Curcumin ameliorate cortical histomorphological deficits in Streptozocin and western diet induced neurodegeneration in Wistar Rats

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Research Article

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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 neuronal density and morphological changes in the neurons of the prefrontal cortex (PFC); evaluated amyloid beta (Aβ) deposits and probed the association between phosphatidylinositol 3-kinase (PI3K), serine/threonin 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) and treated with: (olive oil) control group; (curcumin) for positive control; High Fat Diet (HFD) and three doses of Streptozotocin (STZ) for diabetic; (HFD, three doses of STZ and concurrent curcumin) preventive; (pre-treatment with curcumin, followed by HFD and three doses of STZ) protective group; (HFD, three doses of STZ followed by curcumin) for therapeutic group.

Cortical sections were stained for histological and histochemical 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 oral curcumin significantly reduced blood glucose and insulin resistance. Insulin resistance was associated with cyto-architectural deficits observed in the PFC, while curcuminn ameliorated observed changes; furthermore, Oral curcumin reduced Aβ deposits in the PFC of the model. The impaired activity of PI3K, AKT and GSK-3β of the model was ameliorated by oral curcumin. The study concluded that oral curcumin showed ameliorative potentials on the PFC of adult male Wistar rats against neuronal death associated with dementia and insulin resistance.

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 2 kg which is approximately 3% of the total body weight is one of the smallest and yet the most complex of the body systems and consumes approximately 20% of the total energy produced in the body (Sonnay *et al.,* 2017). 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 (Sonnay *et al.,* 2017). An imbalance in glucose homeostasis leads to metabolic disorders, 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 as seen in Type I diabetes mellitus (T1DM) or non-insulin dependent as in Type II diabetes mellitus (T2DM) (Duarte *et al*., 2013). T2DM is characterized by hyperglycemia and hyperinsulinamia which leads to insulin resistance, accumulation of defective proteins in brain regions and ultimately, neuronal disorders (Duarte *et al*., 2013).

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 (WHO 2021). Uloko *et al.,* 2018 estimated the prevalence of DM to be 5.77% which translates to about 11.2 million Nigerians, averagely one out of every 17 adults in Nigeria is diabetic. This epidemic further aggravated by the fact that T2DM which is previously believed to be adult-onset diabetes

is fast becoming more common in young people, challenging the idea that it usually occurs in people older than 30 years of age (Carvalho *et al*., 2012).

Dementia is a brain illness associated with a loss of cerebral ability severe enough to truncate usual occupational functioning and/or typical social activities (Hugo and Ganguli, 2014). 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 (Sonnay *et al.,* 2017). These highlight the key regulatory role insulin play in energy balancing and maintaining of glucose usage 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 (Sonnay *et al.,* 2017).

The occurrence of dementia heightens exponentially as the population age; it ranges from 10% in the age group of 60-65 years to an alarming 38.6% in the age group of 90-95 years (Manish *et al.,* 2006). Alzheimer's disease (AD), because of steady and progressive nature is most studied of all dementias (Manish *et al.,* 2006).

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. 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, as a result, AD pathology (Pritchard et al., 2011). Tau proteins bind to tubulin to stabilize microtubules and vesicular transport in the normal state. Tau proteins that have been hyperphosphorylated and aggregated form neurofibrillary tangles. Tau becomes insoluble and loses its affinity for microtubules when it is hyperphosphorylated, resulting in neurodegeneration (Iqbal et al., 2005).

Insulin resistance enhances 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, on the other hand, remains unknown.

Similarly, to T2D, impaired insulin function has been also increasingly demonstrated in AD, suggesting that decreased brain insulin levels/action may constitute the link between both pathologies (Duarte *et al.,* 2012). 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. Insulin resistance reduces cellular sensitivity to insulin, resulting in hyperinsulinemia, and this impairment in insulin signaling has a role in AD pathogenesis, manifesting as brain inflammation, oxidative stress, changes in amyloid beta (Aβ) levels, and finally cell death (Van Pragg et al., 2014). Drugs that control insulin resistance have been found in human and experimental animal trials to diminish Aβ build-up in the brain as well as the cognitive impairments associated with Alzheimer's disease. 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.

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.

Materials & 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 *et al.,* 2018) 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: Negative control received 1ml of olive oil daily for 21 days, Curcumin group received 200mg/kgbw of curcumin orally for 21 days, Diabetic group rats were fed with high fat diet and treated with three doses of 40mg/kgbw of STZ, Protective group received a concurrent treatment of three doses of 40mg/kgbw STZ, 200mg/kg of curcumin and were fed with HFD for 60 days, Preventive group rats were pre-treated with 200mg/kg of curcumin for 21days followed by 60 days exposure to high fat diet and three doses of 40mg/kg STZ, therapeutic group rats were fed high fat diet and three doses of 40mg/kg STZ for the 60 days then they were treated with 200mg/kg of curcumin for 21 days. After all treatments have been concluded, rats were anaesthetized and sacrificed. Rats were weighed forth-nightly; Gallenkamp (FA2104A, England) digital weighing balance was used, starting from the first day of treatments. Changes in weight within the group was then calculated and compared to changes in other groups. 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 analysed using GraphPad (Version 6) 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

Insulin resistance is associated with increased weight in the diabetic/insulin resistant models (Tab. 2)

Although diabetes is known to cause weight loss, poorly managed diabetes precisely, the hyperinsulinemic state in type 2 diabetes with resultant insulin resistance increases weight gain leading to obesity and worsening insulin resistance. 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 associates with a high insulin resistance index and trepidation of insulin signaling and action in the brains of the insulin resistant models (Fig. 1a & b)

Streptozotocin and HFD exposure initiated as cascade of events that led to the confirmed hyperglycemic, hyperinsulinemic and consequent insulin resistant state of our untreated diabetic 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 ($p \leq 0.05$).

Elevated glucose level and insulin insensitivity associates with the disruption of the insulin signaling pathway, evident in the trepidation in the expression of proteins in the glucose-insulin signaling pathway by insulin resistance (Fig. 2a, b & c, respectively)

The PI3K/AKT signaling pathway is required for energy metabolism; it is able to achieve this through its key role in glucose level balancing, protein synthesis, lipid metabolism, cell proliferation and survival. Imbalance in this important pathway has been implicated in the promotion of several metabolic diseases such as obesity and type 2 diabetes mellitus. Our insulin resistant model showed an imbalance in the PI3K/AKT pathway while Curcumin treatment significantly increased the level of prefrontal Phosphatidylinositol-3-Kinase and Protein kinase B, (Fig. 2a, and 2b) this increase led to the inhibition and observed decrease the activities of Glucose 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 Glucose Synthase Kinase β level in diabetic rats relative to the level in the control and therapeutic group rats ($p \leq 0.05$).

Neuronal cytoarchitectural deficits (Fig. 3) and chromatolytic changes (Fig. 4) in the prefrontal cortex were associated with disruption in insulin signaling caused by STZ and HFD induced insulin resistance

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 and reduced cellular density, 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, staining intensity 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 and staining intensity relative to the controls and the rats that took curcumin intervention. Figure 8 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 than the untreated diabetic rats.

Amyloidogenesis (Fig. 5) and astrogliosis (Fig. 6) in the prefrontal cortex are associated with with disruption in insulin signaling caused by STZ and HFD induced insulin resistance

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.

Discussion

Curcumin was studied to explore its ability to influence and enhance neuronal survival in a rat model of insulin resistance and associated neurodegenration similar to what presents 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. 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 (Wang et

al., 2017). Curcumin is reported to have the ability to regulate glucose metabolism by initiating glucose uptake and improving insulin signaling (Wang et al., 2017).

In this study, the effect of curcumin is studied on neurodegeneration in a model of neuronal deficits and insulin resistance in Wistar rats induced with streptozotocin (STZ), a diabetogenic drug, which leads to a disruption in energy metabolism and progressive cognitive impairment and a formulation of High Fat Diet (Tab. 1) 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 has been employed by researchers in the creation of animal models of T1DM and T2DM, this is achieved by initiating the death of pancreatic β cells via alkylation of DNA. Administration of a High-dosage of STZ will adversely inhibit insulin secretion and consequently mirror T1DM, while 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 neurodegeneration. HFD and STZ offers a simple, inexpensive, yet effective means of model creation for diabetic and neurodegenerative studies, enabling researchers to check the therapeutic efficacies of innumerable compounds with therapeutic potentials against diabetes and insulin resistance. This study investigated the histological, histochemical, immunohistochemical alterations and 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., 2019) 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, Ejaz et al., (2009) suggested that suppression of angiogenesis in

adipose tissue, down regulating preadipocyte differentiation, and upregulating adipocyte energy metabolism and apoptosis by curcumin as the probable mechanism through which curcumin achieves these body fat and by extension weight reduction effects (Ejaz et al., 2009).

The body weight of rats treated to various treatment regimens increased, according to our findings. Rats treated with HFD and STZ without curcumin intervention had a significant increase in body weight, though the difference was not statistically significant when compared to the rats in the control groups. Rats treated with curcumin only had a lower body weight post-treatment, indicating that curcumin may have a weight control/reduction property. This pattern was previously reported by Kothari, et al., 2017, 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 (Kothari *et al.,* 2017)**.** Curcumin through it regulation of glucose metabolism, further regulates energy usage and conversion and thus controls weight (Kothari *et al.,* 2017; Wang *et al.,* 2017).

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 (Akinola et al., 2011). This study confirmed that oral curcumin administered to diabetic Wistar rats resulted in significant decrease in blood glucose concentration as shown in (Fig. 1a). This further support the claim that curcumin has ameliorative effects on hyperglycaemia, corroborating the report of Ghorbani et al., (2014) 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.

Microscopic investigation was done on the prefrontal cortex to examine cortical cytoarchitecture, protein synthesis, amyloid deposition and Astrocyte integrity, the external granular layer (L2) of the dorsolateral Prefrontal cortex (Fig. 3) presents with characteristic staining morphology in the cortices of the control rats which present with typical histoarchitectural definition with proper delineation and staining characteristics. The cellularity, neurophilic morphology and cellular density appear characteristically normal with no apparent histopathological alteration. The High fat Diet plus Streptotozin treated rats present with 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. Rats pre-treated with curcumin and those treated with curcumin after the model has been established presents with histomorphology similar to the controls, the cellularity, cellular delineation, staining intensity and cortical histomorphology appear characteristically normal, however, they 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. Mishra and Palanivelu (2008) reported that neurodegeneration in nerves 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. (Mishra and Palanivelu, 2008).

(Fig. 4) shows the dorsolateral PFC 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.,* (2020) 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., (2019) 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. Therefore, in our study 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.,* (2019) who reported that vitamin E which is a potent antioxidant prevented scopolamine-induced congophilic amyloid plaque accumulation and neurofibrillary tangles in the hippocampus.

In this study, Glia fibrillary acidic protein was studied to establish the pattern of astrogliosis after administration of STZ and HFD and the putative ameliorative effects, curcumin may have on the process in the studied regions of the brain, the prefrontal cortex of adult male Wistar rats. (Fig. 6) shows 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, glia scar formation is also an evidence of the extreme astrogliosis. 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. The rats that received STZ and HFD start, followed by curcumin showed mild expression of reactive astrocytes, no glia scar was noticed and this was largely due to the reparative effects of curcumin, neurons appeared mostly normal with a few astrocytes expressed, curcumin here helped reduce the progression of astrocytic proliferation and prevention of glia scar formation when 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 Gontier et al., (2015), 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 determination of the HOMA-IR index across the various treatment groups reveals that, comparing the STZ and HFD treated rats to the controls, it was determined that there was hyperglycemia and hyperinsulinemia in the STZ and HFD treated rats. The levels of serum glucose and insulin and therefore insulin resistance were lowered in varying degrees in the three groups that took curcumin intervention, with curcumin proving to be most beneficial in the therapeutic group rats. These findings agree with the finding of (Kothari *et al.,* 2017) 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.,* 2017; Sunil *et al.,* 2017) 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 shown in this study (Kothari *et al.,* 2017) reported a higher HOMA-IR index in High fat and Liquid Sugar treated rats compared to C57BL/6NHsd mice.

In the prefrontal cortex it was observed (Fig. 2a) that curcumin intervention 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. 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 as seen (Fig. 2b), 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 (GSK3), 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. 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.

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 and dispersal was also greatly reduced, amyloid plaques was reduced in the rats that took curcumin interventions, there was also less proliferation of reactive astocytes hence Astrogliosis was reduced in the rats that had curcumin intervention. Curcumin initiated the activation of the PI3K/AKT pathway and down regulated the activities of GSK3β.

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Tables

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

Table 1: Composition of 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

Tab 2: Body weight change of rat across groups. STZ+HFD as well as Concurrent STZ+HFD+Cur treatments caused an increase in rat weight relative to the Negative control group. Further analysis showed no significant correlates within periods (* significant level of difference at $p<0.05$)

Chats

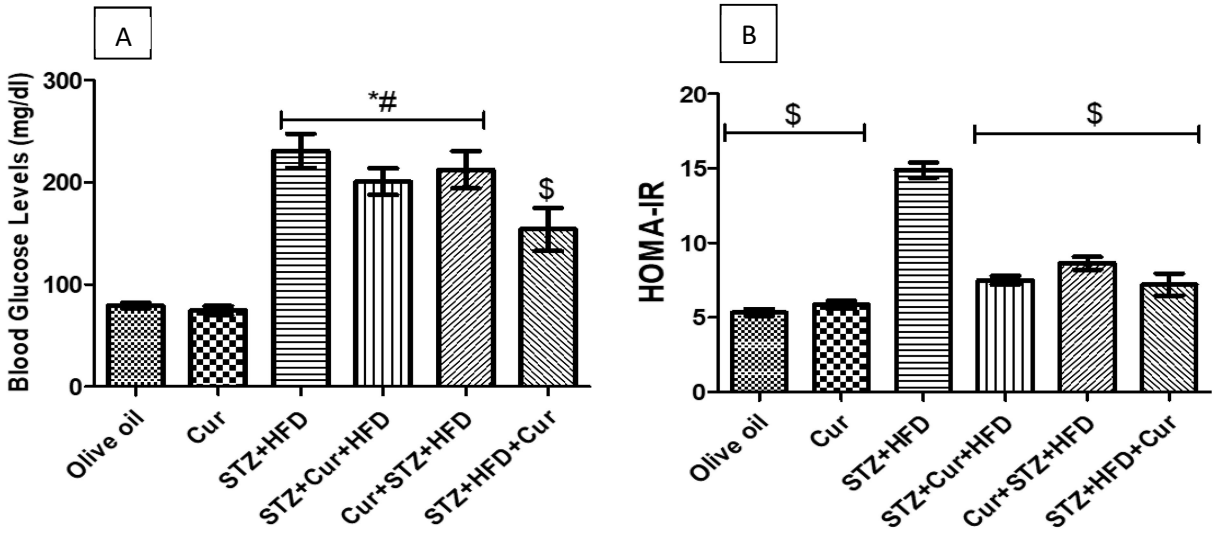

Figure 1 A & B: 60 days Fasting Blood Glucose (A) as well as Insulin resistance as assessed by the HOMA-IR Methods (B) Fasting blood glucose levels showed remarkable reduction in therapeutic group when compared to the untreated diabetic rats. '$'compared to the STZ+HFD group, '#' compared to the Cur group. The HOMA –IR index increase significantly in the diabetic compared to the treated groups and control groups. STZ=Streptozotocin, HFD=High Fat Diet, Cur=Curcumin Data were mean ± SEM ($p<0.05$) n=6

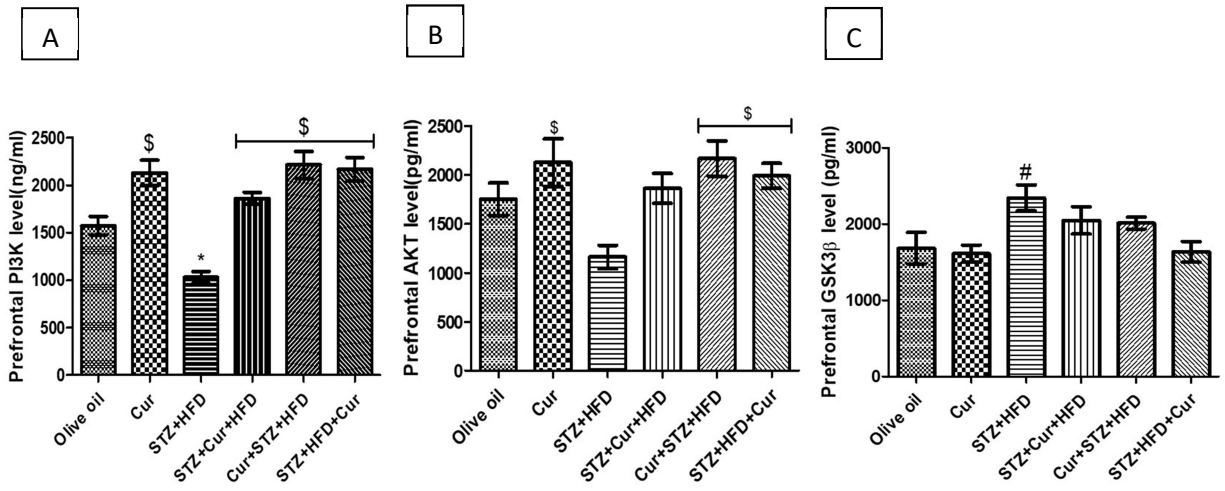

Fig. 2 PI3K (A) AKT (B) and GSK3β (C) Activities quantified in the Pre-frontal Cortex of Rats, ELISA results revealed that the controls and Curcumin treated rats recorded an increase in the level of PI3K, AKT and a decrease in the level of GSK3β compared with the STZ+HFD treated rats. '#'compared to the Cur group. STZ=Streptozotocin, HFD=High Fat Diet, Cur=Curcumin, Data were mean ± SEM ($p<0.05$) n=4

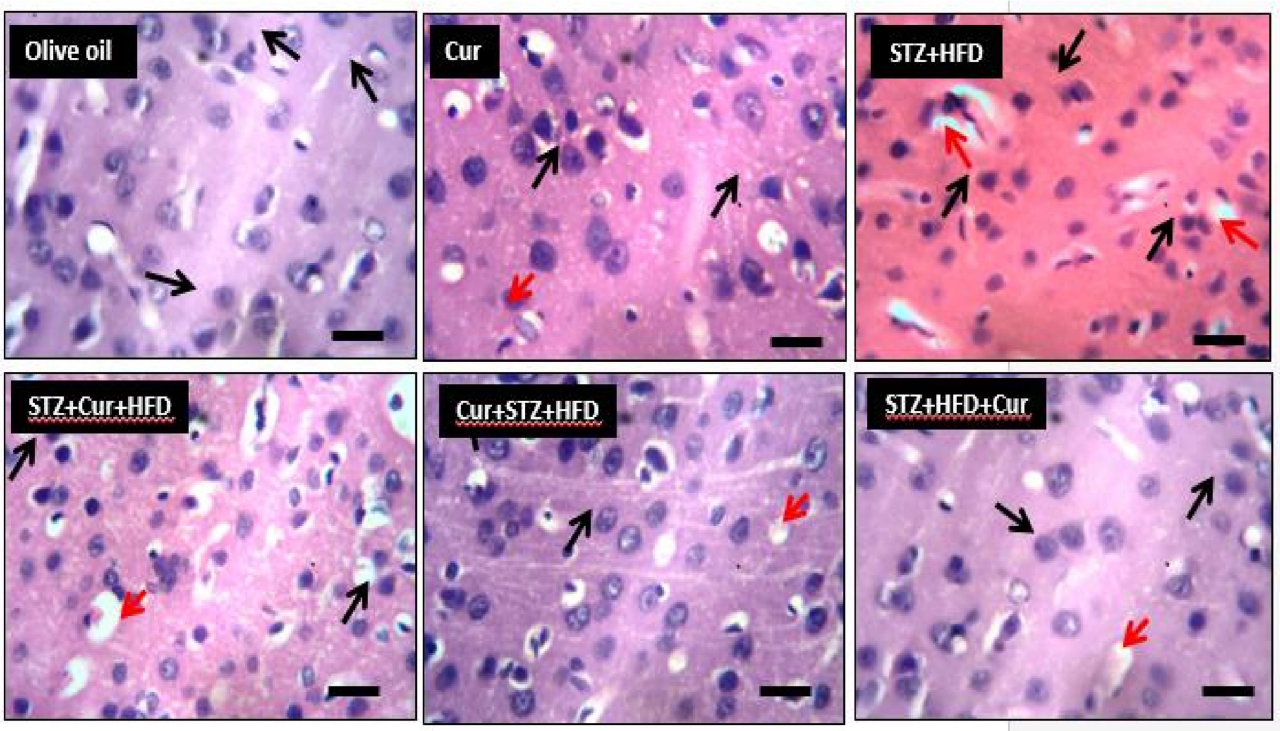

Fig. 3 Demonstration of cortical morphology control, diabetic and curcumin treated rats, showing the external granular layer cytoarchitecture. There are observable neuronal distortions, shrunken nucleus, vacuolation and pyknosis (red arrows) in the diabetic group. Contrariwise, cortical cytoarchitecture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

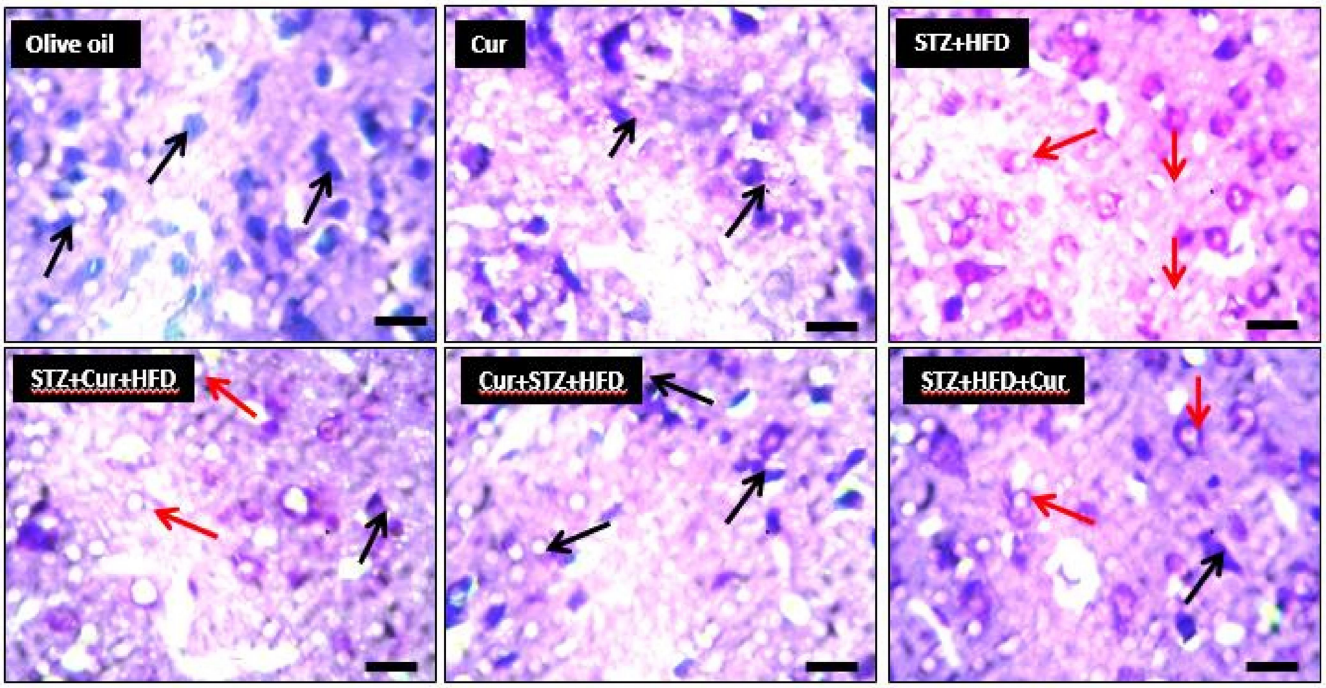

Fig. 4 Demonstration of cortical Nissl substance profiles of rats across the different treatment regimen, Similar to histological observations, cortical external granular layer of control and curcumin treated rats presents with deeply stained Nissl substances, while the diabetic/insulin resistant rats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

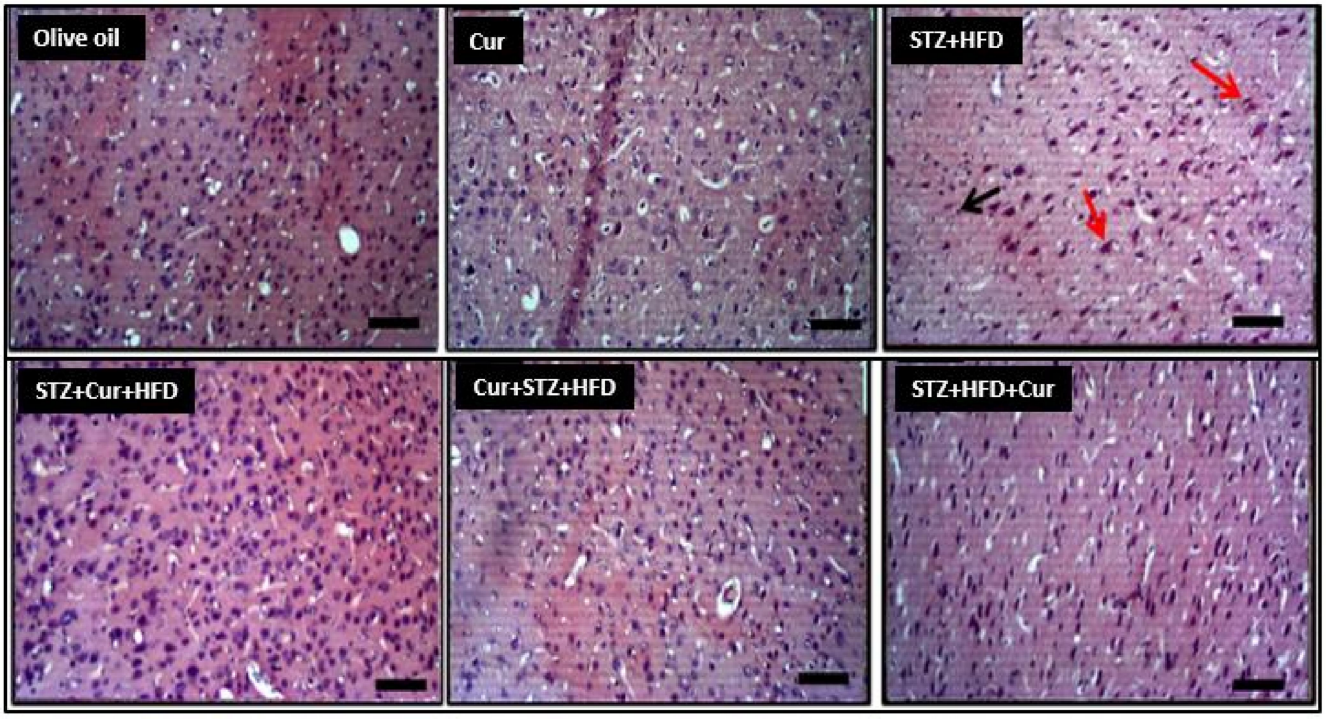

Fig. 5 Demonstration of amyloidogenesis in the rats exposed to the different treatment regimen, cortical external granular layer of control and curcumin treated rats appeared normal with no apparent amyloid deposits. However, the diabetic/insulin resistant rats presents with pockets of amyloid deposits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

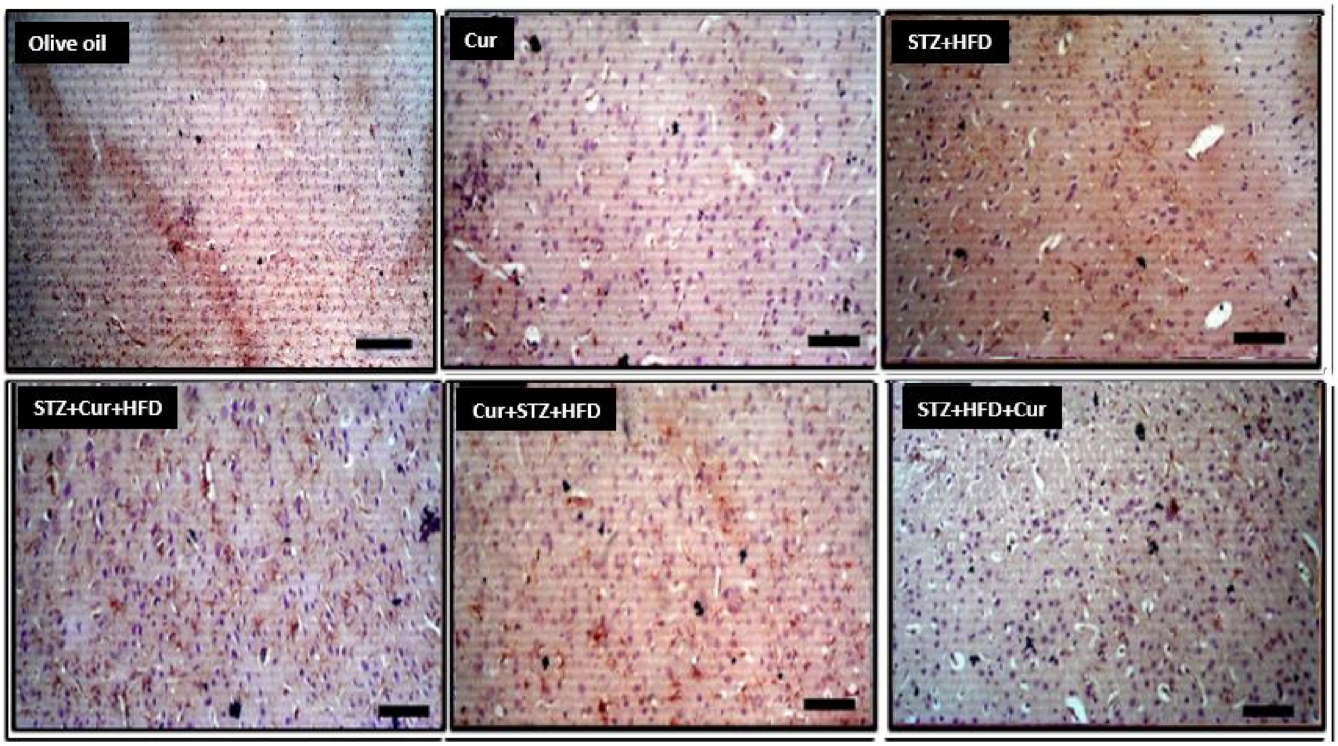

Fig. 6 Activated astrocyte profile was measured via GFAP immunolohistochemical study of the prefrontal cortex across the different treatment groups, Observably, the diabetic/insulin resistant, protective and preventive presents with increased GFAP positivity cells relative to the control, curcumin only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

Statements & Declarations

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Competing Interests

The authors declare no actual nor potential conflict of interest.

Author Contributions

Oluwole B. Akinola is the research principal investigator (P I) responsible for the design, manuscript draft, proofread for final corrections, and publication, while Abdullahi A. Mohammed was responsible for the implementation of the experiment, analysis of results, and manuscript writing.

Data Availability

The datasets generated and analysed during the current study are not publicly available due to protection from unauthorized usage of data, but are available from the corresponding author on reasonable request.

Ethics approval

This research was conducted in line with ethical principles as approved by Ethical review committee of the University of Ilorin, Nigeria with ethical approval with number (UERC/ASN/2016/654) obtained from the postgraduate ethical review committee of the University of Ilorin, Nigeria.