Effect of Different Doses of Unripe Plantain (Musa Paradisiaca) or their Combinations with Ginger (Zingiber Officinale) on some Biochemical Parameters, Hepatic and Pancreatic Histology of Streptozotocin induced Diabetic Rats


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Abstract
Background: To determine the effect of different doses of unripe plantain (200, 400 and 600 g/kg) or their combinations with ginger (25 g/kg) on some biochemical parameters, hepatic and pancreatic histology of streptozotocin induced diabetic rats, and to analyze the bioactive constituents of the diets using standard techniques. Method. Following induction of diabetes mellitus, forty-five male albino rats (6 weeks old) were divided into nine groups of five rats each as follows: Groups 1 and 2 (control and diabetic) were fed rat pellets; group 3 (diabetic rats fed with 200 g/kg unripe plantain incorporated feed (UPIF)); group 4 (diabetic rats fed with 400 g/kg UPIF); group 5 (diabetic rats fed with 600 g/kg UPIF); group 6 (diabetic rats fed with 200 g/kg unripe plantain + 25 g/kg ginger incorporated feed (GIF)); group 7 (diabetic rats fed with 400 g/kg unripe plantain + 25 g/kg GIF); group 8 (diabetic rats fed with 600 g/kg unripe plantain + 25 g/kg GIF) and group 9 (diabetic rats fed rat pellets and administered glibenclamide (2.5 mg/kg body weight). The study lasted for 28 days. Results. STZ administration induced hyperglycemia, systemic oxidative stress, inflammation and also altered the liver and pancreas histology of the rats. Conclusion. Supplementation with unripe plantain (400 g/kg) and ginger (25 g/kg) diet mitigated most of these changes better than other treatment diets or glibenclamide which could be attributed to the bioactive compounds in it as seen from the Gas Chromatography Mass Spectrophotometric analysis.

Categories: Endocrinology/Diabetes/Metabolism, Pathology, Nutrition
Keywords: phytochemicals, nutrition, inflammatory mediators, oxidative stress, hyperglycemia

Introduction
Diabetes mellitus (DM) is a group of metabolic diseases that is characterized by hyperglycemia due to insufficient production of insulin or its inefficient utilization by the body [1, 2]. A total of 425 million adults have been estimated to be living with DM worldwide, and this is estimated to rise to 629 million patients by 2045 [3].

In Nigeria, the current prevalence of DM among adults aged 20-69 years is reported to be 1.7% [3]. It is widely perceived that prevalence figures reported by the International Diabetes Federation (IDF) grossly under-report the true burden of DM in Nigeria, given that they are derived through the extrapolation of data from other countries. In modern medicine, no satisfactory effective therapy is still available to cure DM [4]. In addition, the currently used therapies for the treatment of DM (eg: insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and glinides) are costly and have undesirable side effects [5].

Because of this, research on natural products with anti-diabetic properties has been on the increase especially due to the lesser side effects of natural products compared with conventional therapies [6].

Plantain (Musa paradisiaca) is a tropical plant native to South East Asia and India. That is consumed as an energy yielding food. Plantain is ranked third after yam and cassava for sustainability in Nigeria [7, 8]. It is a rich source of carbohydrates, iron, potassium and vitamin A, but low in fat and protein [9]. Its anti-diabetic properties in experimental animals have been reported [10, 11].

Ginger (Zingiber officinale) is one of the most important and popular spices that is widely utilized to add flavor to foods, in addition to its nutritional and medicinal benefits. It is indigenous to South-East Asia but is also cultivated in various countries around the world [12], as well as in the tropics for its edible rhizome. The anti-diabetic properties of ginger in experimental animals have also been reported [13]. In many homes in Nigeria and in different parts of Africa, it is common to see ginger being added as a spice (at doses not...
exceeding 30%) in different foods such as plantain porridge, rice, beans, yam, etc.

In our earlier study on the possibility of a dietary combination of unripe plantain and ginger for the management of DM in experimental rats [13], our study found that a combination of unripe plantain and ginger (at the ratio, 710: 10 g/kg) could not mitigate hyperglycemia, induced by streptozotocin (STZ) in experimental rats, and we attributed this to the ratio of the combination of unripe plantain with ginger. Moreover, the effect of combined feeding of unripe plantain and ginger on systemic oxidative stress and inflammatory markers in diabetic humans or animals has not been reported in literature, and oxidative stress and inflammation have been implicated in the development of DM and its complications.

In a bid to further explore the possibility of achieving glycemic control with a combination of unripe plantain and ginger, and to understand the biochemical basis for the glycemic control, we varied the doses of combination of unripe plantain and ginger. Therefore, the objectives of this study were as follows:

Objectives of the study:

To determine the effect of combination of unripe plantain and ginger (at varying doses) on the body weights, relative liver and pancreas weights of STZ induced diabetic rats

To analyze the effect of the dietary combination on the blood glucose, insulin, homeostatic model assessment of insulin resistance (HOMA-IR) and homeostatic model assessment of pancreatic β-cell (HOMA-β) function of the rats

To investigate the effect of the diets on the total proteins, antioxidant and oxidative stress indices (superoxide dismutase, (SOD), glutathione peroxidase (GPx), catalase (CAT), reduced glutathione (GSH), malondialdehyde (MDA), inflammatory markers (tumor necrosis factor alpha (TNF-α), interleukins 6 and 10) and hepatic function markers (aspartate amino transaminase, AST), alanine amino transaminase (ALT) and alkaline phosphatase (ALP) in the sera of the rats.

To determine the effect of the diets on the liver and pancreas histology of the rats.

To determine the bioactive constituents in the rat feeds and most effective test feeds using gas chromatography mass spectrophotometry (GCMS).

Materials And Methods

Chemicals

The chemicals/reagents that were used for this study include: streptozotocin (Sigma and Aldrich, USA), formalin (May and Baker, England), Chloroform (AR) [Guangdong Guangha] Company Ltd), Nicotinamide adenine dinucleotide phosphate reduced, Nitrotetrazolium Blue Chloride, L-Glutathione reduced, L-methionine, urea hydrogen peroxide and ammonium molybdate (Sigma and Aldrich, USA). Other chemicals that were used for this study, which were not stated here, were also of analytical grade.

Selection of plant materials

The unripe plantain (variety- Seed Plantain) and ginger rhizomes were obtained from Akpan Ndem Market in Uyo, Akwa Ibom State, Nigeria. These were identified by Mr Anthony Asuquo Udoekong of the Forestry Department, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

Processing of plant materials

The samples were washed, cut into small pieces, air dried for 48 h to a constant weight and then ground into fine powder. They were subsequently pelletized using the feed formulation shown in Table 2.1, oven dried at 70°C to a constant weight before being fed to rats. The composition of the unripe plantain and ginger-based diets is shown in Table 1.

Ingredients used in the formulation

Corn oil

The corn oil that was used was bought from a supermarket in Uyo, Akwa Ibom State, Nigeria and it was a product of Federated Group, Inc. IL 60005, USA (www.myparadebrand.com).

Defatted soybean flour

The soy beans that were used were bought from Akpan Ndem Market in Uyo, Akwa Ibom State, Nigeria.
Contaminants in the soybeans were removed. Thereafter, they were dehulled by soaking in tap water at room temperature for 12 hours, followed by hand-rubbing to remove the testa. The floating testa was removed by decanting the water until no testa was present. Thereafter, the dehulled seeds were boiled for 30 mins with tap water at 100°C, to inactivate trypsin inhibitor that is present in soybean seeds. The boiled samples were then dried in an oven (Blast air oven, KX350A, Kenxin International Co., Ltd) at 70°C to constant weight. They were then ground in a mill and defatted using N-hexane by cold extraction method. Thereafter, the defatted soybean flour produced was dried in the oven at 60°C to constant weight, cooled, and packaged in airtight plastic container until its usage for dietary formulation.

Vitamin/mineral mixture

The vitamin/mineral mixture that was used was purchased from a Company (Unfailing Vet. Services) in Uyo, Akwa Ibom State, Nigeria.

Animals

Seventy-two adult male albino rats of the wistar strain obtained from the animal house of the Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria, were used for the study. The animals were kept in ventilated cages. They were acclimatized for two weeks prior to the commencement of the experiment and maintained under standard conditions. Ethical approval for this study was granted by the Board of the Department of Biochemistry, University of Uyo, Uyo, Akwa Ibom State, Nigeria. The National Institutes of Health Principles of Laboratory Animal Care [14] were observed.

Induction of diabetes

Freshly prepared solution of streptozotocin (0.1 g dissolved in 5 mL of freshly prepared sodium citrate buffer 0.1 M, pH 4.5) was injected intraperitoneally to 67 of the rats at a single dosage of 65 mg/kg body weight at fasting state while the remaining rats (5) served as the control group. Blood was collected from the tail and base line blood glucose concentrations in the STZ administered rats were measured at fasting state prior to STZ administration and thereafter at day three post STZ administration. The STZ administered rats with fasting blood glucose concentrations ≥ 200 mg/dL three days post STZ induction were considered to be diabetic and were used for the study [15].

Experimental procedure

The diabetic rats were then divided into eight subgroups (groups 2 to 9) comprising of five rats per group while the control formed the first group as follows:

Group 1. Normal rats fed standard rat pellets (Control)
Group 2. Diabetic rats which also received standard pellets (Diabetic group)
Group 3. Diabetic rats fed with 200 g/kg unripe plantain incorporated feed
Group 4. Diabetic rats fed with 400 g/kg unripe plantain incorporated feed
Group 5. Diabetic rats fed with 600 g/kg unripe plantain incorporated feed
Group 6. Diabetic rats fed with 200 g/kg unripe plantain + 25 g/kg ginger incorporated feed
Group 7. Diabetic rats fed with 400 g/kg unripe plantain + 25 g/kg ginger incorporated feed
Group 8. Diabetic rats fed with 600 g/kg unripe plantain + 25 g/kg ginger incorporated feed
Group 9. Diabetic rats fed standard rat pellets and administered glibenclamide (2.5 mg/kg body weight) (Positive control).

All administrations were done once daily with ad libitum provision of food and water throughout the experimental period. The body weights and feed intakes of the rats were recorded on a daily basis using an electronic weighing balance (Model Scout Pro, Ohaus Corporation, USA). Rats were subjected to overnight fast on the 28th day and on the 29th day, their final body weights and fasting blood glucose concentrations were determined using a blood glucose meter (Fine test glucometer). Thereafter, blood was drawn from their hearts under chloroform anaesthesia into non-anticoagulant tubes and allowed to clot. Serum was obtained from the clotted blood samples following centrifugation at 3,000 x g for 10 min and it was analyzed for AST,
ALT, ALP, total protein, MDA, GSH, SOD, CAT, GPx, TNF-alpha, IL-6 and IL-10 concentrations. The liver, and pancreas were removed, weighed and fixed in 10% neutral buffered formaldehyde for histopathological studies.

The relative organ weights of the rats were calculated and reported as g/100 g as follows:

Relative liver weight (g/100 g) = Total liver weight x 100
Final body weight

Relative pancreas weight (g/100 g) = Total pancreas weight x 100
Final body weight

The percentage change in fasting blood glucose (FBG) was calculated as:

Percentage change in FBG = Final FBG - Initial FBG x 100
Final FBG

The percentage change in weight was calculated as:

Percentage change in weight = Final weight - Initial weight x 100
Final weight

Assay of serum total proteins

The total protein content in the sera of the rats was determined using assay kits from Agappe Diagnostics Ltd [16].

Assay of serum liver enzymes

The AST, ALT and ALP activities in the sera of the rats were estimated following the method of Reitman and Frankel [17].

Assay of antioxidant and oxidative stress markers in the sera

Superoxide dismutase activity was determined using the method of Sun and Zigma [18]. The assay was based on the ability of SOD to inhibit the auto-oxidation of epinephrine which was measured by an increase in absorbance at 480 nm. Results were reported as units/mL. Catalase activity was determined according to the method of Sinha [19] and results were reported as units per mL. GPx activity was determined using the method of Rotruck et al. [20] and results were reported as units per mL. Reduced glutathione content as non-protein sulfhydryl was estimated according to the method described by Sedlak and Lindsay [21] and results were reported as µmol/mL. Malondialdehyde concentration was determined using the method of Buege and Aust [22] and results were also reported as µmol/mL.

Determination of serum insulin concentration, insulin sensitivity, insulin resistance index and pancreatic β-cell function

The serum insulin concentration was determined using assay kits from Monobind Inc [23].

Homeostatic model assessment (HOMA-IR and HOMA-β) scores were determined mathematically using fasting insulin and final blood glucose levels according to the method of Matthews et al. [24], as shown below:

\[
\text{HOMA - IR} = \frac{\text{Insulin (μIU/mL) x Blood glucose (mmol/L)}}{22.5}
\]

\[
\text{HOMA - β} = 20 \times \text{Insulin (μIU/mL)} - 3.5
\]

Blood glucose (mmol/L)
Assay of inflammatory markers in the sera

The TNF-α, IL-6 and IL-10 concentrations in the sera of the rats were determined using ELISA kits from Bioassay Technology Laboratory (BT Lab) and results were reported as ng/L for TNF-α and IL-6 and pg/mL for IL-10.

Histopathological studies

After fixation, the tissues were embedded in paraffin; solid sections were cut at 5 µm and stained with haematoxylin and eosin. The sections were then be examined under a light microscope and their photomicrographs were be taken [25].

Phytochemical analysis of rat and test feed

The gravimetric method of Harbone [26] was used to determine the alkaloid contents of the rat feed and unripe plantain (400 g/kg) + ginger (25 g/kg) feed while the method of AOAC [27] was used to determine the flavonoid, saponin, and tannin contents of the rat feed and unripe plantain (400 g/kg) + ginger (25 g/kg) feed.

GCMS analysis of rat and test feeds

Plant extraction prior to GCMS analysis was carried out using the method of Al Harthi et al. [28] with modification. To five grams of the flours of the rat feed and unripe plantain (400 g/kg) + ginger (25 g/kg) feed, was added 200 mL of absolute ethanol and the mixture was left overnight in a container that was tightly covered. The next day, the mixture was filtered with a muslin cloth. The filtrate was centrifuged for 10 min at 4000 g and the supernatant was analyzed for the bioactive compounds in it using GCMS.

Analysis of the bioactive compounds in the rat feed and unripe plantain (400 g/kg) + ginger (25 g/kg) feed was done using the method of Ganesh and Mohankumar [29] with modifications. The GC-MS analysis was done using a SHIMADZU gas chromatography (model: 5890-11, Japan) interfaced with a mass spectrophotometer equipped with a GC column (OV-101) and coated with polymethyl silicon (0.25nm x 50m). For GC-MS quantitation, an electron ionization system with ionizing energy of 70 eV and helium (as the carrier gas) at a flow rate of 1 mL/min, with an injection volume of 2 µL of the extract, which was injected into the chromatogram at a split ratio of 1:75. The injector temperature was set at 250oC, the temperature of the ion source was maintained at 200oC. The oven temperature was programmed to increase from 110oC to 200oC at 10 C/min, and to 280oC at 5 C/min, and it was held at this temperature for 10 min. The scan parameters of the MS included electron impact ionization voltage of 70 eV and a mass range of 50-500 m/z.

Statistical analysis

Statistical analysis was carried out using the statistical package for social sciences (SPSS) software, version 23.0 (SPSS, Inc., Chicago, IL, USA). Data obtained were reported means ± standard deviation. One-way analysis of variance (ANOVA) followed by Duncan Multiple Range test was used for comparisons of means. Results were considered to be significant at P<0.05.

Results

Fasting blood glucose concentrations of rats

Table 2 shows the fasting blood glucose concentrations of the studied rats. At the end of the experiment, the diabetic group had a significant increase (p<0.05) in their fasting blood glucose concentrations when compared to the control. In contrast, the diabetic rats fed with unripe plantain (200, 400 and 600 g/kg) or unripe plantain (200, 400 and 600 g/kg) and ginger (25 g/kg) had a significant reduction (p<0.05) in their fasting blood glucose concentrations when compared to the diabetic group at the end of the experiment. The diabetic rats treated with glibenclamide also had a significant reduction (p<0.05) in their fasting blood glucose concentrations at the end of the experiment, when compared to the diabetic group (Table 2).

Serum insulin concentration, HOMA-IR and HOMA-β in rats

Table 3 shows the serum insulin concentration, HOMA-IR and HOMA-β values in the studied rats. There were significant decreases (p<0.05) in the serum insulin concentrations of the diabetic group compared with the control. Whereas there were no significant differences (p>0.05) in the serum insulin concentrations of the diabetic rats fed with unripe plantain (200, 400 and 600 g/kg) or unripe plantain (200 and 400 g/kg) and ginger (25 g/kg) compared with the diabetic group, there were significant increases (p<0.05) in the serum
insulin concentrations of the diabetic rats fed with unripe plantain (600 g/kg) and ginger (25 g/kg) as well as the diabetic rats treated with glibenclamide compared with the diabetic group (Table 3). There were significant increases (p<0.05) in the HOMA-IR values in the diabetic group compared with the control while significant decreases (p<0.05) were obtained in the HOMA-IR values of the diabetic rats fed with unripe plantain (200, 400 and 600 g/kg) or unripe plantain (200, 400 and 600 g/kg) and ginger (25 g/kg) compared with the diabetic group. In contrast, there were no significant differences (p>0.05) in the HOMA-IR values of the diabetic rats treated with glibenclamide compared with the diabetic group (Table 3).

There were significant decreases (p<0.05) in the HOMA-IR value of the diabetic rats fed with unripe plantain (200 and 400 g/kg) or treated with glibenclamide compared with the diabetic group. Further, there were significant increases (p<0.05) in the HOMA-IR values of the diabetic rats fed with unripe plantain (600 g/kg) or unripe plantain (200, 400 and 600 g/kg) and ginger (25 g/kg) compared with the diabetic group (Table 3).

**Body weights of rats**

Table 4 shows the body weights of the studied rats. As shown in Table 4, there were no significant differences (P>0.05) in the body weights of the rats at the beginning of the study. At the end of the study, there were significant decreases (p<0.05) in the body weight of the diabetic rats compared with the control. Additionally, there were no significant differences (p>0.05) in the final body weight of the diabetic rats fed with unripe plantain (200 g/kg) or unripe plantain (200, 400 and 600 g/kg) + ginger (25 g/kg), or treated with glibenclamide compared with the disease group. In contrast, the final body weights of the diabetic rats fed with unripe plantain (400 and 600 g/kg) were significantly increased (P<0.05) compared with the diabetic group. In addition, the final body weights of the diabetic rats fed with 400 and 600 g/kg unripe plantain, 400 g/kg unripe plantain + 25 g/kg ginger feeds, or treated with glibenclamide were not significantly different (p>0.05) from that of the control.

The control had 18% increase in body weight; the diabetic group had -4.82 decrease in body weight; the diabetic rats fed with 200, 400 and 600 g/kg of unripe plantain recorded 3.16, 9.42 and 8.22 increases in body weights respectively. Whereas the diabetic rats fed with 200 g/kg unripe plantain + 25 g/kg ginger, 600 g/kg unripe plantain + 25 g/kg ginger, or treated with glibenclamide had 5.36%, 6.88% and 2.10% decreases in body weights, the diabetic rats fed with 400 g/kg unripe plantain + 25 g/kg ginger recorded 1.34% increase in body weight.

**Relative liver and pancreas weights of rats**

Table 5 shows the relative organ weights of the rats that were studied. There were significant increases (p<0.05) in the relative liver weights of the diabetic group when compared with the control. Further, there were no significant differences (p>0.05) in the relative liver weights of the diabetic rats fed with unripe plantain (200 g/kg), unripe plantain (200 g/kg) + ginger (25 g/kg) or the diabetic rats treated with glibenclamide when compared with the diabetic group. In contrast, the relative liver weights of the diabetic rats fed with unripe plantain (400 and 600 g/kg) or unripe plantain (400 and 600 g/kg) + ginger (25 g/kg) were significantly decreased (P<0.05) compared with the diabetic group.

As shown in Table 5, there were no significant differences (p>0.05) in the relative pancreas weights of the diabetic group compared with the control. There were also no significant differences (p>0.05) in the relative pancreas weights of the test groups compared with the diabetic group.

**Inflammatory markers in the sera of rats**

Table 6 shows the inflammatory markers in the sera of the rats that were studied. As presented in the table, there were significant increases (p<0.05) in the concentrations of TNF-α in the sera of the diabetic group when compared with the control. On the other hand, there were significant decreases (p<0.05) in the concentrations of TNF-α in the sera of the test groups or the diabetic rats treated with glibenclamide, compared with the diabetic group.

There were significant increases (p<0.05) in the concentrations of IL-6 in the sera of the diabetic group when compared with the control. In contrast, significant decreases in the concentrations of IL-6 in the sera of the test groups were obtained when compared with the diabetic group. Further, the serum IL-6 concentrations of the diabetic rats fed with 400 and 600 g/kg unripe plantain, 400 g/kg unripe plantain + 25 g/kg ginger, 600 g/kg unripe plantain + 25 g/kg ginger and the diabetic rats treated with glibenclamide, were not significantly different (P>0.05) from that of the control.

Data presented in Table 6 showed significant elevation (p<0.05) in the serum concentrations of IL-10 in the diabetic group when compared with the control. Whereas the serum IL-10 concentrations of the diabetic rats fed with 200 g/kg unripe plantain or 200 unripe plantain + 25 ginger were not significantly different (P>0.05) from that of the diabetic group, the serum IL-10 concentration of the diabetic rats fed with 400 and 600 g/kg
unripe plantain, 400 g/kg unripe plantain + 25 g/kg ginger, 600 g/kg unripe plantain + 25 g/kg ginger and the diabetic rats treated with glibenclamide, were significantly decreased (P<0.05) in relation to the diabetic group. In addition, the serum IL-10 concentrations of the rats in the 400 and 600 g/kg unripe plantain, 400 g/kg unripe plantain + 25 g/kg ginger, 600 g/kg unripe plantain + 25 g/kg ginger and the glibenclamide groups were not significantly different (P>0.05) from that of the control.

Antioxidant markers in the sera of rats

Table 7 shows the antioxidant markers in the sera of the rats that were studied. As shown in Table 7, there were significant increases (p<0.05) in the SOD, GPx and CAT activities in the sera of the diabetic group compared with the control, but significant decreases (p<0.05) were obtained in the SOD, GPx and CAT activities in the sera of the diabetic rats fed with unripe plantain (200, 400 and 600 g/kg) or unripe plantain (200, 400 and 600 g/kg) and ginger (25 g/kg) or the diabetic rats treated with glibenclamide, compared with the diabetic group. Furthermore, there were significant increases (p<0.05) in the serum GSH concentrations of the diabetic group compared with the control. Whereas there were no significant differences (p>0.05) in the GSH concentrations of the diabetic rats fed with unripe plantain (200, 400 and 600 g/kg) or unripe plantain (200, 400 and 600 g/kg) and ginger (25 g/kg) compared with the diabetic group, there were significant decreases (p<0.05) in the GSH concentrations of the diabetic rats treated with glibenclamide compared with the diabetic group.

Oxidative stress index in the sera of rats

The MDA concentrations in the sera of the studied rats are presented in Figure 1. As shown in Figure 1, there were significant increases (p<0.05) in the MDA concentrations of the diabetic group compared with the control. In contrast, there were significant decreases in the MDA concentrations in the sera of the diabetic rats fed with the test diets or the diabetic rats treated with glibenclamide, compared with the diabetic group. In addition, the MDA concentrations of the diabetic rats fed with 400 unripe plantain + 25 ginger, 600 unripe plantain + 25 ginger or the diabetic rats treated with glibenclamide, were not significantly different (p>0.05) from that of the control.

Liver function parameters in the sera of rats

Table 8 shows the liver function markers in the sera of the rats that were studied. As shown in Table 8, there were significant increases (p<0.05) in the serum AST activities of the diabetic group compared with the control but no significant differences (p>0.05) in the serum AST activities of the diabetic rats fed with unripe plantain (200, 400 and 600 g/kg), unripe plantain (200, 400 and 600 g/kg) and ginger (25 g/kg) and the diabetic rats treated with glibenclamide, compared with the diabetic group. There were no significant differences (p>0.05) in the serum ALT activities of all the rats across the groups. Further, there were no significant differences (p>0.05) in the serum ALP activity of the diabetic group compared with the control. Whereas the serum ALP activities of the diabetic rats fed with unripe plantain (200 g/kg), unripe plantain (200, 400 and 600 g/kg) + ginger (25 g/kg) were not significantly different (p>0.05) from that of diabetic group, the serum ALP activities of the diabetic rats fed with unripe plantain (400 and 600 g/kg) or the diabetic rats treated with glibenclamide, were significantly increased (p<0.05) when compared with the diabetic group.

Total protein concentration in the sera of rats

Figure 2 shows the total protein concentrations in the sera of the rats that were studied. As shown in Figure 2, there were no significant differences (p>0.05) in the serum total protein concentrations of the diabetic group compared with the control. There were also no significant differences (p>0.05) in the serum total protein concentrations of the diabetic rats fed the test diets or the diabetic rats administered glibenclamide, compared with the diabetic group.

Histology results

The results of the histology of the liver of the rats that were studied are shown in Figure 5. (1) Histology of the liver of the control showed normal hepatic architecture with normal hepatic artery. (2) Histology of the liver of the diabetic group showed severe degeneration with severe necrotic changes, intra hepatic inflammation and fatty changes. (3) Histology of the liver of the rats fed 200 g/kg unripe plantain showed mild necrotic nuclear of hepatocytes and lymphoid aggregates. (4) Histology of the liver of the rats fed 400 g/kg unripe plantain showed mild inflammatory cells in their portal triad. (5) Histology of the liver of the rats fed 600 g/kg unripe plantain showed severe degeneration with severe necrotic changes and hemorrhage. (6) Histology of the liver of the rats fed 200 g/kg unripe plantain and 25 g/kg ginger showed mild necrotic nuclear of hepatocytes and inflammatory cells. (7) Histology of the liver of the rats fed 400 g/kg unripe plantain and 25 g/kg ginger showed improved portal triad with bile duct and congested portal vein. (8) Histology of the liver of the rats fed 600 g/kg unripe plantain and 25 g/kg ginger showed severe necrosis with degenerating bile duct, degenerating portal vein, degenerating hepatocytes, inflammatory cells and...
degenerating sinusoid. (9) Histology of the liver of the rats administered glibenclamide showed severe necrosis with necrotic nuclear of hepatocytes, degenerating bile duct and hemorrhage.

The results of the histology of the pancreas of the rats that were carried out are shown in Figures 4. (1) Histology of the pancreas of the control showed normal cytoplasm of pancreatic islets, serous acini, pancreatic islets and intralobular duct. (2) Histology of the pancreas of the diabetic group showed necrotic pancreatic islets, serous acini and degenerating and distorted pancreatic islets. (3) Histology of the pancreas of the diabetic group showed necrotic pancreatic islets, serous acini and degenerating and distorted pancreatic islets. (4) Histology of the pancreas of the rats fed 200 g/kg unripe plantain showed mild necrotic pancreatic islets, degenerating and distorted pancreatic islets. (5) Histology of the pancreas of the rats fed 400 g/kg unripe plantain showed mild necrotic pancreatic islets and improved serous acini. (6) Histology of the pancreas of the rats fed 600 g/kg unripe plantain showed mild necrotic pancreatic islets and improved serous acini. (7) Histology of the pancreas of the rats fed 200 g/kg unripe plantain and 25 g/kg ginger showed improved serous acini and pancreatic islet. Histology of the pancreas of the rats fed 400 g/kg unripe plantain and 25 g/kg ginger showed improved pancreatic islets and serous acini. (8) Histology of the pancreas of the rats fed 600 g/kg unripe plantain and 25 g/kg ginger showed improved serous acini and pancreatic islet. (9) Histology of the pancreas of the rats administered glibenclamide showed degenerating and distorted pancreatic islets.

Phytochemical composition of test and standard feeds

Table 9 shows the phytochemical composition of the test diets and standard rat feed. As shown in Table 9, the tannin, flavonoid, alkaloid and saponin contents of the standard rat feed were significantly lower (p<0.05) than that of unripe plantain feed (200, 400 and 600 g/kg) or unripe plantain (200, 400 and 600 g/kg) + ginger (25 g/kg).

Table 10 shows the results of the GCMS analysis of the bioactive compounds in 400 g/kg unripe plantain + 25 g/kg ginger. While Figure 5 shows the GCMS Chromatogram of 400 g/kg unripe plantain + 25 g/kg ginger. GCMS analysis of 400 g/kg unripe plantain + 25 g/kg ginger showed the presence of eight compounds.

Compound 1 was 4-hydroxy-4-methylpentane-2-one with a RT of 3.616 and a percentage area of 1.78. Compound 2 was diethyl 2,6-dimethyl-3,5-pyridine dicarboxylate with a RT of 14.808 and a percentage area of 14.31. Compound 3 was 3,5-diethoxycarbonyl-2,6-dimethyl pyridine with a RT of 14.895 and a percentage area of 54.77. Compound 4 was identified as n-hexadecanoic acid with a RT of 16.815 and a percentage area of 4.62. Compound 5 was identified as 9,12-Octadecadienoic acid (Z,Z)- with a RT of 18.431 and a percentage area of 10.02.

Compound 6 was identified as cis-13-Octadecenoic acid with a RT of 18.481 and a percentage area of 7.71. Compound 7 was identified to be (-/+-)-alpha.-tocopherol acetate with a RT of 26.235 and a percentage area of 2.08 while Compound 8 was identified to be gamma.-sitosterol with a RT of 26.975 and a percentage area of 4.71.

Table 11 shows the results of the GCMS analysis of the bioactive compounds in the standard rat feed while Figure 6 shows the GCMS Chromatogram of the standard rat feed. GCMS analysis of the standard rat feed showed the presence of eight compounds. Compound 1 was 2-Pentanone, 4-hydroxy-4-methyl with a RT of 3.622 and a percentage area of 0.40. Compound 2 was Dodecanoic acid with a RT of 12.621 and a percentage area of 1.10. Compound 3 was Hexadecanoic acid with a RT of 16.874 and a percentage area of 11.04. Compound 4 was identified as Linolealaidic acid with a RT of 18.566 and a percentage area of 55.88. Compound 5 was identified as 6-Octadecenoic acid with a RT of 18.614 and a percentage area of 27.16.

Compound 6 was identified as Octadecanoic acid with a RT of 18.726 and a percentage area of 5.74. Compound 7 was identified to be Stigmast-5-en-5-ol, (3.beta.)- with a RT of 26.971 and a percentage area of 1.55 while Compound 8 was identified to be 1(2H)-Naphthalenone, 7-(2,5-dimethoxy-9-acridinyl)-3,4-dihydra with a RT of 30.122 and a percentage area of 4.71.

**Discussion**

**Fasting blood glucose levels of rats**

The increased fasting blood glucose concentrations of the diabetic group, indicates successful induction of DM in this group while the decreased fasting blood glucose concentrations of the diabetic rats fed with unripe plantain (200, 400 and 600 g/kg), unripe plantain (200, 400 and 600 g/kg) + ginger (25 g/kg) or treated with glibenclamide, indicate the anti-hyperglycemic properties of the test diets or glibenclamide. Our study further revealed that all doses of unripe plantain or a combination of unripe plantain and ginger as used in this study, produced a blood glucose lowering effect in the diabetic rats in a manner that was similar to glibenclamide.

**Serum insulin level, resistance, and pancreatic β-cell function in rats**
In this study, the observed significant decrease in the serum insulin concentrations of the diabetic group could be due to destruction of their pancreatic β- cells by STZ [30]. The decreased serum insulin concentration in the diabetic rats was abrogated following feeding with a combination of unripe plantain (600 g/kg) and ginger (25 g/kg), as well as treatment with glibenclamide but not feeding with unripe plantain (200, 400 and 600 g/kg) or unripe plantain (200 and 400 g/kg) and ginger (25 g/kg). Indeed, glibenclamide is a sulfonylurea whose mechanism of antidiabetic action involves stimulation of the secretion of insulin from the pancreatic beta cells. Therefore, the increased serum insulin concentrations of the diabetic rats fed unripe plantain (600 g/kg) and ginger (25 g/kg), and in a manner, akin to glibenclamide, suggests stimulation of insulin secretion by the pancreatic beta cells as a potential mechanism of the anti-diabetic action of unripe plantain (600 g/kg) and ginger (25 g/kg) feed, as seen in this study.

The observed significant increase in the HOMA-IR values of the diabetic group is an indication of induction of insulin resistance (IR) [31]. Interestingly, feeding with unripe plantain (200, 400 and 600 mg/dL) or unripe plantain + ginger (at all doses used) mitigated the HOMA-IR values in the rats but not glibenclamide. Whereas glibenclamide mitigates hyperglycemia by targeting insulin secretion (not IR), metformin, the first line drug for diabetes mellitus targets IR (not insulin secretion) [32]. Our study clearly indicates that unripe plantain (200, 400 and 600 g/kg) mitigates hyperglycemia via attenuation of IR while unripe plantain (400 g/kg) and ginger (25 g/kg) mitigate hyperglycemia by stimulating insulin secretion and abatement of IR.

The significant decrease in the HOMA-β values of the diabetic group reflects the presence of impaired pancreatic β- cell function. HOMA-β cell function index is a valid and widely used index that is used to assess insulin secretion in DM [33]. In this study, unripe plantain (600 g/kg) or unripe plantain (200, 400 and 600 g/kg) and ginger (25 g/kg) demonstrated better capacities than glibenclamide to repair the damaged pancreatic β- cell function as seen from the significant elevation of their HOMA-β values compared to the diabetic group, unlike glibenclamide.

Body weight of rats

The significant reduction of body weight of the diabetic group as observed in this study could be due to degradation of structural proteins as a result of hyperglycemia and decreased insulin secretion [34]. Insulin as an anabolic hormone triggers the synthesis of proteins. Therefore, due to deficiency of insulin, protein content is decreased in muscular tissue by proteolysis [35]. In this study, feeding with 200 mg/kg unripe plantain or 200, 400 and 600 g unripe plantain + ginger (25 g/kg) as well as treatment with glibenclamide did not significantly increase the body weights of the rats, unlike feeding with 400 and 600 g/kg unripe plantain. This might be explained by the preference of the rats for the 400 and 600 g/kg of unripe plantain diets and not necessarily due reduction of hyperglycemia since all diets fed or drug administered attenuated hyperglycemia in the rats.

Relative organ weights of rats

Alteration in relative organ weights may primarily indicate toxicity or pathology occurring to these organs [36]. The liver as the major organ that is involved in the metabolism of xenobiotics is prone to toxicity either from the drugs, chemicals, extracts and their metabolites [37]. The observed alterations in the relative liver weight of the diabetic group could have arisen from STZ toxicity to the rats’ liver [36] or it could also be due deposition of fat in the liver due to hyperglycemia. The decreased relative liver weights of the diabetic rats fed with unripe plantain (400 and 600 g/kg) or unripe plantain (400 and 600 g/kg) + ginger (25 g/kg) inferred that the test diets were able to alleviate the negative effect of STZ diabetes or STZ toxicity on liver of the rats. The non-significant change in the relative liver weights of the diabetic rats fed with 200 g/kg unripe plantain, 200 g/kg unripe plantain + ginger (25 g/kg), or treated with glibenclamide indicate that the test diets and drug could not alleviate the effect of STZ diabetes or STZ toxicity on the liver of the rats at the doses that were used in this study.

Surprisingly, STZ administration did not significantly affect the relative pancreatic weights of the rats and feeding with the test diets or treatment with glibenclamide, did not also affect the relative pancreatic weights of the rats and this is noteworthy.

Inflammatory markers in the sera of rats

One of the key processes of pancreatic β cell destruction and the development of diabetes is inflammation [39]. It was reported that DM is an inflammatory disease that triggers an elevation in the concentrations of pro-inflammatory cytokines such as IL6 and TNF-α in the serum of diabetic patients [40]. Hyperglycaemia and oxidative stress activate nuclear factor kappa B and activator protein-1, triggering the transcription of cytokines. TNF-α and IL-6 are cytokines that are up-regulated by activation of NF-kB. Moreover, in a diabetic condition, macrophage migration induces the release of cytokines, which in turn triggers induction of oxidative stress. It has been reported that TNF-α is involved in β-cell dysfunction and death, while raised concentration of IL-6 is known to be a predictor of future development of T2DM and also strongly lowers insulin sensitivity in liver cells [41]. Therefore, the increased circulating concentrations of the inflammatory cytokines- TNF-α and IL6 in the diabetic group, suggest that diabetes induction in rats,
triggered the release of these cytokines into circulation, leading to a pro-inflammatory state [42]. The decreased TNF-α and IL 6 concentrations in the sera of the diabetic rats fed with unripe plantain (200, 400 and 600 g/kg), unripe plantain (200, 400 and 600 g/kg) + ginger (25 g/kg) and glibenclamide, suggest mitigation of systemic inflammation as a contributory mechanism of anti-hyperglycemic actions of the test diets or glibenclamide.

Contrary to the study of Acharya et al. [43] where decreased IL-10 concentrators were reportedly elevated in diabetic untreated rats, a significant increase of serum IL-10 level concentrations was obtained in the sera of the diabetic group. This finding agrees with the studies of Djouwoug et al. [44] and Ren [45] that reported an increase in IL-10 concentrations in diabetic untreated rats. This increase in the circulating concentrations of IL-10 (including TNF-α and IL-6) in the diabetic group, suggests the release of cytokines from the interleukin family as a result of STZ diabetes induced inflammation.

Whereas unripe plantain (200 g/kg) or unripe plantain (200 g/kg) and ginger (25 g/kg) did not significantly affect the IL-10 concentrations of the rats compared with the diabetic group, the decreased IL-10 concentrations of the diabetic rats fed with unripe plantain (400 and 600 g/kg) or unripe plantain (400 and 600 g/kg) and ginger (25 g/kg), or the diabetic rats treated with glibenclamide further suggest mitigation of inflammation as a contributory mechanism of anti-diabetic action of unripe plantain, unripe plantain and ginger and glibenclamide at the doses used in this study.

Antioxidant markers and MDA concentration in the sera of rats

In this study, induction of STZ increased the activities of the enzymatic antioxidant defence systems- SOD, GPx, CAT, GSH and the lipid peroxidation maker-MDA in the sera of the rats.

Antioxidant enzymes like SOD, CAT and GPx have been shown to be vital in eliminating reactive oxygen species. SOD is an important enzyme that scavenges superoxide radical (O2•−) to H2O2 and molecular oxygen [46], while CAT and GPx are involved in the conversion of H2O2 to H2O, without which the generated H2O2 will destroy membrane lipids, releasing large amounts of MDA as a by-product of lipid peroxidation [47].

There are contrasting reports in literature on the concentrations of antioxidants in circulation in the diabetic state. Whereas some studies that evaluated the antioxidant status and SOD activity in patients with diabetes compared with healthy controls found no significant differences between controls and diabetic groups in terms of SOD activity [48], some other studies reported decreased serum activities of SOD and catalase in diabetic rats [49-50]. The study by Obasi and Ogugua [47] reported increased catalase, SOD, vitamin C and MDA but decreased GPx concentrations in the sera of diabetic rats compared with the controls whereas the study by Djouwoug et al. [44] reported increased CAT activity in diabetic rats.

Previous studies reported that increased levels of free radicals in diabetes may increase the activities of antioxidant enzyme that are activated to abrogate them [47, 51]. Therefore, higher SOD, GPx, CAT, GSH and MDA concentrations in the sera of the diabetic group is an indication of oxidative stress in the diabetic group.

The decreased SOD, GPx, CAT and MDA concentrations of the rats fed the test diets or treated with glibenclamide might suggest attenuation of oxidative stress by the test diets or glibenclamide as contributory to their antidiabetic properties.

In this study, glibenclamide decreased the GSH concentrations of the diabetic rats compared with the diabetic group unlike the diabetic rats that received the test diets, a further indication of attenuation of systemic oxidative stress by glibenclamide. Current study therefore reveals that glibenclamide demonstrated better attenuation of STZ diabetes induced oxidative stress than the test diets.

Liver function parameters in the sera of rats

Liver marker enzymes such as ALP, AST, and ALT are commonly utilized to evaluate liver injury caused by drug toxicity or harmful chemicals [52]. ALP is a membrane bound enzyme while ALT and AST are cytosolic enzymes and high levels of ALP, ALT and AST in the serum are indicators of cell membrane permeability and damage to the liver [53]. The heart, liver, skeletal muscle, and red blood cells are the sources of AST while the liver is the primary source of ALT [54].

In this study, elevation in the AST activities of the diabetic group was obtained while there were no significant changes in the ALT and ALP activities of the diabetic group compared with the control. Further, the test diets or glibenclamide did not significantly affect the AST and ALT activities of the rats. Given that elevations in serum ALT are considered to be specific for hepatic disease or damage while AST could be elevated in other conditions such as myocardial infarction, renal disorder and muscle necrosis [54], the presence of hepatocellular damage or liver disease could not be established in the diabetic group. However,
while unripe plantain (200 g/kg) or unripe plantain (200, 400 and 600 g/kg) + ginger (25 g/kg) did not significantly affect the serum ALP activities of the diabetic rats, unripe plantain (400 and 600 g/kg) feed, or glibenclamide significantly elevated the ALP activities of the diabetic rats relative to the diabetic group and this is noteworthy.

**Total protein concentration in the sera of rats**

The effect of diabetes on serum total proteins gave contrasting results. Whereas some studies reported increased serum concentrations of total proteins [55], some studies reported decreased total protein concentrations [56], while others found no significant changes in the serum concentrations of total proteins in diabetic animals [57-59], a finding that is consistent with our findings. The non-significant change in the total protein concentrations of the diabetic group could partly be explained by the increased antioxidant enzymes in the diabetic groups, which activities could have compensated for the loss of structural proteins in the diabetic group.

Our study further found that feeding with the test diets or glibenclamide did not also significantly affect the total protein concentrations of the rats and this is also noteworthy.

**Histology of the liver and pancreas of rats**

Although histology of the liver of the diabetic group showed some pathological changes, interestingly these changes did not increase the activities of the ALT and ALP in the sera of the diabetic group, suggesting that the hepatic tissues of the diabetic rats were in early stage of liver disease, wherein serum activities of liver enzymes might be within the normal range [60]. Interestingly, while unripe plantain (400 g/kg) + ginger mitigated the pathological changes in the liver histology of the rats, there were still evidence of either inflammatory, fatty or necrotic changes in the liver histology of the rats fed unripe plantain (200, 400 and 600 g/kg) or unripe plantain (200 and 600 g/kg) + ginger (25 g/kg).

The pathological changes that were seen in the pancreas histology of the diabetic group corroborate our biochemical results and they may explain the altered insulin secretion, HOMA-IR and HOMA-β values that were obtained in the diabetic group. Interestingly, supplementation with unripe plantain (200, 400 and 600 g/kg) and ginger (25 g/kg) mitigated these pancreatic pathological changes unlike supplementation with unripe plantain (200, 400 and 600 g/kg) or treatment with glibenclamide.

**Phytochemicals play key contribution to the medicinal properties of plants.** Flavonoids, alkaloids, tannins, and saponins, as polyphenolic compounds, have been associated with hypoglycemic activity [38]. The inhibition of the glycolytic activity of brush border enzymes by polyphenolic compounds seems to be one of the factors that stimulates hypoglycemic action in some medicinal plants [58]. Flavonoids are potent water-soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong antioxidant activity. Certain flavonoids, e.g., quercetin, glycoside and phytol have been known to be able to regenerate pancreatic β-cells [61-62]. Alkaloids possess anti-inflammatory antioxidant, antidiabetic and many other pharmacological properties [63-64]. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues. Tannins have received considerable attention in the fields of nutrition, health and medicine, largely due to their pharmacological properties such as antioxidant, antimicrobial and anti-inflammatory properties [65]. Saponins have been reported to have insulin sensitization effect in diabetic rats [66].

The higher contents of tannins, flavonoids, alkaloids and saponins in the unripe plantain and unripe plantain + ginger feeds compared with the standard rat diet, suggest that these polyphenols could have contributed in the anti-hyperglycemic properties of the unripe plantain and unripe plantain + ginger feeds. In all, unripe plantain (400 g/kg) + ginger (25 g/kg) diet demonstrated better antidiabetic properties, pancreatic beta cell repair, mitigation of insulin resistance and hepatoprotective properties than other test diets or glibenclamide and this led to the characterization of its bioactive constituents using GCMS.

GC-MS analysis of the ethanol extract of the unripe plantain (400 g/kg) + ginger (25 g/kg) diet showed the presence of a plethora of bioactive compounds in it. n-hexadecanoic acid that made up 4.62% of the extract was reported to possess anti-inflammatory properties [67].

9, 12-Octadecadienoic acid (Z, Z)-, methyl ester that made up 10.02% of the extract was also reported to possess anti-inflammatory properties in addition to analgesic, and ulcerogenic properties [68], alpha-tocopherol acetate that constituted 2.08% of the extract was reported to possess antioxidant activity [69]. Interestingly, γ-Sitosterol that made up 4.71% of the extract was the bioactive compound in the extract with antihyperglycemic property. This compound was reported to exert antihyperglycemic properties by increasing insulin secretion in response to hyperglycemia and inhibition of gluconeogenesis [70-71]. The presence of this compound in the unripe plantain (400 g/kg) + ginger (25 g/kg) diet may explain the anti-hyperglycemic, insulin secretion, and modulation of HOMA-IR and HOMA-β values in the diabetic rats supplemented with this test diet.
Finally, the presence of the n-hexadecanoic acid, 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester, alpha-tocopherol acetate in the extract and other phenolic phytochemicals in the test diet may have contributed to the anti-diabetic, antioxidant and anti-inflammatory properties of unripe plantain (400 g/kg) + ginger (25 g/kg) diet as seen in this study.

Conclusions
Our study showed that combination of unripe plantain (400 g/kg) and ginger (25 g/kg) diet demonstrated better anti diabetic properties, pancreatic beta cell repair, mitigation of insulin resistance and hepatoprotective properties than 200 g/kg unripe plantain, 400 g/kg unripe plantain, 600 g/kg unripe plantain, 200 g/kg unripe plantain + ginger (25 g/kg), 600 g/kg unripe plantain + ginger (25 g/kg) and glibenclamide, as was also supported by pancreas and liver histology. The diet also demonstrated antioxidant and anti-inflammatory properties in the diabetic rats.

The antidiabetic, antioxidant and anti-inflammatory properties of the unripe plantain (400 g/kg) and ginger (25 g/kg) diet as seen in this study, could be attributed to the plethora of bioactive compounds in it.

Additional Information
Disclosures
Human subjects: All authors have confirmed that this study did not involve human participants or tissue.
Animal subjects: Board of the Department of Biochemistry Issued protocol number 16/PG/BMS/SC/001.
Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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