

Blood Glucose Concentration, Pancreatic Histology and Insulin-Expression following Metformin and Glibenclamide Administration in Diabetic Rats

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ABSTRACT

Introduction: Introduction: Metformin and glibenclamide are used in the management of type 2 diabetes. There is paucity of information on the efficacy of insulin expression by these antidiabetic drugs. Aim: This study investigated the hypoglycemic, pancreatic histomorphological and insulin alterations following administration of metformin and glibenclamide in a type 2 diabetic model.

Materials and Methods: Thirty Wistar rats were divided into 6 groups of 5 animals each. Two groups served as normal and diabetic controls respectively. Diabetes was induced with streptozotocin (STZ). Two diabetic groups received 1.43 and 2.86 mg metformin per kg (body weight, bw), while another 2 groups received 0.07 and 0.14 mg glibenclamide per kg bw respectively. Treatments lasted for 4 weeks, after which animals were fasted over-night before determination of final blood glucose levels. Pancreas was dissected for histological study.

Results: Hypoglycemic effect of glibenclamide was higher than that of metformin. The histological features of glibenclamide-treated rats demonstrated severe distortions, while metformin-treated rats had mild distortions of the pancreatic islets. Insulin expression was strongly enhanced by metformin than by glibenclamide.

Conclusion: The findings of this research may suggest the use of metformin as better alternative to glibenclamide in the amelioration of hyperglycemia, especially in the long term.

Keywords: Diabetes, Glibenclamide, Metformin, Blood glucose, Insulin, Pancreas

Introduction

Diabetes mellitus is a serious chronic disease that occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the insulin it produces. Diabetes is an important public health condition and one of the 4 priority non-communicable diseases (NCDs) targeted for action by world leaders. The global estimates of diabetes have risen from 108 million in 1980 to 422 million as of 2014,¹ a prevalence rising more rapidly in low and middle-income countries, attributable to the adoption of western lifestyle especially in the diet.

A first-line medication for the treatment of type 2 diabetes, particularly in people who are overweight is metformin, marketed as Glucophage®.² Metformin decreases blood glucose levels by decreasing hepatic glucose production (gluconeogenesis), decreasing the intestinal absorption of glucose, and increasing insulin sensitivity by increasing peripheral uptake and utilization of glucose.³ The drug has cardiovascular protective effects independent of glucose-lowering activity,⁴ and is sometimes co-administered with glibenclamide.

Glibenclamide (also known as Glyburide) is an antidiabetic drug in a class of medications known as sulfonylureas, closely related to sulfonamide antibiotics. It is the most popular sulfonylurea used in the treatment of type 2 diabetes in the United States⁵ It is not as good as either metformin or insulin in those who have gestational diabetes.⁶ The drug has been reported to be a major cause of drug-induced hypoglycemia,⁷ and may cause acute hemolysis, if given to persons having glucose-6-phosphate dehydrogenase deficiency.⁸ Glibenclamide works by binding the ATP-sensitive potassium channels in pancreatic β -cells, which results in an increase in intracellular calcium in the β -cells of the pancreas and subsequent stimulation of insulin release.⁹

Diabetes can be induced in rodents by streptozotocin acting by inhibition of β -cell O-linked N-acetylglucosamyl hydrolase¹⁰ and donation of nitric oxide (NO), involved in many physiological and pathological processes in the body.¹¹ Beta cells are clusters of cells in the pancreatic islets of Langerhans that secrete insulin, the antihyperglycemic hormone. This study investigated the effects of metformin and

glibenclamide on blood glucose levels, pancreatic histology and insulin immunohistochemical expression in an experimental diabetic model.

Materials and Methods

Experimental animals

Thirty (30) adult male Wistar albino rats were obtained from the Faculty of Basic Medical Sciences Animal House and acclimatized for 2 weeks before use, under optimum environmental conditions of temperature 25 ± 5 °C with 12 hours light and dark cycle. The animals were allotted into well-maintained plastic cages. The animals were fed with pelletized growers mash (Grand Cereal Vital® Feed Ltd., Jos, Nigeria) and given tap water *ad libitum*. The ethics of animal care were adopted following the "Guide for the Care and Use of Laboratory Animals".¹²

Drug Acquisition

Streptozotocin (300 mg) was obtained from Santa Cruz Biotechnology, Inc., U.S.A. Metformin (Glucophage) was obtained from Merck S. L. Poligono Merck Ltd., Barcelona, Spain. Glibenclamide was obtained from Nigeria German Chemical Plc, Otta, Ogun State, Nigeria. Citrate buffer was obtained from Nanjing Shuguang Silane Chemical Co., Ltd., 5611EH Eindhoven, Netherlands. Normal saline and distilled water were obtained from the laboratory of the Department of Biochemistry, University of Uyo, Nigeria.

Induction of Type 2 Diabetes Mellitus

Diabetes was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of freshly prepared streptozotocin (STZ) at 50 mg/kg body weight (bw) dissolved in 0.1M citrate buffer (pH 4.5), as earlier described.¹³ Control animals were injected intraperitoneally with citrate buffer alone at a single dose of 1.2 ml/kg bw. Diabetes was confirmed 3 days after STZ administration. Thereafter, fasting blood glucose levels of the animals were measured using a Glucometer (ACCU-CHECK Advantage II, Roche Diagnostics, GmbH, Germany). Following the criteria of previous workers, rats with blood glucose levels of more than 198 mg/dL were considered to be diabetic and therefore used for the study.^{14, 15}

Experimental Design

The duration of the experiment was 28 days. The 30 rats were allotted into 6 groups of 5 rats each. Group 1 served as the normal control (NC), which received 0.5 ml normal saline. Groups 2 to 6 received intraperitoneal injection of STZ (50 mg per kg bw) per animal. Group 2 served as the diabetic control (DC), which did not receive any drug treatment. Groups 3 and 4 (D_{LM} and D_{HM}) received 1.43 and 2.86 mg/kg metformin, while groups 5 and 6 (D_{LG} and D_{HG}) received 0.07 and 0.14

mg/kg glibenclamide respectively. Additionally, 0.5 ml normal saline was administered to animals in all test groups.

Determination of Blood Glucose

Blood glucose concentration was estimated by glucose oxidase method,¹⁶ using a reagent kit from Randox Laboratory Ltd, UK.

Histological Studies

Excised pancreas tissues were fixed in 10 % neutral buffered formalin (NBF). After 24 hours, the tissues were processed following standard protocol,¹⁷ and embedded in paraffin wax. Transverse sections of 5 μ m thickness were cut with the rotary microtome (Microtome Thermo Scientific - Microm HM 325, England), and used for hematoxylin and eosin staining. Insulin antibody expression was evaluated with the scoring system reported by Klien *et al.*¹⁸ Photomicrographs of all slides were obtained under a light microscope (Olympus - CX31, Japan), Images were obtained with Amscope digital camera (MU 1000, China) attached to the microscope, and were blindly assessed by 3 independent histopathologists.

Statistical Analysis

Statistical analyses were performed using the Primer of Statistics software version 3.01. Data in this study were expressed as means \pm standard error of mean (SEM) and analyzed using one-way analysis of variance (ANOVA) to determine the difference between the test groups compared to control, and the post-hoc test (Student - Newman Keuls) for comparison between groups. Values were regarded as statistically significant at $p < 0.05$.

Results

Effect of Metformin and Glibenclamide on Body Weights

There was a marked percentage gain in body weight of animals (g) in NC (+19.47) when compared with DC (+0.55), D_{LM} (+ 8.15), D_{HM} (+ 13.73), D_{LG} (- 2.7) and D_{HG} (+ 13.7), as shown in Table 1.

Effect of Metformin and Glibenclamide on Blood Glucose Levels

The STZ-induced diabetes was evident from blood glucose readings of the day, obtained 72 hours post-induction after overnight fasting of the rats (Figure 1), showing a significant increase in blood glucose in the test groups compared to NC. However, consequent administration of metformin and glibenclamide to STZ-treated groups showed trends of blood glucose attenuation, though rats were still considered diabetic. Overall, glibenclamide elicited more hypoglycemic effect than metformin in this study.

Table 1. Effect of Metformin and Glibenclamide Administration on Total Body Weights (g)

Body Weight (g)	Experimental Group					
	NC	DC	DLM	DHM	DLG	DHG
Initial	127.00 ± 5.81	178.20 ± 5.84	160.40 ± 3.89	142.00 ± 0.63	157.00 ± 0.84	145.60 ± 5.88
Final	160.20 ± 3.99	179.00 ± 4.68	174.20 ± 5.54	164.60 ± 4.49	152.80 ± 11.21	168.80 ± 16.70
Weight change (%)	+ 19.47	+ 0.55	+ 8.15	+ 13.73	- 2.7	+ 13.7

Data are expressed as Means ± SEM (n =5 rats per group).

Legend: NC = Normal Control

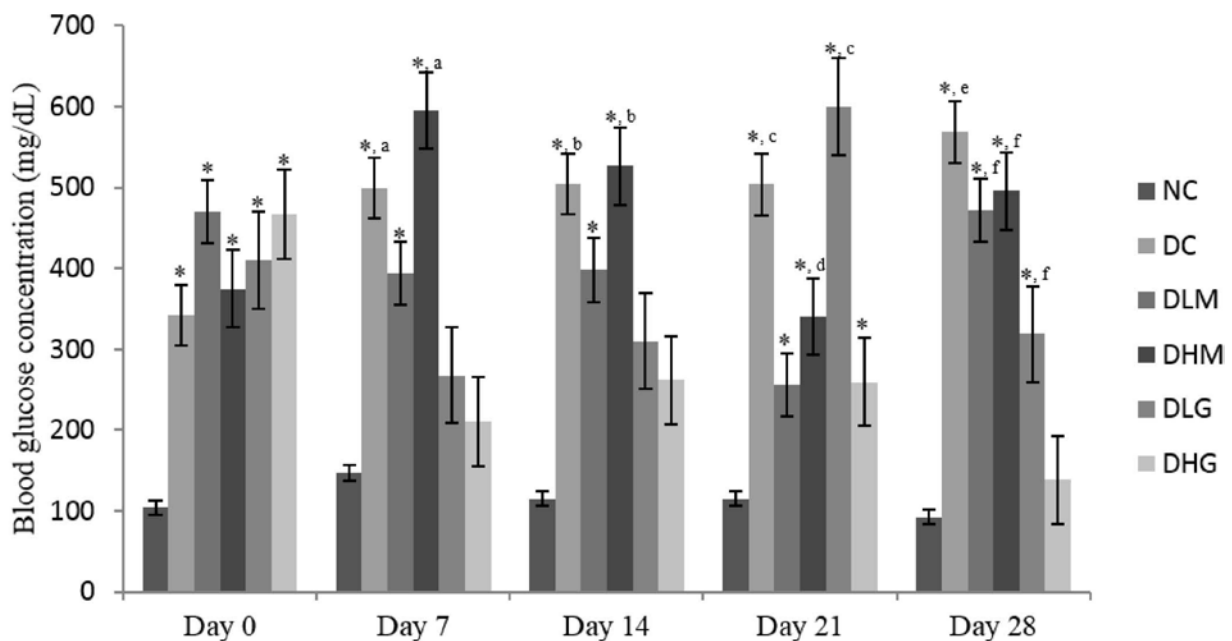
DC = Diabetic Control

DLM = Diabetic rats treated with low dose metformin 1.43 mg/kg body weight (bw)

DHM = Diabetic rats treated with high dose metformin 2.86 mg/kg bw

DLG = Diabetic rats treated with low dose glibenclamide 0.07 mg/kg bw

DHG = Diabetic rats treated with high dose glibenclamide 0.14 mg/kg bw

**Figure 1:** Effects of Metformin and Glibenclamide Administration on Blood Glucose Levels

Data are expressed as Means ± SEM (n =5 rats per group).

* = Significantly increased compared to NC (p < 0.05)

a = Significantly increased compared DLG and DHG (p < 0.05)

b = Significantly increased compared to DHG (p < 0.05)

c = Significantly increased compared to DLM, DHM, and DHG (p < 0.05)

d = Significantly increased compared to DLM (p < 0.05)

e = Significantly increased compared to DLG and DHG (p < 0.05)

f = Significantly increased compared to NC and DHG (p < 0.05)

Legend: NC = Normal Control

DC = Diabetic Control

DLM = Diabetic rats treated with low dose metformin 1.43 mg/kg body weight (bw)

DHM = Diabetic rats treated with high dose metformin 2.86 mg/kg bw

DLG = Diabetic rats treated with low dose glibenclamide 0.07 mg/kg bw

DHG = Diabetic rats treated with high dose glibenclamide 0.14 mg/kg bw

Effect of Metformin and Glibenclamide on Pancreatic Histology and Insulin expression

Histologically, NC group demonstrated well stained pancreatic islets (Pi) with good nuclei morphology and cytoplasm (Fig 2 [NC]). The DC rats had hypertrophied Pi nuclei, atrophied Pi cytoplasm and few necrotic nuclei of Pi with vacuolations (Fig 2 [DC]). The DM groups presented few hypertrophied, atrophied and polymorphic nuclei of Pi, with prominent vacuolations. The DM groups exhibited numerous Pi nuclei and

decreased Pi cytoplasmic volume (Fig 2 [D_{LM} & D_{HM}]). The rats in the group D_{LG} indicated fatty necrosis especially on serous acini with vacuolations, while D_{HG} rats had hypertrophied Pi nuclei (Fig 2 [D_{LG} & D_{HG}]).

Immunohistochemically, the insulin antibody expression revealed (as shown in Fig 3) that there was high insulin expression in the NC, D_{LM} and D_{LG} groups compared with low insulin expression in the DC, D_{HM} and D_{HG} groups. The corresponding immunohistochemical scores are presented in Table 2.

Histological Observations

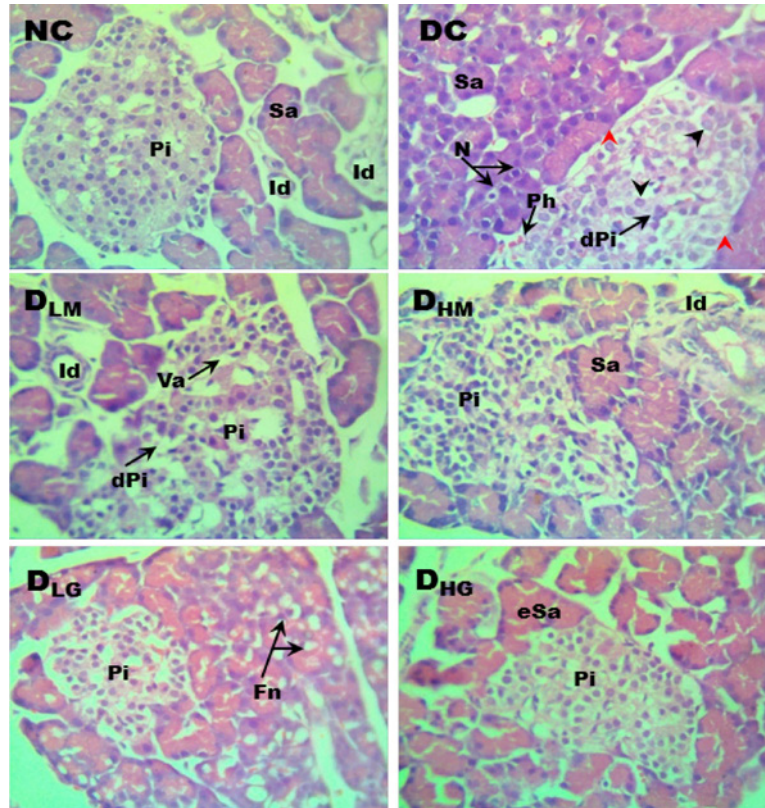


Figure 2. Photomicrographs of transverse section of the pancreas (H & E) x400
 NC = Normal control (appears normal); DC = Diabetic control (severely affected)
 DLM = Diabetic rat treated with low metformin 1.43 mg/kg body wt (bw) (moderately affected) DHM = Diabetic rat treated with high dose metformin 2.86 mg/kg bw (moderately affected)
 DLG = Diabetic rat treated with low dose glibenclamide 0.07 mg/kg bw (severely affected)
 DHG = Diabetic rat treated with high dose glibenclamide 0.14 mg/kg bw (moderately affected)
 Key: Red arrow head - oedema, black arrow head - hypertrophied nuclei, N = necrosis; Sa = Serous acini; eSa = Eosinophilic Serous acini; Pi = Pancreatic islets; dPi = degenerating and distorted Pancreatic islets; Fn = Region of fat necrosis; Id = Intralobular duct; Va = Vacuolation.

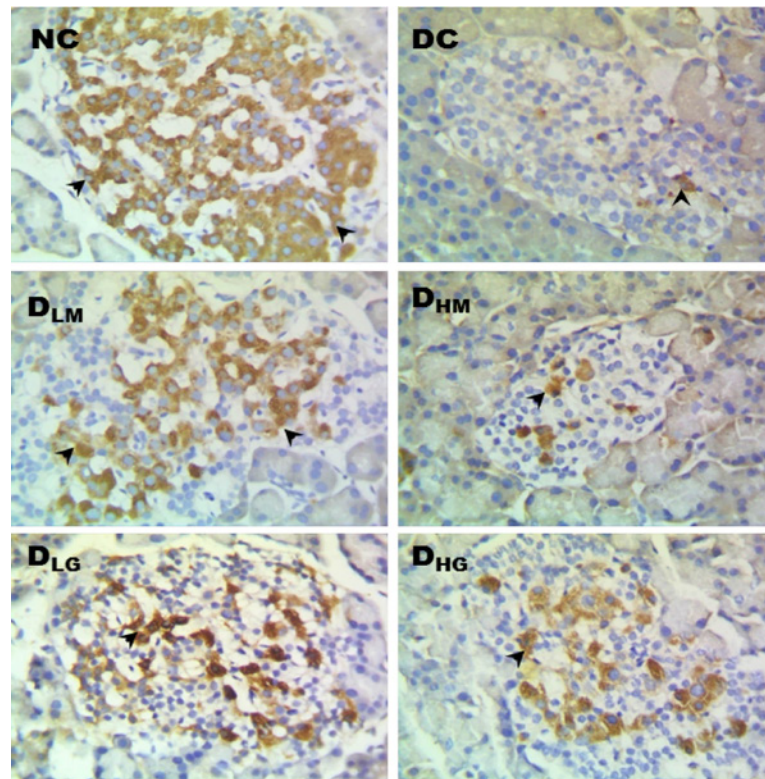


Figure 3. Photomicrographs of transverse section showing insulin antibody expression (black arrow head). Strongly expressed in NC, DLM, DLG and DHG. Weakly expressed in DC and moderately expressed in DHM (at x400).

Table 2. Effect of Metformin and Glibenclamide Administration on Insulin Expression

Group	(A) % of IHC	(B) Intensity of IHC	Final score (A+B) for Insulin Antibody Expression*	Degree of Insulin Antibody Expression
NC	> 60 (3)	Strong (3)	6	High
DC	< 30 (1)	Mild (2)	3	Low
D _{LM}	> 60 (3)	Strong (3)	6	High
D _{HM}	<30 (1)	Mild (2)	3	Low
D _{LG}	<30 (1)	Mild (2)	3	Low
D _{HG}	> 60 (3)	Strong (3)	6	High

Values in parenthesis indicate score for % and intensity of IHC.

Legend: IHC = Immunohistochemistry

NC = Normal Control

DC = Diabetic Control

D_{LM} = Diabetic rats treated with low dose metformin 1.43 mg/kg body weight (bw)

D_{HM} = Diabetic rats treated with high dose metformin 2.86 mg/kg bw

D_{LG} = Diabetic rats treated with low dose glibenclamide 0.07 mg/kg bw

D_{HG} = Diabetic rats treated with high dose glibenclamide 0.14 mg/kg bw

Key: % IHC: 0 = 0%, 1 = < 30%, 2 = 30 – 60 %, 3 = < 60 %

Intensity of IHC: 0 = No reaction, 1 = Weak, 2 = Mild, 3 = Strong

* Final Score: A+B (Range from 0 to 6): 0/6 = Negative Reaction; 1/6 to 3/6 = Low expression; 4/6 to 6/6 = High expression.

Discussion

Well-known anti-diabetic drugs metformin and glibenclamide were investigated to compare their effects on blood glucose levels, pancreatic microanatomical alterations and intensity of insulin expressed by the pancreatic β -cells, as detected with insulin antibody marker in STZ diabetic rat model.

The blood glucose levels of the STZ - induced diabetic-treated groups were statistically reduced ($p < 0.05$) compared to the DC group. This result indicates that metformin and glibenclamide have antidiabetic potentials as earlier documented.¹⁹ This is consistent with the report of Okonkwo and Okoye²⁰ of a reduction in blood glucose levels in diabetic rats treated with metformin. The blood glucose lowering effect of metformin is dependent on the presence of insulin.²¹ This correlates with the histological result of DC group, which demonstrated that administering STZ to rats did not completely degenerate pancreatic β -cells (solely responsible for insulin production), as insulin was expressed albeit in low intensity (Fig. 3 [DC]).

Histologically, the STZ-induced diabetic group revealed features of acute pancreatitis (Fig. 2.). Morphologic alterations of acute pancreatitis span from inflammation and edema to marked necrosis and hemorrhage. These morphologic alterations may show as: (i) microvascular leakage causing edema, (ii) necrosis of fat by lipolytic enzymes and (iii) an acute inflammatory reaction. The alterations demonstrated in this study were ameliorated in the drug-treated groups, however not to the same extent.

Metformin promotes insulin binding to insulin receptors leading to a reduction in blood glucose levels, suggesting that pancreatic β -cells are not completely destroyed in diabetes mellitus (DM) type 2. Metformin has been reported to have antioxidant activity against

oxidative damage caused by reactive oxygen species (ROS).²² The evidence of the attenuation of STZ-induced β -cell toxicity following metformin administration is demonstrated in the intensity of insulin antibody expressed (Fig. 3 [D_{LM} & D_{HM}]).

Glibenclamide showed strong hypoglycemic potential. Insulin expression was strongly demonstrated in NC, D_{LM}, D_{HM} and D_{HG} groups. However, there were severe pancreatic islet distortions (acute pancreatitis) and high insulin expression in the glibenclamide-treated group (Fig. 3 [D_{HG}]), when compared with the metformin group (Fig. 3 [D_{HM}]).

Glibenclamide reportedly stabilizes blood glucose by binding to and inhibiting the ATP-sensitive potassium channels through the inhibitory regulatory subunit, sulfonylurea receptor 1 (SUR1) in pancreatic β -cells. The inhibition causes cell membrane depolarization, opening of voltage-dependent calcium channel which increases intracellular calcium in the β -cells, and subsequent stimulation of insulin release.⁹

Conclusion

The present study may suggest that the hypoglycemic effect of glibenclamide was higher than that of metformin. The histological features of glibenclamide-treated rats demonstrated severe distortions, while metformin-treated rats had mild distortions of the pancreatic microanatomy (islets of Langerhans). Insulin expression was strongly enhanced by glibenclamide than by metformin. The mechanism of action of metformin (as regards insulin utilization) is presumed to be direct and a less energy-dependent pathway, whereas the inverse is the case for glibenclamide. Thus the findings of this research may suggest the use of metformin as a better alternative to glibenclamide in the amelioration of hyperglycemia, especially in the long term.

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