300 mg/kg). Lower concentrations can activate or inhibit one or

more signal transduction pathways in cells[16,17]. Curcumin modulates the expression of various molecular targets, such as transcription factors, enzymes, cytokines, cell cycle proteins, receptors and adhesion molecules[18]. CUR may antagonize the deficit of glucose energy metabolism or oxidative stress related to cognitive impairment, as seen in AD[19].

The aforementioned suggests CUR could be beneficial in ameliorating neuronal deficits and insulin resistance induced by HFD and STZ. Increasing empirical evidence supports an association between insulin resistance and the pathogenesis and progression of neurodegenerative disease, however the molecular basis of the association between insulin resistance and Alzheimer's dementia as well as the potential ameliorative effects of oral curcumin (CUR) on insulin resistance-induced neurodegenerative changes characteristic of Alzheimer's disease requires scientific validation, hence the purpose for this research endeavor.

MATERIALS AND METHODS

Animal Model and Materials Used: Adult male Wistar rats weighing 170±30g were housed in the animal holdings of the Faculty of Basic Medical Sciences, University of Ilorin in accordance with the ethical rules of University of Ilorin, as granted in the approval designated number: UERC/ASN/2016/654 and the NIH Guide for the Care and Use of Laboratory Animals. The rats were housed in plastic cages under typical laboratory settings and were allowed free access to standard rat chow, HFD and water according to the grouping. Streptozotocin was obtained from Sigma Aldrich (USA). High fat diet fed to the rats was formulated using the composition adapted from small and collegues[20], the composition is described in Table 1, while curcumin was also a product of Sigma Aldrich. The Olive oil used was a product of Goya® procured from a local vendor. Abcam's products are; colorimetric assay kits for insulin and glucose. Antibodies rats' (Anti-GFAP) was bought from Cell Signaling® USA.

Animal Grouping and Treatments: A total of thirty-six (36) adult male Wistar rats used for this study were randomized into six groups of 6 rats each (n=6); treatment is as described below: control group rats received 1ml olive oil daily throughout the experiment; the curcumin group received 200 mg/kg BW curcumin; the diabetic model received HFD for 60 days, then three doses of 40mg/kg BW of streptozotocin (STZ); protective group were administered HFD, three doses of 40mg/kg BW of STZ and a concurrent treatment with 200mg/kg BW curcumin within 60 days; The preventive group rats received a pretreatment of 200mg/kg BW of curcumin, HFD for 60 days and three doses of 40mg/kg BW of STZ; the therapeutic group rats were administered HFD, three doses of 40mg/kg BW of STZ within 60 days, followed by 200mg/kg BW of curcumin for 21 days; After all treatments have been concluded, the rats were euthanized. Gallenkamp (FA2104A, England) digital weighing balance was used to measure the weight of rats across the groups and weight change was calculated from the obtained data. The fasting blood glucose level was checked weekly using a digital glucometer (Accu-Check, Roche, Belgium), and Homeostatic model assessment of insulin resistance (HOMA-IR) was evaluated by first measuring the level of expression of

fasting plasma glucose and fasting plasma insulin, then ultimately determining the HOMA-IR index across the various groups. HOMA-IR was calculated using the formula = (glucose in nmol/L x insulin in mU/L)/22.5.

Induction of Hyperglycaemia: Hyperglycaemia was induced by multiple low-doses (three doses in all; one dose every 48 Hours) at 40 mg/kg streptozotocin dissolved in chilled, buffered sodium citrate, It was administered through the intraperitoneal route each day of treatment after each rat has been fasted overnight. Fasting blood glucose readings were determined by means of the glucose oxidase method using a glucometer 72hours after STZ injection. Rats with fasting blood glucose concentrations of 200 mg / dl and above were included in the study.

Sacrifice and Tissue Excision for Processing: Rats were euthanized with (20 mg/kg) ketamine given intramuscularly for rats used for histological, histochemical and immunohistochemical assessments, sacrifice was followed then by an incision and opening of the abdominal and thoracic cavity to allow access for transcardial perfusion fixation. After the animal has been perfused, the whole brain tissues were excised and post fixed in 4% paraformaldehyde for 24 hours and after which they were equilibrated in 30% sucrose solution. Cervical dislocation method was used for the rats used for colorimetric assays. Tissues fixed in paraformaldehyde were then embedded in paraffin wax; thin coronal sections (10 μm) of the prefrontal cortex were obtained, and processed to demonstrate amyloid beta deposits by Congo red stain, Cresyl fast violet staining was used to demonstrate Nissl substances, the general cytoarchitecture was demonstrated using Hematoxylin & Eosin stain, and Glia fibrillary acidic protein immunohistochemical assay demonstrated astrocytic distribution and forms

Colorimetric Assay for Hormonal Studies: Adopting rat insulin as the standard the manufacturer's instructions was followed to determine the fasting plasma insulin concentrations using rat ELISA insulin kit (Mercodia, Sweden). Similarly, the fasting plasma glucose was also measured following the manufacturer's guide in the kit from Span Diagnostics (India).

Light Microscopy: Histological, histochemical and immunohistochemical cortical photomicrographs were captured and analysed using an Amscope Camera mounted on an Olympus binocular light microscope and attached to a computer with Windows operating system for image capturing.

Brain Homogenate for Protein Assays: Portions of the pre-frontal cortex were excised and weighed, 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 PI3K, AKT and GSK3β ELISA assays, assessments were done following manufacturer's guide included in kits from Cusabio (USA).

Data Analysis: The quantitative data were analyzed using GraphPad, version 6 (GraphPad Software Inc. California U.S.A.

using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test and data were presented as mean ± SEM, (n=6) $P<0.05$ was considered statistically significant.

RESULTS

Effect of Insulin Resistance on Weight Gain in the Diabetic/Insulin Resistant Models: Morphometric results from Table 2 show that there was a significant increase in the weight of the rat in the diabetic model and flavonoids in curcumin reduced weight gain in the rat model that received curcumin intervention ($p\leq 0.05$)

Elevated Glucose Level and Insulin Insensitivity in the Brains of the Insulin Resistant Models: Figure 1a shows a significant increase in the blood glucose levels of the untreated diabetic rats relative to control and curcumin treated in the therapeutic group ($p\leq 0.05$). Curcumin treatment significantly reduced HOMA-IR Index of rats that took the intervention. Figure 1b showed control rats and curcumin treated rats with Insulin resistance (5.33±0.48 and 5.85±0.53 respectively), significant increase was recorded in diabetic rats (14.89±1.01), while a significant reduction in the insulin resistance was recorded in the therapeutic group 7.20±1.48 ($p\leq 0.05$).

Effect of Curcumin on Cortical Expression of PI3K, AKT and GSK-3β.: Fig. 2a, and 2b shows a significant increase in the expression of PI3K and AKT respectively in the curcumin treated groups compare with the untreated insulin resistant models, this increase led to the inhibition and observed decrease in the activities of Glycogen synthase kinase (Fig 2c). Curcumin raised the PI3K level in the therapeutic group. A similar trend was observed in Figure 2b, as AKT activity was also increased in the curcumin treated model relative to the untreated insulin resistant rats, However Figure 2c shows a significant increase in Glycogen Synthase Kinase-3 β level in diabetic rats relative to the level in the control and therapeutic group rats ($p\leq 0.05$).

Cortical Cytoarchitectural and Nissl Staining in the Insulin Resistant Model: Characterization of the histology of sections of the prefrontal cortex (Fig. 3) showed that sections from Olive oil and curcumin treated rats presented with typical histoarchitectural definition of the cortical layers with proper delineation and staining characteristics. The cellularity, neurophilic morphology and cellular density appear characteristically normal with no apparent histopathological alteration. Sections from the STZ+HFD treated rats present with poor histomorphological delineation, reduced and shrunken granular cells indicated by their small stained nuclei (black arrow) as well as degenerative propensities indicated by the white halo-spaces (red arrows). Sections from the curcumin treated rats present with histomorphology similar to the control. The cellularity and cortical histomorphology appear characteristically normal. However, they appear to have reduced cellular density and halo-spaced neutrophils as well as few darkly stained nuclei.

The Nissl profile of sections of the prefrontal cortex were examined microscopically, (Fig. 4) sections from the STZ+HFD treated rats revealed a reduction in chromatogenic properties (pale purple) and staining intensity (White hallow spaces) relative to the controls and the rats that took

curcumin intervention. Figure 4 also revealed that Olive oil and Cur treated rats present with characteristic staining intensity with Nissl intactness. Sections from the untreated diabetic rats present with reduced staining intensity across the cortical layers, section from the curcumin treated rats present with restoration of chromatogenic properties and better Nissl staining intensities (Blue) than the untreated diabetic rats.

Cortical Amyloid Aggregation and Astrogliosis in Insulin Resistant Models: Amyloid plaque pathology was evaluated in the prefrontal cortex of the rats using the Congo red staining technique, (Fig. 5) revealed that STZ+HFD treated rats presented with deposits of amyloid (red arrows), The STZ+HFD+Cur treated rats presented with healthy granular cells in the cortical areas devoid of amyloid deposits, and this is similar to the cellular intactness and amyloid deposit-free observation in the cortical sections of the control rats. The astrocytic profile of cortical sections of the rats were also examined using the GFAP immunolocalization, (Fig. 6) reveals that, Olive oil and Cur treated rats present with normal granular cells and normal appearance of supporting astrocytes. STZ+HFD treated rats present with astrocytes appearing activated, noticeably larger in size and invaded the granular cells. However, the prefrontal cortex of curcumin treated rats show less activated astrocytes accumulation in amyloid deposit vicinity. The astrocytic profile of curcumin treated rats appear similar to that observed in the olive oil with no observable astrocytic hypertrophy, astrocyte activation or astrogliosis.

Table 1: Composition of High Fat Diet feed

Constituents	Standard Rat Diet (%)	High Fat Diet (%)
Lard	-	17.5
Beef tallow	-	17.5
Full fat soya	-	40
Fish meal	20	2
Ground nut cake	10	10
Palm kernel cake	-	3
Wheat bran	15	-
Limestone	0.3	0.4
Soya bean	12	4
Di-calcium Phosphate	1	1
Premix	0.6	0.5
Salt	0.1	0.1
Bone meal	1	-
Maize	40	4

Composition of standard rat chow and High-fat diet fed to rat model of diabetes, formulation was modified from Small *et al.*, 2018).

Table 2: Weight changes in the rats in the treatment and control groups

Groups	Initial weight (g)	Final weight (g)	Weight Difference (g)
Negative control (olive oil**)**	147.40±8.58	177.57±6.19	29.83±1.22
Positive control (curcumin**)**	156.88±6.92	184.28±8.22	28.66±3.50
Diabetic group (STZ+HFD**)**	158.13±8.35	203.71±7.53*	45.16±1.98
Protective group (concurrent STZ+HFD+Curcumin**)**	147.65±4.92	185.40±3.47*	37.83±2.38
Preventive group (Curcumin+STZ+HFD**)**	139.44±7.37	172.18±5.36	32.66±0.94
Therapeutic group (STZ+HFD+Curcumin**)**	156.28±2.41	188.41±3.68	31.33±2.18

Values are expressed as mean ±SD, N=6 in each group. *= significant difference when compared to the control group ($p<0.05$)

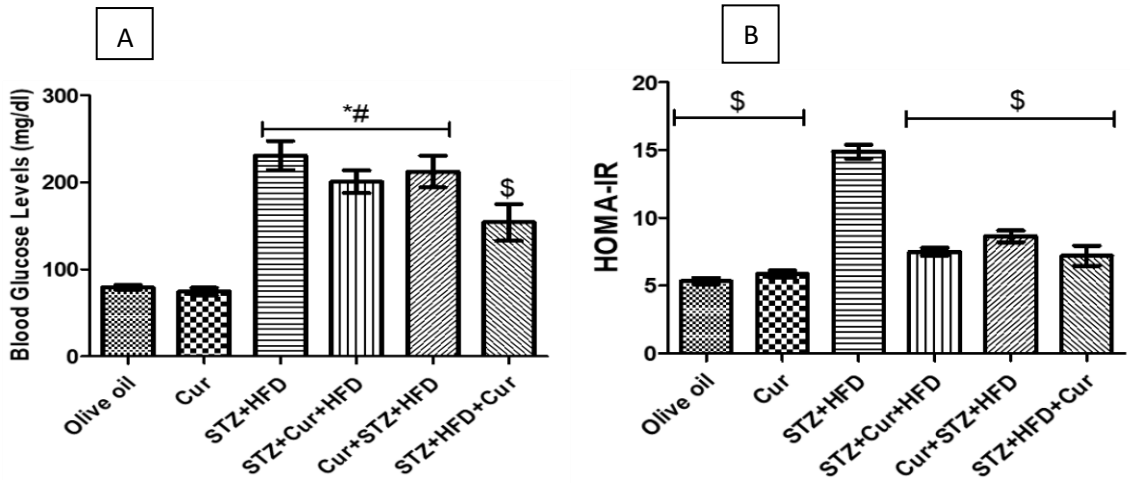

Figure 1: A & B: 60 days Fasting Blood Glucose (A) as well as Insulin resistance as assessed by the HOMA-IR Methods (B) Fasting blood glucose levels across the groups. (n=4), Values are expressed as mean ± SEM, ($, # and *) represents significance difference ($p<0.05$) compared to the STZ+HFD group, Cur group and olive oil group respectively. STZ=Streptozotocin, HFD=High Fat Diet, Cur=Curcumin

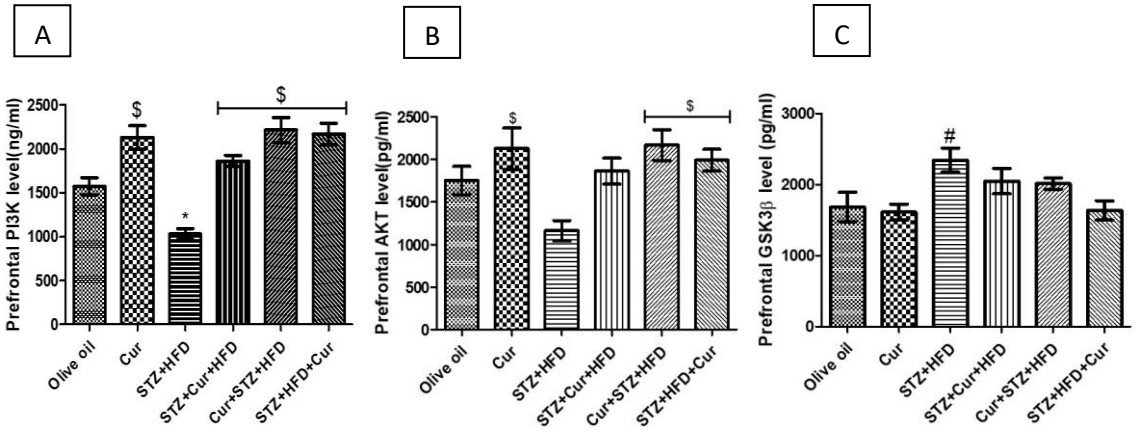

Figure 2: PI3K (A) AKT (B) and GSK3β (C) Activities quantified in the Pre-frontal Cortex of Rats, (n=4), Values are expressed as mean ± SEM, ($, # and *) represents significance difference ($p<0.05$) compared to the STZ+HFD group, Cur group and olive oil group respectively. STZ=Streptozotocin, HFD=High Fat Diet, Cur=Curcumin

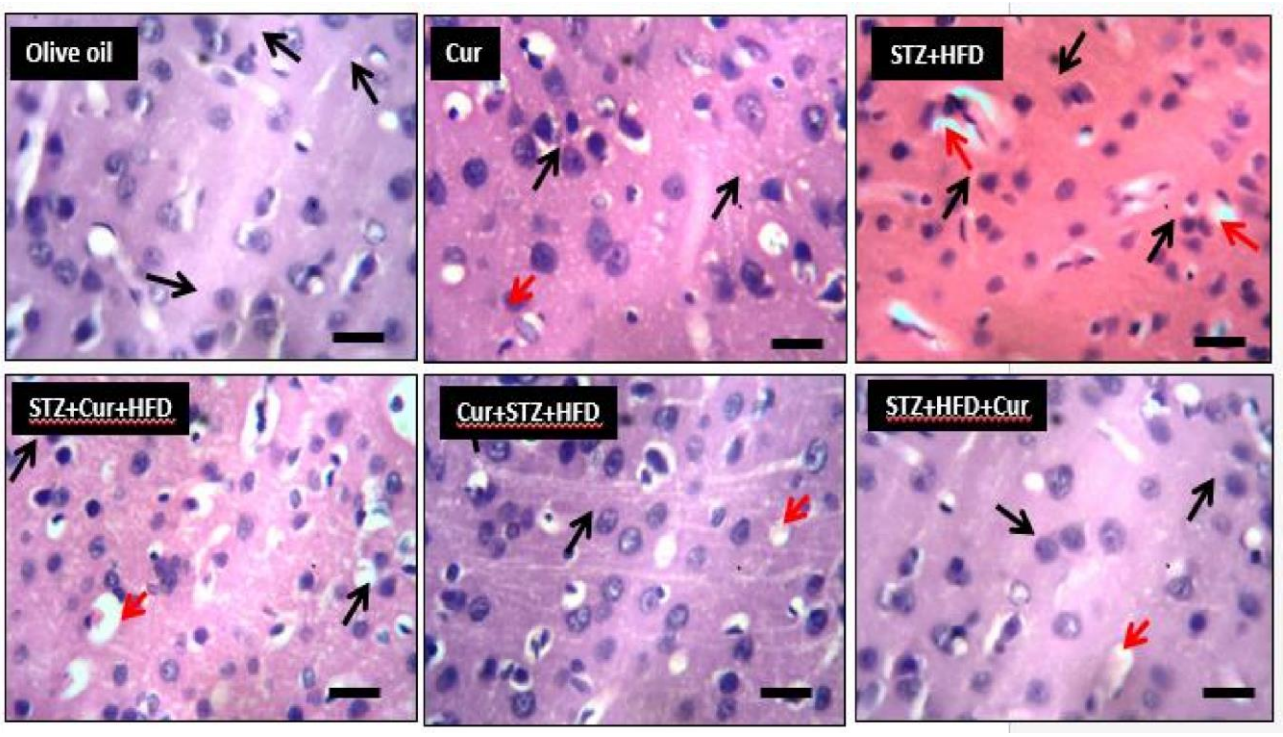

Figure 3: Demonstration of cortical morphology control, diabetic and curcumin treated rats, showing the external granular layer cytoarchitecture. There are observable neuronal distortions, shrunken nucleus, vacuolation and pyknosis (red arrows) in the diabetic group. Contrariwise, cortical cytoarchitecture in control and curcumin treated rats appeared normal. H&E staining; scale bar = 50 μm

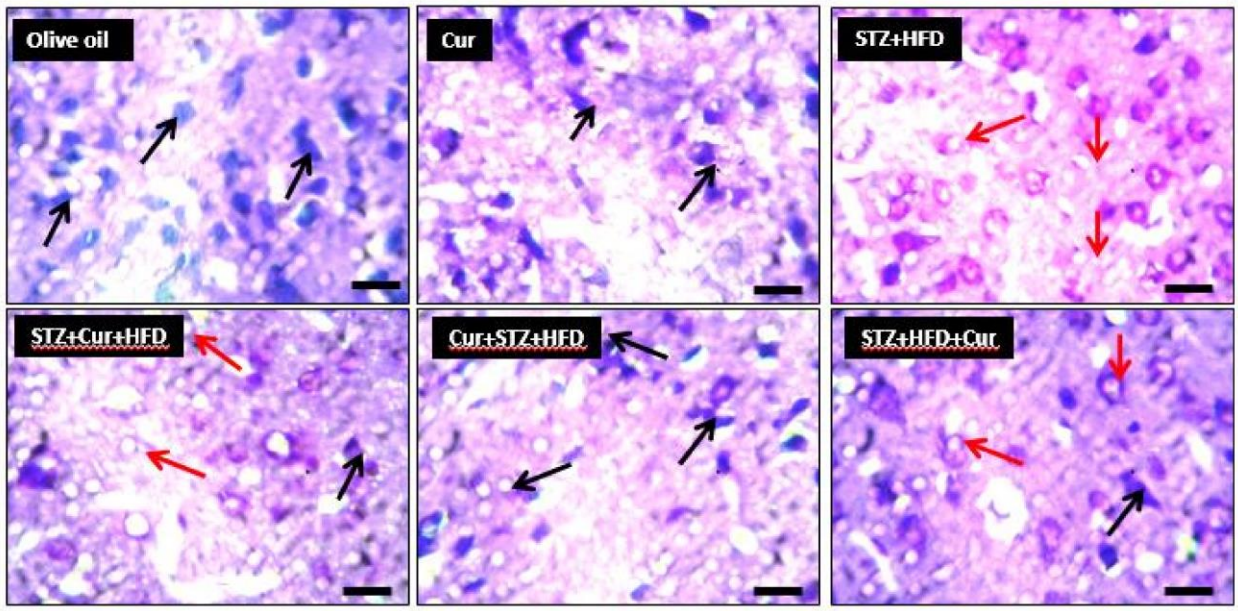

Figure 4: Demonstration of cortical Nissl substance profiles of rats across the different treatment regimen, Similar to histological observations, cortical external granular layer of control and curcumin treated rats presents with deeply stained Nissl substances, while the diabetic/insulin resistant rats presents with diffused Nissl substance (red arrows) due to chromatolysis, CFV stain, scale bar = 50 μm

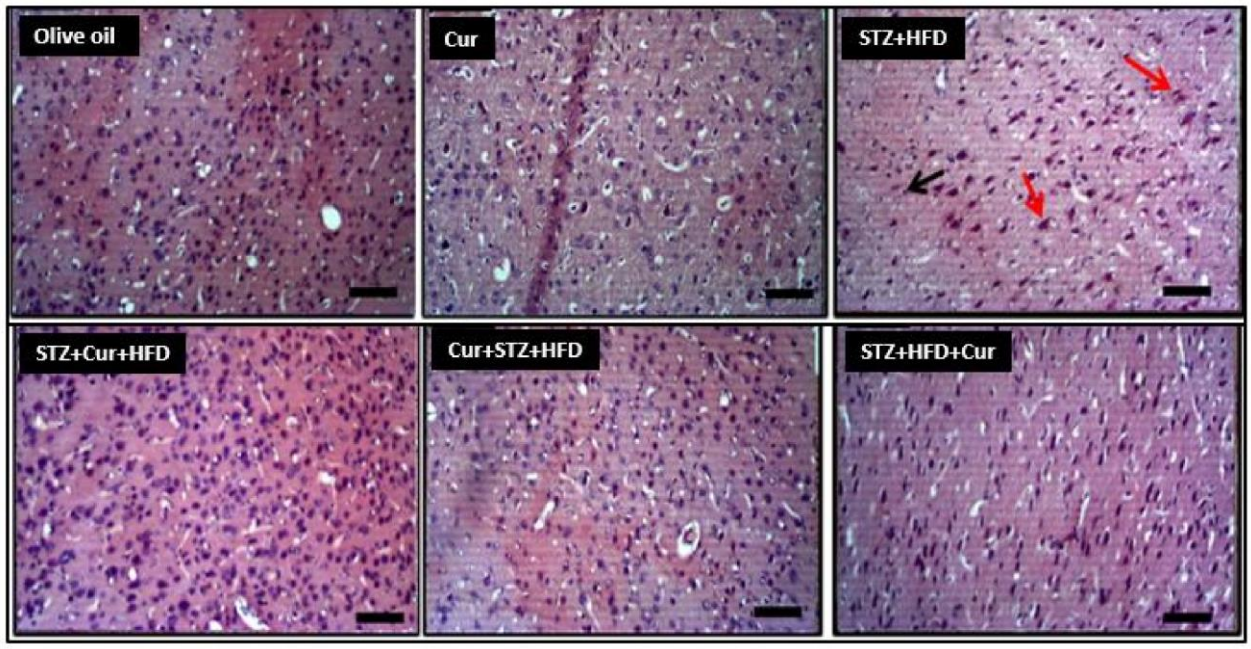

Figure 5: Demonstration of amyloidogenesis in the rats exposed to the different treatment regimen, cortical external granular layer of control and curcumin treated rats appeared normal with no apparent amyloid deposits. However, the diabetic/insulin resistant rats presents with pockets of amyloid deposits (red arrows). Congo red stain, scale bar = 50 μm

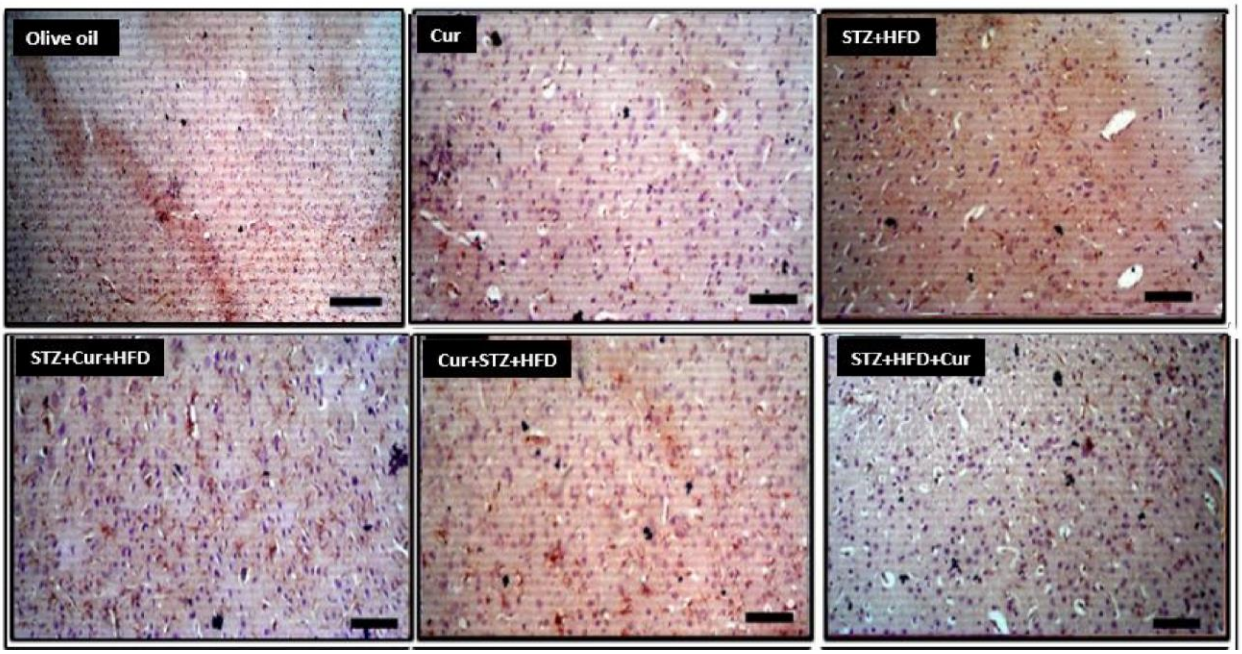

Figure 6: Activated astrocyte profile was measured via GFAP immunohistochemical study of the prefrontal cortex across the different treatment groups, Observably, the diabetic/insulin resistant, protective and preventive presents with increased GFAP positivity cells relative to the control, curcumin only and therapeutic group rats ($p < 0.05$) GFAP, Scale bar = 100 μm

DISCUSSION

Curcumin was studied to explore its ability to influence and enhance neuronal survival in a rat model of insulin resistance and associated neurodegeneration similar to the presentations in Alzheimer's disease. AD is a neuropathological disorder characterized by abnormal accumulations of extracellular amyloid plaques and intracellular neurofibrillary tangles throughout cortical and limbic brain regions[8]. Cognitive deficits in AD are widely believed to result from progressive synaptic dysfunction and neurodegeneration initiated by soluble aggregated β amyloid peptide 1–42 (Aβ42) and further involving aggregates of hyperphosphorylated tau, a principal component of intracellular neurofibrillary tangles[18]. Curcumin is reported to have the ability to regulate glucose metabolism by initiating glucose uptake and improving insulin signaling[18].

In this study, we established a link between disruption in energy metabolism and progressive cognitive impairment which has been implicated in obesity associated with insulin resistance (IR) and other chronic, diet related illnesses, including Alzheimer's disease type of dementia. The combination of STZ and HFD we used to be previously employed by researchers in the modeling of T1DM and T2DM; the reported mechanism of action is through the initiation of pancreatic β cells death via DNA alkylation[21]. Administration of multiple low doses of STZ as employed in this study led to a reduction of insulin secretion and action, mimicking T2DM with associated insulin resistance, metabolic imbalance and

resultant neurodegenerative deficits. HFD and STZ offers a simple, inexpensive, yet effective means of modeling diabetes and neurodegenerative defects, enabling researchers to check the therapeutic efficacies of innumerable compounds with potentials against diabetes and insulin resistance. This study investigated the histological, histochemical, immunohistochemical alterations as well as the biochemical activity of molecules of the PI3K/AKT pathway in the prefrontal cortex of adult male Wistar rats, it also examined the protective or ameliorative effects and mechanisms through which curcumin exerts its effects.

Akbari et al.,[22] reported that curcumin intake resulted in a detectable reduction in body mass index, weight, Waist Circumference and leptin level, they also reported a significant increase in adiponectin levels, Kasprzak-Drozd *et al.,*[23] suggested that suppression of angiogenesis in adipose tissue, down regulating preadipocyte differentiation, and up-regulating adipocyte energy metabolism and apoptosis by curcumin as the probable mechanism through which curcumin achieves these body fat lowering and by extension weight reduction effects[23].

Our study confirms that curcumin possesses a weight control/reduction property; this fact was previously reported by Kothari, et al.,[24], where weight was monitored. A 93.9% increase in liver weight and fat mass was observed in the HFD group as compared to the control group at the end of 14 weeks of diet[24]. Curcumin through it regulation of glucose metabolism, further regulates energy usage and conversion and thus controls weight[18,24].

The damaging effects of fasting blood hyperglycaemia on cognition in the diabetic rats has been reported severally and may be facilitated through increased generation of free radicals, furthermore, Increased cortical oxidative damage is purportedly associated with cognitive decline and Alzheimer's disease[25]. This study confirmed that oral curcumin administered to diabetic Wistar rats resulted in significant decrease in blood glucose concentration. This further support the claim that curcumin has ameliorative effects on hyperglycaemia, corroborating the report of Vafaeipour et al., [17], who reported that Curcumin reduced blood glucose and HbA1c level by the reduction in hepatic glucose production and glycogen synthesis and suppression of hyperglycemia-induced inflammatory state, stimulation of insulin secretion from pancreatic tissues, improvement in pancreatic β cell function, Increase phosphorylation of protein kinase B (AKT), insulin receptor β and reduction of insulin resistance[17].

Microscopic examination of cortical cytoarchitecture, protein synthesis, amyloid deposition and astrocyte integrity, reveals that High fat Diet and Streptotozin administration lead to poor histomorphological delineation, reduced layer of neuronal cell, altered neuronal morphology and reduced cellular density which may suggest pathological alteration, neuronal degeneration with pyknotic nuclei and vacuolations were also observed. Curcumin treatment facilitated histomorphology similar to the control. The cellularity, cellular delineation, staining intensity and cortical histomorphology appear characteristically normal, however, the treated models appear to have reduced cellular density and halo-spaced neuropils

as well as numerous darkly stained nuclei, curcumin appeared to have been beneficial in salvaging and preserving the neurons and reducing the deleterious effect of STZ and HFD in the rats that took curcumin intervention.

Assi *et al.*,[26] reported that neurodegeneration in brain tissues is consistent with chronic inflammation of the neurons, with associated inflammatory changes such as astrocytosis and microgliosis accompanied by hallmarks like amyloid-β deposition and mis-folded tau proteins, curcumin is able to mediate and ameliorate these deficits through its anti-inflammatory, antioxidant properties, furthermore curcumin decreases the formation of microglia through it anti-proliferative effects on microglia, curcumin also helps through it lipophilic effects by passing through membranes and exerting its effects in the intracellular environment[26].

In our study, the dorsolateral PFC presents with characteristic Nissl profile of the external granular layer (L2). The rats in the control group present with characteristic staining intensity which is densely stained and it suggests Nissl intactness, this confirms that the neurons of rats are healthy and synthesizing proteins effectively. The metabolic imbalance created by the STZ and HFD resulted in poor Nissl staining, dispersed and chromatolytic Nissl substance, which is reflective of oxidative stress, neuroinflamation and is a precursor for apoptosis. These findings are in agreement with reports from Adebola *et al.*,[27] stating that STZ caused a reduction in staining intensity which is suggestive of chromatolysis and decreased synthetic activity hence leading to neuronal damage and apoptosis resulting in possible impairment of cognitive function in the rats.

Jahanshahi et al.,[28] reported that Aβ plaques and neurofibrillary tangles are the two major neuropathological indications of AD, the accumulation of Aβ in brain areas initiates a pathological cascade of other deleterious events in this disease. The neurotoxic effects of Aβ in AD include impairing synaptic plasticity, apoptosis, oxidative stress and stimulating tau phosphorylation. Although we were unable to explore Tau protein profile in this study due to constraints in our laboratory, the expression of amyloid plaques was studied in the external granular layer (L2). It was observed that STZ and HFD caused dark stained cellular components to aggregate, also amyloid angiopathy was seen as Congo red–positive amyloid deposits around and within the walls of small cortical blood vessels this is also seen in varying degrees in the rats that received curcumin intervention but the aggregates were smaller and less conspicuous, curcumin was able to reduce the amyloid deposit in the group that received it when compare to the diabetic rats, this is in agreement with the study Jahanshahi *et al.*,[28] who reported that vitamin E which is a potent antioxidant prevented scopolamine-induced congophilic amyloid plaque accumulation and neurofibrillary tangles in the hippocampus.

Our assessment of Glia fibrillary acidic protein revealed that rats treated with STZ and HFD showed expression of numerous reactive astrocytes in the granular layer of the PFC, this is indicative of reactive astrogliosis which is resultant of the healing of neurons after significant injury. However, Curcumin as a pretreatment was able to offer some protection to the cells although

the protection did not totally deter the deleterious effects of STZ and HFD. curcumin treatment resulted in mild expression of reactive astrocytes and no glia scar formation, neurons appeared normal with a few astrocytes expressed, curcumin here helped reduce the progression of astrocytic proliferation and prevention of glia scar formation compared to the untreated diabetic rats. In the PFC of adult male Wistar rats, STZ and HFD were able to trigger considerable progressive astrocyte proliferation and, in some cases, glia scar formation. These findings are in line with previous research by Sohrabi *et al.,*[29] who found that GFAP-positive astrocytes increased, by 14% in the cortex of female ADINKO (AD and inducible neuronal IGF-1R knock-out) mice compared to controls, whereas controls remained unaltered in the cortex.

An evaluation of the level of expression of serum glucose and insulin and ultimately, the determination of HOMA-IR index across the various treatment groups reveals that, a regimen of STZ and HFD successfully induced hyperglycemia and hyperinsulinemia in the rat model. The levels of serum glucose and insulin and therefore insulin resistance were lowered to varying degrees in the three groups that took curcumin intervention, with curcumin proving to be most beneficial in the therapeutic group rats. Our findings agree with that of Kothari *et al.,*[24] which stated that changes in circulating glucose and insulin levels was induced by high fat diet and liquid sugar drink, it was reported that this effect were reflected in a statistically significant increase in the HOMA-IR index, a quantitative measure of IR in their study.

The HOMA-IR index in the untreated diabetic rats was significantly higher than that of all the curcumin treated rats and controls, this indicates that STZ and HFD disrupted the metabolic balance and initiated a series of other reaction that led to insulin insensitivity and thus cell resistance to insulin action. HOMA-IR in the rats that took curcumin intervention showed that curcumin has the potential to alleviate the disruption and restore insulin action. The HOMA-IR index in the rats that were pre-treated with curcumin was significantly lower than that of the STZ and HFD treated rat, a similar low index was observed in the concurrently treated rats and the rat treated after the assault of STZ and HFD. This trend is consistent with the findings of Kothari *et al.,*[24] and Sunil *et al.,*[14], where it was reported that diet rich in fructose and fat decreased insulin sensitivity, and the observed changes in glucose metabolism and insulin sensitivity indicated systemic IR, similar to what we observed in this study, Kothari *et al.,*[24] reported a higher HOMA-IR index in High fat and Liquid Sugar treated rats compared to C57BL/6NHsd mice.

A peep at the mechanistic insight of curcumin action shows that the oral curcumin regimen caused significant increase in the level of expression of PI3K, this is indicative that curcumin activated the PI3K/AKT pathway downstream, and produced a regulative effect that would have been achieved in the presence of normal insulin function[30]. This increased level of PI3K level and activity is absent in the untreated diabetic rats, this suggest that STZ and HFD modulate and downgrade the activity of this important pathway, hence compromised neuronal survival. Enhanced activity of PI3K in the prefrontal cortex of

control and curcumin treated rats translated to the improved expression of AKT, however the untreated diabetic rats had lower expression of AKT when compared to the control rats, this further shows the ameliorative potential of curcumin in eliciting positive, and protective tendencies around the cells by recruiting AKT. Consequently the activated AKT enters the cytoplasm, where it evokes the phosphorylation and inactivation of glycogen synthase kinase 3 (GSK3β), which promotes glycogen synthesis. The level of expression of GSK3β in the untreated diabetic rats was significantly higher compared to the controls, where significantly lower expression was observed. The rats that took curcumin intervention also showed lower expression when compared with untreated diabetic rats. This suggests that curcumin, through the activation of AKT was able to cause the phosphorylation and consequent inhibition of the GSK3β, thereby activating the anti-apoptotic sequence of events and promote glucose usage and regulation[30]. Furthermore, these events stimulate the reduction of the accumulation of defective tau proteins and beta amyloid, all of these leads to a healthier cell environment and ultimately neuronal survival[15].

CONCLUSION

Curcumin played an ameliorative role against the dementing deficits caused by Streptozocin and High fat diet in the prefrontal cortex of the treated animal models. Curcumin was able to reduce the extent of nuclei fragmentation, Nissl bodies' disintegration, amyloid aggregation, and astrogliosis. Curcumin achieved the aforementioned by activating the PI3K/AKT pathway and inhibiting GSK3β.

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