Full Length Research Paper

Unripe *Musa paradisiaca* Fruit Diet Ameliorates Impaired Glucose Regulation Caused by Iron-Induced Oxidative Stress

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ABSTRACT

Excess iron impairs glucose regulatory mechanisms through an increase in oxidative stress. Unripe Musa paradisiaca fruit (UMP) diets have been reported to alleviate diabetes and exert antioxidant effects. In this study, some glucose regulatory indices were investigated in Wistar rats with iron-induced oxidative stress and maintained on UMP-diet. Thirty-rats were divided into five equal groups. Group 1 (control) received standard rat chow only. Oxidative stress was induced with ferrous-sulphate (3mg/kg, i.p.) in groups 2–5 and animals were simultaneously maintained on standard rat chow (group2), 20%UMP-diet (group3), 40%UMP-diet (group4) and 80%UMP-diet (group5) respectively, for 28days. Phytochemical, mineral and proximate analysis of UMP were evaluated. Blood glucose was monitored on days 0,7,14,21 and 28 respectively using the tail tipping method. At day28 post-treatment, blood samples were obtained from each animal; serum was extracted and assayed for iron, total iron binding capacity (TIBC), insulin and interleukin-6 levels. Muscle glycogen was determined using the anthrone method; insulin resistance, pancreatic beta cell function and transferrin saturation levels were mathematically calculated. Pancreatic samples were histologically evaluated using H and E stains. Phytochemical screening showed the presence of flavonoids, saponins, phenols, tannins, coumarins, steroids and alkaloid. Mineral and proximate analysis indicate the presence of proteins, fibre, sodium, iron, zinc and potassium. Blood glucose on day28 was unchanged in control, increased in group2 and decreased in groups 3-5 respectively compared to day 0. Beta cell function, insulin and TIBC were decreased while transferrin saturation was increased in group2 compared to all other groups. Serum iron and interleukin-6 was increased while muscle glycogen was reduced in group2 compared to groups4 and 5 respectively. Group2 animals had pancreas with necrotic acinar cells and ill-defined islet of Langerhans. These pathologies were not observed in normal and UMP treatment groups. This study suggests that unripe Musa paradisiaca fruit diet may reduce the deleterious effects of iron-induced oxidative stress on glucose regulatory indices.

Keywords: *Unripe Musa paradisiaca, glucose, iron, iron-induced oxidative stress*

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INTRODUCTION

Iron is essential for life. It is an essential component of haemoglobin as well as a key component and cofactor of major enzymes and metalloproteins (Vaziri *et al*., 2003). When in excess (iron overload), it causes excessive production of free radicals that impairs the oxidative balance in the body resulting in a state of oxidative stress (Lee *et al*., 2015). In Nigeria, iron supplements are randomly prescribed during pregnancy and in conditions of moderate to severe anemia (Ugwu *et al*, 2014). Furthermore, red blood cell (RBC) transfusion therapy remains germane in the management of sickle cell disease thus predisposing carriers to iron overload and increased oxidative stress (Otaigbe 2013). Oxidative stress has also has been implicated in the apoptosis of pancreatic islets, decrease in insulin secretory capacity, insulin deficiency (Cooksey *et al*., 2004), hepatic dysfunction, insulin resistance, impaired glucose metabolism and regulation.

Musa paradisiaca (plantain) is a crop belonging to Musa genus and is reported to be indigenous to the tropical and subtropical regions of the world (Alabi *et al*., 2013). It has been reported to promote healthy digestion, improve affective state, help in the retention of and serve as good sources of potassium, calcium, phosphorus and nitrogen, iron, and vitamins (Imam and Akter, 2011). The fruits can be consumed when ripe or unripe, cooked, roasted, steamed, baked or grilled. Green plantain has also been observed to be high in total dietary fibre content (Kirtikar and Basu, 1991), which suggests that it may lower glycaemic response by forming a physical barrier to enzymatic hydrolysis of starch.

In folklore medicine amongst Nigerians, unripe plantain meal has been observed to be useful in the management of diabetes, treatment of anemia, and liver disorders (independent of diabetes) (Eleazu and Okafor, 2012). Furthermore, Nigerian diabetics have also been reported to reduce postprandial glucose by consuming unripe plantain meal (Edo *et al*., 2011). The antioxidant potential of the unripe plantain meal in diabetic subjects has also been reported (Eleazu and Okafor, 2012).

In this study, we investigated the effects of varying compositions of unripe *Musa paradisiaca* fruit (UMP) diet on glucose regulatory indices in Wistar rats with iron-induced oxidative stress.

MATERIALS AND METHODS

Plant Preparation: Commercially available dried sliced unripe *M. paradisiaca* (UMP) fruits were purchased from Oje market in Ibadan metropolis. The dried fruits were milled into powdery form and compounded with the standard rat chow at different percentages of 20%, 40% and 80% respectively.

Phytochemical Screening, Mineral and Proximate Analysis: Phytochemical analysis of *M. paradisiaca* fruits was carried out for flavoniods (alkaline reagent test – Tiwari *et al*., 2011), saponins (frothing test – Banso and Adejumo, 2006), phenols (lead acetate test – Tiwari *et al*., 2011), coumarins (filter paper chromatography – Zohra *et al*., 2012), anthraquinones (Borntragers test – Evans, 2002), steroids (Salkowski test – Ayoola *et al*., 2008) and alkaloids (Wagners test – Joshi *et al*., 2013) using standard phytochemical screening methods. Mineral and proximate analysis was carried out according to the procedure of the Association of Official Analytical Chemist (AOAC, 1990) to determine the moisture content, crude fiber, protein, crude fat, carbohydrate and electrolyte components of unripe *M. paradisiaca* fruits.

Formulation of Diet Composition: Standard rat chow was obtained from Ladokun feed, Ibadan, Nigeria (carbohydrate 67%, protein 21%, fat 3.5%, fiber 6%, calcium 0.8%, phosphorus 0.8%). The chow were ground into powdery form and mixed with dried ground UMP fruits in the ratio 80% standard feed: 20% UMP; 60% standard feed: 40% UMP; and 20% standard feed: 80% UMP respectively. This was then reconstituted into pellets and given to the experimental animals.

Animals and Experimental protocol: Thirty (30) female Wistar rats, weighing between 140-170g were housed in well-ventilated cages, exposed to alternate light and dark cycles, maintained at 25-28ºC, low relative humidity, fed on standard rat chow, allowed free access to drinking water and acclimatized to standard laboratory conditions for two weeks prior to experimental procedures. All experiments were performed according to the guidelines for the care and use of laboratory animals in the University of Ibadan, Nigeria and that of the National Research Council, USA (NRC, 1996). The animals were randomly divided into five (5) groups of six (6) animals each. Group 1 was control and maintained on standard rat chow throughout the duration of study. Oxidative stress was induced with daily intraperitoneal administrations of ferrous sulphate (3mg/kg) (Abd Allah *et al*, 2014) in groups 2 – 5 and these animals were simultaneously maintained on standard rat chow (group 2), 20% UMP-diet (group 3), 40% UMP-diet (group 4) and 80% UMP-diet (group 5) respectively for 28 days.

Blood collection biochemical and histopathological analysis: Blood samples were obtained by the tail tipping method on days 0, 7, 14, 21 and 28 respectively for determination of fasting blood glucose level (Hoff *et al*, 2000). The glucose level was assessed using an Accu-Check active glucometer (Tack *et al*., 2012) (Roche, Germany), based on the glucose oxidase method (Barham and Trinder, 1972). On day-28 post treatment, blood samples were obtained from the retro-orbital sinus after light di-ethyl ether anesthesia into plain sample tubes. The blood was allowed to stand at room temperature to obtain serum and thereafter centrifuged at 3000rpm for ten minutes to isolate the serum.

Biochemical analysis: The serum samples obtained was analyzed for iron using Atomic Absorption Spectrometry, (Analytical Methods for Atomic Absorption Spectrometry, 2000); total iron binding capacity (Fortress diagnostics, UK), insulin (CalBiotech, USA) and interleukin-6 (Biolegend Max, San Diego) were analyzed using commercially available kits. Muscle glycogen content was determined by reacting fresh muscle homogenates with anthrone reagent to form a blue-coloured solution that was read with a spectrophotometer at 630nm. The reading obtained was compared with that of known glycogen standards on a line graph to determine the actual glycogen concentration in each sample (Seifer *et al*., 1950; Jermyn, 1975). Insulin resistance and beta cell function was determined mathematically using the Homeostasis Model Assessment Insulin Resistance (HOMA-IR) and Pancreatic Beta Cell Function (HOMA-β) equations (Mathews *et al*., 1985). Percentage transferrin saturation was also estimated mathematically (Adams *et al*., 2007).

Histopathological analysis: Pancreatic samples were also excised from animals in each experimental group and analysed for histological and structural changes using Haematoxylin and Eosin stains.

Statistical Analysis
Data were expressed as Mean ± SEM and analysed using One-way ANOVA while Newman-Keuls' Post-hoc test was used to establish the statistical significance at $P<0.05$.

RESULTS

Phytochemical Screening, Mineral and Proximate analysis of dried unripe *Musa paradisiaca* fruits (UMP).: Phytochemical screening of unripe M. paradisiaca fruit indicates the presence of flavonoids, saponins, phenols, tannins, coumarins, steroids and alkaloid**.** The presence of anthraquinones was however not detected (Table 1). Mineral analysis showed the presence of sodium (204.6mg/g), iron (83.8mg/g), manganese (9.7mg/g), zinc (6.0mg/g) and copper (3.2mg/g). Other minerals identified were calcium (0.024%),

magnesium (0.075%) and potassium (0.880%)(Table 2). Proximate analysis of the dried unripe fruits of M. paradisiaca also showed the presence of proteins (5.25%), ash (2.90%), fat (2.5%), crude fiber (2.95%) and dry matter (93.3%) (Fig. 1).

Body weight and blood glucose levels in the different experimental groups: Significant increases (P<0.05) in body weight (g) was observed in control (17.2%), iron-induced oxidative stress only (12.0%), 20% UMP-diet (17.9%) and 40% UMP-diet (13.9%) groups compared to their individual day 0 values. Body weight values obtained in the 80% UMP-diet) at the end of the experiment were not significantly different from their day 0 values (Table 3). Blood glucose level (mg/dl) was unchanged in control (63.2±2.9 vs. 61.4±3.3), increased in iron-induced oxidative stress only (54.8±5.0 vs. 97.4±2.8) and reduced in the 20% UMP-diet (76.8±2.6 vs.61.2±3.7), 40% UMP-diet (72.4±6.8 vs. 59.0±2.4) and 80% UMP-diet (79.0±4.5 vs. 65.0±5.1) groups respectively compared to their initial (day 0) values (Table 4).

Table 1.
Phytochemical screening of *Musa paradisiaca*

Parameter	Methanol Medium	Aqueous Medium
Flavonoids	-	++
Saponins	+++	+++
Phenols	+++	+++
Tannins	+++	++
Coumarins	++	+
Antrhraquinones	-	-
Steroids	+	+
Alkaloids	++	++

KEY:
- = Absent
+ = Present in trace amounts
++ = Present
+++ = Very abundant

Table 2.
Mineral evaluation of unripe *Musa paradisiaca fruits*

Mineral	Content
Ca (%)	0.024
Mg (%)	0.075
K (%)	0.880
Na (mg/g)	204.630
Mn (mg/g)	9.722
Fe (mg/g)	83.796
Cu (mg/g)	3.241
Zn (mg/g)	6.019

Key: Ca = Calcium; Mg = Magnesium; K = Potassium; Na = Sodium; Mn = Manganese; Fe = Iron; Cu = Copper; Zn = Zinc.

Serum Iron, total iron binding capacity (TIBC) and percentage transferrin saturation in control and experimental animals: Serum iron level (mg/l) was significantly increased (P<0.05) in Iron-induced oxidative stress group (46.5 ± 2.0) and 20% UMP-diet (46.1 ± 1.3) compared to control (37.4 ± 2.2), 40% UMP-diet (16.5 ± 2.8)

and 80% UMP-diet (25.4 + 2.3) groups respectively (Table 6). Total iron binding capacity (µg/dl) was reduced (P<0.05) in iron-induced oxidative stress group (365.6 ± 39.0) compared to control (557.7 ± 55.6), 20% UMP-diet (500.8 ± 87.0), 40% UMP-diet (552.3 ± 73.3) and 80% UMP-diet (624.6 ± 106.4) respectively while percentage transferrin saturation was significantly increased in the iron-induced oxidative stress group compared to all other groups respectively (Table 5).
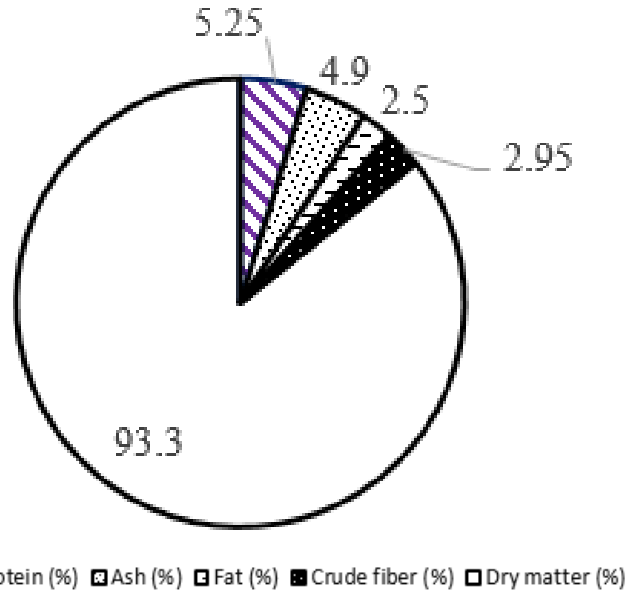

Figure 1:
Proximate analysis of unripe *Musa paradisiaca* fruits

Table 3
Body weight (g) changes in control and experimental groups

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
1	149.8 ±4.3	168.5 ±7.3	172.5 ±6.1	173.8 ±6.7	175.5 ±6.3
2	166.2 ±7.9	174.6 ±7.4	181.4 ±6.7	186.2 ±5.6	186.2 ±6.0
3	165.6 ±0.7	179.2 ±8.7	188.2 ±8.1*	196± 8.2*	195.2 ±7.6*
4	166.8 ±3.3	178.8 ±3.5*	184.4 ±4.8*	188.2 ±6.3*	189.8 ±7.5*
5	146.4 ±6.0	165.0 ±6.0	160.8 ±6.0	151.4 ±11.1	142.0 ±4.8

Values are Mean ± SEM.
* indicates values that are significantly different from control. Group 1 = Control (Normal diet only); Group 2 = Iron-induced oxidative stress only; Group 3 = Iron-induced stress fed 20% UMP-diet group; Group 4 = Iron-induced stress fed 40% UMP-diet group; Group 5 = Iron-induced stress fed 80% UMP-diet group.

Insulin level, Insulin resistance and pancreatic beta cell function in control and experimental groups: Insulin levels (pmol/L) in group 2 (Iron-induced oxidative stress) (2.51 ± 0.65) was significantly reduced compared to control (4.15±0.81), 40% UMP-diet (3.90 ± 0.85) and 80% UMP-diet (7.01± 1.0) treatment groups respectively. Insulin values

obtained in the iron-induced oxidative stress only and 20% UMP-diet groups (2.51 ± 0.65 vs. 3.15 ± 0.64) were comparable (Fig. 2). No significant difference was observed in insulin resistance (HOMA-IR values) between control and the 20%, 40% and 80% UMP-diet groups (Table 7). Insulin resistance in the 80% UMP-diet group was significantly increased compared to all other groups respectively. Pancreatic beta cell function (HOMA-B values) was increased in control, 20%, 40% and 80% UMP-diet groups compared to the iron-induced oxidative stress group (Table 6).

Table 4.
Blood glucose level (mg/dl) in control and experimental groups

Groups	Day 0	Day 7	Day 14	Day 21	Day 28
1	63.2 ±2.9	76.2 ±3.1	63 ±2.5	69.8 ±2.3	61.4 ±3.3
2	54.8 ±5.0	75.6 ±3.2	80.8 ±2.4	85.6 ±1.6	97.4 ±2.8*
3	76.8 ±2.6	60.2 ±3.8	53.8 ±2.0	56.0 ±2.3	61.2 ±3.7*
4	72.4 ±6.8	75.6 ±4.4	52.2 ±2.7	69.4 ±4.9	59.0 ±2.4*
5	79.0 ±4.5	69.5 ±3.5	51.5 ±4.7	60.75 ±4.3	65.0 ±5.1*

Values are Mean ± SEM.
* indicates values that are significantly different from control.
# Indicates values that are significantly differently from animals in group 2. Group 1 = Control (Normal diet only); Group 2 = Iron-induced oxidative stress only; Group 3 = Iron-induced stress fed 20% UMP-diet group; Group 4 = Iron-

induced stress fed 40% UMP-diet group; Group 5 = Iron-induced stress fed 80% UMP-diet group.

Table 5
Iron level, Total iron binding capacity and percentage transferrin saturation in control and experimental animals

Groups	Serum iron level (mg/l)	Total iron binding capacity (µg/dl)	Percentage Transferrin saturation (%)
1	37.4±2.2	557.7±55.7	6.7
2	46.5±2.0	365.6±39.0	12.7
3	46.1±1.3	500.8±87.0#	9.2#
4	16.5±2.8*#	552.3±73.3#	3.0#
5	25.4±2.3*#	624.6±106.4#	4.1#

* Values are Mean ± SEM.
* indicates values that are significantly different from control.
# Indicates values that are significantly differently from animals in group 2. Group 1 = Control (Normal diet only); Group Group 2 = Iron-induced oxidative stress only; Group 3 = Iron-induced stress fed 20% UMP-diet group; Group 4 = Iron-induced stress fed 40% UMP-diet group; Group 5 = Iron-induced stress fed 80% UMP-diet group.

Skeletal muscle glycogen, serum triglyceride and total cholesterol level in control and experimental groups
There was significant increase in skeletal muscle glycogen content (mg/100g muscle wt.) in groups 1 (control), 4 (40% UMP-diet) and 5 (80% UMP-diet) compared to group 2 (Iron-induced oxidative stress only). Glycogen values observed in group 3 (20% UMP-diet) were significantly reduced compared to group 2 (Table 6).
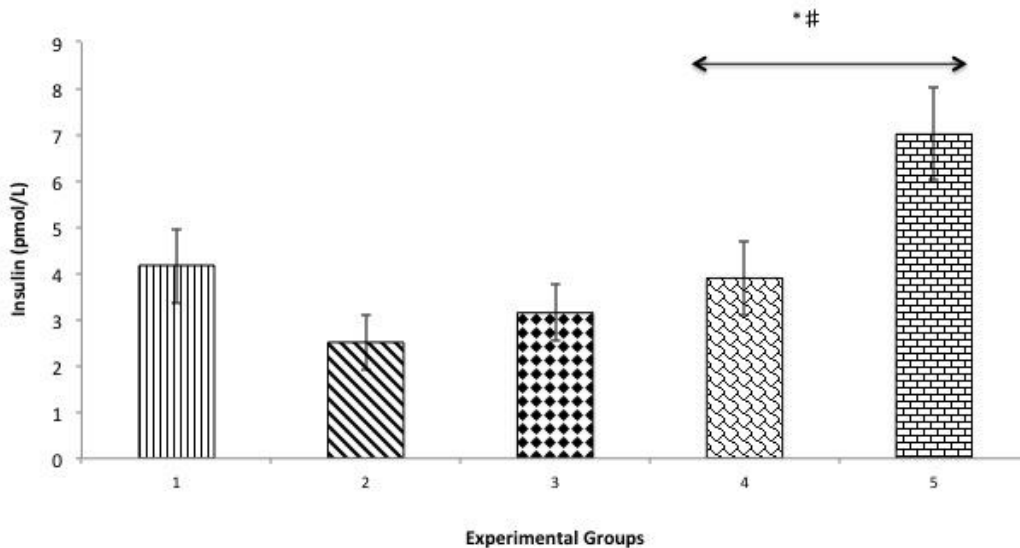

Figure. 2
Insulin level in control and experimental groups. Values are Mean ± SEM. * indicates values that are significantly different from control. # Indicates values that are significantly differently from animals in group 2. Group 1 = Control (Normal diet only); Group 2 = Iron-induced oxidative stress only; Group 3 = Iron-induced stress fed 20% UMP-diet group; Group 4 = Iron-induced stress fed 40% UMP-diet group; Group 5 = Iron-induced stress fed 80% UMP-diet group.
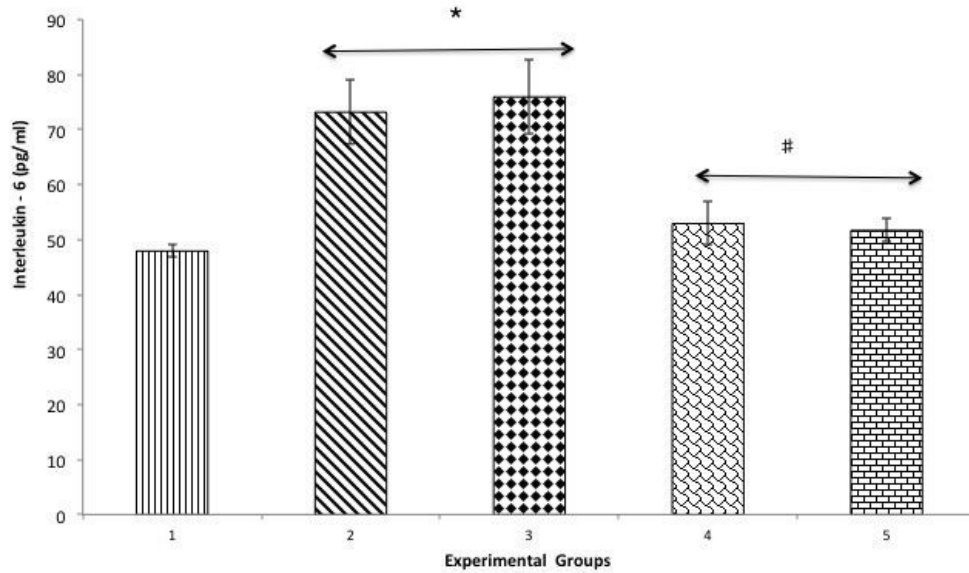
Figure 3
Interleukin 6 level in control and experimental groups. Values are Mean ± SEM. * indicates values that are significantly different from control. # Indicates values that are significantly differently from animals in group 2. Group 1 = Control (Normal diet only); Group 2 = Iron-induced oxidative stress only; Group 3 = Iron-induced stress fed 20% UMP-diet group; Group 4 = Iron-induced stress fed 40% UMP-diet group; Group 5 = Iron-induced stress fed 80% UMP-diet

Table 6
Effect of unripe *M.sapientum* fruits diet on some glucose regulatory indices

Groups	Insulin resistance	Pancreatic beta cell function	Skeletal muscle glycogen (mg/100g fresh muscle weight)
1	0.63±0.12	24.5±4.9	802.1±41.8
2	0.62±0.15	9.1±2.0	516.6±72.5
3	0.48±0.12	18.6±3.3#	237.8±26.8*#
4	0.60±0.30	22.6±9.9#	1017.2±116.9*#
5	1.14±0.21*#	38.9±6.4*#	817.5±74.2#

* Values are Mean ± SEM.
* indicates values that are significantly different from control.
# Indicates values that are significantly differently from animals in group 2. Group 1 = Control (Normal diet only); Group 2 = Iron-induced oxidative stress only; Group 3 = Iron-induced stress fed 20% UMP-diet group; Group 4 = Iron-induced stress fed 40% UMP-diet group; Group 5 = Iron-induced stress fed 80% UMP-diet group
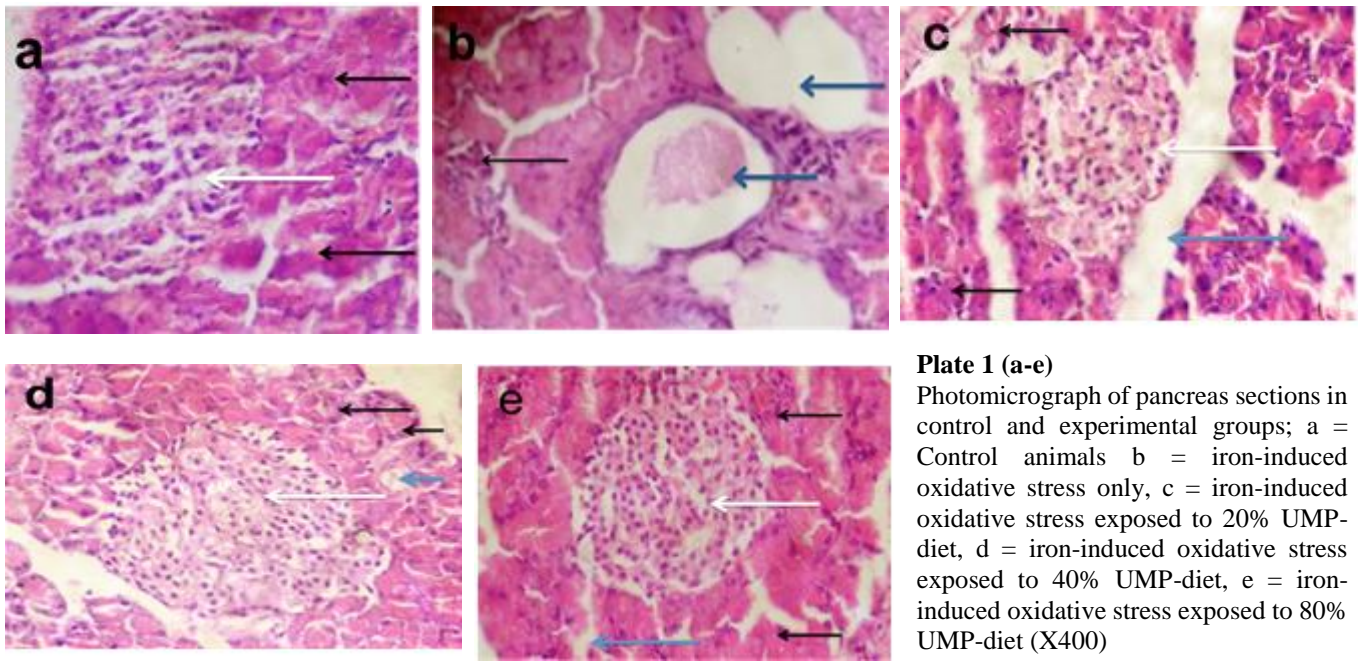
Histological evaluation of the pancreas in control and experimental groups
Control animals had pancreas that showed normal architecture with the parenchyma of the pancreas showing normal serous acinar and zymogenic cells (slender black arrow) that contain abundant granular eosinophilic cytoplasm, normal interlobular connective tissues and septa. There are large islets of Langerhans (slender white arrow) consisting of round to oval collections of endocrine cells. Group 2 animals (Iron-induced oxidative stress only) had pancreas showing moderate architecture; the parenchyma of the pancreas also shows moderate acinar cells necrosis that contain abundant granular eosinophilic cytoplasm. Moderately dilated interlobular duct (blue arrow) and moderately dilated vessel are also seen. Animals in this group also had diffuse islets with ill-defined islets of Langerhans (slender black arrow). Pancreatic samples in groups 3 (20% UMP-diet), 4 (40% UMP-diet) and 5 (80% UMP-diet) showed normal architecture with the parenchyma of the pancreas showing normal acinar cells (slender arrow) that contain abundant granular eosinophilic cytoplasm, normal interlobular duct and septa noted (blue arrow). The islets of Langerhans seen in these groups also appear normal (white arrow).

DISCUSSION

Increase in the prevalence of iron overload has been reported in Nigerian women and it has been suggested that laboratory investigations to ascertain iron status should be carried out routinely in women (Fashola *et al* 2013). Iron overload has been reported to result in impairment of glucose metabolism, which in turn affects several iron metabolic pathways resulting in both insulin resistance and increased ferritin (an acute phase pro-inflammatory reactant) synthesis. These two effects increase the predisposition towards type-2-diabetes mellitus. This study shows an increase in body weight in the iron-induced oxidative stress group (group 2) at the end of the experiment compared to their initial values (Table 2). The weight gain in this group was also comparable to that in the control animals. This observation is consistent with the report of Wu *et al* (1990) who also showed weight gain in the control and the group with dietary iron overload.

Plate 1 (a-e)
Photomicrograph of pancreas sections in control and experimental groups; a = Control animals b = iron-induced oxidative stress only, c = iron-induced oxidative stress exposed to 20% UMP-diet, d = iron-induced oxidative stress exposed to 40% UMP-diet, e = iron-induced oxidative stress exposed to 80% UMP-diet (X400)

The weight gain in the overloaded rats was found to be associated with marked increases in extra hepatic and hepatic iron concentrations. It is also likely that iron-induced oxidative stress caused an increase in weight gain that may be associated with type 2 diabetes mellitus. Animals in groups 3 and 4 (20% UMP diet and 40% UMP diet) also had an increase in body weight, which may be due to the high protein content of UMP diets that have been reported to improve nutritional status and increase body weight in experimental diabetic animals. However, animals in group 5 (80% UMP diet) had a reduction in body weight (Table 3). Unripe *Musa paradisiaca* (UMP) fruit meal has been reported to have a low glycemic index, high fiber content and cause a reduction in nutrient assimilation. It is therefore likely that these effects of UMP fruit meal might have caused a reduction in body weight in this treatment group as the concentration of UMP fruit in the diet increased. It may therefore be likely that UMP-dietary supplementation at 80% may be counteracting the weight gain observed in the other treatment groups. The observation is further confirmed by the presence of flavonoids, saponins, phenols, tannins, coumarins and alkaloids in the phytochemical screening of UMP in this study (Table 1). These phytochemical principles have been reported to either control weight gain or restore lost weight in different experimental studies (Bertoia *et al* 2016; Marelli *et al*., 2016). Animals in group 2 (iron-induced oxidative stress) had elevated serum levels of iron, an increase in percentage transferrin saturation and a reduction in total iron binding capacity (TIBC) (Table 6) thus suggesting that some degree of iron overload may exist in this animal group. This observation is in accordance with the reports of Abd Allah *et al*., (2014) who also observed these symptoms in animals that received similar treatment. Animals in this group also showed reductions in pancreatic beta cell function, serum insulin,

muscle glycogen as well as an increase in blood glucose level and serum interleukin – 6 level (Table 4 and 7, Fig 1 and 2) which suggest possible impairment in glucose regulation as a result of iron-induced increase in oxidative stress. Insulin resistance in this group (iron-induced oxidative stress only) was comparable to control, 20% and 40% UMP diet but reduced compared to the 80% UMP-diet groups. Pancreatic beta cells have been reported to be particularly sensitive to reactive oxygen species (ROS) because of the low expression of antioxidants such as catalase and superoxide dismutase in these cells (Azevedo-martins *et al*, 2010). Increased ROS in the pancreas causes beta cell dysfunction by multiple mechanisms including decreased insulin gene expression secondary to decreased expression of transcription factors necessary for beta cell differentiation, maintenance and insulin gene transcription (Cernea and Dobreanu, 2013). Some of these pancreatic abnormalities were seen in the iron-induced oxidative stress group (group 2), which had moderate acinar cell necrosis, moderately dilated interlobular ducts and diffuse islets with ill defined islet of Langerhans (Plate 2a and 2b).

Unripe *Musa paradisiaca* meal has been reported to control postprandial hyperglycemia, exert hypoglycemic and antioxidant effects (Ayodele and Godwin, 2010; Edo *et al*., 2011). These observations were also evident in this study as animals in the UMP diet group had reduced blood glucose, serum iron levels (40% and 80% UMP only) as well as increased TIBC and percentage transferrin saturation level (20%, 40% and 80% UMP diet) respectively (Table 6) thus suggesting a reduction or attenuation of iron-induced oxidative stress in these groups. These animals also exhibited an increase in insulin output and a reduction in serum in Il-6 level (40% and 80% UMP-diet), which further suggests an attenuation of oxidative stress in the pancreas, and serum. Histological evaluation of the pancreas in the UMP dietary

supplemented groups also suggests a reversal in the deleterious effects caused by iron-induced oxidative stress (Plate 1b), as they showed normal acinar and islet of Langerhans cells (Plate1 c-e) similar to control group (Plate 1a).

It is well known that the most reliable method for evaluating insulin sensitivity and resistance is by using the hyperinsulinemic euglycemic glucose clamp (HEGC) (DeFronzo *et al* 1979; Antunes *et al*., 2016). Factors such as high-cost, need for pump-infusion equipment and considerable expertise have been reported to considerably limit its clinical applicability (Muniyappa *et al*., 2008). This has led to the development of other methods such as the homeostasis model assessment equations for insulin resistance (HOMA-IR) and pancreatic beta cell function (HOMA-B), which has been largely validated against the HEGC (Antunes *et al*., 2016). However, though some researchers report a correlation of interpretation of results using the HOMA equations in both humans and rodent experiments (Antunes *et al*, 2016), other reports suggest a poor validation in rodents when using HOMA equations for experimental results. This may thus form one of the limitations in this study as insulin resistance and pancreatic beta cell function were assessed using the HOMA equations, which is based on the premise that fasting circulating glucose/insulin levels are determined by a crosstalk between the liver and the pancreas. This is reported to reflect changes in hepatic insulin-sensitivity, but is limited for reflecting changes in peripheral insulin sensitivity (Antunes *et al*, 2016). Even though the HOMA equations can be considered a good predictor of total insulin-sensitivity care should be taken when extrapolating results to humans and vice versa more so that there are metabolic differences between humans and rodents, (Cacho *et al*., 2008).

Increases in serum iron level have been associated with damage to major tissues involved in glucose and lipid metabolism (pancreatic β cells, liver, muscle, and adipose tissue) and organs affected by chronic diabetic complications (Fernández-Real and Manco, 2014)). There is also some evidence that iron overload also affects skeletal muscle, the main effector of insulin action (Duffy *et al*., 2001). However, its exact mechanism of action is yet to be fully elucidated. In this study, skeletal muscle glycogen level was reduced compared to control suggesting either an increase in glycogen storage depletion or a reduction in the ability of the skeletal muscle in the iron induced oxidative stress group to store glucose as glycogen. The muscle glycogen level in the 40% and 80% UMP-diet groups had increased glycogen stores, which suggests that the dietary formulations at these percentages either facilitated an increase in glucose storage as glycogen or prevented the breakdown of glycogen stores in the skeletal muscle. These presumptions however need to be further investigated. It is likely that the phytochemical constituents of *M. paradisiaca* maybe facilitating the activity of skeletal muscle insulin receptors and thus facilitate the entry of glucose in to the cell for onward conversion to glycogen.

In summary, this study suggests that unripe *M. paradisiaca* rich diet may reduce iron induced glucose deregulations. It is likely the diet formulations causes reduced postprandial hyperglycemia and iron-induced oxidative stress as well as facilitate an increase in glucose storage as glycogen in the skeletal muscle.

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