Studies on Cardiac Cytoarchitectonic and Biochemical Indices in Pregnant Streptozotocin-Induced Diabetic Rats Treated with Gliclazide and Insulin

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Abstract: Introduction: GDM (gestational diabetes mellitus) is a common complication of pregnancy, and prevalence has become alarming. Insulin remains the cornerstone in management of diabetes mellitus but oral agents have been increasingly viewed as potential alternatives to insulin because it's economically low in cost, easier to administer and affordable to monitor compare to insulin. Aim: This study aimed to determine the effects of Gliclazide on the heart cytoarchitectonic and some biochemical indices of pregnant STZ (streptozotocin)-induced diabetic rats compare with insulin. Methods: Twenty (20) pregnant Sprague Dawley rats weighing between 130 g and 150 g were used for the experiments. The rats were divided into four (4) groups: Group 1 (Non-diabetic), Group 2 (Diabetic), Group 3 (Diabetic + Gliclazide) and Group 4 (Diabetic + Insulin). The glucose levels and body weights were monitoring daily. The experimental rats were sacrificed on the 19th on gestational period, blood samples and organs were collected for biochemical and histo-morphological examinations. Results: Gliclazide group rats showed significant increase in body weight compared with diabetic group ($p \leq 0.05$). The blood glucose level of rats in gliclazide group was significantly reduced compared with other groups. There was significant increase in reduced GSH (glutathione) of gliclazide and insulin groups compared with diabetic group. MDA (malondialdehyde) and CAT (catalase) levels activities were significantly increase in diabetic group compared with other groups. Hormonal profiles and hematological parameters are significantly increased in gliclazide, and insulin group compared with diabetic group. There were distortions in the structural organization of heart of diabetic group while gliclazide and insulin groups showed remarkable improvements of the degenerative changes of the myocardium. Conclusions: Gliclazide glycemic control has shown beneficial effects on the cardiomyocytes damage in STZ-induced diabetic rats by maintaining the histological integrity of the heart leading to reduced degenerative changes in the myocardium.

Key words: Gliclazide, Gestational diabetes mellitus, insulin, cardiomyocytes, oxidative stress.

1. Introduction

DM (diabetes mellitus) is a chronic metabolic disease, and represents one of the most challenging public health problems of the 21st century and is reaching epidemic levels globally [1]. The overall risk of dying among people with diabetes is at least double the risk of their peers without diabetes. Diabetes can be broadly classified as either Type 1 or Type 2 [2], but

another form of diabetes, Gestational diabetes, has not received comparable level of attention despite its severity as an important cause of maternal and neonatal death [3].

Gestational diabetes is hyperglycemia with blood glucose values above normal but below those diagnostic of diabetes, occurring during pregnancy [4]. Women with gestational diabetes are at an increased risk of complications during pregnancy and at delivery. The prevalence of DM keeps increasing at alarming rate. WHO (world health organization) projects of diabetes

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will be the 7th leading cause of death in 2030 [5].

The cornerstone of management of a diabetic pregnancy is an attempt to keep maternal glucose as close to normal as possible, and the standard therapy for women with gestational diabetes requiring drug treatment is insulin. However, since the seminal trial by Langer et al. [6] comparing glibenclamide (glyburide) with insulin oral agents has been increasingly viewed as potential alternatives to insulin because they are economically of low cost, easier to administer, better acceptance and affordable to monitor compared to insulin [7].

Gliclazide is a second-generation sulfonylurea antihyperglycemic agent [8] that stimulates insulin secretion from pancreatic β cells by inhibiting ATP-dependent potassium channels. ATP-dependent potassium channels also mediate a variety of functions in heart and blood vessels [9], thus it is possible that sulfonylurea agents would have some effects on the vascular function aside from their effects on glycemic control [10]. In diabetic animal models, it has also been reported that gliclazide potentially benefits the vasculature through improvements in plasma lipids and in platelet function [11]. Mechanisms may include the ability of the drug to increase tissue plasminogen activator, and its properties as a free radical scavenger [12-15]. Therefore, this research work has the ability of giving approval to the use of gliclazide during pregnancy, which is economically of low cost, easier to administered and affordable to monitor comparing to insulin. It's also imperative to know that acceptability of these OHA can lead to reduction in maternal and foetal mortality and mobility in GDM. Evidence shows that patients with diabetes have an increased level of low-grade inflammation of their arterial lining, a process that initiates the blood vessel changes leading to heart disease [11].

2. Materials and Methods

2.1 Experimental Animals

Thirty (30) Female Sprague Dawley rats weighing

between 130 g and 150 g and Ten (10) giant fertile Male Sprague-Dawley weighing between 230 g and 250 g were procured from the Animal Laboratory Centre of the College of Medicine of the University of Lagos and maintained under standard laboratory conditions for an acclimatization period of Two (2) weeks in a wire-mesh cages at the Animal Holdings of Anatomy Department, College of Medicine, University of Lagos. This research work was conducted between May and November, 2015 in the Laboratories of Anatomy department, College of Medicine of the University of Lagos.

2.2 Determination of the Oestrous Cycle

Vaginal smear from each rat were examined daily for 15 days, between the hours of 7 am and 9 am using the tip of a 3 inches' borosilicate glass medicine dropper that was filled with approximately 0.2 mL of normal saline (sterile water) and inserted approximately 2-3 mm into the rat's vagina [8] and those rats that exhibited at least three consecutive 4-day regular oestrous cycles were included in this research [9, 10]. Twenty (20) rats with regular cycling were used for the experiment. These rats were identified by slight cut on their ears as; RF (right front), RM (right middle), RB (right back), LF (left front) and LB (left back).

2.3 Mating of the Rats

The female rats were caged with non-diabetic male rats of known fertility in the ratio of 2:1 in the evening of proestrous. They were examined the following morning for the presence of sperms. The presence of a vaginal plug had on the following morning indicated that mating had occurred, and the rats that exhibited thick clumps of spermatozoa in the vaginal smear are separated, and that day was regarded as day 0 of pregnancy [11, 12].

2.4 Experimental Design (Cyesis + DM)

Twenty female rats (6-8 weeks old) were used for

this experiment. Vaginal smear from each rat was examined daily for 15 days, and those rats that exhibit at least three consecutive 4-day regular oestrous cycles were included in this segment [9, 10].

The rats were weighed and randomly divided into 4 groups of 5 rats each.

The blood assays of glucose, insulin were conducted on all the rats at recruitment so as to have the base-line values of all the indices.

2.5 Induction of Diabetes

STZ (streptozotocin) was purchased from Energy Bond Chemicals, Ilorin, Nigeria. Modified protocol to induce diabetes was followed [13]. Rats were allowed to fast for 12 hours prior to the administration of STZ. STZ was freshly prepared by dissolving in 0.01 M sodium citrate buffer solution, pH 4.5 and was used within 15 min. Low multiple doses at 45 mg/kg and 30 mg/kg body weight STZ were intraperitoneally administered [14, 15] between 9 am and 10 am on day 1 and 2 of pregnancy respectively.

2.6 Weight Assessment

The weight of each rat was monitored daily as an index of the physical status of the animals over the period of study using compression spring balance (BAW-660-M).

2.7 Blood Glucose Test

The glucose levels were measured on the 2nd day of pregnancy using a glucometer (Acucheck), strips (Lifescan) and lancet from the blood samples collected from the tail vein of the rats and were recorded. Rats with blood glucose level greater than 300 mg/dL were considered manifestly diabetic and were administered the test drugs daily from day 2 of gestation. The monitoring of the blood glucose level and body weight were done daily throughout the period of experiment

2.8 Grouping of the Rats and Test Drugs

The experimental rats were divided into four (4)

groups using table of random number and the grouping was done as follows:

Group 1 (Control) was non-diabetic rats and were given distilled water only;

Group 2 (Diabetic) was diabetic rats without treatment, taken only distilled water;

Group 3 (Diabetic+ gliclazide) received test drug Gliclazide (4.57 mg/kg/day);

Group 4 (Diabetic + insulin) received test drug insulin (0.1 IU/kg/day).

2.9 Sacrifice of the Rats

The experimental rats were sacrificed at day 19 of the pregnancy while still under anesthesia and the organs were exercised (harvested).

2.10 Hematoxylin and Eosin Staining for Histological Studies

The tissue samples in 10% buffered formalin were sliced to approximately 1 cm thick, and placed into the cassettes. Then, the cassettes are placed in a tissue processor machine, which comprise of dehydration with alcohol, clearing with xylene and wax, and impregnating process automatically overnight (14 h). The cassettes were embedded in molten paraffin, which later cooled down to formed blocks of paraffin. Each block was trimmed then sectioned about 5 μ m by using a microtome [16]. H & E (hematoxylin and eosin) dye, which mounted with DPX for microscopic observations.

2.11 Blood Sample Collection

At the end of experiment, animals were anesthetized by infusion of ketamine (60 mg/kg) and xylazine (10 mg/kg). Five (5) mL of blood was collected from each animal. Part of the blood sample was put in an EDTA bottles for hematological determinations [17].

2.12 Hematological Analysis

The CBC was performed on an automated hematology analyzer using well mixed whole blood to

which EDTA was added to prevent clotting. CBC count was measured by Sysmex XS800i, hematology analyzer with fluorescence technology (Diamond Diagnostics-USA).

2.13 Hormonal Profile Analysis

Blood was collected in tubes containing anticoagulants and centrifuged within 2 h. Blood was collected and the separated plasma was kept in a freezer at -20 °C for hormonal determination (FSH (follicle-stimulating hormone), LH (luteinizing hormone), estradiol, progesterone). Hormonal assays were done by ELISA Kit obtained from Cayman Chemical Company, USA [18].

2.14 Blood Sampling and Preparation of Serum

The blood samples were drawn in the fasting state of the experiment rats. Serum was separated within 2 h of collection to prevent artificial changes in concentration of HDL. The blood was centrifuged at 5,000 rpm for 10 min. The supernatant clean serum was then pipette out using dry piston pipettes with disposable tips and stored in dry thin walled vials at 4 °C. The samples were analyzed the same day. Care was taken to exclude the hamolyzed serum [19].

2.15 Laboratory Procedure

The lipid and lipoprotein assay was done using Dr. Lange LP 700 equipment [19].

2.16 Oxidative Stress Markers

2.16.1 Method for Homogenizing Sample

The rats were dissected and some hearts were removed for the purpose of these analyses while other was used for histological tissue processing, the fleshes of the animal were also cut. The post mitochondria fraction of the organs of the animal was prepared as fellow:

The hearts of the animal were washed in an ice cold 1.15% KCl solution, blotted and weighed. They were then homogenized with 0.1 M phosphate buffer

(pH 7.2), putting the organs each into the mortar; laboratory sand was added to it (acid washed sand) and it was blended in the mortar with pestle together. The resulting homogenate was centrifuge at 2,500 rpm speed for 15 min then it was removed from the centrifuge and the supernatant was decanted and stored -20 °C until analysis.

2.16.2 Antioxidant Enzymes Assay

The following antioxidant enzymes activities were determined spectrometrically as follows.

2.16.3 Determination of SOD (superoxide dismutase) activity

SOD activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm as described by Sun and Zigma (1978). The reaction mixture (3 mL) contained 2.95 mL 0.05 M sodium carbonate buffer pH = 10.2, 0.02 mL of heart homogenate and 0.03 mL of epinephrine in 0.005 N HCl was used to initiate the reaction. The reference cuvette contained 2.95 mL buffer, 0.03 mL of substrate (epinephrine) and 0.02 mL of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min, $\Sigma = 4,020 \text{ M}^{-1} \cdot \text{cm}^{-1}$.

2.16.4 Catalase Activity Determination

Catalase activity was determined according to Sinha, et al. (1972). It was assayed colorimetrically at 620 nm and expressed as $\mu\text{moles of H}_2\text{O}_2$ consumed/min/mg protein at 25 °C. The reaction mixture (1.5 mL) contained 1.0 mL of 0.01 M phosphate buffer (pH 7.0), 0.1 mL of tissue homogenate and 0.4 mL of 2 M H_2O_2 . The reaction was stopped by the addition of 2.0 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). $\Sigma = 40 \text{ M}^{-1} \cdot \text{cm}^{-1}$.

2.16.5 Reduced Glutathione Determination

The reduced GSH (glutathione) content of heart tissue as non-protein sulphhydryls was estimated according to the method described by Sedlak and Lindsay (1968). To the homogenate 10% TCA was added, centrifuged. And 1.0 mL of supernatant was

treated with 0.5 mL of Ellman's reagent (19.8 mg of 5,5-dithiobis nitro benzoic acid (DTNB) in 100 mL of 0.1% sodium nitrate) and 3.0 mL of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. $\Sigma = 1.34 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$.

2.16.6 Lipid Peroxidation

MDA—an index of lipid peroxidation was determined using the method of Buege and Aust (1978). 1.0 mL of the supernatant was added to 2 mL of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24 N HCl and 15% TCA) tricarboxylic acid-thiobarbituric acid-hydrochloric acid reagent boiled at 100 °C for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3,000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA—complex of $1.56 \times 10^5 \text{ M}^{-1} \cdot \text{CM}^{-1}$.

2.16.7 Determination of Glutathione S-Transferase Activity

Glutathione S-transferase activity was determined by the method according to Habig et al. (1974).

(1) Principle

This based on the fact that all known glutathione S-transferase demonstrate a relatively high activity with CDNB (1-Chloro-2,4-dinitrobenzene) as the second substrate. Consequently, the conventional assay for glutathione S-transferase activity utilizes CDNB as substrate. When this substrate is conjugated with reduced GSH, its absorption maximum will shift to a longer wavelength. The absorption increases at the new wavelength of 340 nm which provides a direct measurement of the enzymatic reaction.

(2) Reagent

(a) 20 mM (CDNB)

3.37 mg of CDNB (Sigma Chemicals Co, London) was dissolved in 1 mL of ethanol.

(b) 0.1 M Reduced Glutathione

30.73 mg (0.0307 g) of reduced glutathione powder (Sigma Chemical Co, London) was

dissolved 100 mL of 0.1 M phosphate buffer pH 6.

(3) Procedure

The medium for the estimation of GST activity was prepared as shown in the table below and the reaction was allowed to run for 60 seconds each time before the absorbance was read against the blank at 340 nm. The temperature was maintained at approximately 31 °C. The absorbance was measured using spectrophotometer.

Reagent	Blank	Test
0.1 M reduced GSH	30 µL	30 µL
20 mM CDNB	150 µL	150 µL
0.1 M phosphate buffer pH 6.5	2.82 mL	2.79 mL
Sample	-	30 µL
Total mixture	3 mL	3 mL

$$\Sigma = 9.6 \text{ M}^{-1} \cdot \text{cm}^{-1}$$

(4) Calculation

$$\text{SOD/CAT/GST} = \Delta A / \text{min} \cdot V_T / \Sigma \cdot V_s$$

$$\text{MDA/GSH} = A \cdot V_T / \Sigma \cdot V_s$$

ΔA = change in absorbance;

V_T = total volume;

V_s = sample volume;

Σ = molar extinction.

2.17 Statistical Analysis

The mean blood glucose value (measured in mg/dL) and the weight (measured in grams) of the experimental rats before and after induction with diabetes were analyzed using ANOVA and were represented in the tables.

All the results were presented as the mean $\pm$ standard error of the mean. Statistical analysis was ANOVA for comparisons between two groups and by analysis of variance for more than two groups and $p < 0.05$ was considered to be statistically significant [20].

3. Results

It was evident from above Table 1 that the weights of diabetic rats were significantly reduced throughout the period of experiment. Diabetic rats treated with gliclazide shows significant increase in body weight at day 6 and from day 10 they maintain constant body

Table 1 Statistics of the mean body weights of animals in (grams, g) across all groups.

Group/days	Day 2	Day 6	Day 10	Day 14	Day 18
Control	151.14 + 2.854	149.71 + 3.352	158.43 + 114.924 ^{ac}	159.57 + 3.101 ^{ac}	165.00 + 3.559 ^{ac}
Diabetic	140.14 + 2.610 ^a	138.43 + 2.637 ^{ab}	138.60 + 3.435	136.00 + 3.536 ^{ab}	136.00 + 3.536 ^{ab}
Gliclazide	144.14 + 39.34 ^c	145.50 + 28.81 ^b	138.14 + 2.035 ^b	138.00 + 2.708 ^{ab}	138.43 + 2.507 ^{ab}
Insulin	146.57 + 9.998	147.50 + 10.310	149.00 + 10.050 ^b	148.40 + 9.659 ^{bc}	148.20 + 10.085 ^{bc}

Sig. $P \leq 0.05$. ^a Significance when compared with Insulin; ^b Significance when compared with control; ^c Significance when compared with diabetic, ($\pm$ SEM).

Table 2 Statistics of the mean blood glucose levelin (mg/dL) of animals across all groups.

Group/days	Day 2	Day 6	Day 10	Day 14	Day 18
Control	79.86 + 6.543 ^{ac}	78.86 + 8.533 ^{ac}	82.14 + 9.477 ^{ac}	80.43 + 6.188 ^{ac}	80.14 + 7.221 ^{ac}
Diabetic	469.57 + 56.471 ^b	478.83 + 51.219 ^{ab}	468.00 + 71.165 ^{ab}	470.40 + 68.966 ^{ab}	467.20 + 65.308 ^{ab}
Gliclazide	391.86 + 74.787 ^{abc}	332.14 + 75.999 ^{abc}	254.14 + 58.082 ^{bc}	188.71 + 33.920 ^{bc}	119.71 + 8.995 ^{bc}
Insulin	470.00 + 45.354 ^b	394.00 + 41.943 ^{bc}	299.40 + 47.321 ^{bc}	222.80 + 35.074 ^{bc}	123.40 + 8.961 ^{bc}

Sig. $P \leq 0.05$. ^a Significance when compared with insulin; ^b Significance when compared with control; ^c Significance when compared with diabetic, ($\pm$ SEM).

weight till the end of the experiment. Diabetic rats treated with insulin shows significant increase in body weight from day 2 till day 10 of the experiment and after that maintain constant body weight till the end of the experiment.

The above Table 2 reveals that the blood glucose levels of control rats were normal. The blood glucose levels of diabetic rats were significantly increased throughout the period of experiment. Diabetic rats treated with gliclazide have the best blood glucose level control, it was reduced drastically. Diabetic rats treated with insulin also have a significant reduction in blood glucose level but not as rats treated with gliclazide.

It was very clear from the above Table 3a that hematological parameters of control rats were normal. The hematological parameters were significantly reduced in diabetic rats. The hematological parameters of diabetic rats treated with gliclazide and insulin were reduced compared with control rats but improved when compared with diabetic rats.

Keywords: RBC (red blood cell), HCT (hematocrit), MCV (mid cell volume), RDW (red cell distribution width), HGB (heamoglobin), MCH (mean cell heamoglobin), MCHC (mean cell haemoglobin concentration), PLT (platelet), PCT (plateletcrit), MPV

(mean platelet volume), PDW (platelet distribution width), LPCR (liposome polymerase chain reaction), WBC (white blood cell), LYM (lymphocyte), GRAN (granulocyte), MID (mid-sized cells (monocytes).

The result of the analysis on hormonal profile from the above Table 4 shows that hormonal profiles of the control rats were normal. The hormonal profiles of diabetic rats were significantly reduced except for PRL which was significantly increased compared with control. Diabetic rats treated with gliclazide have significantly reduced hormonal profile when compared with control and insulin. Diabetic rats treated with insulin have significantly reduced hormonal profile when compared with control but increase when compared with gliclazide.

Keywords: LH (luteinizing hormone), FSH (Follicle stimulating hormone), PRL (prolactin), PROG (Progesterone), E2 (estradiol).

The Table 5 above shows the result of the lipid profile. Levels of CHOL were higher in both rats treated with gliclazide, insulin and control rats when compared with diabetic rats. Control rats have low level of TG, and TG levels were significantly high in rats treated with gliclazide when compared with control, insulin and diabetic. HDL level was significantly high in rats treated with insulin, gliclazide

Fig. 1 Showing the histogram of the mean blood glucose level across the groups.

Table 3a Statistical summary mean hematological parameter of animals across all groups.

Drugs/P	RBC (mL/kg)	HCT (g/dL)	MCV (η^3)	RDW($10^3/\eta L$)	HGB (g/dL)	MCHC (g/dL)	PLT ($10^3/\eta L$)
Control	7.07 $\pm$ 0.48 ^c	49.20 $\pm$ 3.92	65.46 $\pm$ 2.62 ^c	27.18 $\pm$ 0.98	14.40 $\pm$ 0.79 ^{abc}	35.68 $\pm$ 2.43 ^c	521.86 $\pm$ 291.15
Diabetic	4.35 $\pm$ 1.566	27.20 $\pm$ 14.10	44.04 $\pm$ 23.66 ^{ab}	21.70 $\pm$ 9.69 ^b	9.96 $\pm$ 1.22 ^{ab}	28.32 $\pm$ 3.30 ^b	350.88 $\pm$ 312.97 ^b
Gliclazide	4.35 $\pm$ 1.566	27.20 $\pm$ 14.10	61.40 $\pm$ 5.88 ^c	21.98 $\pm$ 7.34 ^b	12.48 $\pm$ 2.57 ^{abc}	31.54 $\pm$ 1.22 ^c	426.00 $\pm$ 189.61 ^b
Insulin	5.82 $\pm$ 1.33 ^{bc}	31.12 $\pm$ 8.25	61.40 $\pm$ 5.88 ^c	21.98 $\pm$ 7.34 ^b	12.48 $\pm$ 2.57 ^{bc}	31.54 $\pm$ 1.22 ^c	426.00 $\pm$ 189.61 ^b

Sig. $P \leq 0.05$. ^a Significance when compared with insulin; ^b Significance when compared with control; ^c Significance when compared with diabetic, ($\pm$ SEM).

Table 3b Statistical summary mean hematological parameter of animals across all groups.

Drugs/par	PCT ($10^3/\eta L$)	MPV (pg/cell)	PDW (g/dL)	LPCR (g/dL)	WBC ($10^3/\eta L$)	LYM (g/dL)	MID (g/dL)
Control	3.14 $\pm$ 6.07 ^{ac}	8.72 $\pm$ 0.55 ^{ac}	8.70 $\pm$ 0.2 ^c	14.56 $\pm$ 0.3 ^{ac}	6.80 $\pm$ 0.25	6.98 $\pm$ 0.13 ^{ac}	0.64 $\pm$ 0.09
Diabetic	0.29 $\pm$ 0.16	4.98 $\pm$ 0.58 ^{ab}	5.88 $\pm$ 0.76 ^{ab}	4.00 $\pm$ 1.12	4.30 $\pm$ 0.29 ^{ab}	2.98 $\pm$ 2.03 ^b	0.40 $\pm$ 0.16
Gliclazide	0.84 $\pm$ 0.04 ^{abc}	7.46 $\pm$ 0.54 ^{bc}	8.64 $\pm$ 0.56 ^c	6.18 $\pm$ 1.61 ^{ab}	4.18 $\pm$ 2.84 ^b	4.22 $\pm$ 0.19 ^b	0.40 $\pm$ 0.27
Insulin	0.55 $\pm$ 0.02	8.02 $\pm$ 0.39 ^{bc}	8.44 $\pm$ 0.3 ^c	9.60 $\pm$ 4.57 ^c	4.58 $\pm$ 1.15	4.46 $\pm$ 2.25 ^b	0.44 $\pm$ 0.06

Sig. $P \leq 0.05$. ^a Significance when compared with insulin; ^b Significance when compared with control; ^c Significance when compared with diabetic, ($\pm$ SEM).

Table 4 Showing Statistical differences in hormonal profile in (mIU/ML) across the treatment groups.

DRUG/P	LH	FSH	PRL	PROG	E2
Control	0.56 $\pm$ 0.09 ^{ac}	1.00 $\pm$ 0.16 ^c	1.00 $\pm$ 0.14	21.75 $\pm$ 3.23 ^a	51.47 $\pm$ 28.26 ^{ac}
Diabetic	0.14 $\pm$ 0.05 ^{ab}	0.20 $\pm$ 0.07 ^b	1.18 $\pm$ 0.08 ^a	12.23 $\pm$ 4.49 ^b	14.52 $\pm$ 0.81 ^b
Gliclazide	0.38 $\pm$ 0.26 ^c	0.28 $\pm$ 0.15 ^b	0.64 $\pm$ 0.15	13.88 $\pm$ 1.77 ^b	21.16 $\pm$ 0.78 ^{ac}
Insulin	0.42 $\pm$ 0.16 ^c	0.72 $\pm$ 0.42	0.42 $\pm$ 0.15 ^c	16.90 $\pm$ 0.44	46.10 $\pm$ 11.02 ^{bc}

Sig. $P \leq 0.05$. ^a Significance when compared with insulin; ^b Significance when compared with control; ^c Significance when compared with diabetic, ($\pm$ SEM).

Table 5 Showing Statistical differences in Lipid profile in (mmol/L or mg/dL) across the treatment groups.

Drug/P	CHOL	TG	HDL	LDL	AST	ALT	ALP
Control	2.08 ± 0.16 ^c	0.68 ± 0.25	0.96 ± 0.18 ^c	0.58 ± 0.08	41.60 ± 11.28 ^c	30.00 ± 11.85 ^c	101.00 ± 13.27 ^a
Diabetic	1.34 ± 0.05 ^{ab}	0.88 ± 0.08	0.54 ± 0.05 ^{ab}	1.00 ± 0.16	65.60 ± 1.52 ^b	17.60 ± 1.52 ^b	109.80 ± 1.30 ^a
Gliclazide	2.14 ± 0.27 ^c	1.54 ± 0.42 ^{abc}	1.04 ± 0.11 ^c	0.76 ± 0.23	55.60 ± 3.05	12.80 ± 0.84 ^{ab}	68.20 ± 18.24 ^{bc}
Insulin	1.94 ± 0.46 ^c	0.76 ± 0.11	1.08 ± 0.18 ^c	0.94 ± 0.74	49.60 ± 14.22	22.80 ± 10.13	74.40 ± 3.44 ^{bc}

Sig. $P \leq 0.05$. ^a Significance when compared with insulin; ^b Significance when compared with control; ^c Significance when compared with diabetic, ($\pm$ SEM).

Table 6 Showing Statistical differences of the oxidative stress markers in (mmol/1mole) across the treatment groups.

Groups/parameter	GSH	SOD	CAT	MDA
Control	8.71 ± 1.51 ^{ac}	94.23 ± 3.74 ^a	428.42 ± 91.89 ^a	0.65 ± 0.32 ^c
Diabetic	0.72 ± 0.58	45.13 ± 7.03 ^a	816.46 ± 42.12 ^a	4.95 ± 0.52 ^a
Gliclazide	4.10 ± 1.16 ^{bc}	78.98 ± 12.21 ^{bc}	655.10 ± 167.42 ^{bc}	3.44 ± 1.88 ^a
Insulin	2.25 ± 0.62 ^c	77.20 ± 9.58 ^{bc}	678.45 ± 52.71 ^{bc}	1.67 ± 0.25 ^c

Sig. $P \leq 0.05$. ^a Significance when compared with insulin; ^b Significance when compared with control; ^c Significance when compared with diabetic, ($\pm$ SEM).

and control when compared with diabetic rats. LDL level in control rats, gliclazide and insulin treated rats were low compared with diabetic rats. The levels of AST were significantly reduced in control rats compared with rats treated with insulin and gliclazide but significantly increased in diabetic rats. Level of ALT was significantly high in control rats compared with diabetic rats and increased in insulin compared with gliclazide. Diabetic rats have the significantly high level of ALP compared with control, insulin and gliclazide.

CHOL (cholesterol), TG (triglycerides), HDL (high density lipoprotein), LDL (low density lipoprotein), AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase).

The results in the above Table 6 show the result of the oxidative stress markers. In control rats, the GSH levels were significantly high compared with insulin and diabetic rats. While the level of GSH in rats treated with gliclazide was significantly high when compared with diabetic but low when compared with control rats. Levels of SOD were significantly reduced in diabetic rats when compared with rats treated with insulin, gliclazide and control rats. There were significant increase in the levels of both CAT and MDA in diabetic rats compared with gliclazide, insulin and control.

GSH (glutathione), SOD (superoxide dismutase), CAT (catalase), MDA (malondialdehyde).

Fig. 2 shows the longitudinal section of well oriented cardiac multicellular muscle fibres and well defined cardiomyocytes that revealed central oval vesicular nuclei (vertical arrows). Cell fibres were connected end to end by intercalated discs as seen in horizontal arrow.

Fig. 3 shows the longitudinal section of diabetes heart with degeneration and apoptosis of some cardiocytes with loss of myofibrils and disarray of muscle fibres (showed by horizontal arrow) and with the nuclei of degenerated cardiac muscle fibres appeared dense, variable in size and shape (seen in vertical arrow).

Fig. 4 reveals the longitudinal section of diabetic rats treated with gliclazide that shows improvement of these degenerative changes of the myocardium as the nuclei appeared nearly equal in size with uniform shape and the cardiac muscle fibres had almost regular array but fairer in insulin and intact myofibrils as shown by horizontal and vertical arrows

Fig. 5 reveals the longitudinal section of treatment of diabetic rats with insulin lente showed marked improvement of these degenerative changes of the myocardium as the nuclei appeared nearly equal in size with uniform shape and the cardiac muscle fibers had

Control Group (Heart)

Fig. 2 Group 1 (control H&E stained $\times 100$).

Diabetic Heart

Fig. 3 Group 2 (STZ-induced diabetic H&E stained $\times 100$).

Gliclazide Group (Heart)

Fig. 4 Group 3 (gliclazide H&E stained $\times 100$).

Insulin Group (Heart)

Fig. 5 Group 4 (insulin treated H&E stained $\times 100$).

regular array and intact myofibrils as shown by horizontal and vertical arrows.

4. Discussion

The focus of this study was to determine the effect of gliclazide and insulin on maternal weight, blood glucose, maternal cardiac cytoarchitectonic, hematological parameters, hormonal profile, lipid profile and oxidative stress in pregnant STZ-Induced diabetic rats treated with gliclazide and insulin. GDM is a problem that affects a significant number of women during pregnancy [21]. GDM can have lasting health impacts on both the mother and the fetus, Gestational diabetes mellitus (GDM) affects between 2% and 5% of pregnant women [21].

It was evident from Table 1 of the results of this finding that mean body weight of rats in control group shows significant increase throughout the period of the experiment, this finding correlates with study that shows that changes of body weight in adult and non-adult diabetic rats varied [22]. Since the non-adult diabetic rats are in the growing age, diabetic loss of weight is not seen in them and they even show a significant weight gain [22]. The body weight of rats in diabetic group shows significant decrease in the first 12 days of the experiment, this failure of STZ-induced diabetic rat to gain weight has already been reported [23, 24]. Table 1 also revealed that rats in insulin group show better improvement in body weight from the beginning of the experiment to the end which is in according with the findings that stated, weight gain of diabetic rats treated with gliclazide alone and of the vehicle-treated diabetic rats during 6 months was less than that of the other groups receiving insulin [25]. It was observed that rats treated with gliclazide show tremendous weight loss in the first 4 days of the experiment but gradually show gradual and significant increase in body weight from day 6 to the end of the experiment and this agrees with the previous study that stated that increased body weight is associated with insulin resistance, type 2 diabetes mellitus, and

increases the risk of cardiovascular disease, though It is widely accepted that medication-induced weight gain is an unfavorable result for patients with type 2 diabetes [26-28].

Blood glucose level of rats in control group is normal throughout the period of experiment as it was shown in Table 2. Rats treated with gliclazide shows significant decrease in blood glucose level than rats treated with insulin which shows that result in Table 2 is in consonant with the study by Guillausseau and Greb (2001) performed in 21 T2DM patients previously treated with diet alone or oral glucose-lowering drugs showed significant decreases in mean daily plasma glucose levels and in plasma glucose levels during the fasting and postprandial periods after 10-week treatment with gliclazide [29]; as well as agreement with another study that says, decrease in HbA1c of $1.0 \pm 1.1\%$ ($p = 0.022$) was observed in drug-naïve patients moreover, gliclazide appears to have several positive effects beyond its glucose-lowering properties [30], another report also supported the findings of this research that Gliclazide reduces blood glucose levels in patients with NIDDM by correcting both defective insulin secretion and peripheral insulin resistance [31].

The hematological parameters were studied and shown in Tables 3a and 3b in order to underscore the effect of insulin and gliclazide on the diabetic rats. It was observed that hematological parameters of control rats were normal. The decrease noticed in hematological parameters of the diabetic rats as it was shown in Tables 3a and 3b especially after administration of STZ is in agreement with a study that called it an indication of abnormal hemoglobin synthesis [32], failure of blood osmoregulation, and plasma osmolarity were also reported by Stookey et al. (2007). More improvement in hematological parameters were noticed in rats treated with insulin and gliclazide which are slightly increase compared with rats in diabetic group as shown in Tables 3a and 3b which are consistence with the previous study that

Short-term administration of gliclazide to patients with T2DM lowers the adhesion of diabetic monocytes, the oxidized low-density lipoprotein and glycated albumin-induced monocyte adhesion to ECs (endothelial cells), known to be a critical step in the atherosclerotic process [33].

The hormonal profiles were studied (LH, PRO, PRL, E2, FSH) and showed in Table 4 which revealed normal hormonal profile levels in control rats. There was significant decrease in the hormonal profiles of the diabetic rats as it was shown in Table 4 and also correlates with the study on hormonal profile of diabetic rats by Salonia et al. [34] (2006) that shows significant decrease in levels of FSH, LH and other hormonal profile. Significant improvement in hormonal profile was observed in rats treated with insulin and gliclazide which were also reported by Salonia et al.

The lipid parameters like HDL, LDL, TG, CHOL, AST, ALT and ALP were all analyzed in order to investigate the effect of insulin and gliclazide on diabetic. The lipid profile of the rats in control was normal as it was revealed in Table 5. As it was reported by Jain et al. (2016) that TG, CHOL, AST, ALT, ALP, TC, LDL-C, and VLDL-C, the lipid profile is higher significantly in diabetes than insulin and gliclazide group and HDL-C were significantly lower in diabetics than control groups [19] and this result that was gotten from Table 5 is in consonant with results of the findings that noticed increased in the levels of triglycerides, LDL and cholesterol in streptozotocin diabetic rats [35, 36].

Table 6 of the result shows the oxidative stress markers like GSH, SOD, CAT and MDA that were all analyzed. GSH and SOD levels were both increased in control group, reduced level of GSH and SOD were noticed in diabetic group while GSH and SOD levels of gliclazide and insulin were improved compared with diabetic group these results from Table 6 were also reported by Djordjevic et al. [37] (2004) in their study which revealed that, SOD and GSH activities was

gradually increased in the group of normal pregnant rats but decreased significantly in the group of diabetic pregnant rats. CAT and MDA levels were significantly reduced in control group but significantly increased in diabetic group, while gliclazide and insulin groups shows improvement as it was shown in Table 6 and in agreement with the previous study MDA and CAT levels were significantly increased in type 2 diabetes mellitus [38] as well as another study that there is consistent evidence that gliclazide may decrease the ongoing oxidative stress, a typical biochemical feature of T2DM patients. Gliclazide has this property because of the free radical scavenging ability of its unique amino azabicyclo-octane ring, which is grafted on to the SU moiety [39].

It was evident from Fig 2 that the histological features of the cardiac tissue of rats in control group shows longitudinal section of well oriented cardiac multicellular muscle fibres and well defined cardiomyocytes that revealed central oval vesicular nuclei and cell fibres were connected end to end by intercalated discs. Fig 3 revealed the histological characteristics of diabetic heart of rats that presents longitudinal section of diabetes heart with degeneration and apoptosis of some cardiocytes with loss of myofibrils and disarray of muscle fibres and with the nuclei of degenerated cardiac muscle fibres appeared dense, variable in size and shape which is in consonant with the previous studies [40-44] and the same findings were reported in the study titled correlation between cardiovascular diseases and diabetes mellitus [45]. It was shown in Fig. 4 that diabetic rats treated with gliclazide showed improvement of these degenerative changes of the myocardium as the nuclei appeared nearly equal in size with uniform shape and the cardiac muscle fibres had almost regular array but fairer in insulin and intact myofibrils. There was marked improvement of the degenerative changes of the myocardium as shown in Fig. 5, moreover, previous study [43] agreed with the findings of this study that demonstrated the diabetic rats treated with insulin

showed marked improvement of the degenerative changes of the myocardium as the nuclei appeared nearly equal in size with uniform shape and the cardiac muscle fibers had regular array and intact myofibrils, this was also observed by Cosyns et al.

5. Conclusions

The present study clearly demonstrate that the structural integrity of the heart of untreated diabetic rats were damaged compared with treated diabetic groups. Therefore, it is concluded that gliclazide has a beneficial effect on the cardiomyocytes damage in STZ-induced diabetic rats by maintaining the histological integrity of the heart lead to reduced degenerative changes in the myocardium.

6. Recommendation

It is recommended that future study should examine the effect of gliclazide on cardiac biology at molecular level.

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