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Cucurbitacin E Modulates Cognitive Impairment Associated with Insulin Resistance-Induced Diabetes via its Anti-Inflammatory Properties and Activation of the PI3K/AKT Signalling Pathway

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ABSTRACT

Type 2 diabetes-induced insulin resistance has been linked to neurodegenerative dementia. Natural plant compounds have been established to influence the phosphatidylinositol 3-kinase (PI3K)-AKT/protein kinase B signalling pathway, offering therapeutic benefits in diseased conditions. Therefore, this study investigated the effects of cucurbitacin E (CuE) on the PI3K/AKT signalling pathway in insulin resistance-related cognitive decline in the prefrontal cortex. Forty-eight rats were divided into six groups: control, streptozotocin (STZ) (60 mg/kg), STZ + CuE (0.5 mg/kg), STZ + metformin (MTF) (150 mg/kg), CuE only, and metformin only. Hyperglycaemia was induced using a single intraperitoneal dose of STZ, followed by 28 days of CuE and MTF treatment. MTF was administered orally with the use of a well-calibrated oral gavage, while STZ and CuE were administered intraperitoneally. Behavioural performance, inflammatory cytokines (interleukin-1β, interleukin-9, and tumour necrotic factor-alpha) and PI3K/AKT gene expression were evaluated. CuE significantly ($p < 0.05$) reduced blood glucose and insulin resistance compared to diabetic rats and improved memory function, as shown by reduced escape latency in the Morris water maze test and discriminatory index in the novel object recognition test. CuE also significantly ($p < 0.05$) attenuated elevated inflammatory markers and upregulated PI3K/AKT expression. These findings suggest that CuE improves cognitive function in diabetic conditions, likely through its anti-inflammatory action and activation of the PI3K/AKT pathway.

Keywords

Prefrontal Cortex, Streptozotocin, Neuroinflammation, Memory, Escape latency, Discrimination index

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterised by persistent high levels of blood glucose (hyper-

glycaemia) due to the body's inability to produce or effectively use insulin. Insulin, a hormone produced by the pancreas, plays a crucial role in regulating blood glucose levels and facilitating the uptake of glucose into cells for energy production. When the normal insulin function is impaired, as in diabetes, an imbalance in blood glucose levels occurs, potentially leading to complications affecting various organs and systems (Westman, 2021). The global prevalence of diabetes is on the rise, making it a major public health concern that impacts millions of individuals 'worldwide (Sun *et al.*, 2022).

Metformin, a medication from the biguanide class, has established itself as the gold standard for managing type 2 diabetes mellitus due to its effectiveness in controlling blood glucose levels and its generally favourable safety profile (Rena *et al.*, 2017). Since its introduction in the 1950s, metformin has been the first-line pharmacological treatment recommended by major diabetes organisations, such as the American Diabetes Association and the European Association for the Study of Diabetes (Bailey, 2024). Despite its benefits, metformin has some limitations such that its prolonged use could lead to abdominal discomfort, vitamin B12 deficiency, or lactic acidosis (Chaudhary and Kulkarni, 2024). In contrast, natural products often have a broader range of biological effects. They can modulate insulin signalling pathways, enhance glucose uptake in muscle cells, and reduce oxidative stress and inflammation factors that significantly contribute to diabetes complications. This comprehensive approach of natural products has the potential to address multiple underlying factors of diabetes, providing a more holistic management strategy.

Dementia, an organic brain disease characterised by a decline in cognitive function to the extent that it interferes with daily activities, becomes more prevalent with advancing age. The prevalence of dementia increases exponentially, ranging from 10% in the age group of 60-65 years to 38.6% in the age group of 90-95 years (Lucca *et al.*, 2015). Numerous studies have highlighted the correlation between insulin resistance and age-related memory impairments, with insulin resistance serving as a risk factor for Alzheimer's disease (AD) (Dineley *et al.*, 2014; Vinuesa *et al.*, 2021). However, the precise molecular and cellular link between insulin resistance and AD remains elusive. Similarly, in type 2 diabetes, impaired insulin function has been increasingly associated with AD, suggesting a potential connection between reduced brain insulin levels/action and the pathologies of both conditions (Cai *et al.*, 2012).

Previous research has discussed the potential mechanisms of metabolic disorders in the development of AD (Arshad *et al.*, 2018). Proper glucose regulation is crucial for maintaining energy, neurogenesis, neuronal survival, and synaptic plasticity, all of which are essential for learning and memory. During insulin resistance, reduced cellular sensitivity to insulin leads to hyperinsulinemia, and this impairment in insulin signalling plays a role in AD pathogenesis, resulting in brain inflammation, oxidative stress, alterations in amyloid beta (Aβ) levels, and ultimately cell death (Akhtar and Sah, 2020). Human and animal studies have demonstrated that drugs targeting insulin resistance can reduce Aβ accumulation in the brain and alleviate cognitive impairments associated with AD (Tyagi and Pugazhenthi, 2021). Therefore, therapeutic approaches aimed at understanding the link between insulin resistance and AD could contribute to the development of future AD treatments.

Medicinal plants have significant importance as potential therapeutic remedies for diabetes-related complications (Saad *et al.*, 2017). Among the vast plant family Cucurbitaceae, encompassing about 125 genera and 960 species, various parts such as fruits, seeds, stems, and leaves have long been used for culinary purposes since ancient times. These cucurbitaceae species have gained recognition for their potential in effectively managing lifestyle diseases like diabetes, obesity, and related disorders (Alzahrani *et al.*, 2022; Wal *et al.*, 2024). Cucurbitacin E (CuE) is a highly oxygenated tetracyclic triterpenoid found in pumpkins, gourds, and medicinal herbs like watermelon and *Ecballium elaterium*. Rich in glucose, fructose, essential amino acids, vitamins, water-soluble polysaccharides, dietary fibres, phenolic glycosides, flavonoids, terpenoids, and minerals, cucurbitaceae plants hold significant promise as therapeutic agents for various health conditions, such as cancer and diabetes.

This present study endeavours to elucidate the potential neuroprotective effects of CuE in insulin-resistance-induced dementia via the phosphatidylinositol 3-kinase (PI3K)-AKT/protein kinase B (PKB) pathway and its ability to mitigate inflammatory activities in the prefrontal cortex

MATERIALS AND METHODS

Experimental Animals and Care

Forty-eight rats, weighing between 170 and 180 g, were obtained from Ladoke Akintola University of Technology School of Medical Laboratory Sciences, Osogbo. Upon acquisition, the rats were housed in the animal room of the Department of Anatomy and allowed to acclimate for one week under standard laboratory conditions with a temperature range of 27-30°C. The animals received ethical treatment in adherence to the protocols outlined in the 'Guide for the Care and Use of Laboratory Animals' and the Animals in Research: Reporting In vivo Experiments (ARRIVE) guidelines (2010). Ethical approval was obtained from the College of Health Sciences Research Ethical Committee with reference no.: UNIOSUNHREC 2022/004A

Preparation of Treatment Solutions

Metformin hydrochloride (Emzophage®-500, Emzor Pharmaceuticals, Nigeria) was dissolved in distilled water. CuE was obtained from Aktin Chemicals, Inc. (cat. no.: CB3372306) and was dissolved in dimethyl sulphoxide (Si *et al.*, 2019). These solutions were freshly prepared each morning of administration and kept at 4°C before use.

Animal Grouping and Treatments

The animals were randomly assigned into six groups of eight rats each. Table 1 shows the grouping and treatments received by each group.

Induction of Hyperglycaemia

Hyperglycaemia was induced by a single dose of 60 mg/kg streptozotocin (STZ) (Sigma Aldrich, St. Louis, USA) (Wu and Huan, 2008) dissolved in cold sodium citrate buffer (0.1 M, pH 7.4) and administered intraperitoneally after fasting the rats overnight. Fasting blood glucose levels were calculated by the glucose oxidase method (Bankar *et al.*, 2008) using a glucometer at 72 h post-STZ injection (Accu-Check, Roche, Belgium). Rats with fasting blood glucose concentrations of at least 200 mg/dL were included in the study.

Table 1: Grouping and Treatments

Animal grouping	Dose concentration given (mg/kg)
Control	The rats received distilled water exclusively.
STZ only	A single dose of 60 mg/kg
STZ + CuE	STZ (single dose of 60 mg/kg) + Cucurbitacin E (0.5 mg/kg)
STZ + MTF	Metformin (150 mg/kg) + STZ (60 mg/kg) in one dose
CuE only	Cucurbitacin E only (0.5 mg/kg)
MTF only	Metformin only (150 mg/kg)

CuE and metformin were administered for 28 consecutive days after induction of hyperglycaemia. Metformin was administered orally with the use of a well-calibrated oral gavage, while STZ and CuE were administered intraperitoneally.

Novel Object Recognition Test

The novel object recognition (NOR) test assesses recognition memory in which rats investigate objects, and greater curiosity in unfamiliar objects suggests memory retention (Antunes and Biala, 2012). Initial habituation sessions during which rats were placed in a wooden box (40 × 60 cm) without the objects were conducted for 5 min, after which the rats were removed from the box. The two similar objects were then placed at least 10 cm from the side wall of the box, and the rat was placed centrally between the two objects for exploration for a duration of 5 min in each session, and exploration time was recorded. After a 15-min interval, the rat encountered a new set of objects: one familiar and one novel. Exploration was typically defined as the time the animal spent sniffing, touching, or interacting with the objects without sitting on them.

Two measures were determined: The time spent exploring the novel object and the discrimination index (DI).

DI, expressed as a percentage, is calculated as follows:

$$D1 = [\frac{(Time\ spent\ exlloring\ novel\ object - Tim\ \ spent\ exploring\ familar\ object)}{(Total\ exploration\ tim\ )}] \times 100$$

Morris Water Maze Test

For this investigation, a substantial water enclosure with dimensions of 100 cm in diameter and a depth of 30 cm was utilised. Within one of the quadrants, an escape platform was positioned just below the water's surface, serving as the focal point. To assist in spatial orientation, visual cues were strategically positioned within this quadrant. Before commencing the primary assessment, a 24-h training period was administered to the rats. During this training phase, each rat was placed in quadrants devoid of the platform for 60 s intervals, with 15 min breaks between quadrant changes. Training persisted until the rats consistently achieved an escape latency period of less than 15 s, signifying successful acquisition of the platform's location. Following the training phase, the rats were subjected to the actual test, which lasted for five days. To reduce visibility, the water in the pool was clouded. The rats were then positioned in the three quadrants that lacked the escape platform. Comparable to the training phase, quadrant changes were spaced 15 min apart. The duration for the rats to reach the escape platform was recorded, defining this as their escape latency period (Othman *et al.*, 2022).

Tissue Collection

The rodents were sacrificed by cervical dislocation, and the whole brain tissue was excised from the animal skull using brain forceps. The prefrontal cortex was isolated and processed for enzymatic studies and real-time polymerase chain reaction analysis. Plasma glucose levels were determined using blood glucose meters employing the glucose oxidase method with blood collected via the tail vein. Insulin concentrations in the blood were estimated using an ELISA kit from eBioscience, USA, following the manufacturer's instructions.

The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the formula:

$$\frac{\text{Plasma glucose (mg/dl)} \times \text{fasting plasma insulin (IU mg/L) in the fasting state}}{405}$$

Immunoassay of Inflammatory Markers

Using enzyme-linked immunosorbent analysis, IL-1β, IL-9, and tumour necrotic factor-alpha (TNF-α) levels in rat prefrontal cortices were quantified (Hawkes *et al.*, 1999). Mouse interleukin-1β (IL-1β) (Cat. No. 432601), mouse interleukin-9 (IL-9, Cat. No. 442704) and mouse TNF-α (Cat. No. 430907) ELISA kits procured from Bio Legend Inc. were used for these assays. Prefrontal cortex samples were weighed and homogenised in ice-cold phosphate-buffered saline using a Teflon-glass homogeniser. The homogenate was centrifuged at 12,000 rpm for 10 min to obtain the supernatant. The supernatant was collected and analysed in accordance with the assay manufacturer's instructions. Absorbance was read at 405 nm with a microplate reader.

Reverse Transcriptase Polymerase Chain Reaction Analysis

Using TRIzol reagent (ThermoFisher Scientific Inc, USA), ribonucleic acid (RNA) was extracted from the prefrontal cortex. The purified RNA, free from deoxyribonucleic acid (DNA) contamination, was promptly converted to complementary deoxyribonucleic acid (cDNA) using the ProtoScript® first strand cDNA synthesis kit (New England Biolabs, USA). The primer sequences are listed in Table 2.

For polymerase chain reaction (PCR) amplification, 5 µL of synthesised cDNA (10 ng) was added to a 50 µL reaction mix containing 10 µL of PCR buffer (10 mM Tris-HCl, pH 8.4; 50 mM KCl; 1.5 mM $MgCl_2$), 2.5 µL of each dNTP (10 mM), and 5 µL each of forward and reverse primers (10 mM). The thermocycling program included an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and

extension at 72°C for 30 s, with a final elongation step at 72°C for 5 min.

PCR products were separated on a 5% agarose gel, and expression bands were imaged using an iPhone 5c camera (Noir filter). Post-processing of gel images was done using Keynote on a MacBook Pro (iOS). Band intensity (densitometry) was analysed using ImageJ software (v2.3.0, Mac), and the resulting gene expression bar graphs were created using GraphPad Prism (v8.03, Mac) (Tokunbo *et al.*, 2023).

Table 2: Primer sequence used for PCR

Gene primer sequence (5'-3')
PI3K F: CCAGAAGAAGGGACAGTGGTAGT
R: TCGTAGCCAATCAGGGTATG
AKT F: ATGGACTTCCGGTCAGGTTCA
R: GCCCTTGCCCAGTAGCTTCA

Statistical Analysis

GraphPad Prism® (version 8) software was used for all statistical analyses. All data were expressed as mean ± SEM. Differences among the groups were analysed by one-way ANOVA using Tukey's post hoc for multiple comparisons. P value < 0.05 was considered statistically significant. Densitometric analysis was done using ImageJ software (2.3.0 V, Mac version).

RESULTS

Elevated Glucose Levels and Insulin Insensitivity were Modulated by CuE in STZ-Induced Diabetic Rats

Blood Glucose Level

As displayed in Figure 1, blood glucose assessment showed that STZ-induced diabetic rats had significantly increased blood glucose level (273.22 ± 1.31) ($p < 0.05$) when compared with the control group (98.03 ± 0.61), STZ+MTF (145.82 ± 0.33), and STZ+CuE (99.02 ± 1.02) groups. CuE significantly modulated the blood glucose level of diabetic rats. However, STZ+MTF rats had a significantly increased ($p<0.05$) blood glucose level when compared with the control and STZ+CuE groups.

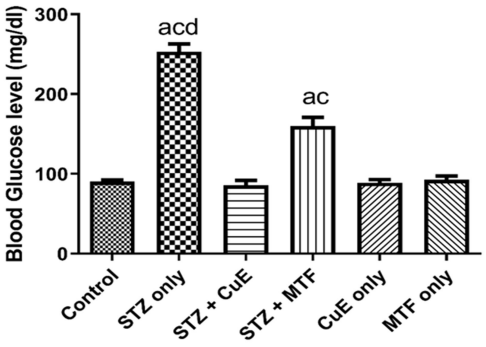

Fig. 1: Blood glucose levels across the experimental groups: a - significantly different compared with the control group; c - significantly different compared with STZ+CuE; d - significantly different compared with STZ+MTF.

HOMA-IR Index

CuE treatment significantly reduced the HOMA-IR index. The STZ rats had a significantly higher HOMA-IR value (12.93 ± 0.42) when compared to the control rats (2.03 ± 0.48). Rats that received CuE (STZ+CuE and CuE groups) had significantly low HOMA-IR values (2.97 ± 0.17 and 2.00 ± 0.28, respectively) when compared with STZ rats. A significant increase was recorded in rats treated with metformin (7.79±1.01) when compared with STZ+CuE animals (Fig. 2).

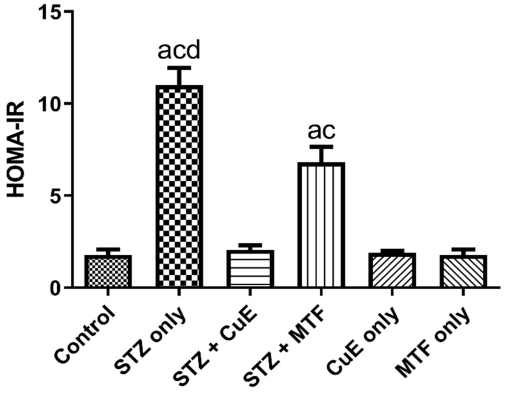

Fig. 2: HOMA-IR values across the experimental groups: a - significantly different compared with the control group; c - significantly different compared with STZ+CuE; d - significantly different compared with STZ+MTF.

Neurobehavioural Evaluation

As shown in Figure 3, rats exposed to CuE treatment had significantly reduced escape latency when compared to the STZ rats. MTF treatment also reduced the escape latency when compared to the diabetic group.

Figure 4 showed that the STZ rats recorded a significant decrease in the time spent exploring the novel object when compared with the control group. Treatment with CuE and MTF significantly increased the time spent exploring the novel object when compared with STZ rats. The discriminatory index shown in Table 3 indicates a negative value of -6.7% for the diabetic rats (STZ). Rats treated with CuE and MTF, as well as the control group, had positive ID values.

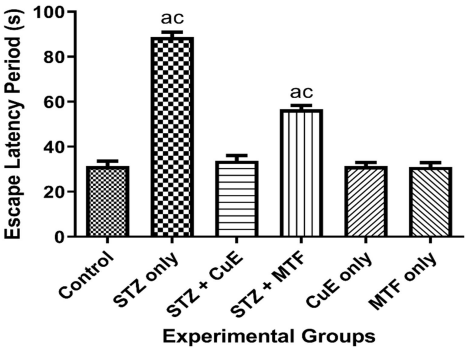

Fig. 3: Escape latency following the Morris water maze test; a - significantly different compared with the control group; c - significantly different compared with the STZ+CuE group; d - significantly different compared with the STZ+MFT group.

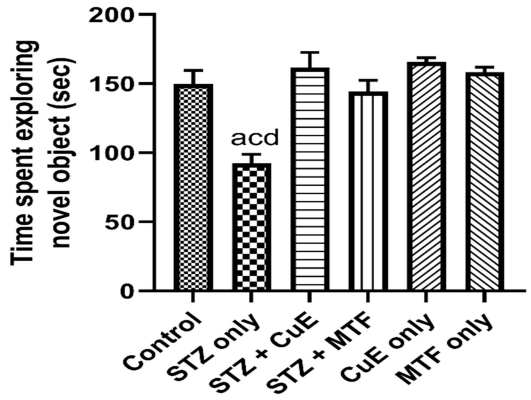

Fig. 4: Time of exploration during the novel object recognition test: a - significantly different compared with the control group; c - significantly different when compared with STZ+CuE; d - significantly different when compared with STZ+MTF.

Table 3: Discriminatory index score following the NOR test

Groups	Control	STZ only	STZ+ CuE	STZ+ MFT	CuE only	MFT only
DI (%)	6.7	-6.7	6.0	4.6	10.7	6.4

Neuroinflammatory Evaluation

The prefrontal cortex activities of interleukin-1β and interleukin-9 are shown in Figures 5 and 6, respectively. IL-1β and IL-9 levels significantly increased in the STZ rats when compared with the control group ($p<0.05$). The Cu treatment (STZ+CuE) resulted in a significant ($p<0.05$) decrease in the activity levels of IL-1β and IL-9 when compared to the STZ rats. Furthermore, IL-1β and IL-9 levels increased significantly ($p < 0.05$) in STZ rats that received the standard drug (STZ+MTF) when compared with rats treated with CuE (STZ+CuE).

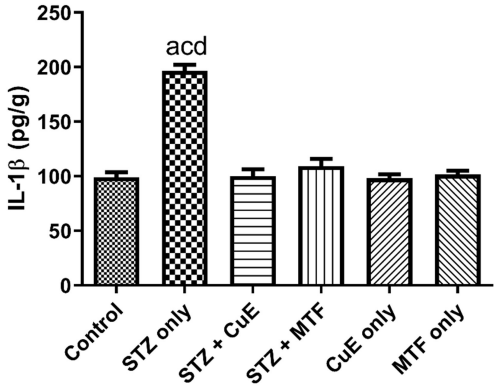

Fig. 5: IL-1β activity in experimental animals: a - significantly different compared with the control group; c - significantly different compared with STZ+CuE; d - significantly different compared with STZ+MTF.

Upregulation of TNF-α in the prefrontal cortex following hyperglycaemia induction is shown in Figure 7. Animals administered with STZ only increased TNF-α levels, which were significant when compared with the control group and other experimental groups treated with CuE ($p<0.05$). Rats exposed to STZ and metformin also had increased TNF-α levels when compared to rats exposed to STZ and CuE.

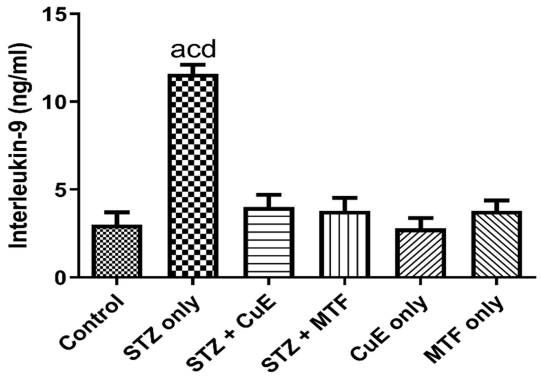

Fig. 6: IL-9 activity in experimental animals: a - significantly different compared with the control group; c - significantly different compared with STZ+CuE; d - significantly different compared with STZ+MTF.

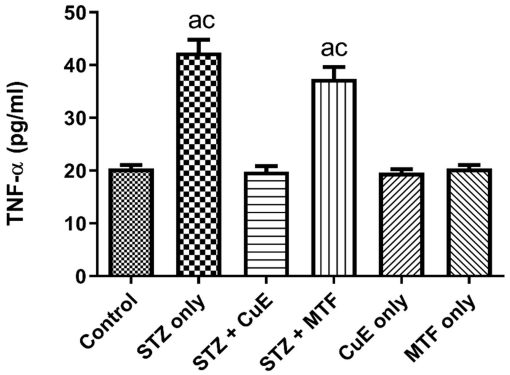

Fig. 7: TNF-α activity in experimental animals. a - significantly different compared with the control group; c - significantly different compared with STZ+CuE; d - significantly different when compared with STZ+MTF.

Expression of PI3K and AKT Genes following STZ exposure and CuE treatment

CuE treatment up-regulated the expression of the PI3K gene in the prefrontal cortex of diabetic rats (Fig. 8). Mean values following densitometry analysis showed a significant ($p<0.05$) down-regulation of the PI3K gene in the untreated diabetic rats (STZ group) when compared with the control group and rats that received CuE only. There was also a significant ($p<0.05$) down-regulation of PI3K protein in the prefrontal cortex of rats treated with metformin (STZ+MTF) when compared with animals treated with CuE. As shown in Figure 9, the AKT gene was significantly ($p < 0.05$) down-regulated in the diabetic group (STZ) when compared to the control rats and those treated with CuE.

DISCUSSION

Neurodegeneration associated with insulin resistance, as observed in conditions like AD, remains a critical public health concern due to its progressive nature and limited therapeutic options. Insulin resistance disrupts neuronal

function by impairing glucose metabolism, exacerbating neuroinflammation, and promoting oxidative stress, all of which contribute to cognitive decline. Current treatment strategies targeting insulin resistance-induced neurodegeneration are inadequate, necessitating the exploration of novel therapeutic agents with neuroprotective potential. One such promising compound is CuE, a tetracyclic triterpenoid known for its anti-inflammatory and metabolic regulatory properties. While previous studies suggest that CuE can enhance glucose uptake and modulate insulin signalling (Kibria *et al.*, 2017), its role in mitigating neurodegenerative diseases remains underexplored. In this study, we investigated the potential of CuE to counteract cognitive impairment in a rat model of insulin resistance-induced neurodegeneration, focusing on its ability to modulate neuroinflammation and activate the PI3K/AKT signalling pathway. Our findings provide new insights into the neuroprotective mechanisms of CuE and highlight its potential as a therapeutic candidate for insulin resistance-related cognitive dysfunction.

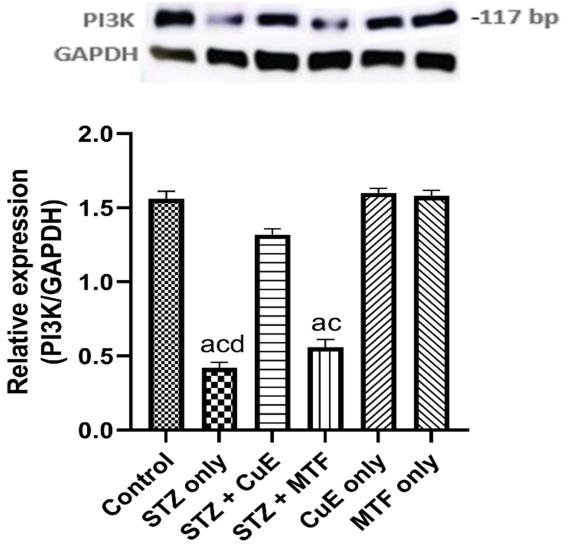

Fig. 8: PI3K gene expression in the prefrontal cortex of experimental rats: a - significantly different compared with the control group; c - significantly different compared with STZ+CuE; d - significantly different compared with STZ+MTF.

In this study, results from the evaluation of the serum glucose level and the HOMA-IR index assessment showed the presence of hyperglycaemia and hyperinsulinaemia in the untreated diabetic rats when compared with the controls. The results of the study revealed that diabetic rats treated with CuE exhibited lower levels of serum glucose and insulin resistance than diabetic rats not treated with CuE. This indicates that CuE promotes pancreatic cell function, resulting in enhanced glucose regulation and decreased insulin resistance in diabetic rats. These findings align with a previous study by Bathina and Das (2018), which reported that the administration of STZ induced changes in circulating glucose and insulin levels, resulting in an insulin-resistant state. In the untreated diabetic rats, the HOMA-IR index was significantly higher compared to all the CuE-treated rats and the control group, indicating that CuE had the potential to alleviate the disruption and restore insulin action in the diabetic rats.

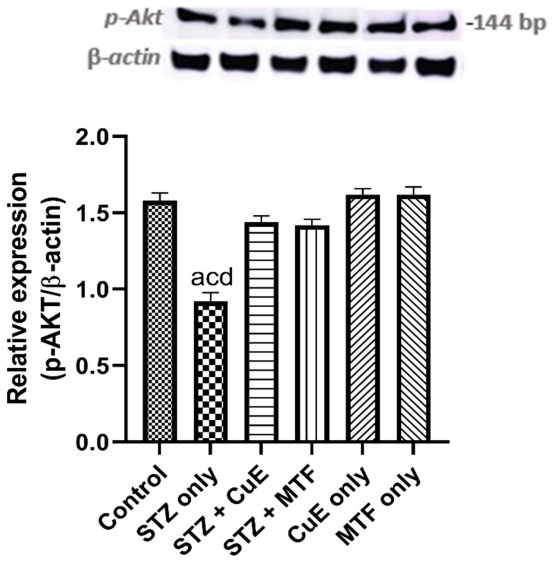

Fig. 9: p-AKT gene expression in the prefrontal cortex of experimental rats: a - significantly different compared with the control group; c - significantly different compared with STZ+CuE; d - significantly different compared with STZ+MTF.

Findings from this study showed that the untreated diabetic rats displayed cognitive impairment. We used NOR test to illustrate that diabetic rats exhibit disruptions in working memory, suggesting potential prefrontal cortex dysfunction associated with diabetes. This exploratory behaviour has been employed to evaluate deficits in recognition memory across various neurodegenerative disease models (Zhang *et al.*, 2012; Grayson *et al.*, 2015). Importantly, we observed that CuE-treated diabetic rats retained the capacity to recognise familiar objects, indicating that the cognitive deficit is attributable to the condition. We suggest that the improved cognitive score in the CuE-treated rats may be attributed to the anti-inflammatory and antioxidant as well as the antidiabetic properties of CuE (Hussein *et al.*, 2017; Silvestre *et al.*, 2022).

We also examined the impact of CuE on spatial memory acquisition in diabetic rats. Untreated diabetic rats exhibited subpar performance in the Morris water maze test, evidenced by longer escape latencies displayed during this test. This performance deviation aligns with findings from prior studies that reported that diabetic conditions can both induce and enhance deficits in the domains of spatial learning and memory acquisition (Wu *et al.*, 2023). Rats that received treatment with CuE and metformin demonstrated preserved spatial memory capabilities. This observation suggests that these treatments may have a positive effect on spatial memory processes in these animals. One potential explanation for this result is that CuE might have neuroprotective properties. CuE has been investigated for its anti-inflammatory and antioxidant properties, which could help protect neurons and promote cognitive function. MTF, a well-known medication for managing type 2 diabetes, shows potential benefits for cognitive function and neuroprotection (Tao *et al.*, 2018; Sportelli *et al.*, 2020). Additionally, these treatments might have a positive impact

on factors like insulin sensitivity and glucose regulation, which are known to affect cognitive function. Diabetes and insulin resistance have been linked to cognitive impairments, so treatments that improve metabolic health could indirectly support better cognitive outcomes (Zhang *et al.*, 2021a).

Furthermore, we observed that the expression of interleukin 1β and interleukin-9 was found to be overexpressed in the untreated diabetic rats, whereas CuE treatment modulated their expression in the prefrontal cortex. Our findings align with previous studies that have demonstrated the triggering of a cascade of inflammatory responses within the brain by hyperglycaemia (Wei *et al.*, 2023; Hernández-Rodríguez *et al.*, 2022). This cascade results in the release of pro-inflammatory cytokines, such as IL-1β and TNF-α. Although these cytokines play a vital role in the body's immune response to injury or infection, their overproduction in the context of hyperglycaemia can have detrimental effects on neuronal health.

In this study, CuE treatment mitigated the inflammatory responses induced by STZ in the prefrontal cortex, as reported in a previous study (Adarmanabadi *et al.*, 2024). The administration of CuE showed remarkable efficacy in regulating the expression of TNF-α, a key signalling protein involved in systemic inflammation. By modulating TNF-α levels, CuE demonstrated its potential as a potent anti-inflammatory agent (Jia *et al.*, 2015), effectively attenuating the damaging effects of STZ-induced inflammation in the brain. The ability of CuE to regulate TNF-α levels is particularly significant due to the crucial role this pro-inflammatory cytokine plays in the immune response. Chronic and excessive production of TNF-α can lead to a state of persistent inflammation, which has been linked to a wide range of pathological conditions, including neurodegenerative diseases, cardiovascular disorders, and autoimmune disorders (Stork *et al.*, 2022; Zhang *et al.*, 2021b). By effectively controlling IL-1β, IL-6, and TNF-α expression, CuE demonstrated its capacity to intervene in inflammatory processes and potentially halt the progression of detrimental inflammatory cascades in the brain triggered by diabetic conditions.

CuE treatment in this study revealed an increase in the expression of PI3K and AKT, which strongly suggests the activation of the PI3K/AKT pathway downstream in the prefrontal cortex. The PI3K/AKT pathway, known for its role in cell survival, growth, and metabolism, plays a critical part in maintaining cellular integrity and function (Kumar and Bansal, 2022). This activation mimics the regulatory effect observed under normal insulin function, indicating that CuE may play a pivotal role in promoting signalling pathways essential for cellular processes and survival. Conversely, the untreated diabetic rats did not exhibit the same elevated levels of PI3K and AKT activity in the prefrontal cortex, implying that STZ influenced and downregulated this crucial pathway. Such dysregulation may have profound implications for neuronal survival, potentially contributing to the pathogenesis of diabetic-related neuronal dysfunctions. These compelling findings highlight the beneficial effects of CuE on the PI3K/AKT pathway in the prefrontal cortex, which may contribute to enhanced neuronal protection and function as reported in other studies (Wang *et al.*, 2023). By modulating these essential signalling pathways, CuE exhibits promising potential as a therapeutic intervention to address neuronal dysfunctions associated with diabetes.

Conclusion

CuE played an ameliorative role against the cognitive impairment caused by STZ-induced hyperglycaemia in the prefrontal cortex, as indicated by prolonged escape latency and a reduced discriminatory index. The neuroprotective effects of CuE in mitigating these cognitive deficits were attributed to its anti-inflammatory properties and its capacity to activate the PI3K/AKT signaling cascade, thus promoting cellular survival and proper functioning. Understanding the intricate mechanisms by which CuE exerts its effects on neuronal pathways could pave the way for innovative strategies to combat the neurodegenerative consequences of diabetes and improve overall neuronal health.

DECLARATION

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Conflict of Interest

None declared.

Ethical Approval

Ethical approval to conduct this research study was obtained from the College of Health Sciences Research Ethical Committee, Osun State University, with reference no.: UNIOSUNHREC 2022/ 004A

Consent to Participate and Publish Data

Not Applicable.

Authors Contributions

TA, OT - Conceptualisation, design of research study and draft manuscript preparation; OO, CA, PO -Data acquisition and preparation of the first draft of the manuscript; TO, DI, OO, IB - Data analysis and interpretation; OO, JF, MA, OT, TA - Critical revision of the manuscript for intellectual content.

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