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Brain activities of streptozotocin-induced diabetic Wistar rats treated with gliclazide: Behavioural, biochemical and histomorphology studies

Moses B. Ekong [a,*], Francis N. Odinukaeze [a], Amaobi C. Nwonu [a], Christopher C. Mbadugha [a], Agnes A. Nwakanma [b]

[a] Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Uyo, Nigeria
[b] Department of Anatomy, Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli, Nigeria

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ABSTRACT

Gliclazide (GLD), a sulphonylurea is efficacious in the treatment of diabetes type-2. However, there is limited information on its activity in the brain, especially in diabetics. This research investigated the brain activities of GLD following streptozotocin-induced diabetes in Wistar rats. Twenty five adult male Wistar rats (200–250 g) were grouped (n = 5) as: Control (distilled water, 5 mL/kg) and GLD (150 mg/kg) groups; and the diabetic groups, untreated streptozotocin (STZ, 35 mg/kg), and STZ (35 mg/kg) treated with GLD (150 mg/kg) for two and four weeks, and already on high fat diet. The animals' body weights and blood glucose levels were checked weekly. After the experimental duration, spontaneous alternation and novel object recognition tests were carried out and the animals sacrificed. Perfusion with phosphate buffered saline preceded brain excision for biochemical analyses, with halves fixed in 10% neutral buffered formalin for histology. Compared with the control, results showed ($p < 0.05$) declined spontaneous alternation and exploratory activities with no preference for familiar or novel objects, body weights loss, raised blood glucose, increased malondialdehyde with decreased superoxide dismutase concentrations, and no apparent adverse effect on hippocampal and prefrontal cortical Nissl substance in the untreated diabetic group. The adverse observations were attenuated in the GLD treated diabetic groups; although the spontaneous alternation in the four weeks GLD treated diabetic group improved ($p < 0.05$), exploration of objects increased ($p < 0.05$) without preference. The present results showed that treatment with GLD for two and four weeks mitigated STZ activities, even though there was less improvement in neurocognitive activities.

1. Introduction

Diabetes mellitus or simply diabetes is a metabolic disorder characterised by hyperglycaemia and also associated with high morbidity and mortality (Daneman, 2001; WHO, 2021). This disorder results from impaired metabolism of carbohydrates, proteins and fats, due to inadequate or inefficient activity of insulin (American Diabetes Association, 2009). Diabetes is a public health challenge, with a global population of about 463 million people and 1.5 million deaths reported in 2019; a global population of over 750 million people with diabetes have been projected for 2045 (International Diabetic Federation, 2021a; WHO, 2021). Nigeria is one of the most affected countries in Africa with 2.7 million people living with diabetes (Uloko et al., 2018; International Diabetic Federation, 2021a, 2021b), requiring a consolidated approach towards tackling.

Diabetes can be classified into three major subtypes: gestational, type-1 and type-2. Gestational diabetes occurs during pregnancy due to factors including hormonal levels, obesity and lifestyle. It usually disappears after delivery, although may predispose one to diabetes type-2 development. Diabetes type-1 most commonly affects children, adolescents or young adults, and characterised by deficiency of insulin resulting from pancreatic beta cells destruction, and may be associated with acidosis or ketosis (Albert and Zimmet, 2004; American Diabetes Association, 2009; WHO, 2021).

In diabetes type-2, insulin resistance, relative insulin deficiency or both are the hallmark. It develops generally in adults, and is associated with lifestyle, genes, overweight, obesity and physical inactivity (Albert and Zimmet, 2004; American Diabetes Association, 2009; WHO, 2021). It is the most prevalent subtype and more predominant in developing countries making up about 85–90% of all cases (International Diabetic

* Corresponding author.
E-mail address: mosesekong@uniuyo.edu.ng (M.B. Ekong).

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Federation, 2021a; WHO, 2021). Its management and treatment requires pharmacological interventions, although other approaches may help. One such pharmacological approach is the use of the sulphonylurea, gliclazide, reported with high efficacy (Landman et al., 2014; Leiter et al., 2018).

Gliclazide is an oral anti-hyperglycaemic second generation sulphonylurea used either in monotherapy or in combination with other anti-diabetics or insulin (Landman et al., 2014; Leiter et al., 2018). It stimulates insulin secretion by binding to the pancreatic beta cell sulphonylurea receptor, probably leading to intracellular calcium transport and insulin exocytosis (Campbell et al., 1991). In addition, gliclazide reduces hepatic glucose production and increase peripheral insulin sensitivity. Like most medications, adverse effects are reported including hypoglycaemia, gastrointestinal disturbance, weight gain, nausea and pancreatitis.

Hyperglycaemia associated with diabetes damages the body systems, particularly the brain cells (Bourne et al., 2013; WHO, 2021), with diabetes type-2 linked to neurodegenerative diseases (Ott et al., 1999; Daneman, 2001; Cox et al., 2005). This neurodegeneration is one of the most common causes of dementia, associated with memory and cognitive impairments (Muonagolu and Ekong, 2016; Yarube and Mukhtar, 2018; Nduohosewo and Ekong, 2020). While it is reported that gliclazide inhibits diabetic neuropathy (Qiang et al., 1998) and stabilizes retinopathy (Ziegler and Drouin, 1994), there is a dearth of information on gliclazide action on cognitive functions. Hence, this study investigated the brain activities of gliclazide following streptozotocin-induced diabetes in Wistar rats.

2. Materials and methods

2.1. Preparation of chemicals

Streptozotocin (Sigma, Germany) solution was prepared by dissolving the powder in 0.1 M citrate buffer (pH 4.5) (Furman, 2021); Gliclazide (Diamicron, Servier, Egypt) was dissolved in distilled water. They were all prepared just before use.

2.2. Animals handling and groupings

A total of twenty five adult male Wistar rats of body weights 200 – 250 g, obtained from the Faculty of Basic Medical Sciences Animal House, University of Uyo, were allowed to acclimatise for a week and then randomly grouped into five (n = 5): Groups 1 and 2 were the control and gliclazide, while diabetes was induced in groups 3–5 rats (Table 1). The animals were housed in well ventilated cages with wire gauze roof. They were exposed to 12 h light/dark cycles and room temperature of 25–28 °C. Animals were handled and cared according to the guidelines of National Research Council of the United States of America (National Research Council, 2011). Ethical approval for the research was obtained from the Faculty of Basic Medical Sciences Research and Ethical Committee of the University of Uyo, Nigeria (UU/FBMSREC/2019/010).

2.3. Diabetes type-2 induction

Diabetes type-2 was induced according to the protocol of de Magalhães et al. (2019). Briefly, groups 3–5 animals were fed with high fat diet for 4 weeks prior to diabetes induction, while groups 1 and 2 were fed normal pellet diet (Table 2). Thereafter, animals in groups 3–5 were fasted overnight for 8 h, and streptozotocin (35 mg/kg) was administered intraperitoneally (i.p.) (Furman, 2021). To avoid fatal hypoglycaemia, the rats were administered glucose solution orally at different time intervals after 6 h of streptozotocin (Huang and Wu, 2005).

To confirm diabetic induction, the animals' fasting blood glucose levels were checked after 72 h of streptozotocin administration: Using a pin prick, a drop of blood was obtained from the animals' tails unto strips and the readings taken in a fine test Auto-coding glucometer (Metrado GmbH, Korea). A second streptozotocin administration was carried out after one week. Rats with blood glucose level of 250 mg/dL and above were considered diabetic, and used for the study. The animals' body weights before the commencement of the experiment, and weekly thereafter were monitored using a digital weighing scale.

2.4. Post-diabetic treatment

The animals were then treated for up to four weeks using the schedule of treatment in Table 1. The control, gliclazide and untreated diabetic groups received distilled water only, while the diabetic treated groups received gliclazide, all by oral gavages and once daily by 8 AM (GMT+1).

2.5. Neurobehavioural tests

The novel object recognition and spontaneous alternation tests were carried out 24 h after the last treatments. All the remaining animals were moved to the test room an hour prior to the start of the first trial to habituate with the condition of the test room. The novel object recognition test followed the protocol of Lueptow (2017): The open field maze used for the novel object recognition test was a white plywood box, with dimensions, 40 × 40 × 40 cm. The objects to be discriminated were the same size, made of plastic, deferring in shape and colour. The test involved three sessions, habituation, training, and test sessions. On day 1 (two days to the test trial), the rats were allowed to explore the box for 5 min, in order to acclimatise. On the training and test days (days 2 and 3), each rat was placed in the box for 10 min and allowed to explore either familiar or familiar/novel objects respectively. Exploration was timed in seconds, when the rats sniffed, gnawed or touched the objects with their nose or head.

The T-maze spontaneous alternation test followed the protocol of Deacon and Rawlins (2006). The maze was made up of white plywood having a "T" shape, with two goal arms and a start arm. The dimensions of the goal arms were 50 × 10 cm, while the start arm was 50 × 16 cm. The central partition extended into the start arm with the dimension of 10 cm, whereas the heights of the walls were 30 cm. The animals were first placed in the start arm: Upon leaving the start arm, the rats chose between entering either the left or the right goal arms. The alternation for five trials per animal was recorded and the percentage of alternation

Table 1
Schedule of treatment of the animals.

Group (n = 5)	Treatment	Duration
Group 1 (normal control)	Distilled water (5 mL/kg)	4 weeks
Group 2	150 mg/kg of Gliclazide (equiv. of 30 mg/day in humans)	2 weeks
Group 3	Untreated diabetics	2 weeks
Group 4	Diabetics treated with 150 mg/kg of Gliclazide	2 weeks
Group 5	Diabetics treated with 150 mg/kg of Gliclazide	4 weeks

Table 2
Compositions of the normal and high fat diets.

Constituent	Normal diet (g/kg)	High fat diet (g/kg)
Corn starch	276	276
Soya beans	180	180
Mineral mix	30	30
Vitamin mix	10	10
Sucrose	80	80
Cellulose	40	40
Lard	0	330

was calculated.

2.6. Termination of the experiment

Immediately after the neurobehavioural tests, the animals from each of the groups were sacrificed after anaesthetising with ketamine hydrochloride (Rotex Medica, Germany, 50 mg/kg i.p.). Each animal's thoracic cavity were opened up and perfused with cold -phosphate buffered saline intracardially (pH 7.35), and whole brains were removed.

2.7. Tissue processing for histological studies

One half of the brains were fixed in 10% neutral buffered formalin for 48 h, and subsequently prepared for paraffin embedding. Paraffin blocks were sectioned at 10 μm using a rotary microtome (HM304E, India), and processed for cresyl violet staining (Zhu et al., 2015). Photomicrographs were analysed for colour intensity using ImageJ software (1.52p).

2.8. Tissue processing for biochemical analysis

The remaining one halves of the brains were homogenised for biochemical assay. The homogenised brains were centrifuged and aliquots of the supernatant were used for biochemical analyses. The protein concentration was estimated using the Biuret reaction method (Bianchi-Bosisio, 2005). The absorbance was read at 540 nm against reagent blank using UV/ visible spectrophotometer.

Monoamine oxidase was assayed using the method of Green and Haughton (1961). The orange-yellow colour that formed was measured at 450 nm. Malondialdehyde was assayed using Wright's method (Wright et al., 1981). The amount of malondialdehyde formed in each of the brain samples was assessed by measuring the optical density of the supernatant at 532 nm. Superoxide dismutase activity was assayed as described by Sun and Zigma (1978). The absorbance was read at regular interval 0.1–5 min at 480 nm.

$$\text{Activity of SOD} = \Delta A/\text{min} \times TV/\varepsilon \times SV$$

Where ΔA = change in the absorbance, TV = total volume, SV= sample volume, ε = molar extraction.

2.9. Statistical analysis

One-way analysis of variance was used to compare the means for all groups' activities, thereafter Bonferroni's Multiple Comparison post-hoc test was carried out, both using Graphpad Prism software (Graphpad 5.0) to find the level of significance at $p < 0.05$. Data are presented as mean ± standard error of mean.

3. Results

3.1. Body weight changes

There was no significant difference ($p > 0.05$) in the initial (baseline) body weights among the groups, except in the untreated diabetic group, which was significantly ($p = 0.0109$, $F = 4.340$) higher than the control. After two weeks of high fat dieting, the body weights of the diabetic groups (3–5) were significantly ($p < 0.0001$, $F = 206.2$) higher than the control (Fig. 1).

After diabetic induction, there was no significant difference ($p = 0.7166$, $F = 0.5279$) in body weights between the diabetic groups and the control. Following treatment with gliclazide for two weeks, the body weights of the gliclazide and untreated diabetic groups were significantly ($p < 0.0001$, $F = 43.03$) lower than the control, but was significantly higher in the diabetic groups treated with gliclazide than the control and untreated diabetic groups. By the fourth week of treatment, the body weights of diabetic group treated with gliclazide for 4 weeks were significantly ($p < 0.05$) higher than the control (Fig. 1).

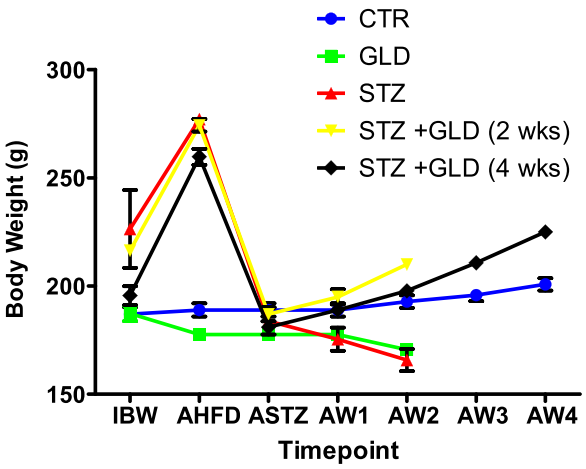

Fig. 1. Body weight changes in the experimental animals. Data presented as mean standard error of mean; CTR – control; GLD – gliclazide; STZ – streptozotocin; STZ + GLD (2 weeks) = 2 weeks of streptozotocin and gliclazide; STZ + GLD (4 weeks) = 4 weeks of streptozotocin and gliclazide; IBW – initial body weight, AHFD – after high fat diet, ASTZ – after streptozotocin, AW1 – after week 1, AW2 - after week 2, AW3 - after week 3, AW4 - after week 4.

3.2. Blood glucose levels

The glucose level increased gradually in high fat dieting ($p < 0.0001$, $F = 145.6$), and steeply after streptozotocin administration ($p < 0.0001$, $F = 593.8$) compared with the control and gliclazide groups. After two weeks of gliclazide treatments, there were significant ($p < 0.0001$, $F = 132.8$) higher glucose levels in the diabetic groups (3–5) compared with the control and gliclazide groups, while the glucose levels were significantly reduced in the diabetic groups treated with gliclazide compared with the untreated diabetic group. After four-weeks of gliclazide treatments, there were significantly ($p < 0.0001$, $F = 89.41$) higher glucose levels in the diabetic group treated with gliclazide compared with the control group, and which were significantly reduced compared with the untreated diabetic group (Fig. 2).

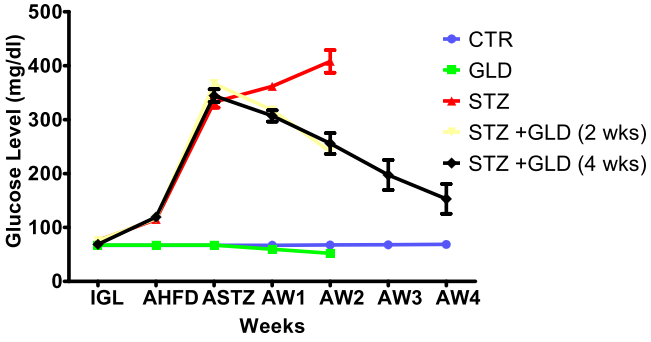

Fig. 2. Glucose level prior and after treatment with gliclazide. Data presented as mean standard error of mean; CTR = control; GLD = gliclazide; STZ = streptozotocin; STZ + GLD (2 weeks) = 2 weeks of streptozotocin and gliclazide; STZ + GLD (4 weeks) = 4 weeks of streptozotocin and gliclazide; IGL – initial glucose level, AHFD – after high fat diet, ASTZ – after streptozotocin, AW1 – after week 1, AW2 - after week 2, AW3 - after week 3, AW4 - after week 4.

3.3. Neurobehavioural analysis

In the training session for the object recognition, the test groups, except the diabetic group treated with gliclazide for 4 weeks spent significantly ($p < 0.0001$, $F = 34.15$ and $p < 0.0001$, $F = 36.62$, respectively) less time exploring objects A1 and A2 compared with the control. The diabetic group treated with gliclazide for four weeks spent significantly more time exploring objects A1 and A2 than the other test groups. However, there was no significant difference ($p > 0.05$) in time spent exploring either objects (Fig. 3).

In the test session, all the test groups spent significantly ($p < 0.0001$, $F = 33.46$ and $p < 0.0001$, $F = 49.63$, respectively) less time exploring objects A and B compared with the control. The diabetic groups treated with gliclazide spent significantly more time exploring objects A and B than the other test groups. The control group spent significantly more ($p = 0.0278$) time exploring object B compared to object A, but there was no difference ($p > 0.05$) among the other groups (Fig. 4).

In the spontaneous alternation test, the test groups had significantly ($p < 0.0001$, $F = 24.00$) less spontaneous alternation compared with the control group. The diabetic group treated with gliclazide for four weeks had significantly more spontaneous alternation than the other groups. However, there was no difference ($p > 0.05$) between the other test groups (Fig. 5).

3.4. Brain tissue biochemical analysis

The monoamine oxidase level was not significantly different ($p = 0.0737$, $F = 2.447$) between the test and control groups, and among the test groups. The malondialdehyde level was significantly higher ($p = 0.0001$, $F = 9.561$) in the untreated diabetic group compared with the control and other test groups. There was however, no significant difference ($p > 0.05$) among the other test groups, and compared with the control. The superoxide dismutase level was significantly lower ($p < 0.0001$, $F = 11.60$) in the untreated diabetic group compared with the control and the other test groups. There was however, no significant difference ($p > 0.05$) among the other test groups, and compared with the control (Table 3).

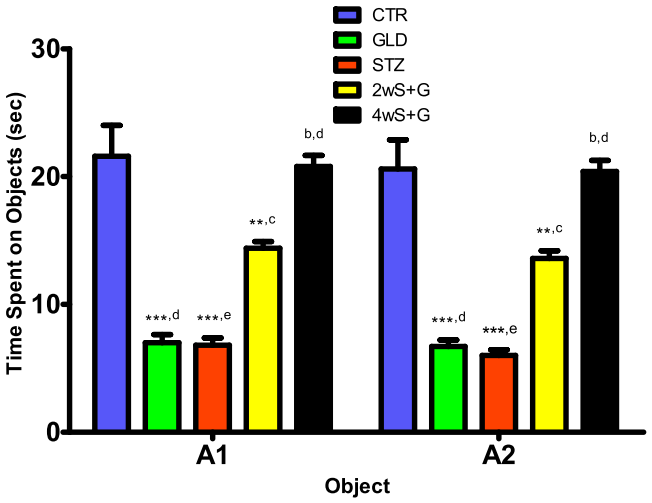

Fig. 3. Training phase: time spent to explore objects A1 and A2 in the open field. Data presented as mean standard error of mean; *** = Significantly different from control group at $p < 0.001$; ** = Significantly different from control group at $p < 0.01$; b = Significantly different from GLD group at $p < 0.05$; c = Significantly different from STZ group at $p < 0.05$; d = Significantly different from 2wS+G group at $p < 0.05$; e = Significantly different from 4wS+G group at $p < 0.05$; A1 - $p < 0.0001$, $F = 34.15$; A2- $p < 0.0001$, $F = 36.62$; CTR = control; GLD = gliclazide; STZ = streptozotocin; 2wS+G = 2 weeks of streptozotocin and gliclazide; 4wS+G= 4 week of streptozotocin and gliclazide.

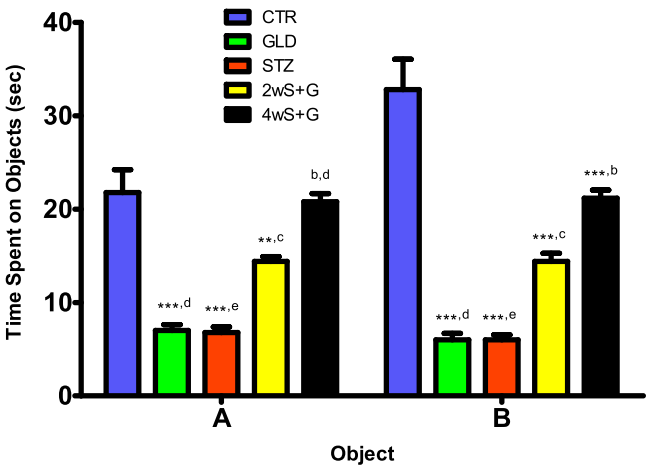

Fig. 4. Test phase: time spent to explore objects A and B in the open field. Data presented as mean standard error of mean; *** = Significantly different from control group at $p < 0.001$; ** = Significantly different from control group at $p < 0.01$; b = Significantly different from GLD group at $p < 0.05$; c = Significantly different from STZ group at $p < 0.05$; d = Significantly different from 2wS+G group at $p < 0.05$; e = Significantly different from 4wS+G group at $p < 0.05$; CTR = control; GLD = gliclazide; STZ = streptozotocin; 2wS+G = 2 weeks of streptozotocin and gliclazide; 4wS+G= 4 week of streptozotocin and gliclazide.

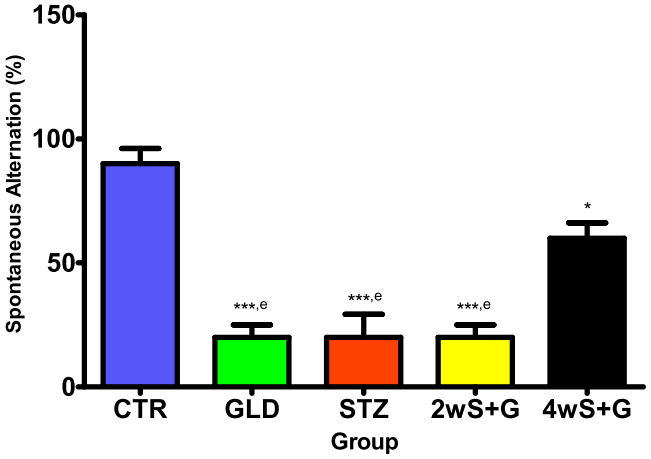

Fig. 5. Spontaneous alternation in the T-maze. *** = Significantly different from Control group at $p < 0.001$; ** = Significantly different from Control group at $p < 0.01$; e = Significantly different from 4wS+G group at $p < 0.05$; CTR = control; GLD = gliclazide; STZ = streptozotocin; 2wS+G = 2 weeks of streptozotocin and gliclazide; 4wS+G= 4 week of streptozotocin and gliclazide.

3.5. Histomorphology results

3.5.1. Hippocampus

The hippocampus of the control group showed Nissl substance distribution in the three cortical layers; outer molecular, middle pyramidal and inner polymorphic. Within the molecular layer were well-stained sparsely distributed cells. In the pyramidal layer and especially in the CA3 region, and there were densely distributed well-stained Nissl substance. The polymorphic layer contained scanty well-stained Nissl substance (Fig. 6a).

The cortical layers of the hippocampus were also observed in the test groups: well stained Nissl substance was observed in the gliclazide group; the untreated diabetic group showed Nissl substance staining, with some containing dark nuclei; the diabetic group treated with gliclazide for two weeks showed Nissl substance staining; the diabetic

Table 3
Biochemical quantification of brain tissue biomolecules.

Group	MAO (pg/mL) p = 0.0737 F = 2.447	MDA (U/mg) p = 0.0001 F = 9.561	SOD (U/mg) p < 0.0001 F = 11.60
CTR	180.3 ± 32.57	0.460 ± 0.03	107.6 ± 3.63
GLD	122.0 ± 1.36	0.439 ± 0.09d	121.9 ± 8.07d
STZ	136.8 ± 10.07	0.903 ± 0.04***	50.74 ± 5.23**
STZ+GLD (2WK)	135.8 ± 9.87	0.469 ± 0.09^{c}	125.0 ± 5.98^{c}
STD+GLD (4WK)	117.1 ± 8.38	0.566 ± 0.03^{c}	111.0 ± 11.74^{c}

CTR = control; GLD = gliclazide; STZ = streptozotocin; MAO = monoamine oxidase; MDA = malondialdehyde; SOD = superoxide dismutase.
*** = Significantly different from control at p < 0.001; ** = Significantly different from control at p < 0.01; c = Significantly different from STZ group at p < 0.05

group treated with gliclazide for four weeks showed stained Nissl substance (Fig. 6b-e); There was no significant difference (p = 0.6891; F = 0.8857) in their staining intensities compared with the control (Fig. 8).

3.5.2. *Lateral prefrontal cortex*

The lateral prefrontal cortex is made up of six cortical layers; the outer three layers are shown in the present study. The control group showed well-stained Nissl substance in the external granular and pyramidal layers (Fig. 7a). The cortical layers of the lateral prefrontal cortex were also observed in the test groups: The gliclazide group showed stained Nissl substance in most of the cells (Fig. 7b), and stained Nissl substance in the untreated diabetic group (Fig. 7c). The diabetic groups treated with gliclazide showed stained Nissl substance (Fig. 7d and e), compared with the control. There was no significant difference (p = 0.2828, F = 0.5675) in their staining intensities compared with the control (Fig. 8).

4. Discussion

The present study investigated the brain activities of gliclazide

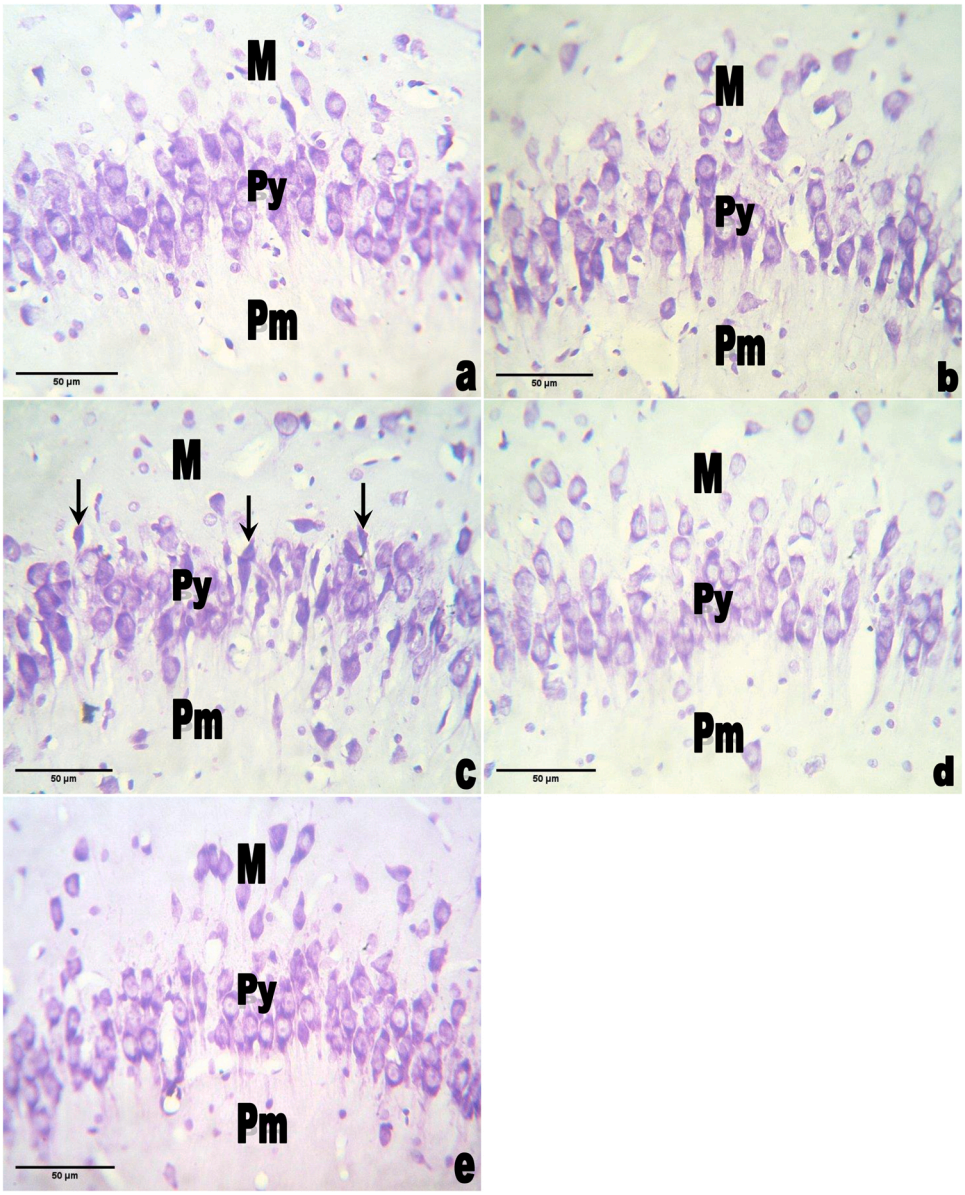

Fig. 6. The sections of the CA3 region of the hippocampus. a. Control group showing well-stained Nissl substance. b. Gliclazide group showing Nissl substance staining. c. Untreated diabetic group showing Nissl substance staining, with some containing dark nuclei (arrow). d. Diabetic group treated with gliclazide for two weeks showing stained Nissl substance. e. Diabetic group treated with gliclazide for four weeks showing stained Nissl substance. M – molecular layer; Py – pyramidal layer; Pm – polymorphic layer; Cresyl violet stain, × 400.

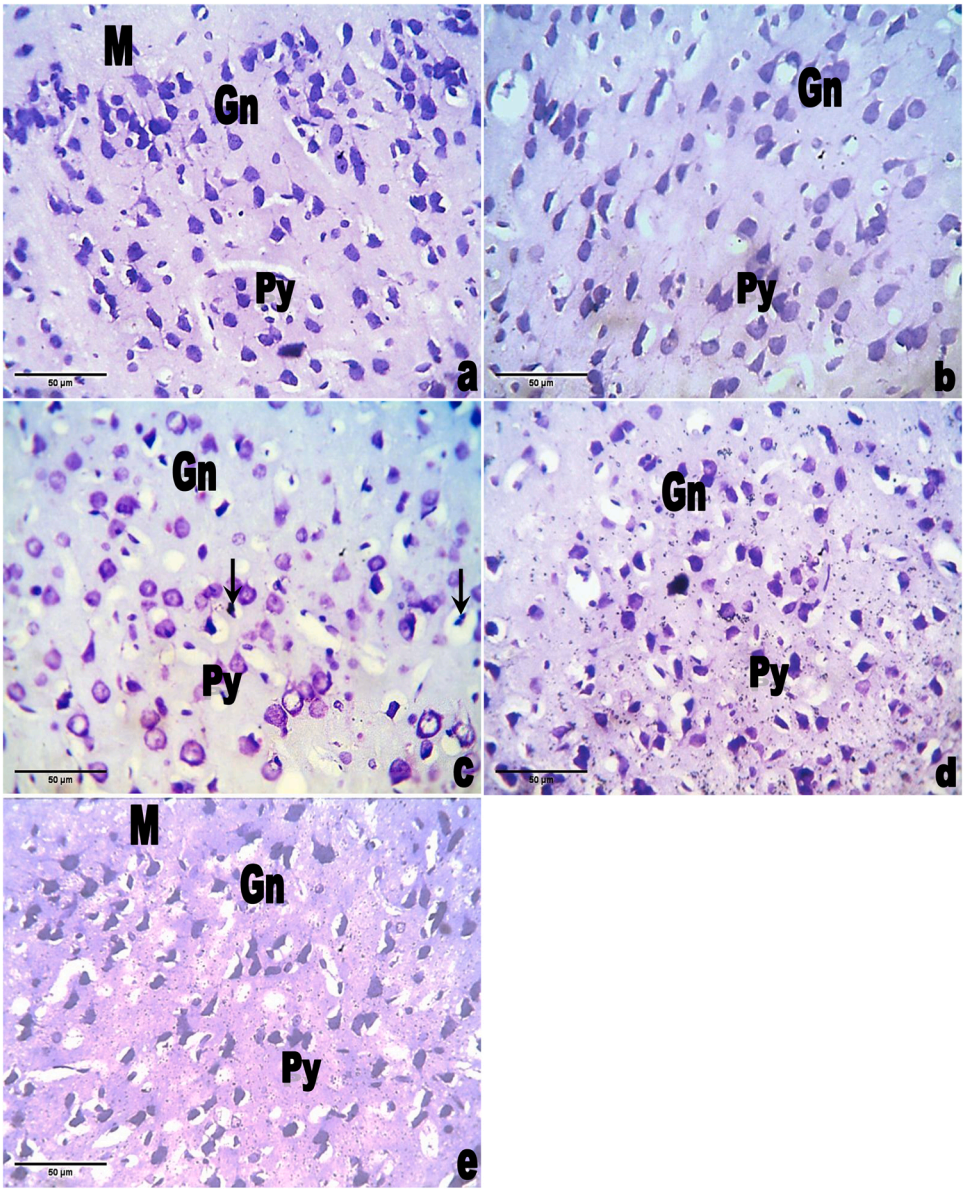

Fig. 7. The sections of the lateral prefrontal cortex. a. Control group showing well-stained Nissl substance. b. Gliclazide group showing Nissl substance staining. c. Untreated diabetic group showing Nissl substance staining, with some containing dark nuclei (arrow). d. Diabetic group treated with gliclazide for two weeks showing stained Nissl substance. e. Diabetic group treated with gliclazide for four weeks showing stained Nissl substance. M – molecular layer; Gn – external granular layer; Py – external pyramidal layer; Cresyl violet stain, × 400.

following streptozotocin-induced diabetes in Wistar rats. It was observed that administrations of streptozotocin and gliclazide resulted in significant changes across the experimental groups: After two weeks of high fat diet, the body weights of animals in groups 3–5 (the diabetic groups) were significantly higher compared with the control and gliclazide groups which were on normal diet. The significant increase in the body weights of animals fed with high fat diet is an indication of increased adipose tissue deposition attributed to the high intake of energy rich saturated fats (Sasidharan et al., 2013), and this may have initiated insulin resistance, which is one of the important features of diabetes type-2 (Do et al., 2018; Ramos-Romero et al., 2018). One week after diabetes induction with streptozotocin, the body weights of animals in the diabetic groups were significantly decreased to the baseline, compared with the control and gliclazide groups, where there were no differences. This is an indication of the deleterious effect of streptozotocin, usually associated with hyperglycaemia (Hirata et al., 2019). By the second week of treatments, the body weights of animals in the gliclazide group showed a decline compared with the control indicating that gliclazide may have affected the body weight of the animals, as previously reported (Seshiah et al., 1993; Leiter et al., 2018). While the untreated diabetic group's body weight was on a continuous decline, the diabetic groups treated with gliclazide increased body weights until the fourth week, indicating that gliclazide may have attenuated the adverse body weight effect of streptozotocin as also reported by Leiter et al. (2018), due to its anti-hyperglycaemic and anti-oxidative effects (McGavin et al., 2002; Sena et al., 2009).

The groups allowed high fat diets (diabetic groups) had significantly higher blood glucose levels compared with the control and gliclazide groups. These differences in glucose level could be as a result of increased dietary intake (high fat diet), which often leads to obesity, insulin resistance and hyperglycaemia, as previously reported (Lang et al., 2019; Abi et al., 2020). There were further significantly increased blood glucose levels in the diabetic groups after induction with streptozotocin compared with the control and gliclazide groups, an indication that streptozotocin exacerbates hyperglycaemia. The transition from pre-diabetes to diabetes may be as a result of declined secretory capacity of pancreatic beta cells to compensate for existing insulin resistance (de Magalhães et al., 2019). After treatment with gliclazide, there were continuous significant reduction in the blood glucose level from the first to the fourth week compared with the control and untreated diabetic groups. The reduction in the blood glucose indicates the anti-hyperglycaemic effect of gliclazide (Leiter et al., 2018), which is

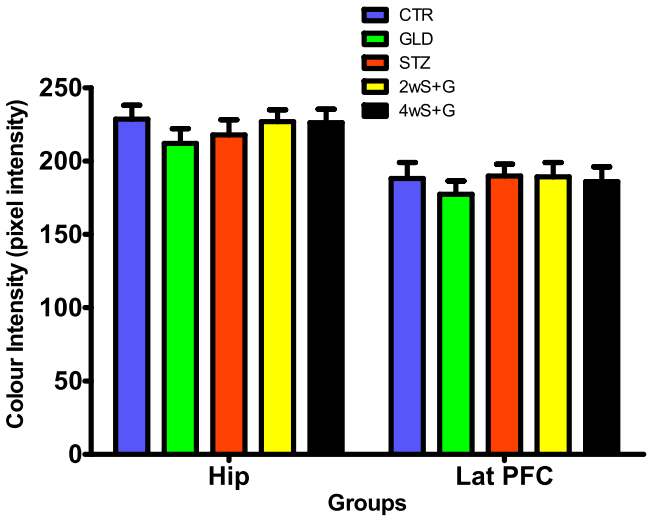

Fig. 8. The intensity of Nissl substance staining in the hippocampus and lateral prefrontal cortex. There was no significant difference in the staining intensity of the hippocampus (Hip) and lateral prefrontal cortex (Lat PFC), $p = 0.6891$, $F = 0.8857$; $p = 0.2828$, $F = 0.5675$ respectively, using ImageJ software; CTR = control; GLD = gliclazide; STZ = streptozotocin; 2wS+G = 2 weeks of streptozotocin and gliclazide; 4wS+G= 4 week of streptozotocin and gliclazide.

similar to reports on sulphonylureas generally (Elmali et al., 2004; Seena et al., 2017).

Novel object recognition and spontaneous alternation tests are considered as valid ethological paradigm for neurocognitive and neurobehavioural assessments. The novel object recognition test is a low stress and efficient test for memory in rat (Akkerman, 2012). The animals in the gliclazide group spent significantly less time exploring the familiar and novel objects, with no difference between the two compared with the control group. These indicate that gliclazide may have negatively impaired learning or memory of the animal as observed in the time, and the non-preference of the objects. Rats have an innate preference for novelty (Sivakumaran et al., 2018), which was not the case in the present study. The animals in the untreated diabetic group spent significantly less time exploring the familiar and novel objects, with no difference between the two, compared with the control. These indicate that streptozotocin may have negatively affected learning or memory of the animal, as observed in the time, and the non-preference of the objects. This is expected as diabetes leads to cognitive deficit (Moheet et al., 2015; Paidi et al., 2015).

The diabetic groups treated with gliclazide for up to four weeks spent significant ($p < 0.05$) time exploring the familiar and novel objects, compared with the gliclazide and untreated diabetic groups, although there was no difference in the latency of both familiar and novel objects. These differences in the latency to explore the objects may indicate improvement in learning or memory. However, the latency with the novel object was less ($p < 0.05$) compared with the control group, which still indicates impairment of this brain function. The present results are at variance with reports of other sulphonylureas, glibenclamide and metformin, that mitigated cognitive impairments (Herath et al., 2016; Esmaeili et al., 2020).

The spontaneous alternation measures exploratory behaviour in animals for central nervous system disorders such as Alzheimer's or other dementia-related diseases, and is based on the willingness of animals to explore a new environment (Wu et al., 2018). The present results showed that the gliclazide group had less spontaneous alternation compared with the control, indicating that gliclazide may have impaired the animals' cognitive functions resulting in deficit learning and memory ability. One adverse effect of continuous gliclazide intake is hypoglycaemia, which is indicted in cognitive decline (van Dalem et al., 2016; Ebadi et al., 2018), and may be a reason for the present result. The untreated diabetic group also had less spontaneous alternation compared with the control, also indicating deficit in learning or memory ability. This deficit in cognitive function may be as a result of oxidative stress (Muriach et al., 2014), as diabetes type-2 is recognised as an independent risk factor for the development of cognitive dysfunction (Moheet et al., 2015; Karvani et al. 2019).

The diabetic groups treated with gliclazide for up to four weeks showed less spontaneous alternation compared with the control, which may be due to the synergistic negative cognitive effect of both streptozotocin and gliclazide as also reported in the novel object recognition test of the present study. However, the diabetic group treated with gliclazide for four weeks showed higher spontaneous alternation compared with the groups with untreated diabetic and the gliclazide treated diabetic for two weeks, suggesting that prolong treatment with gliclazide may reverse this cognitive actions of streptozotocin.

The level and activities of brain enzymes reflects the state of such brain. In the present study, the monoamine oxidase activity was not different in the gliclazide group compared with the control, indicating the non-adverse effect of gliclazide on this enzyme activity. In the untreated diabetic group, monoamine oxidase activity was not different compared with the control, indicating that streptozotocin may not have affected this enzyme, and is in line with the report of Ashafaq et al. (2014). This result is at variance with Gupta et al. (2014), who reported decreased monoamine oxidase activities, and this may be attributed to the species, as mice was used. There was no difference in monoamine oxidase activity in the diabetic groups treated with gliclazide for up to four weeks compared with the control and the untreated diabetic groups, indicating that both gliclazide and streptozotocin may not have affected this enzyme activity.

In the present study, there was no difference in the malondialdehyde activity between the gliclazide and control groups, indicating that gliclazide may not have caused oxidative stress. Malondialdehyde is one of the products of polyunsaturated fatty acids peroxidation in cells widely used in the evaluation of oxidative stress, a product of lipid peroxidation and free radical formation, which accumulates in the presence of oxidative stress and antioxidants like superoxide dismutase and catalase are used up for scavenging. The present result supports the report of Jennings and Belch (2000), who reported that gliclazide has anti-oxidative role.

The untreated diabetic group showed significant increased malondialdehyde activity compared with the control, indicating that streptozotocin may have caused oxidative stress, a result of lipid peroxidation and free radicals formation (Desai et al., 2014). The present result is in agreement with previous reports of increased malondialdehyde activity in diabetic animals (Akinola et al., 2011; Seena et al., 2017).

Malondialdehyde activity decreased significantly in the diabetic groups treated with gliclazide for up to four weeks compared with the untreated diabetic group, and was not different with the control group. The decreased activities of malondialdehyde in these groups indicate that treatment with gliclazide may have inhibited lipid peroxidation. Gliclazide is reported to prevent lipid oxidation due to the presence of a unique aminoazabicyclo-octane ring (Jennings and Belch, 2000), and this may be a reason for the present result.

In the present study, superoxide dismutase activity in the gliclazide group was not different compared with the control, indicating that gliclazide may not have affected the brain's superoxide dismutase activity. Superoxide dismutase activity in the untreated diabetic group was significantly reduced compared with the control group. This reduction of superoxide dismutase activity may be due to the damaging effects of free radicals generated by streptozotocin as this leads to oxidative stress. The result supports a previous study by Nazaroglu et al. (2009), who reported that decreased activity of superoxide dismutase in diabetes condition is attributable to oxidative stress.

There was a significant increased superoxide dismutase activity in the diabetic groups treated with gliclazide for up to four weeks compared with the streptozotocin group, but was not different from the

control group. These increased superoxide dismutase indicate that gliclazide may have attenuated the free radicals generated by streptozotocin. Superoxide dismutase acts as a defence system against reactive oxygen species by catalysing the dismutation of two molecules of superoxide anion to hydrogen peroxide (Wang et al., 2018), thereby rendering them less harmful. It is used up when there is oxidative stress, *vis-à- vis* accumulating when oxidative stress is absent. Gliclazide has a free radical scavenging ability (Jennings and Belch, 2000), which may have stimulated the superoxide dismutase defence system and the resultant increased superoxide dismutase accumulation. This agrees with previous works that reported gliclazide action through dynamic-related protein 1 mediated oxidative stress (Qiang et al., 1998; Wu et al., 2012).

The morphological appearance of the brain also gives insight to its state. In the present study, the hippocampus and lateral prefrontal cortex of the diabetic group treated with gliclazide for two weeks showed no adverse histological effect. This may be attributed to the gliclazide activity which plays anti-oxidative and anti-hyperglycaemic roles. The hippocampus and lateral prefrontal cortex of the group administered gliclazide alone showed stained Nissl substance, indicating no apparent adverse effect. The untreated diabetic group's hippocampus and lateral prefrontal cortex showed stained Nissl substance, indicating no apparent adverse histological streptozotocin activity. The present result is at variance with a report that untreated streptozotocin-induced diabetics present with prefrontal cortical weak Nissl staining (Akinola et al., 2011).

The hippocampus and lateral prefrontal cortex of the diabetic group treated with gliclazide for four weeks showed stained Nissl substance, indicating no adverse morphological effect. Gliclazide attenuates hyperglycaemia and oxidative stress (Jennings and Belch, 2000), which are hallmarks in diabetes and neurodegeneration (Yarube and Mukhtar, 2018). The present result is not similar to that of Akinola et al. (2011), who reported that the sulphonylurea, metformin ameliorated streptozotocin-induced diabetic Nissl deficit.

The hippocampus and the lateral prefrontal cortex are the parts of the forebrain: While the hippocampus is responsible for memory consolidation and learning (Eichenbaum, 2017), the lateral prefrontal cortex subserves working memory and executive functions (Wagner et al., 2001; Lara and Wallis, 2015). These functions may have been impaired in the resulting diabetic condition, which gliclazide may not have successfully attenuated probably due to the short duration of treatment.

5. Conclusion

The present study showed that normoglycaemic gliclazide-treated animals had declined spontaneous alternation and exploratory activities with no preference for familiar or novel objects, as well as body weights loss. On the other hand, streptozotocin-induced diabetes caused declined spontaneous alternation and exploratory activities with no preference for familiar or novel objects, body weights loss, elevated blood glucose, increased lipid peroxidation, but no apparent Nissl substance deficit in the hippocampal and prefrontal cortex. These adverse observations were attenuated with gliclazide treatments, although the spontaneous alternation in the four weeks GLD treated diabetic group improved ($p < 0.05$), exploration of objects increased ($p < 0.05$) without preference. The present results showed that treatment with gliclazide for two and four weeks mitigated streptozotocin activities, even though there was less improvement in neurocognitive activities. Therefore, gliclazide activities on cognitive functions still need further investigation.

CRediT authorship contribution statement

Moses B. Ekong, Francis N. Odinukaeze: Conceptualization. **Francis N. Odinukaeze, Amaobi C. Nwonu**: Data curation. **Moses B. Ekong**: Formal analysis. **Francis N. Odinukaeze, Amaobi C. Nwonu**: Investigation. **Francis N. Odinukaeze, Amaobi C. Nwonu**: Methodology. **Moses B. Ekong, Christopher C. Mbadugha**: Project administration. **Moses B. Ekong, Christopher C. Mbadugha**: Supervision. **Moses B. Ekong**: Validation. **Moses B. Ekong**: Visualization. **Moses B. Ekong, Agnes A. Nwakanma**: Roles/Writing – original draft. **Moses B. Ekong**: Writing – review & editing.

Conflicts of Interest

Authors declare no conflict of interest.

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