Comparative effects of glibenclamide, metformin and insulin on fetal pancreatic histology and maternal blood glucose in pregnant streptozotocin-induced diabetic rats

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Abstract

Background: Oral hypoglycemic agents use during pregnancy was assumed to cause fetal macrosomia and skeletal deformities, and maternal complications due to significant transfer across placenta or ineffective control of blood glucose.

Objective: This study investigated effects of insulin, metformin and glibenclamide on maternal blood glucose; and fetal crown-rump length, gross malformation and pancreatic histology in pregnant streptozotocin-induced diabetic rats.

Methods: Twenty-five pregnant rats of groups 1 to 5 as normal and diabetic controls; and diabetic treated with insulin, metformin and glibenclamide were used. Experimental GDM was induced using 45 and 35mg/Kgbw of intraperitoneal streptozotocin.

Results: Metformin, Insulin and Glibenclamide significantly reduced maternal glucose by 140.6mg/dL, 103.2mg/dL and 98.54mg/dl; respectively and showed islets with regular interlobular ducts, islets with some irregular interlobular ducts, and islets with many irregular interlobular ducts in histological fetal pancreatic photomicrographs respectively. This depicts metformin having highest ameliorative effect. There were no significant differences in maternal and fetal body weights, maternal blood glucose between diabetic groups, and fetal gross examination.

Conclusion: At the doses used in this research, metformin and glibenclamide showed no adverse effects on maternal and fetal features in the treatment of GDM. Thus, they can be used as safe and inexpensive alternatives to insulin.

Keywords: Gestational diabetes mellitus, oral hypoglycemic agents, blood glucose, fetal malformation and fetal pancreatic histology.
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Introduction

Diabetes mellitus is a prevalent medical disorder in pregnancy and its incidence is increasing. Gestational diabetes mellitus (GDM) has been recognized for decades[1]. While its optimal monitoring and treatment has been controversial, it is apparent that even a mild degree of maternal hyperglycemia may result in fetal defects[2,3].

During pregnancy, proper control of maternal blood glu-

cose level is important for the mother and the fetus[4,5]. This is because the maternal blood glucose level determines the fetal blood glucose level[6] and the adverse consequences of GDM on the fetus and the mother increase linearly with increasing maternal glucose[7]. Thus, failure of proper control of maternal glucose level during pregnancy can increase the risk of teratogenicity, stillbirth and neonatal morbidity[4].

The primary treatment of hyperglycemia or GDM during pregnancy is insulin after diet and physical exercise[8,9,10,11]. Although insulin has been shown not to cross the placenta to affect the fetuses directly[6,12,13], it can lead to maternal hypoglycemia which can then lead to fetal defects[14,15,16], depending on the dosage and period of exposure[17].

Another common mode of treatment of GDM is the use of some common oral antidiabetic agents (ODAs) or oral hypoglycemic agents (OHA) but some of the commonly used OHAs have been contraindicated in pregnancy because they can cross the placenta into the fetal circulation and are believed to cause fetal defects[18,19,20]. Even though a lot of research, reviews and clinical trials have been carried out on the glibenclamide and metformin as commonly used OHAs[11], the evidence supporting their use in the treatment of GDM is still controversial.

While some researchers believed that there may be minimum placenta transfer of OHAs which could be teratogenic to the developing baby, some argued that OHAs may not be able to effectively reduce the blood glucose level thereby causing macrosomia and others reported that no serious safety concerns[11]. Therefore, this study was designed to compare the effect of insulin with that of glibenclamide and metformin in the treatment of GDM and the GDM's level of detriment to the fetuses.

Materials and methods

Experimental animal

Thirty-five adult female and thirteen adult male Sprague-Dawley rats weighing between 120-160g and 300-350g respectively were procured from the Animal Laboratory of College of Medicine, University of Lagos. All the animals were housed in wire-mesh cages in the animal room of the Department of Anatomy, University of Lagos. The female rats were put in a cage partitioned into five compartments of the same size containing seven rats each. The bottom of the cage was made to allow passage of the rats' urine and fecal pellets into a removable tray put underneath it. The rats were allowed to acclimatize for three weeks. In addition, they were handled in accordance with the standard guide for the care and use of the animal laboratory, which was maintained at a temperature of 26-28^{o}C and 12:12 light: dark cycle. The animals were fed with astandard diet of rat pellets and water was provided ad libitum.

Determination of the oestrous cycle

During the period of acclimatization, the phases of the oestrous cycle (proestrus, estrus, metestrus and diestrus)[21] of the female rats were determined by daily examination of vaginal smear as described[22,21]. Briefly, 0.2ml of normal saline was drawn into a borosilicate glass medicine dropper and the tip of the dropper was carefully and gently inserted into the vaginal orifice of each rat at a depth of approximately 5mm. The saline was then flushed into the vagina from the dropper and immediately drawn back into it[21]. The vaginal fluid from each rat was placed on a separate untreated glass microscopic slide and viewed while still wet under a light microscope at 100x magnification. The vaginal smears were taken once daily between 8 a.m and 10 a.m[23] while holding each rat behind the shoulder blades in the supine position. The female rats with an oestrous cycle of 4 - 5 days were then selected for the study.

Pregnancy

Out of the 35 female rats, those with 4-5 day oestrous cycle were mated overnight with the male rats on the evening of the proestrus stage of their oestrous cycle and the mating was confirmed the following morning, denoted as day 0 of the pregnancy, by the presence of sperms in the vaginal smear[24,25]. Twenty-five pregnant rats randomly divided into five groups (containing five rats each) were used for the study.

Induction of Experimental Gestational Diabetes Mellitus (GDM)

The type 1 diabetic condition (Gestational Diabetes Mellitus) was induced in the groups 2 to 5 of the pregnant rats by intraperitoneal injection of double dosage of freshly prepared streptozotocin (STZ) (Sigma, St. Louis, MO, USA) in 0.1M sodium citrate buffer (pH 4.5). The rats were fasted overnight from the night of day 0 of

pregnancy and the initial dose of 45mg/Kg b.w.[26] was injected on day 1 of pregnancy. The rats were fasted overnight again from the night of day 1 and the second dose of 35mg/Kg b.w.[27,28] was injected on day 2. The use of the two doses was previously described and the group 1 rats (non-diabetic control) were injected an equal volume of citrate buffer only[24,29]. Fasting blood glucose level (diabetic status) was measured 48 h after STZ treatment (day 4 of pregnancy) using One Touch Ultra Mini Glucometer (Accu-chek, Roche, Germany) with a drop of blood obtained by tail vein puncture. The pregnant rats with fasting blood glucose value above 120 mg/dL were considered diabetic as recommended by WHO[30].

Drug treatment and grouping

Drug treatment began after the pregnant rats have been confirmed diabetic on day 4. The rats were either administered distilled water, insulin, or oral hypoglycemic agents (metformin and glibenclamide) once daily using oral cannula except for insulin, which was administered intraperitoneally. The animals were grouped and treated for 2 weeks (day 4 to day 17) as follows:

Group1: Non-diabetic control + distilled water (0.5ml)
Group 2: Diabetic control + distilled water (0.5ml)
Group 3: Diabetic + insulin (1 IU once daily)
Group 4: Diabetic + metformin (36.43 mg/kg b.w)
Group 5: Diabetic + glibenclamide (0.26 mg/kg b.w)

Maternal body weights and fasting blood glucose

All the rats were weighed and their fasting blood glucose was taken before STZ administration, before the commencement of drug administrations on day 4 of the pregnancy, after 1 week (day 11) and after 2 weeks of the treatment just before sacrifice (day 18). On day 4, the diabetic rats were grouped in such a way that the rats with higher glucose levels were randomly assigned to the insulin, metformin and glibenclamide treated groups while those with lower glucose levels were put in the diabetic untreated group. This regrouping is important to prevent the death of the diabetic untreated rats before the end of the experiment since the highly diabetic rats rarely survive for a longer period of time without treatment[77]. Also, putting the highly diabetic rats in drug-treated groups will enhance the effect of insulin[78] and probably the OHAs.

Sacrifice and sample collection

A day after the completion of the treatment (day 18), the rats were weighed and their fasting blood glucose was taken. Then, they were anaesthetized with diethyl ether (TKM PharmaPvt, Ltd., Andhra Pradesh, India). After becoming unconscious, the blood was collected from each of the rats into separate plain tubes followed by dissection of each of them. After being dissected, the fetuses were removed. Each of the fetuses was examined for any morphological deformity and later dissected and its pancreas excised.

Fetal examination

The fetuses were examined for gross deformity using a hand lens. Then, their average number, weight and crown-rump length were determined for each group.

Histology of the fetal pancreas

The pancreas excised from each fetus was rinsed in ice-cold 1.15% KCl, blotted and weighed for sub-Cellular fraction preparation. A section of the pancreas was cut and introduced into 10% formaldehyde (formalin) solution to preserve the tissues for histopathology.

Hematoxylin and Eosin (H & E) Staining of the pancreatic tissue 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 were placed in a tissue automatic processor machine, in which dehydration was done with graded alcohol (70%, 90% and 100%), clearing was done with xylene in order to remove fat from tissues and increase the refractive index, the infiltration was carried out with paraffin wax at about 60^{o}C, and finally embedding and blocking process was done. The whole procedure was done automatically by the processor machine overnight for about (14 h). Each block was trimmed and then sectioned about 3µm by using a microtome. Then, Hematoxylin and eosin (H & E) staining procedure was done as follows: the sectioned tissues were deparaffinized by placing the slide on the burner with regulated temperature and placed in the xylene. This was followed by hydration where the tissue sections passed through decreasing concentration of alcohol baths and water (100%, 90%, 80%, 70%). The first staining was done with hematoxylin for 3-5 minutes and then washed in running tap water until sections "blue" for 5 minutes or less, it was then differentiated in 1% acid alcohol (1% HCl in 70% alcohol) for 5 minutes. Washing was done in running tap water

until the sections were again blue by dipping in an alkaline solution (e.g. ammonia water) followed by tap water wash. The second stain was done in 1% Eosin Y for 0 minutes, washed in tap water for 1-5 minutes, dehydrated in increasing concentration of alcohols and cleared in xylene. The prepared slides were mounted in DPX for microscopic observations.

Statistical analysis

All the quantitative results were presented in a tabular form as mean ± Standard Error of Mean (SEM). The maternal body weight and blood glucose levels were analyzed with GraphPad Prism version 7 (GraphPad Software Inc., CA. USA)using repeated measures ANOVA while the fetal parameters were analyzed using One-way ANOVA. Multiple comparisons were done using Tukey's test. A p-value of $p<0.05$ was considered to be statistically significant.

Results

Effect of induced GDM and drug treatment on maternal blood glucose: Table 1

Table 1: Maternal blood glucose in mg/dL and its reduction (in bracket) due to the administration of hypoglycemic agents at different days of pregnancy

Days of pregnancy	Normal Control	Diabetic Control	Diabetic + Insulin	Diabetic + Metformin	Diabetic + Glibenclamide
Baseline (Before STZ)	91.6 ± 1.7	93.8 ± 3.1	91.8 ± 3.8	91.4 ± 3.7	95.2 ± 1.6
Day 4 (just before beginning the drug treatment)	91.2±1.6	164±13.4[a]	237.2±18.6[a,b]	280.6±51.1[a,b]	216.8±38.9[a]
Day 11 of pregnancy	82.4 ± 5.1	152.8 ± 8.6[a]	183.4 ± 12.3[a] (53.8 mg/dL, 22.7%)	190.8 ± 24.1[a,c] (89.8 mg/dL, 32%)[c]	151.2 ± 22.1[c] (65.6 mg/dL, 30.3%)[c]
Day 18 of pregnancy	89.8±3.9	141.2 ± 9.0	134 ± 11.2[c] (103.2 mg/dL, 43.5%)[c]	140 ± 7.6[c] (140.6 mg/dL, 50.2%)[c]	118.26 ± 7.0[c] (98.54 mg/dL, 45.5%)[c]

Values are presented as mean (± SEM) and are significant at $p<0.05$. N= 5.
[a] Significant compared with the normal control. [b] Significant compared with diabetic control
[c] Significant compared to day 4 for each of the treated groups.

Effect of GDM

There was no observed significant difference in the blood glucose levels within the normal control group and within the diabetic control group throughout the days of the experiment. The GDM significantly increased blood glucose levels of all the diabetic rats on day 4 of pregnancy compared to the normal control. Accordingly, all the drug-treated rats showed a rise in glucose levels which were significant in both the insulin-treated ($p=0.0372$) and metformin-treated ($p=0.0001$) rats on day 4 of pregnancy compared with diabetic control.

Effect of insulin

Insulin reduced blood glucose by the mean values of 53.8 mg/dL (22.7%) and 103.2 mg/dL (43.5%) on the days 11 and 18 respectively but the reductions were only significant on day 18 ($p<0.0001$) compared with day 4.

Effect of metformin

When compared with day 4, metformin significantly reduced blood glucose level by a mean value of 89.8 mg/dL (32%) on day 11 ($p=0.0003$) and by the mean value of 140.6 mg/dL (50.2%) on day 18 ($p<0.0001$).

Effect of glibenclamide

When compared with day 4, glibenclamide significantly reduced blood glucose level by a mean value of 65.6 mg/dL (30.3%) on day 11 ($p=0.0129$) and by the mean value

of 98.54 mg/dL (45.5%) on day 18 ($p<0.0001$).

NOTE: Each of the drugs caused further reduction of the blood glucose between days 11 and 18 but the reductions were not significant for all of them. Also, there was no significant difference in the maternal blood glucose levels between all the diabetic (treated and untreated) groups at the end of the experiment.

Effect of induced GDM and drug treatment on maternal body weight: Table 2

There was no significant weight difference between all the groups and within the groups throughout the treatment days except in the glibenclamide group. In the glibenclamide group, there was a significant weight increase on day 18 compared with day 4 ($p = 0.0216$) and day 11 ($p = 0.0181$).

Table 2: Maternal body weight (g) across all the groups at different days of pregnancy

Days of pregnancy	Normal Control	Diabetic Control	Diabetic + Insulin	Diabetic + Metformin	Diabetic + Glibenclamide
Baseline (before STZ)	127.2 ± 9.7	130 ± 8.1	125.36 ± 7.1	126.32 ± 10.7	133.32 ± 10.7
Day 4 of pregnancy (beginning of drug treatment)	136.2 ± 11.2	136.04 ± 6.9	120.24 ± 6.1	116.62 ± 10.0	126.08 ± 10.0
Day 11 of pregnancy	154.64 ± 12.5	138.58 ± 5.7	120.18 ± 6.9	119.92 ± 13.9	125.66 ± 8.6
Day 18 of pregnancy	149.7 ± 13.4	127 ± 9.5	129 ± 6.1	123.12 ± 15.9	145.08 ± 7.6[c,d]

Values are presented as mean (± SEM) and are significant at $p<0.05$. N=5.
[c] Significant compared to day 4 for each of the treated groups.
[d] Significant compared to day 11 for each of the treated groups.

Physical examination of fetal parameters: Table 3

There was no significant difference in the examined physical parameters of the fetuses across all the groups including the control group.

Table 3: Average fetal physical parameters – average number, body weight (g) and Crown-rump length (cm) across all the groups

Groups	Body Weight (g)	Crown-rump length (cm)	No. of Pups
Normal Control	6±0.32	2.2±0.07	6±0.45
Diabetic Control	6.2±0.37	2.1±0.13	5.8±0.37
Diabetic + Insulin	6.4±0.4	2.08+0.04	5.8±0.37
Diabetic + Metformin	6.4±0.25	2.02±0.07	6.2±0.37
Diabetic + Glibenclamide	6.4±0.4	2.12±0.05	5.8±0.2

Discussion

This research was designed to compare the safety and effectiveness of insulin and two commonly used oral hypoglycemic agents/ oral antidiabetic agents (metformin and glibenclamide)[11] in the treatment of gestational diabetes mellitus and its complications in both the mother and the fetus using streptozotocin (STZ) – induced diabetic pregnant rats. Diabetes in pregnancy is associated with serious complications for both the mother and the fetus, and the adverse consequences on the fetus and the mother increase linearly with increasing maternal blood glucose[7]. Some of the fetal risks are spontaneous abortion, intrauterine death, stillbirth, congenital malformation, shoulder dystocia and birth injuries, neonatal hypoglycemia and infant respiratory distress syndrome (RDS) while maternal risks include hydramnios, hypertension and preeclampsia, prolonged labour, obstructed labour,

infections, assisted delivery, uterine atonia and postpartum hemorrhage[31]. Thus, good maternal blood glucose control before conception and throughout pregnancy reduces the risks substantially[32,33]. Therefore, management of maternal GDM is one of the priorities of WHO[31] because its incidence is increasing[34].

After diet control and physical exercise in the treatment of GDM, insulin remains the gold standard[10]. Taking insulin as the gold standard was based on unparalleled efficacy standard and because of lack of any well-studied alternative[11]. However, disadvantages of insulin use such as multiple daily injection sites, maternal weight gain, the risk of hypoglycemia, high cost and handling have led to consideration of oral hypoglycemic agents (OHA) especially, metformin and glibenclamide, as preferred alternatives[9]. The use of OHAs raised a concern about the risks of fetal teratogenicity and neonatal hypoglycemia as a result of their 10-16% maternal to fetal transfer rate[8,35]. Therefore, this research was carried out to compare the effects of insulin, metformin and glibenclamide in STZ-induced diabetic pregnant rats. The effect of the drugs on maternal blood glucose and body weight, as well as fetal malformation and fetal pancreas, was considered.

Maternal blood glucose and body weight

On day 4, the diabetic rats were grouped in such a way that the rats with higher glucose levels were randomly assigned to the insulin, metformin and glibenclamide treated groups while those with lower glucose levels were put in the diabetic untreated group. This regrouping is important to prevent the death of the diabetic untreated rats before the end of the experiment since the highly diabetic rats rarely survive for a longer period of time without treatment[78], and it will enhance the effect of insulin[78] and probably the OHAs. At the end of the research (day 18), all the drugs reduced maternal blood glucose by 140.6 mg/dL (50.2%), and 103.2 mg/dL (43.5%) and 98.54 mg/dL (45.5%) for metformin, insulin and glibenclamide respectively (table 1). The blood glucose reductions occurred without the risk of hypoglycemia (blood glucose < 3.9 mmol/L or 70 mg/dL) at the drug doses used in this research and this is in agreement with the previous report[71]. Since the management of GDM is primarily aimed at the glycemic target to reduce the incidence of fetal and maternal complications[8,36], metformin and glibenclamide can be used as safe alternatives to insulin. This is supported by the fact that there was no significant difference in the blood glucose levels between all the treated groups at the end of this research and it corroborates with the previous reports from the review of randomized controlled trials which showed that there was no significant difference in maternal fasting glucose between glyburide (glibenclamide) and insulin although there was slightly lower fasting blood glucose in the insulin group than in glyburide group[37], and between OHAs and insulin[8]. It was even showed that maternal complications were higher in insulin-treated GDM women compared to metformin and glibenclamide[8]. More reduction in fasting blood glucose observed in the metformin-treated group than the glibenclamide treated group is supported by the report of Mansour and colleagues[59], who also reported higher and significant reductions in fasting blood glucose and glycosylated haemoglobin (HbA1c) in metformin-treated rats than in glibenclamide-treated rats. In addition, Atsuo et al. reported that insulin, glibenclamide and metformin caused significantly improved glucose tolerance in mildly diabetic rats while only insulin and metformin but not glibenclamide significantly improved glucose tolerance in severely diabetic rats[60].

Although there was no significant maternal weight difference across all the groups, the glibenclamide-treated group showed a significant weight increase at the end of the research compared to the previous days (table 2). This is supported by the previous report that oral anti-diabetic drugs like sulfonylurea including glibenclamide, glinides, and glitazones as well as insulin increase body weight within years by up to 8 kg while metformin decreases it[58] and this may be a call for careful monitoring of glibenclamide use in GDM.

Fetal gross/physical examination

The results obtained from the fetal gross examinations showed that there were no statistically significant differences in body weight, crown-rump length and number of fetal across all groups (treated and control) (table 3). This result is in line with the previous reports concerning the use of oral hypoglycemic agents during pregnancy. According to Tran et al.[18], the risk of embryo-fetal harm with biguanides appears to be very low or non-existent. Also, the birth defect in newborns of mothers who had used one of the oral hypoglycemic agents was thought to be the result of uncontrolled diabetes but not to the drugs taken[12]. In addition, it was reported that metformin did not result in major malformation or significant changes in mouse embryonic growth[67] and that metformin also protected neural cells against apoptosis and neural de-

Table 3: Average fetal physical parameters – average number, body weight (g) and Crown-rump length (cm) across all the groups

Groups	Body Weight (g)	Crown-rump length (cm)	No. of Pups
Normal Control	6±0.32	2.2±0.07	6±0.45
Diabetic Control	6.2±0.37	2.1±0.13	5.8±0.37
Diabetic + Insulin	6.4±0.4	2.08+0.04	5.8±0.37
Diabetic + Metformin	6.4±0.25	2.02±0.07	6.2±0.37
Diabetic + Glibenclamide	6.4±0.4	2.12±0.05	5.8±0.2

fects caused by high glucose challenge in rats[68] and mice[69].

Fetal pancreas

In the control (non-diabetic) group, the pancreatic histology showed an intact fetal pancreatic β and acini cells with regular interlobular ducts (Figure 1). Group 2 (diabetic without treatment) pancreas showed large irregularly shaped degenerating Islets of Langerhans with irregular interlobular ducts (Figure 2). Diabetes mediated hyperglycemia has been shown to generate a high level of reactive oxygen species (ROS) from cells causing oxidative stress[38,39,40]. Exposure to chronic oxidative stress destroys body cells. β cells are especially vulnerable to attacks by ROS because expression of antioxidant enzymes in pancreatic islets is low[41,42] and β cells have a high oxidative energy requirement. Hyperglycemia and increased ROS impair glucose-stimulated insulin secretion[43,44], decrease expression of key β cell genes[45-49] and induce cell death[50,51], worsening the diabetic condition related com-

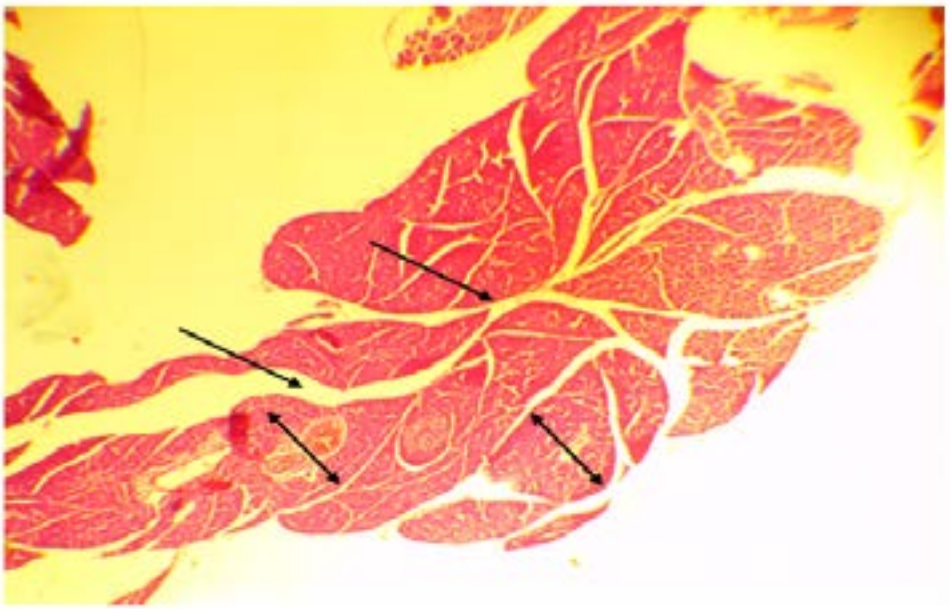

Figure 1: Photomicrograph of the non-diabetic control (group 1) fetal pancreas using H&E (x100), showing normal distribution of islets of Langerhans (↔) with well-developed interlobular ducts (→) interspersed among the pancreatic lobules.

plications.

Since maternal blood glucose level determines fetal blood glucose level[6] and because adverse consequence of increased maternal blood glucose on the fetus and the mother increases linearly with maternal glucose[7], degenerating Islets of Langerhans in fetal pancreas of this diabetic group can be linked to maternal hyperglycemia which in turn resulted in high ROS generation in the fetal pancreatic cells leading to subsequent fetal islet of Langerhans degeneration (Figure 2).

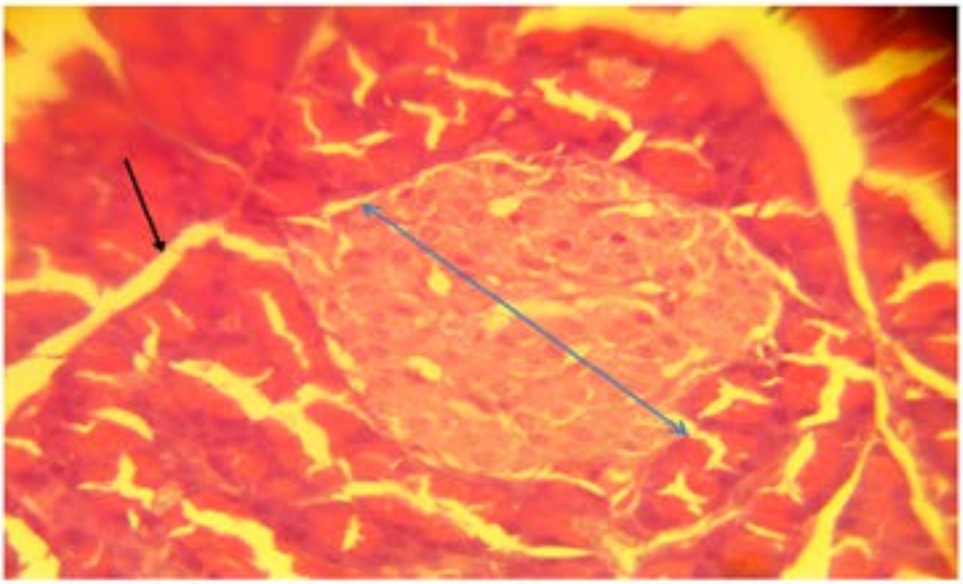

Figure 2: Photomicrograph of the diabetic control (group 2) fetal pancreas using H&E (x100), showing a large irregularly shaped degenerating islets of Langerhans (↔) with irregular interlobular ducts (→).

Figure 3 showed developing fetal pancreatic Islets of Langerhans with some irregular interlobular ducts following treatment with insulin. This might result from the ability of insulin treatment to rescue β cells and facilitate their recovery from hyperglycemia-induced destruction due to the insulin's effects as anti-hyperglycemic, anti-in-

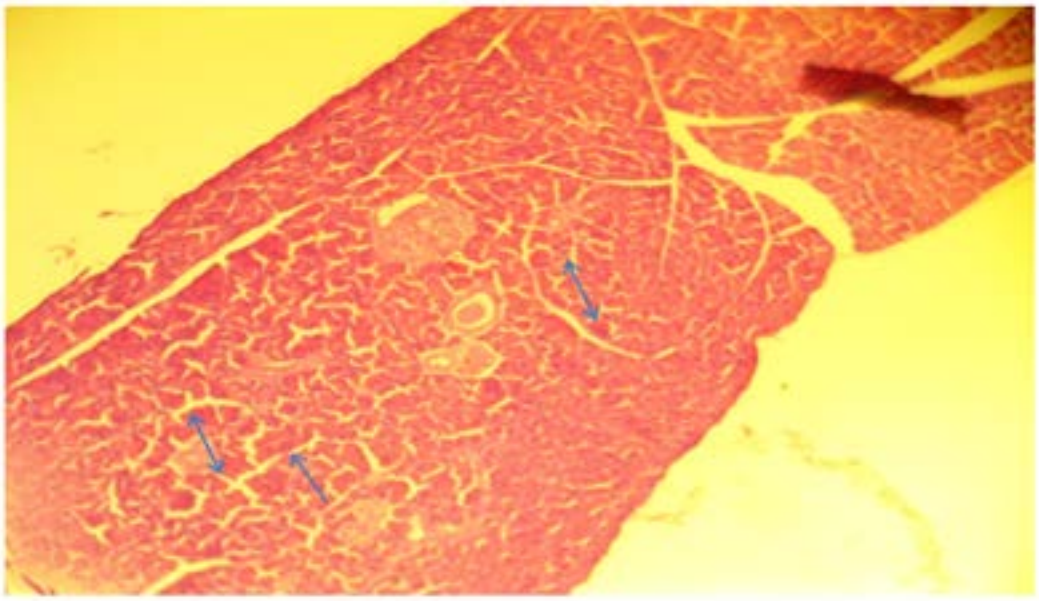

Figure 3: Photomicrograph of the diabetic + insulin (group 3) fetal pancreas using H&E (x100), showing normal developing islets of Langerhans (↔) and some irregular interlobular ducts.

flammatory and anti-oxidant agents[52].

In figure 4, the fetal pancreatic histology showed the well-rounded distribution of Islets of Langerhans with regular interlobular ducts implying that the pancreatic recovery was more than that of insulin. In a recent meta-analysis, metformin performed well compared to insulin as it was associated with significantly less maternal weight gain, a trend towards a lower rate of any neonatal hypoglycemia,less pregnancy-induced hypertension and less severe neonatal hypoglycemia[53]. This might be due to the higher antihyperglycemic effect of metformin more than that of insulin (table 1) and its higher weight reduction effect (table 2) as well as its higher antioxidant effect. Although it was previously reported that metformin impaired insulin secretion in primary human and mouse islets, and in rat pancreatic beta cell lines in a normoglycemic environment[61,62], it has been reported that metformin enhanced mouse pancreatic progenitor cells[63] and protected mouse pancreatic beta cells exposed to fatty-acid induced stress[64] and high glucose[65]. Therefore, metformin overuse without metabolic challenge could cause beta cell toxicity[66] but in a hyperglycemic environment, as in this

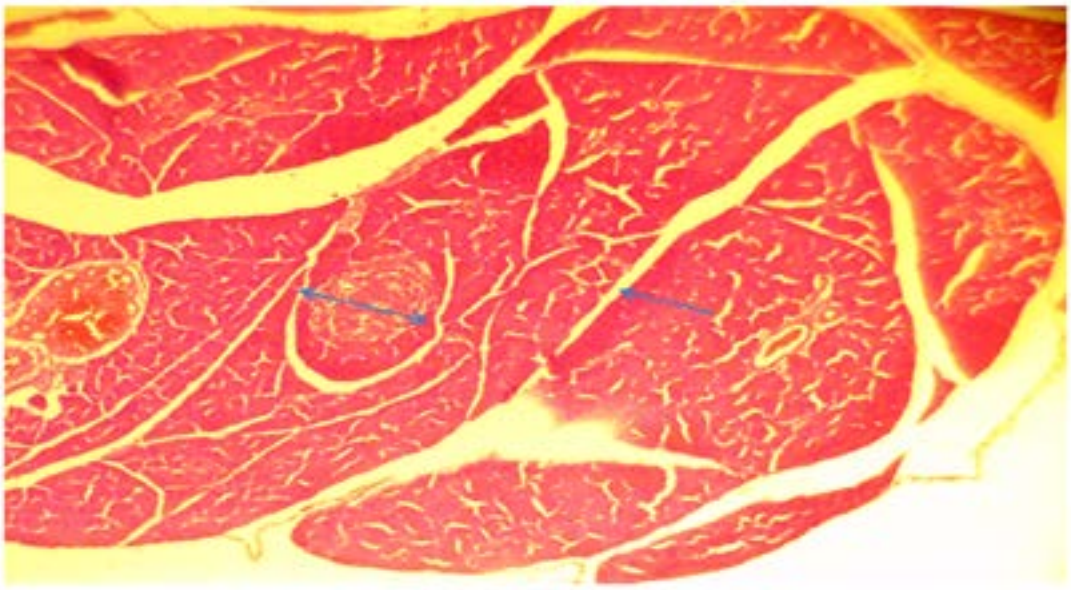

Figure 4: Photomicrograph of the diabetic + metformin (group 4) fetal pancreas using H&E (x100), showing the well-rounded distribution of islets Langerhans (↔) with regular interlobular ducts (→) within the developed pancreatic cells.

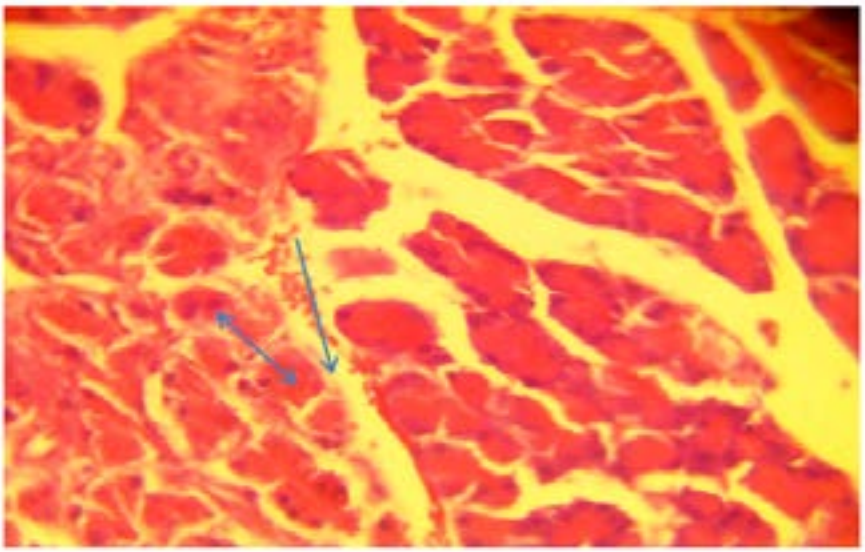

Figure 5: Photomicrograph of the diabetic + glibenclamide (group 5) fetal pancreas using H&E (x100), numerous developing islets Langerhans (↔) with irregular interlobular ducts (→) slightly less developed than that of the insulin group within the developing pancreatic cells

research, metformin is protective to the pancreas.
Figure 5 showed numerous developing Islet cells with many irregular interlobular ducts following treatment with glibenclamide. The level of Islets' development was less than those of insulin and metformin (Figure 5). This can be due to the relatively lower ability of glibenclamide to reduce maternal blood glucose and body weight compared to metformin and insulin (tables 1 & 2). The poor-pancreatic recovery with the glibenclamide treatment could also be due to its lower antioxidant effect as previously reported[54,55].

The results of this research show that metformin and glibenclamide are safe alternatives to insulin in GDM at the doses used. Although head-to-head comparison of insulin and OHA use in pregnancy is still scanty in animal research, there are reviews, meta-analyses and randomized clinical trials[37,8,71,72,73,74] whose authors reported metformin and glibenclamide as being safe and effective alternatives to insulin in the treatment of GDM with minimal or no complications in both the fetus and mothers. Direct comparison of metformin and glibenclamide showed higher glycemic control by metformin than glibenclamide[75,57]. Improved type 2 diabetes mellitus condition using combined insulin and metformin has been reported[56] while combining insulin with metformin treatment, to improve glycaemic control towards the end of the pregnancy, showed more favourable results than insulin alone[57,76]. In addition, the rate of severe hypoglycemia and weight gain was reported to be reduced in metformin-treated GDM patients than insulin-treated patients[77,78].

However, this research adds that metformin is more effective than both the glibenclamide and insulin in the treatment of GDM with no risk of hypoglycemia and fetal malformation, as well as in the improvement of fetal and maternal outcomes at the doses used. The use of metformin and glibenclamide as safe and inexpensive alternatives to insulin is especially favoured by the fact that no significant difference was observed between all the groups treated with each of the drugs in all the measured maternal and parameters at the dosage used in this research. Nevertheless, more research is still needed in testing these drugs on more fetal parameters and in comparing the antioxidant effects of metformin and glibenclamide with insulin in GDM patients or in laboratory animals.

Conclusion

At the doses used in this research, metformin and glibenclamide use in the treatment of gestational diabetes mellitus showed no adverse effects on maternal and fetal features. Metformin had the highest effect in ameliorating the adverse effects of gestational diabetes than insulin and glibenclamide without causing hypoglycemia.

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Conflict of interest

There is no conflict of interest to declare.

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