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# A comparison of the anti-diabetic potential of D-ribose-L-cysteine with insulin, and oral hypoglycaemic agents on pregnant rats

Abraham A.A. Osinubi[a], Leke Jacob Medubi[a], Edidiong N. Akang[a,*], Lawal K. Sodiq[a], Titilola A. Samuel[b], Taiwo Kusemiju[a], James Osolu[c], Danladi Madu[d], Olufemi Fasanmade[d]

[a] Department of Anatomy, College of Medicine of the University of Lagos, Lagos, Nigeria
[b] Department of Biochemistry, College of Medicine of the University of Lagos, Lagos, Nigeria
[c] Ajah Primary Healthcare Centre, Eti-Osa East, LCDA, Lekki, Lagos, Nigeria
[d] Department of Medicine, Lagos University Teaching Hospital, Lagos, Nigeria

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ABSTRACT

Over 18% of pregnant women are affected by diabetes mellitus (DM) and Insulin has been the commonest drug used in its treatment. There are reports of noncompliance to insulin due to trypanophobia, with suggestions for the use of oral hypoglycaemic agents (OHAs). However, the opposing views about the benefits and risk of oral hypoglycaemic agents (OHAs) warrant a continuous search for an alternative regimen. Therefore, this study is aimed at comparing the antidiabetic effects of D-ribose-L-cysteine (riboceine) with vildagliptin, glibenclamide, metformin, glipizide and insulin in diabetes in pregnancy. Forty (40) female Sprague-Dawley (SD) rats were mated with twenty (20) male SD rats. Diabetes was induced by streptozotocin and the female SD rats were divided into 8 groups of five (5) rats each. The animals were administered either of the OHAs vildagliptin, glibenclamide, metformin, glipizide and riboceine for a period of 19 gestational days. The results showed that streptozotocin (STZ) significantly ($p < 0.05$) decreased the weights of the animals, increased malondialdehyde, blood glucose levels and altered reproductive hormones. These effects of STZ were better ameliorated in animals that received insulin and riboceine compared to the other OHAs. While progesterone levels were significantly ($p < 0.05$) higher in animals that received riboceine compared to insulin. Glibenclamide increased ($p < 0.05$) foetal weights compared to non-diabetic animals. In conclusion, glibenclamide may be a threat to mother`s life in the management of diabetes in pregnancy however, riboceine as well as vildagliptin, metformin and glipizide are effective oral hypoglycaemic agents which could serve as a potent adjuvant comparable to insulin in the management of diabetes during gestation.

## 1. Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both [1,2]. It is the 7th leading cause of death in the USA and a major cause of heart disease and stroke [3]. Diabetes Mellitus has a global prevalence of 8.3% and 415 million persons are currently affected [4]. It is estimated that by 2030 over 439 million adults aging between 20–79 will be living with diabetes, signifying over 69% and 20% rise from 2010 in developing and developed countries respectively [1,5]. This growing burden of diabetes has necessitated great concern in the management of diabetes.

A vast majority with DM fall under two categories: type 1 (T1DM) and type 2 (T2DM) [1]. T1DM is as a result of shortage in the production of insulin due to the destruction of pancreatic β cells [6] while the T2DM is as a result of tissue insensitivity to insulin, a situation known as insulin resistance [7]. It results in high blood glucose due to the inability of the tissues to convert the available glucose into consumable energy, a situation known as glucose intolerance, which leads to β cell compensation: delayed insulin secretion. Prolonged glucose intolerance will ultimately lead to β cell dysfunction [7]. About 95% of diabetic cases in developing countries are of the T2DM [1].

DM also occurs for first time during pregnancies [8]. Such diabetes in pregnancy is diagnosed if a fasting plasma glucose level equal to 5.1–6.9 mmol/L (92–125 mg/dL) is measured or a 75 g glucose load produces a 2-hour plasma glucose equal to 8.5–11.0 mmol/L (153–199 mg/dL) [8,9]. Over 18% of pregnant women develop diabetes; a condition regarded as gestational diabetes mellitus [3]. These

* Corresponding author at: Department of Anatomy, College of Medicine, University of Lagos, Idi-Araba, P.M.B. 12003, Lagos, Nigeria. Tel.: +234 8065299278.
*E-mail address:* enakang@cmul.edu.ng (E.N. Akang).

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women with gestational diabetes have a 35%–60% chance of developing diabetes (usually T2DM) in the next 10–20 years [3].

The safety of Oral hypoglycaemic agents (OHAs) in pregnancy has always been a source of concern to both the clinicians and patients. Specifically, concerns over teratogenicity due to the possible placental transfer of antidiabetics as well as maternal and neonatal outcomes have continued to generate so much interest [10–12]. Some authorities believe that OHAs have been almost universally endorsed as first line drugs in the treatment of diabetes in pregnancy. In the past few years, OHAs have been considered as an alternative to insulin therapy in the treatment of diabetes in pregnancy [13,21]. To this group of clinicians, these agents have an efficacy comparable with insulin in their ability to facilitate achievement of targeted levels of glycaemic control on all DM severity levels and in obese patients. Some are, however, cautious and believe that information is inadequate to evaluate the risk of some of these agents [14] in pregnancy. Others [15–17] hold that the use of OHAs in pregnancy is not recommended because of reports of foetal anomalies and other adverse outcomes in animal studies and in some human cases. Some researchers have questioned the basis for this fear and attribute any teratogenicity in such pregnant women more to poor glycaemic control [18,19]. Early case reports and small-scale studies suggested an association between oral hypoglycaemic agents and congenital anomalies. This anecdotal evidence was even translated into guidelines by the American College of Obstetricians and Gynecologists and the American Diabetes Association, among others. Unfortunately, these guidelines were based largely on a retrospective study involving 20 women with type 2 diabetes—all with glycosylated/glycated haemoglobin A1c (HA1c) concentrations exceeding 8%—in whom there was an increased rate of anomalies [20]. However, the fact that maternal hyperglycaemia existed prior to conception makes it impossible to determine whether the increased rate of anomalies was the result of medication or elevated glucose levels.

It is, however, the consensus of physicians and researchers that there is still little information available on the safety of these drugs during pregnancy [21]. All agree that more comprehensive studies are needed to ensure the safety and efficacy of these drugs in pregnancy [11]. These additional studies are also needed to better define the benefits and risks of OHAs in pregnancy. A few studies have examined the effects of these oral agents on pregnancy outcome most of them albeit retrospectively. The opposing views of benefits/risks have therefore not been subjected to objective scientific tests as ethical clearance for such testing in pregnant women using OHAs cannot be easily obtained for obvious reasons.

Another great concern frequently overlooked is trypanophobia. Trypanophobia or needle phobia affects at least 10% of the population, and it is likely that the actual number is larger, as the most severe cases are never documented due to the tendency of the sufferers to simply avoid all medical treatment [22]. Needle phobia is both inherited and learned. Needle phobia is highly associated with avoidance behaviour [23]. The fear of needles is known to partly account for rate of dropouts from follow-up and compliance to insulin therapy [24]. In extreme situation, trypanophobia is capable of instigating tocophobia: morbid fear of getting pregnant. A credible alternative to insulin therapy will therefore be of immense help to this large group of individuals, in whom aversion to the use of injectables encourages follow-up dropouts, precludes good DM medication compliance, and promotes development of tocophobia.

Oxidative stress has been reported to play a major role in chronic diabetes complications [25,26]. This is linked to the disproportionate formation of free radicals by glucose oxidation, nonenzymatic glycation of proteins, and oxidative degradation of glycated proteins [27]. Thus, though speculative but it is plausible that the mitigation of oxidative stress may decrease complications resulting from diabetes in pregnancy.

D-ribose is a prodrug from L-cysteine known to aid the elevation of intracellular levels of glutathione (GSH) [28]. GSH is the coenzyme that mediates the protection against free radicals generated during oxidative metabolism of acetaminophen by the hepatic cytochrome P-450 system [29].

In view of the discussed above, this study aimed at determining the antidiabetic potential of Metformin, Glibenclamide, Glipizide, Vildagliptin and D-ribose-L-cysteine (riboceine) in comparison with Insulin in pregnant streptozotocin (STZ) -induced diabetic rats.

## 2. Material and method

### 2.1. Animals

Forty (40) Virgin female and twenty (20) male Sprague-Dawley (SD) rats weighing between 130–160 g were procured from Animal laboratory Centre of College of Medicine, University of Lagos. The Animals were housed in wire-mesh cages in the animal room of the Department of Anatomy of the College of Medicine, University of Lagos in 12:12 light–dark cycles at room temperature. All procedures guiding the use of the animals were in accordance with the standard international guidelines on the use of animals for research. Approval for the study was obtained from the Departmental Ethics Committee and also granted by the Health Research Ethics Committee on Animals Use, College of Medicine, University of Lagos, Nigeria with a protocol number CM/HREC/010/16/056. After 2 weeks of acclimatization the female SD rats were randomly distributed into 8 groups (A–H) of 5 each. Detachable sieves were placed underneath every cage for the collection and separation of urine and faeces.

### 2.2. Determination of cyclicity/mating

The oestrous cycle of each animal was characterized for two weeks, using vaginal lavage obtained between 8:00-9:00 am before the commencement of the experiment. Cyclicity was determined by the modification of the method previously reported by Bazzano et al. [30]. Briefly, fresh normal Saline was drawn into a fresh plastic Pasteur pipette which was inserted into the vaginal canal 1 mm deep and irrigated. The lavage was then smeared on a microscopic slide and viewed under microscope, before it dried. The presence of large nucleated cells with a few leucocytes on the slide was marked the pre-estrous day of the cycle. On the Pre-estrous day of each rat's cycle, a marked male was introduced into a marked female cage at a 1:2 ratio. These mating animals were left together overnight. Vaginal lavage was taken on the morning (estrous day of the cycle) following pairing between 8:00–9:00 a.m. The presence of spermatozoa in the lavage was marked as day 1 of pregnancy.

### 2.3. Induction of diabetes mellitus

STZ-induced diabetes mellitus was produced in a batch of normoglycaemic pregnant (Day 1 of gestation) SD rats (fasting blood glucose level of 75 ± 5 mg/dl). STZ freshly dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, was immediately injected intraperitoneally (60 mg/kg) [31]. This dose of streptozotocin (STZ) monohydrate (Sigma, St. Louis, MO, USA) produced diabetes mellitus after 24 h (Day 2 of gestation) of injection and this diabetic state is maintained throughout the experimental schedule.

Glucose levels were tested by using One Touch Ultra Mini Glucometer (Accu-Chek, Roche, Germany) with a drop of blood obtained by tail vein puncture. Rats with blood glucose values of 126 mg/dl in fasted state were considered diabetic.

### 2.4. Dosage of test Agents/Treatment

Treatment of animals began at Day 2 of gestation after the rats have been confirmed diabetic. Animals in group A remained as non-diabetic (negative) control and were administered 0.5 ml of distilled water,

while groups B–H were diabetic. Group B diabetic (positive control) received 0.5 ml of distilled water, Group C received 1 IU of insulin, Group D received 30 mg/kg of riboceine, Group E received 1.43 mg/kg of vildagliptin, group F received 0.29 mg/kg of glibenclamide, group G received 36.43 mg/kg of metformin and group H received 0.57 mg/kg of glipizide. Insulin was administered intraperitoneally while the rest of the animals were treated via oral gavage, once daily until the 19th day of gestation when the animals were euthanized. All drugs were purchased from a local pharmaceutical store in Lagos Nigeria.

### 2.5. Blood sample collection

At the end of experiment, animals were anesthetized by infusion of ketamine (60 mg/kg) and

xylazine (10 mg/kg). Blood was collected from each animal via cardiac puncture, centrifuged for 10 min at 3000 rpm for sera collection.

### 2.6. Oxidative stress markers analysis

#### 2.6.1. Processing and preparation of sample

The blood sample was collected into lithium heparin bottles and centrifuged at 3000 rpm for 10 min at 4 °C in a refrigerated centrifuge. The resulting supernatant (plasma) was used for the assay of activities of antioxidant enzymes and Malondialdehyde (MDA) concentrations.

The lipid peroxidation products were estimated by measuring TBARS and were determined by modifying the method of Niehaus and Samuelsson [32]. Antioxidants including Superoxide dismutase (SOD) and Catalase (CAT) were estimated by employing modified methods of, Rukmini et al. [33] and Sinha [34] respectively.

### 2.7. Reproductive hormones

The serum levels of progesterone (PROG), Oestrogen, luteinizing hormone (LH), Follicle stimulating hormone (FSH) and prolactin (PRL) were measured using commercially available enzyme-linked immunoassay kit (Abcam) according to manufacturer's instructions.

### 2.8. Weight measurement

Initial body weights of animals on the 2nd day of pregnancy and body weights on the 18$^{th}$ day of pregnancy were measured and represented accordingly. Likewise, foetal, placental, ovarian and empty uterine weights were measured using a sensitive electronic balance (Zeiss, West Germany (Pty) Ltd; 0.000 g).

### 2.9. Statistical analysis

Paired *t*-test was used for the initial and final weight difference using a Graphpad Prism 5.03. While one-way ANOVA and a Tukey posthoc Test was used for the rest of the analysis using IBM SPSS statistics 24.

## 3. Results

### 3.1. Effects of OHAs, Riboceine or insulin on body weights

There was a significant increase ($p < 0.0001$) in final body weight of non-diabetic negative control compared to their initial body weight. There was also a significant a significant decrease ($p < 0.0001$) in final body weight of diabetic positive control compared to their initial body weight. Whereas the riboceine, vildagliptin, glibenclamide, metformin, glipizide and Insulin groups had no significant difference in their initial and final weights as seen in Fig. 1.

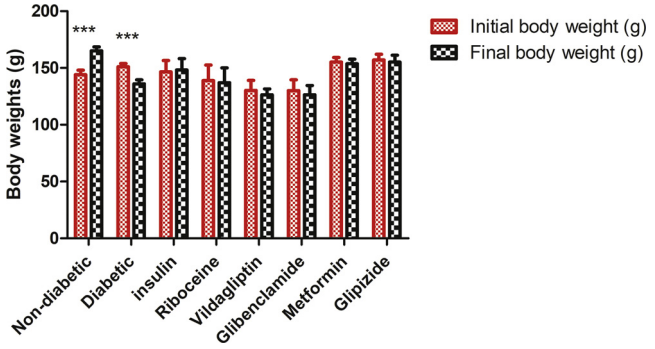


**Fig. 1.** Showing Initial and final body weights. ***$p < 0.0001$ within the group.

### 3.2. Effects of OHAs, Riboceine and insulin on blood glucose levels

The blood glucose levels increased significantly ($p < 0.0001$) after the induction of diabetes but after the administration of insulin, riboceine, vildagliptin, glibenclamide, metformin, and glipizide the blood glucose levels also decreased significantly ($p < 0.0001$) compared to the diabetic positive control (group B). There was an observable significant decrease ($p < 0.0001$) between the animals that received riboceine, and insulin compared to those that received vildagliptin, glibenclamide, metformin and glipizide. However, blood glucose levels also reduced further ($p < 0.05$) in the animals that received insulin compared to riboceine. There was a significant decrease ($p < 0.05$) in blood glucose levels of animals that received metformin, and glipizide compared to the animals that received vildagliptin, and glibenclamide as depicted in Figs. 2 and 3.

### 3.3. Effects of OHAs, Riboceine and insulin on reproductive hormones

Progesterone and Oestrogen levels decreased significantly ($p < 0.05$) in animals that received Insulin compared to non-diabetic control (group A). There was no significant difference in progesterone levels of animals that received Riboceine, vildagliptin, glibenclamide and metformin compared with group A, but it increased significantly ($p < 0.05$) compared to diabetic positive control and insulin groups. Oestrogen reduced significantly ($p < 0.05$) in all groups except in riboceine and metformin groups compared to negative and positive controls. Conversely, It increased significantly ($p < 0.05$) in riboceine compared to insulin, negative and positive control groups (Table 1).

LH levels increased significantly ($p < 0.05$) in insulin group compared to negative and positive control groups. However, those animals administered Riboceine, glibenclamide and glipizide had significantly decreased ($p < 0.05$) levels of LH compared to negative control. LH also increased significantly in vildagliptin group compared to positive control.

FSH decreased significantly ($p < 0.05$) in positive control and

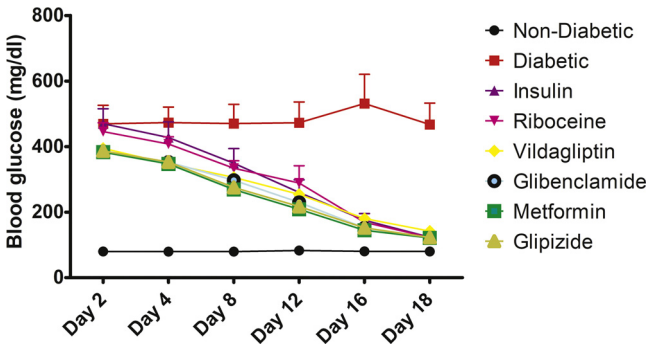


**Fig. 2.** Showing progressive changes in blood glucose levels.

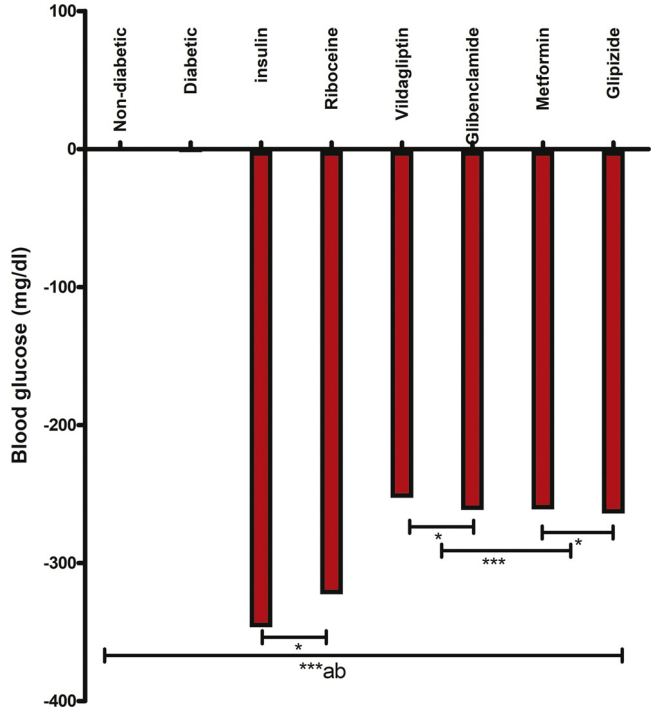


**Fig. 3.** Showing the difference in blood glucose levels between groups. *$p < 0.05$ between groups; ***$p < 0.0001$ between groups; ***ab $p < 0.0001$ comparing groups with non-diabetic negative control and diabetic positive control groups.

metformin groups compared with non-diabetic negative control. There was no significant difference in FSH of negative control and Riboceine groups, however, there was a significant increase of FSH in animals that received Riboceine compared to diabetic groups.

Prolactin levels decreased significantly ($p < 0.05$) in groups that received insulin, glibenclamide and glipizide compared to positive control. Prolactin levels in animals that received vildagliptin increased significantly compared to insulin, negative and positive control groups as seen in Table 1.

### 3.4. Effects of OHAs, Riboceine and insulin on maternal oxidative stress markers

MDA levels increased significantly in diabetic positive control (B), Riboceine (D), vildagliptin (E) and glibenclamide (F) groups compared to non- diabetic negative control group (A) as seen in Table 1. Whereas when compared to diabetic positive control group, those animals that received insulin, Riboceine, vildagliptin, glibenclamide, metformin and glipizide, MDA decreased significantly ($p < 0.05$).

CAT increased significantly ($p < 0.05$) in all the groups excluding Vildagliptin compared to negative control but on comparison with diabetic positive control group, CAT decreased ($p < 0.05$) in all groups.

SOD increased ($p < 0.05$) in all groups compared to negative control, and increased significantly ($p < 0.05$) in diabetic positive control compared to insulin, vildagliptin, glibenclamide and glipizide groups (Table 1).

### 3.5. Effects of OHAs, Riboceine and insulin on maternal reproductive organs

There was no statistically significant difference in foetal number across the groups. However, weight of foetuses decreased significantly ($p < 0.05$) in vildagliptin and metformin groups compared to non-diabetic and insulin groups. Whereas, weight of foetuses of animals that received glibenclamide increased significantly ($p < 0.05$) compared to non-diabetic, diabetic positive control and insulin groups (Table 1).

The weight of placenta of glibenclamide group decreased significantly ($p < 0.05$) compared to group insulin (Table 1). The weight of the empty uterus decreased significantly ($p < 0.05$) in diabetic positive control, riboceine and glibenclamide groups compared to insulin group. Whereas, animals that received metformin increased significantly ($p < 0.05$) compared to the non-diabetic negative and diabetic positive control groups (Table 1).

Weight of the ovaries increased significantly ($p < 0.05$) in diabetic positive control animals compared to non-diabetic negative control, insulin, metformin and glipizide groups. In addition, the weight of the ovaries of animals that received Riboceine, vildagliptin and glibenclamide increased significantly compared to those that received insulin. Likewise, vildagliptin group increased significantly compared to non-diabetic animals (Table 1).

There was a significant increase ($p < 0.05$) in crown-rump lengths the foetuses from glipizide group compared to insulin. The other foetuses had no significant difference (Table 1).

## 4. Discussion

The toxic effects of STZ on the pancreatic beta cells has been reported [35,36]. Thus, these effects lead to a decline in insulin production leading to less cellular absorption of glucose. Consequently, hyperglycemia will lead to the increase in reactive oxygen species which will lead to glucose toxicity further destroying pancreatic beta cells [37,38]. It is on this basis that Insulin is used to treat patients with T1DM [39]. to prevent ravaging effects of glucose toxicity.

Similarly, T2DM which results from insensitivity of cells to insulin will also lead to hyperglycemia, and glucose intolerance. Thus, leading to glucose toxicity, increased ROS and beta cells apoptosis. Hence, the combination of chemical substances that will aid the cellular sensitivity to insulin will ameliorate and prevent glucose toxicity [40].

This study mimicked DM in pregnant women using pregnant SD rats by knocking off pancreatic beta cells using STZ to create a hyperglycaemic condition and treating this condition using insulin, riboceine, vildagliptin, glibenclamide, metformin, and glipizide.

In this study, the decrease in body weights of the animals under the influence of streptozotocin must be due to the cytotoxic effects of streptozotocin on cells. It has been reported that streptozotocin increases necrosis of pancreatic beta cells thereby resulting in insulinopenia and a concomitant hyperglycaemic condition [41]. This excess glucose can undergo autoxidation and generate OH radicals [42]. In addition, glucose can react with proteins in a non-enzymatic manner leading to the development of Amadori products followed by the formation of advanced glycation end-products AGEs; ROS is generated at multiple steps during this process [43]. Elevated lipid peroxidation in tissues will result in a concomitant decrease in body weights [26] as seen in this study. Insulin, Riboceine and the OHAs used maintained the body weights of the animals protecting them from the cytotoxic effects of streptozotocin. This suggests a possibility of these substances to either improve pancreatic beta cells function or prevent lipid peroxidation by impairing the formation of ROS or increasing the production of antioxidants to neutralize ROS. Metformin has been reported to reduce hepatic glucose output and improves insulin sensitivity [44,45]. Vildagliptin improves β cell sensitivity to glucose which results in glucose-sensitive modulation of insulin secretion, improving both fasting and postprandial glycaemia [45,46].

Increased oxidative stress and changes in antioxidant capacity as observed in this study has been implicated in the aetiology of chronic diabetes complications [25,26]. This study demonstrated that streptozotocin increased lipid peroxidation, though this was with a concomitant increase in antioxidant enzymes, the antioxidant enzymes were not sufficient to prevent lipid peroxidation or oxidative stress from occurring. This confirms some level of dependence on non-enzymatic

**Table 1**
Effects of Riboceine, OHAs and insulin on reproductive hormones, maternal oxidative stress markers and maternal reproductive organs.

| | A (Non-diabetic) | B (Negative control) | C (Insulin) | D (Riboceine) | E (Vildagliptin) | F (Glibenclamide) | G (Metformin) | H (Glipizide) |
|---|---|---|---|---|---|---|---|---|
| Effects of Riboceine, OHAs, and Insulin on Reproductive Hormones | | | | | | | | |
| Prog (pg/ml) | 21.75 ± 3.23 | 16.90 ± 0.44 | 13.88 ± 1.77[a] | 24.67 ± 4.27 [b,c] | 22.99 ± 2.51[b,c] | 22.16 ± 5.73[b,c] | 23.74 ± 8.30[b,c] | 15.24 ± 2.02[b] |
| Oestrogen (pg/ml) | 46.10 ± 11.02 | 14.52 ± 0.81[a] | 21.16 ± 0.78 [a] | 94.43 ± 11.30[a,b,c] | 20.98 ± 0.84[a] | 21.05 ± 0.82[a] | 41.22 ± 27.05[b,c] | 21.95 ± 1.45[a] |
| LH (mlU/ml) | 0.42 ± 0.16 | 0.14 ± 0.05[a] | 0.56 ± 0.09 [a,b] | 0.14 ± 0.05 [a,c] | 0.60 ± 0.35[b] | 0.18 ± 0.08[a,c] | 0.32 ± 0.23[c] | 0.16 ± 0.09[a,c] |
| FSH (mlU/ml) | 1.00 ± 0.16 | 0.20 ± 0.07[a] | 0.72 ± 0.42 | 1.00 ± 0.16[b] | 0.56 ± 0.45 | 0.58 ± 0.36 | 0.26 ± 0.09[a] | 1.90 ± 1.14[a,b,c] |
| Prolactin (ng/ml) | 1.00 ± 0.14 | 1.18 ± 0.08 | 0.42 ± 0.15[b] | 1.00 ± 0.16 | 2.18 ± 1.72[a,b,c] | 0.28 ± 0.13[a,b] | 0.54 ± 0.09 | 0.28 ± 0.08[a,b] |
| Effects of Riboceine, OHAs, and Insulin on Maternal Oxidative Stress Markers | | | | | | | | |
| SOD (min/mg/protein) | 45.13 ± 7.03 | 94.23 ± 3.74[a,c] | 77.20 ± 9.58[a,b] | 84.78 ± 3.04[a] | 79.24 ± 12.89[a,b] | 77.26 ± 7.08[a,b] | 86.92 ± 6.77[a] | 77.64 ± 11.49 [a,b] |
| CAT (min/mg/protein) | 428.42 ± 91.89 | 816.46 ± 42.12[a,c] | 678.45 ± 52.71[a,b] | 670.06 ± 35.84[a,b] | 528.34 ± 62.29[b,c] | 623.09 ± 39.02[a,b] | 629.33 ± 91.33[a,b] | 625.64 ± 103.00[a,b] |
| MDA (nmol/ml) | 0.65 ± 0.32 | 4.95 ± 0.52[a,c] | 1.67 ± 0.25[b] | 2.60 ± 0.10[a,b] | 2.52 ± 0.29[a,b] | 5.69 ± 1.58[a,c] | 1.51 ± 0.49[b] | 1.86 ± 0.66[b] |
| Effects of Riboceine, OHAs, and Insulin on Maternal Reproductive Organs | | | | | | | | |
| Number of Foetuses | 9.40 ± 2.36 | 9.40 ± 1.84 | 8.80 ± 2.25 | 9.20 ± 3.54 | 8.00 ± 2.32 | 9.20 ± 2.30 | 8.00 ± 2.32 | 8.20 ± 2.61 |
| Weight of foetus (g) | 3.09 ± 0.15 | 3.02 ± 0.25 | 2.95 ± 0.27 | 2.81 ± 0.11 | 2.52 ± 0.08[a,c] | 3.56 ± 0.19[a,b,c] | 2.54 ± 0.43[a,c] | 2.67 ± 0.46 |
| Weight of placenta (g) | 0.64 ± 0.07 | 0.63 ± 0.06 | 0.68 ± 0.08 | 0.64 ± 0.05 | 0.70 ± 0.08 | 0.58 ± 0.06[c] | 0.64 ± 0.06 | 0.65 ± 0.05 |
| Weight of empty uterus (g) | 0.66 ± 0.11 | 0.64 ± 0.06[c] | 0.73 ± 0.05[b] | 0.65 ± 0.10[c] | 0.69 ± 0.07 | 0.65 ± 0.04[c] | 0.74 ± 0.10[a,b] | 0.71 ± 0.06 |
| Weight of ovaries (g) | 0.29 ± 0.06 | 0.38 ± 0.11[a,c] | 0.24 ± 0.02[b] | 0.35 ± 0.05[c] | 0.39 ± 0.10[a,c] | 0.52 ± 0.08[a,b,c] | 0.23 ± 0.02[b] | 0.24 ± 0.02[b] |
| Crown rump length (cm) | 4.14 ± 0.263 | 4.02 ± 0.35 | 3.70 ± 0.532 | 4.10 ± 0.294 | 4.14 ± 0.350 | 4.16 ± 0.556 | 3.97 ± 0.478 | 4.27 ± 0.427[c] |

A (Non-diabetic), B (Diabetic + Distilled water), C (Diabetic + Insulin), D (Diabetic + 30 mg/kg of Riboceine), E (Diabetic + 1.43 mg/kg of Vildagliptin), F (Diabetic + 0.29 mg/kg of Glibenclamide), G (Diabetic + 36.43 mg/kg of Metformin), H (Diabetic + 0.57 mg/kg of Glipizide).
(PROG: Progesterone, E2: Estradiol, LH: Luteinizing hormone, FSH: Follicle stimulating hormone, PRL: Prolactin, SOD: superoxide dismutase; Cat: Catalase: MDA: Malondialdehyde).

[a] Significantly ($p < 0.05$) different from Negative control group.
[b] Significantly ($p < 0.05$) different from diabetic positive control group.
[c] Significantly ($p < 0.05$) different from insulin group.

enzymes in the prevention of oxidative stress [25,47,48]. In our previous study, Riboceine attenuated aluminium –induced testicular damage [49]. This corroborates with the findings of this study, in addition, all the OHAs used in this study exemption of glibenclamide had similar effects with insulin in impeding lipid peroxidation. Balsells et al. [50] Meta-analytical study reported untoward effects of glibenclamide in gestational diabetes. The elevated levels of oxidative stress markers has been associated with hyperglycaemia which is due to the shortfall in insulin as a result of beta cells dysfunction [51,52]. This anomaly has been associated with the complications reported in DM including cardiovascular diseases. [26,53,54]. These negative effects of streptozotocin were ameliorated by riboceine, vildagliptin, metformin and glipizide as well as insulin. Since streptozotocin is known to destroy pancreatic beta cells leading to a concomitant increase in glucose availability- hyperglycemia [41], we speculate that the possible mechanism of action of riboceine, vildagliptin, metformin and glipizide is mediated through influencing glucose uptake/utilization by tissues and probably regeneration of beta cells. Protracted hyperglycemia may result in glucose toxicity and increase in ROS activity as well as increased lipid peroxidation especially in the pancreas which lacks sufficient antioxidants. This is in tandem with the increased blood MDA levels of the diabetic positive controls in this study. Furthermore, the decreased MDA levels and the concomitant increase in CAT and SOD antioxidants as seen in animals that received Riboceine, vildagliptin, metformin and glipizide shows an abrogation of cellular redox. N-acetyl-L-cysteine has been reported to neutralize ROS and preventing glucose toxicity [55]. Antioxidants are important for the prevention of pancreatic beta cells apoptosis [56]. Riboceine an analog of L-cysteine has been reported to improve the delivery of L-cysteine (a limiting component for glutathione synthesis) in the liver, thus, increasing glutathione levels [57,58].

Lebovitz and Feinglos [59], reported that mechanism for glipizide`s activity is significantly greater on peripheral uptake of glucose than suppression of hepatic glucose production. On the contrary, metformin lowers the fasting plasma glucose and insulin concentrations, improves oral glucose tolerance, and decreases plasma lipid levels independent of changes in body weight. The improvement in fasting glucose results from a reduction in basal hepatic glucose production. Metformin per se does not enhance tissue sensitivity to insulin in DM subjects [60]. Vildagliptin also inhibits hepatic glucose production, mainly through changes in islet hormone secretion, and improves insulin synthesis and sensitivity, as determined with a variety of methods [61]. These effects underlie the improved glycaemia with low risk for hypoglycaemia.

Riboceine, insulin and streptozotocin had no observable changes in the foetal weights. This may be due to effective placental barrier regulation of exogenous and endogenous maternal-foetal exchange [62]. The significant increase of foetal, and ovarian weight by glibenclamide may be signs of fatty accumulation in relation to glibenclamide which further disapproves its usage in the management of pregnancy in diabetes. This study did not show any anomaly in crown-rump lengths. Howbeit, the lack of evidence of anomaly in the foetuses does not necessarily translate to evidence of lack of anomaly, more foetal parameters should be assessed.

The significant decrease in oestrogen levels in diabetic positive control animals depicts a reduction in the synthesis of oestrogen by the ovarian follicles [63]. We speculate that the decreased oestrogen levels affected the formation of trophoblast decreasing the overall progesterone of pregnancy [64]. This negative feedback of oestrogen downregulated the synthesis of FSH and LH in the anterior pituitary gland [65,66]. This study also showed a significant increase in oestrogen in diabetic pregnant rats that received riboceine, and metformin as compared to insulin. This signifies the varying mechanisms of action by which the trio operate. The increased antioxidant levels by riboceine and metformin will enhance the production of FSH and LH by the anterior pituitary gland [67] which promotes follicle development and oestrogen synthesis. Moreover, oestrogen has also been reported to increase superoxide dismutase and total antioxidant capacity [68]. The interplay between insulin and oestrogen synthesis requires more illumination. Albeit, oestrogen deficiency and insulin resistance has been reported as concomitant disorders in T2DM patients [69]. It is therefore suggestive that insulin antagonises oestrogen receptors, the compensatory effects downregulates oestrogen synthesis.

## 5. Conclusion

To the best of our knowledge, this study is the first to introduce the potential benefits of D-ribose-L-cysteine (riboceine) as an effective oral hypoglycaemic agent which could serve as a potent adjuvant in the management of diabetes in pregnancy. We therefore suggest further studies and possible clinical trials on riboceine. In addition, this study highlights the possibility of glibenclamide leading to overweight of foetus thereby endangering mother`s life. Other antidiabetic agents including insulin, vildagliptin, metformin and glipizide also showed good potentials of mitigating diabetes in pregnancy.

## Conflict of interest

None.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2018.08.003.

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