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Evaluation of Acetylcholinesterase Activity in Diabetic Male Wistar Rats Treated with Glibenclamide

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ABSTRACT

Diabetes is linked to cognitive decline via cholinergic neurodegeneration, and while neuronal loss cannot yet be halted, acetylcholinesterase inhibitors remain central to therapy. Glibenclamide, an antidiabetic sulfonylurea, was recently reported to potently inhibit acetylcholinesterase *in vitro*, suggesting potential for treating cognitive impairment. However, its *in vivo* activity on this enzyme remains underexplored. This study investigated the effects of orally administered glibenclamide on the peripheral and central cholinergic system in a diabetic rat model. Diabetes was induced in male Wistar rats by a single, intraperitoneal injection of 65 mg/kg streptozotocin (STZ). Diabetic animals were orally administered vehicle (distilled water) or glibenclamide (1.2, 5, or 20 mg/kg) for fourteen consecutive days. A normal, non-diabetic group also received the vehicle. Body weight and fasting blood glucose (FBG) were monitored. After training, memory was tested using the Y-maze and Morris water maze (MWM). On day 15, following the MWM probe trial, animals were euthanized, plasma and hippocampus homogenates assayed for acetylcholine (ACh), acetylcholinesterase (AChE), and insulin concentrations. STZ-induced hyperglycemia was associated with an increase in plasma AChE activity (control group, 0.15 ± 0.02 U/L vs diabetic control 0.4 ± 0.04 U/L, p =0.02), which was reduced by glibenclamide 20 mg/kg, p = 0.0319. Correspondingly, plasma ACh concentrations increased significantly in the glibenclamide 20 mg/kg group (p=0.004) without changes in brain cholinergic signalling despite improvement in short-term spatial memory as assessed in the Y-maze. Glibenclamide demonstrates a dose-dependent inhibitory effect on plasma acetylcholinesterase; further studies should assess its therapeutic potential for diabetic neuropathy.

Keywords: glibenclamide, acetylcholinesterase, cholinergic system, cognitive dysfunction, neuroprotection.

INTRODUCTION

Diabetes mellitus is a major global health crisis, affecting an estimated 589 million people in 2025, with projections rising to 852 million by 2050[1]. In Nigeria, approximately 10 million adults aged 18 and older are living with the disease, yet about 60% remain undiagnosed[2]. Placing them at a high risk for severe complications. Diabetes is associated with well-known vascular and non-vascular complications[3] including stroke, heart attack, diabetic neuropathy, nephropathy, and retinopathy, as well as leg amputations. Beyond these well-known diabetic complications, there is a significant risk of developing dementia and cognitive dysfunction, which may include Alzheimer's disease (AD)[4]. Chronic hyperglycemia in diabetes induces cellular stress pathways that, in addition to glial activation, neuroinflammation, insulin signalling impairment, and progressive vascular damage, contribute to neurodegeneration in critical brain regions, including the cortical cholinergic system[4]. The cortico-cholinergic pathway comprises neurons from the basal forebrain projecting to the cortex. This pathway plays a vital role in memory and learning[5]. These presynaptic neurons synthesize and release acetylcholine during memory formation and consolidation, particularly during encoding new memories or long-term potentiation[5]. The action of acetylcholine is thereafter terminated by enzymatic hydrolysis into acetate and choline, catalyzed by acetylcholinesterase. In AD and diabetes, there is a progressive loss of these cholinergic neurons, reaching a 50-90% decline in late AD, and it is associated with significant dementia[6]. However, whilst neuronal loss remains unabated, acetylcholinesterase enzyme inhibitors can prolong the synaptic availability of existing acetylcholine and thus improve cognition. Therefore, inhibiting AChE activity presents a logical and promising therapeutic strategy to improve cognitive impairment. Indeed, the therapeutic agents used in AD to delay the progressive cognitive decline are acetylcholinesterase inhibitors.

The sulfonylureas are the first-line antidiabetic drugs in Nigeria. Glibenclamide is a potent, insulinotropic, antidiabetic sulfonylurea used as the preferred treatment for certain monogenic diabetes mellitus and as a second-line agent for type 2 diabetes[7,8]. The insulinotropic action is achieved via binding to and inhibiting the sulfonylurea receptor (SUR1) subunit of the Adenosine triphosphate-sensitive potassium (KATP) channel in pancreatic β-cells. The sulfonylurea receptor (SUR) is part of the ATP-binding cassette proteins, with four SUR subunits that combine with four pore-forming, inward-rectifying receptors (Kir6) to create ATP-sensitive K channels. Inhibition of the channel results in decreased potassium efflux, membrane depolarization, calcium influx, actin reorganization, and insulin exocytosis. Although primarily expressed in the pancreas, KATP channels are functionally expressed in virtually all excitable tissues and adipocytes, retina, cardiac, and smooth muscles[9–11]. Physiologically, SUR1 expression in the brain is low[12]. However, it becomes markedly upregulated during traumatic brain injuries, such as cerebral oedema following haemorrhage[13], COVID-19-induced neuroinflammation[14], and neonatal-onset monogenic diabetes[15]. Sulfonylureas have been found to promote neuronal survival and neuroprotection in these clinical conditions[16]. A recent *in vitro* study reports that sulfonylureas possess anticholinesterase (AChE) inhibitory activity, with glibenclamide being the most potent (IC50 ~ 0.74 ± 0.02 μM), comparable to the approved acetylcholinesterase inhibitor, donepezil, used in Alzheimer's disease[17]. Donepezil improves cholinergic signalling in the brain,

improving memory. This raises the possibility of repurposing glibenclamide for cognitive dysfunction, particularly in patients with comorbid diabetes. However, whether this enzymatic inhibition observed *in vitro* translates to a functional impact on cholinergic signalling *in vivo*, particularly under the pathological conditions of diabetes, remains unexplored. Therefore, this study was designed to investigate the effects of glibenclamide on cholinergic signalling in a diabetes animal model. We assessed whether glibenclamide inhibits AChE in blood and brain and whether this translates to cognitive improvements in a rodent model of diabetes.

MATERIALS AND METHODS

Chemicals and reagents

Streptozotocin, ELISA kits for acetylcholinesterase, choline acetyltransferase, and colorimetric kits for insulin, acetylcholine, and calcium were all purchased from ElabScience (China). Glibenclamide was sourced from Hovid Pharmaceuticals (Malaysia), and ketamine was obtained from Jawa (India). Accu-Chek strips and glucometers were from Roche. Unless stated otherwise, all other reagents or chemicals are of pharmaceutical grade.

Animal experiments

All experiments followed the US National Institutes of Health Guide for the Care and Use of Laboratory Animals and received ethical approval from the University of Ilorin Ethical Review Committee (EERC/ASN/2024/2694). Male Wistar rats (8–9 weeks old) were procured from Mctemmy Animal Farm (Ogbomosho, Nigeria). The animals were housed at the animal facility of the Faculty of Basic Clinical Sciences, University of Ilorin, in standard cages (five animals per cage) with wired gauze and a base filled with wood shavings, acclimatized for two weeks during which they were fed standard chow and tap water *ad libitum* with twelve (12) hours of light and dark cycle.

Induction of experimental diabetes

Diabetes was induced by a single intraperitoneal (i.p.) injection of streptozotocin (STZ; 65 mg/kg, freshly prepared in 0.1 M citrate buffer, pH 4.5). Fasting blood glucose was measured from the tail vein using an Accu-Chek glucometer (Roche Diagnostics, Germany) following a 14-h fast. Animals with fasting blood glucose levels above 250 mg/dl 48 hours after STZ injection were considered diabetic and included in the study.

Drug administration

Diabetic rats were randomly assigned to treatment groups and administered either vehicle (distilled water) or glibenclamide (1.2, 5, or 20 mg/kg) daily for 14 consecutive days via oral gavage. All treatments were administered between 08:00 and 10:00 h. A control group of non-diabetic rats also received the vehicle.

Behavioral assessments

Spatial working memory and recognition were evaluated using the Y-maze spontaneous alternation task. In contrast, the Morris water maze assessed spatial learning and reference memory, as described[18]. Briefly, during a 5-minute test in a Y-maze (day 10), the total number of arm entries, consecutive entry in an arm (alternation), spontaneous alternation (ratio of actual and possible alternations), and percentage alternation (actual alternations/spontaneous alternations X100) were determined for each animal. The Morris water maze (MWM) assessed spatial learning and memory using a circular pool with opaque water and a hidden platform. Training lasted 3 days (days 12-14) with three trials daily from different quadrants. Escape latency was recorded up to 60 s; rats failing to find the platform were guided to it. A probe trial 24 h after training removed the platform, allowing free swim for

60 s. Swim path and time spent in the target quadrant were recorded to assess memory.

Biochemical analysis

Following the final behavioral test, animals were euthanized using intraperitoneal 300 mg/kg ketamine and 30 mg/kg xylazine, followed by bilateral thoracotomy. Whole brain tissue was rapidly dissected on ice, and the hippocampus was excised and homogenized. The serum and hippocampal tissue homogenate levels of acetylcholine (ACh), acetylcholinesterase (AChE), and choline acetyltransferase (ChAT) were quantified using commercially available colorimetric or ELISA kits, according to the manufacturer's protocols.

Data and statistical analysis

The data are presented as mean ± standard error of mean (SEM). Statistical analyses were performed with GraphPad Prism 8.0 software (GraphPad Software, La Jolla, CA. A one-way ANOVA, followed by Tukey's post hoc test, was conducted to analyze changes over time for datasets with more than two groups. Statistical significance was set at $p \leq 0.05$.

RESULTS

Effects of glibenclamide on metabolic parameters (body weight, glycemia, insulin)

The results show that body weight was significantly reduced in all diabetic groups compared to the standard control after 14 days (Figure 1a). Glibenclamide dose-dependently prevented weight loss ($p < 0.05$) compared to diabetic untreated animals. Similarly, One-way ANOVA analyses followed by Tukey's post hoc test revealed no significant changes in hyperglycemia (Figure 1b) and plasma insulin (Figure 1c) in glibenclamide-treated diabetic rats compared to control diabetic rats.

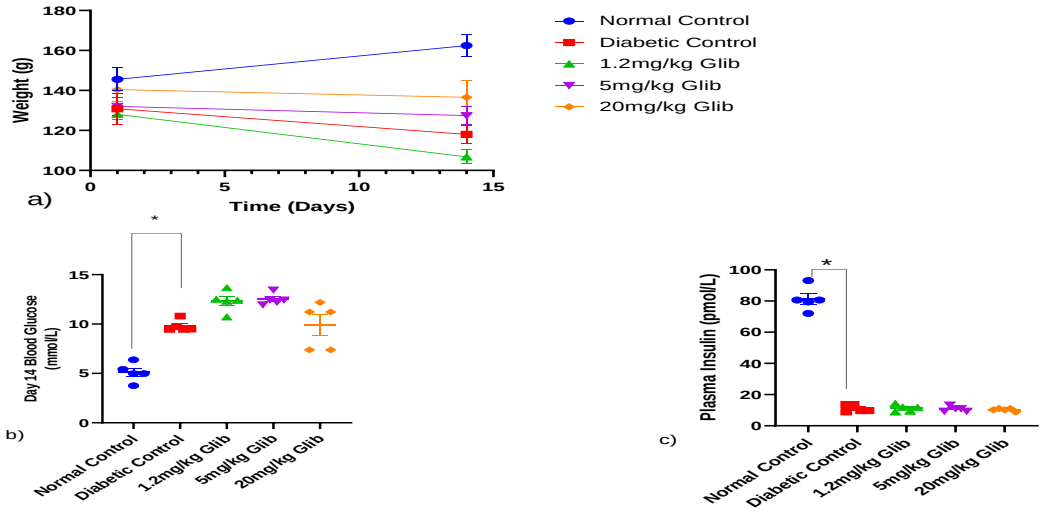

Figure 1. Effects of glibenclamide on a) body weight (g), (b) Day 14 FBG, and c) plasma insulin (pmol/L) in experimental animals (n=5/group). STZ-induced diabetes caused significant weight loss ($p \leq 0.0001$ vs normal), which was dose-dependently attenuated by glibenclamide. Glibenclamide (20 mg/kg) did not significantly reduce hyperglycemia compared to untreated diabetics ($p>0.05$). Nor did it restore plasma insulin. * Indicates $p \leq 0.05$ vs untreated diabetic. FBG = fasting blood glucose, STZ = streptozotocin

Peripheral and central cholinergic markers (plasma AChE and acetylcholine) To investigate the impact of glibenclamide on cholinergic function in diabetic rats, we quantified acetylcholinesterase (AChE) activity and acetylcholine (ACh) concentration in plasma and hippocampal homogenates. Plasma AChE activity was significantly elevated in diabetic rats compared with non-diabetic controls, but this increase was attenuated by the highest glibenclamide dose ($p = 0.0319$; Fig. 2a). While elevated AChE activity in untreated diabetics did not substantially alter plasma ACh levels, glibenclamide induced a dose-dependent rise in plasma ACh, reaching ~10-fold higher levels than in untreated diabetic rats ($p = 0.0004$; Fig. 2b). In contrast, no significant changes were observed in hippocampal insulin content, AChE or choline acetyltransferase (ChAT) activity, or ACh concentration following glibenclamide treatment (Figs. 2c–f; $p > 0.05$). These findings suggest that glibenclamide selectively modulates peripheral cholinergic markers without influencing central cholinergic activity, indicating a possible systemic mechanism of action that spares hippocampal neurotransmission. This peripheral selectivity may have implications for therapeutic strategies aiming to improve metabolic control while minimizing central cholinergic side effects.

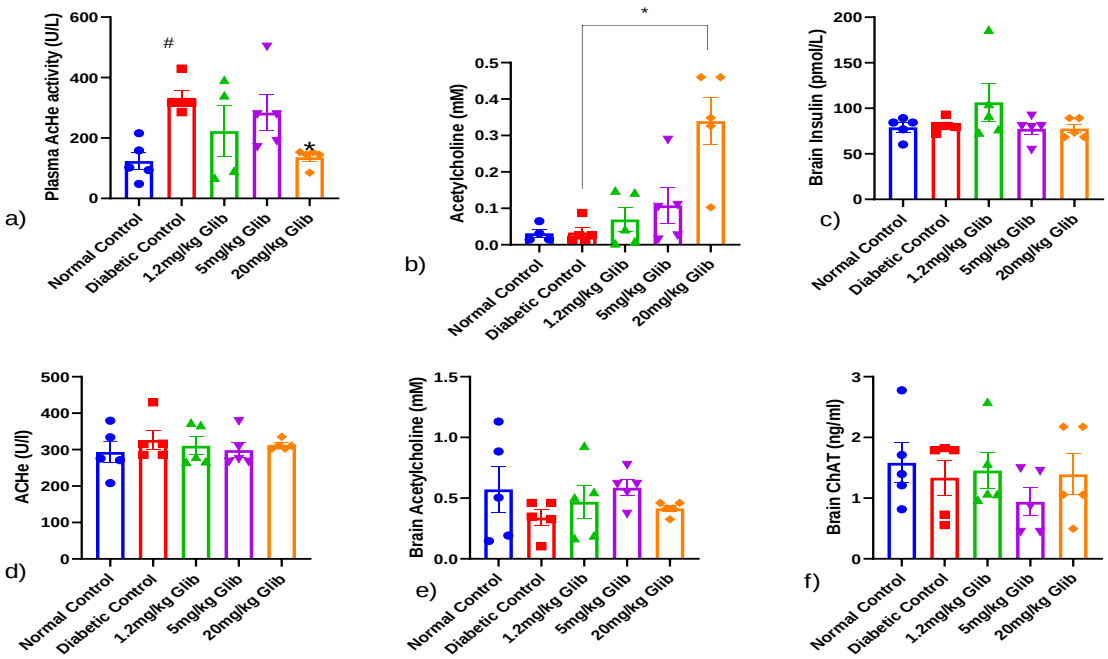

Figure 2: Effect of glibenclamide on (a) plasma AChE activity (U/L), (b) plasma acetylcholine (mM) (c) hippocampal insulin (pmol/L), (d) hippocampal acetylcholinesterase (AChE) activity (U/L), (e) hippocampal acetylcholine (mM), and (f) hippocampal choline acetyltransferase (ChAT) activity (ng/mL), in experimental animals (n = 5/group). In plasma, diabetes significantly increased AChE activity compared to normal controls ($p \leq 0.05$), while glibenclamide 20 mg/kg significantly reduced AChE (#$p \leq 0.05$ vs diabetic), which was associated with a concomitant increase in plasma acetylcholine concentration ($^{\#}p \leq 0.05$). In the hippocampus, neither diabetes nor glibenclamide treatment significantly altered insulin, AChE activity, acetylcholine, or ChAT activity. Data are mean ± SEM (n=5/group). "*" denotes $p \leq 0.05$ vs normal; "#" denotes $p \leq 0.05$ vs untreated diabetic.

Spatial memory (Y-maze alternation, Morris water maze)

Rats were subjected to the Y-maze and Morris water maze to assess short-term spatial memory and learning. Diabetic rats exhibited significantly fewer arm alternations and a lower percentage of alternation than normal ($p \leq 0.05$ vs control) (Figure 3a). Glibenclamide did not affect the total number of alternations but significantly improved the alternation percentage compared to untreated diabetics ($p \leq 0.05$). In the Morris water maze, no significant group differences were observed in escape latency or path length during learning or probe trials: diabetic and glibenclamide-treated rats performed similarly to controls (Figure 3b). Thus, glibenclamide selectively rescued a working memory deficit in the Y-maze but did not affect water-maze performance.

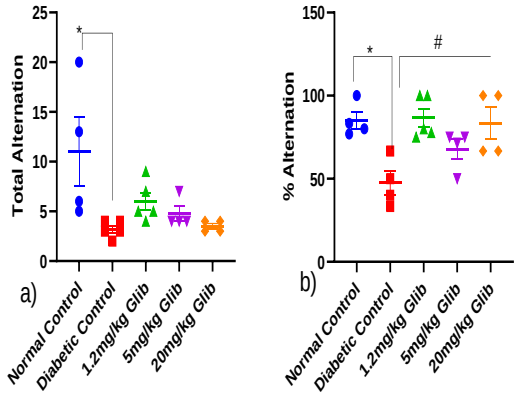

Figure 3: *Spatial memory tests.* A) total alternation counts and b) alternation percentage in the Y-Maze test. Diabetic rats had significantly lower total and per cent alternations than normal (*$p \leq 0.05$ vs normal). Glibenclamide 20 mg/kg markedly improved alternation percentage compared to diabetic controls ($p \leq 0.05$), whereas lower doses had no effect. (c) Morris water maze: escape latency during acquisition and probe performance (target quadrant time). No significant differences were observed among any groups in maze performance. Data are presented as mean ± SEM (n=5). *$p \leq 0.05$ vs normal; $p \leq 0.05$ vs untreated diabetic.

Histological changes

No significant neuronal loss, cell shrinkage, or neurodegeneration was observed in this 14-day streptozotocin-induced diabetic model (Figure 4). This corroborates the biochemical data obtained from these study animals in which no significant changes in metabolic or neurochemical parameters were observed.

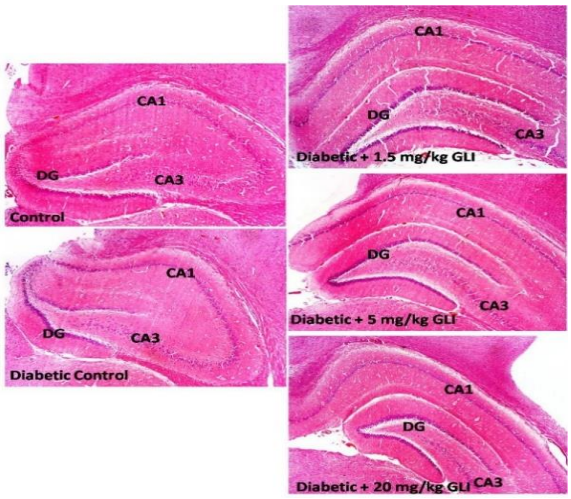

Figure 4: Representative photomicrographs of Hematoxylin and Eosin (H&E) stained sections of the dorsal hippocampus. The images show the primary subfields involved in memory: the Dentate Gyrus (DG), CA1, and CA3. No widespread neuronal loss, pyknosis (cell shrinkage), or other overt signs of neurodegeneration were observed in the diabetic control group compared to the healthy control group. Similarly, treatment with glibenclamide at 1.5, 5, or 20 mg/kg did not produce any visible changes in hippocampal morphology compared to the diabetic control group, suggesting the drug is not overtly neurotoxic at these doses.

DISCUSSION

This study investigated the effects of 14-day oral glibenclamide administration on metabolic, cholinergic, and cognitive parameters in STZ-induced diabetic rats. As expected, neither fasting blood glucose nor plasma insulin levels were significantly altered in glibenclamide-treated diabetic animals relative to diabetic untreated animals. In pancreatic β-cells, glibenclamide binds to the sulfonylurea receptor of ATP-sensitive K^+ (K_ATP) channels, triggering insulin release and lowering blood glucose. However, because STZ-treated rats exhibit near-complete β-cell destruction and negligible insulin secretion, glibenclamide did not increase insulin levels or meaningfully reduce hyperglycemia compared with untreated diabetic controls.

Glibenclamide treatment partially attenuated diabetes-induced weight loss in a dose-dependent manner. This modest weight-sparing effect likely reflects improvements in peripheral glucose metabolism independent of insulin, given that insulin levels remained low. Indeed, sulfonylureas exert extra-pancreatic actions, including reduced insulin clearance, suppression of glucagon secretion, and enhanced insulin sensitivity[19]. In this streptozotocin-induced diabetic model, a significant elevation in plasma acetylcholinesterase (AChE) activity was observed. This finding aligns with previous reports in type 2 diabetic models, suggesting that hyperglycemia consistently upregulates AChE activity regardless of the diabetic phenotype[20,21]. Glibenclamide treatment decreased AChE activity while concurrently increasing acetylcholine concentration. This reduction in acetylcholinesterase activity may reflect direct enzymatic inhibition by glibenclamide, as earlier reported *in vitro*[17]. These findings suggest glibenclamide may enhance peripheral cholinergic tone by limiting ACh breakdown. While this peripheral cholinergic modulation could influence autonomic or immune functions, its cognitive implications remain uncertain. In contrast to peripheral changes, no significant alterations in insulin levels, AChE activity, ACh concentration, or choline acetyltransferase (ChAT) activity were detected in the hippocampus of diabetic rats. The absence of central effects may be attributed to the study's relatively short duration, which may not have allowed sufficient time for diabetes-related neurochemical changes to manifest. Similarly, the lack of glibenclamide-induced modulation of central cholinergic markers likely reflects limited drug penetration into the brain. Lahmann *et al*.[22] demonstrated that systemically administered glibenclamide remains virtually undetectable in rodent brain or cerebrospinal fluid due to rapid efflux by transporters, with even supra-therapeutic doses and P-glycoprotein inhibition failing to enhance central drug levels. Thus, despite its potent *in vitro* activity, glibenclamide may not reach effective CNS concentrations to inhibit brain AChE. This “peripheral-only” cholinergic action is supported by our findings of reducing AChE activity in plasma while hippocampal AChE activity remains unchanged in glibenclamide-treated diabetic rats. Despite the lack of central AChE inhibition, glibenclamide did improve one aspect of cognitive function. Diabetic rats showed impaired working memory in the Y-maze, while the highest glibenclamide dose significantly rescued the alternation percentage without affecting locomotor activity. This suggests a beneficial effect on hippocampal-dependent memory. One plausible mechanism involves KATP channels in brain neurons: Stefani and Gold demonstrated that septal administration of glibenclamide or glucose enhances alternation performance, suggesting a role for KATP channels[23]. Although we administered glibenclamide orally, our treatment likely

yielded much lower brain levels than in the Stefani and Gold study. Yet, it is possible that at higher doses, some glibenclamide reached neural targets. The increased hippocampal ACh release with KATP blockade suggests that the cognitive benefit of glibenclamide could involve enhanced excitability or neurotransmitter release in memory circuits. Previous studies have shown that changes in acetylcholine concentrations do not always correlate with behavioral changes, implying additional factors beyond acetylcholine[23]. In our case, memory improvement occurred without measurable changes in basal brain AChE/ACh, indicating a complex interplay beyond cholinergic modulation. The dissociation between peripheral and central cholinergic effects has implications for developing novel drugs. The potent AChE inhibition by glibenclamide suggests it could be explored as an anti-Alzheimer's agent. However, our findings show that systemic glibenclamide may not effectively penetrate the brain to enhance synaptic acetylcholine or interact with central SUR1; however, the observed improvement in Y-maze performance suggests that some degree of central SUR1–K_ATP modulation may have occurred. This raises the possibility that structurally modified sulfonylureas or alternative delivery strategies could improve brain targeting. Notably, intranasal administration can facilitate direct drug delivery to the CNS[24,25]. This suggests that intranasal glibenclamide may achieve therapeutic brain concentrations capable of modulating central targets. In conclusion, glibenclamide significantly modulated peripheral cholinergic activity in diabetic rats but showed no effect on central cholinergic markers, likely due to limited brain penetration. Nonetheless, its cognitive benefits and dual metabolic-cholinergic actions highlight its potential for further investigation in diabetes-associated neurodegenerative models.

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Conflict of interest: The authors have no conflicts of interest to declare.

Authors' contribution: AA-O, OA & EOI: concept; IA & ALO: design; MFA & HOA: data collection; HAA, AA, SMG & NAB: data analysis & interpretation; AA-O, IA & MFA: drafting the manuscript. All author/s critically revised the manuscript for intellectual content and approved the final version for publication.

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