ANTIDIABETIC, ANTIHYPERLIPIDEMIC, ANTIOXIDATIVE EFFECTS OF RIDA HERBAL BITTERS IN STREPTOZOTOCIN-INDUCED DIABETIC MALE WISTAR RATS

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ABSTRACT

This study aimed at investigating antidiabetic, antihyperlipidaemic and antioxidant profile of RIDA herbal bitters in diabetic male Wistar rats. Thirty-two (32) male Wistar rats were randomly divided into control and experimental groups of n = 8. The experimental rats were administered intraperitoneal single dose of freshly prepared streptozotocin (50 mg/kg b.w) dissolved in 0.1M citrate buffer (pH 4.5) after 12 h fasting period. On the establishment of diabetes, food consumption was monitored daily. Fasting blood glucose level and body growth rate were determined on 1st, 3rd and 7th day. Biomarkers of oxidative stress, lipid peroxidation markers kidney and liver function tests were determined post treatment. Pancreas were excised for histological evaluation. The administration of RIDA bitters significantly reduced (p<0.05) TC, LDL, TG, and significantly increase (p<0.05) HDL-C in the RIDA and GMP treated groups compared to STZ group. There were significant increases (p<0.01) in enzyme activity of SOD, GPx, GSH, CAT and significant reduction (p<0.01) of MDA concentration in RIDA treated group compared to STZ group comparable to GMP. Urea, uric acid, and creatinine concentrations were significantly reduced (p<0.01) after RIDA treatment compared to STZ group. In addition, AST, ALT, ALP were significantly (p<0.01) reduced in the RIDA group compared to STZ which were comparable to the GMP. Conclusion: This result indicates that RIDA bitters has antidiabetic, antihyperlipidemic and antioxidative properties that could be useful in the management of diabetes mellitus.
KEYWORDS: Diabetes mellitus; RIDA bitters; Hyperglycaemia; Oxidative stress; Lipid profile.

INTRODUCTION
Diabetes mellitus is a varied disorder usually with manifestations of hyperglycaemia and glucose intolerance. It is an endocrinological and/or metabolic disorder that arises due to lack of insulin and/or substandard insulin action, or action of both. The global prevalence and incidence of diabetes mellitus increases substantially. Diabetes mellitus is associated with the risk of cardiovascular, peripheral vascular and cerebrovascular diseases and several pathogenetic processes which involves destruction of pancreatic β-cells. Complications in
diabetes mellitus that includes the catabolism and anabolism of carbohydrates, lipids and proteins can be resulted from storage and mobilization of metabolic fuels stemmed from defective insulin secretion, insulin action, or both as a result of irrationalities in the regulatory systems.$^{[4,5]}$

Diabetes mellitus can be classified base on the cause and clinical presentation as; type I diabetes mellitus, type II diabetes mellitus, and gestational diabetes types.$^{[1]}$ Type I diabetes mellitus is known as a major type of diabetes affecting younger age groups which increases in both rich and poor countries with an imminent shift.$^{[1]}$ It is a condition accounted for a minority of the entire burden of diabetes in a population.$^{[1]}$ In Type II diabetes, there are about 85 to 95% increased varied disorder dominance in developing countries. This is caused by a combination of hereditary factors related to impaired insulin secretion, insulin resistance and environmental factors such as obesity, over eating, lack of exercise, aging and stress.$^{[6]}$

The epidemiological estimation of diabetes mellitus have been shown to affect 8.4% globally, and is predicted to rise to 9.9% by the year 2045.$^{[8]}$ More so, the effects of diabetes mellitus include various organ failures and progressive metabolic complications such as retinopathy, nephropathy, and/or neuropathy.$^{[9]}$

Results from Meta-analyses demonstrations shows that life style control including diet and physical activity is known as essential part in prevention of diabetes and cardiovascular disease.$^{[10]}$ It is shown to cause 63% decrease in diabetes occurrence in those at high risk.$^{[10]}$ It has been reported that dietary management proffer a progressive consequence on long term health and quality of life due to metabolic control (balance between food intake, physical activity, and medication) to avoid complications of diabetes mellitus. However, in type II diabetes, dietary management proffer appropriate objective including weight loss, enhanced glycemic and improved lipid levels.$^{[9]}$

In spite of the underscored importance of lifestyle measures in diabetes therapy, the value of pharmacotherapy to achieve target glucose concentrations cannot be escape by most diabetic patient. However, at the vital verge in diabetes mellitus, different oral hypoglycemic agents have been in use to aid the maintenance of blood glucose level through distinct mechanisms.$^{[11]}$ Several sulfonylureas and the nonsulfonylurea secretagogues have been reported to establish normal glucose level through variable endogenous insulin secretion, alpha-glucosidase inhibitors and thiazolidinediones (TZDs).$^{[12]}$ Metformin works by
decreasing hepatic gluconeogenesis while at times also increasing peripheral glucose mobilization and disposal. In the study of Curtis, (2007)\cite{12} it has been shown that 5-10% of the population experience secondary failure of the oral hypoglycemic agents available to manage diabetes. It is as a result of deteriorating beta cell function, poor compliance to treatment, weight gain, reduced exercise, dietary changes, or illness.\cite{12}

However, due to poor compliance to treatment of the conventional hypoglycemic agent, the use of plant derived medications have been shown to found immense practice in the management of diabetes mellitus\cite{13,14} in other to avoid the adversative effects associated with conventional hypoglycemic agents. The primary health care needs and the use of medicinal plants have been shown to increase immensely to about 80% of the world population. Studies have shown that many plant species have been used in management of life-threatening diseases including diabetes mellitus.\cite{13} In the study of Ogbonnia et al., (2010)\cite{15}, bitters including Angelica root (Angelica archangelica)\cite{16}, Senna leaves (Cassia senna)\cite{17}, Turmeric (Curcuma longa syn. C. domestica)\cite{18} and Aloe (Aloe vera syn. A. arbadensis)\cite{19} generally have been reported to avert kidney and bladder infections, regulation of blood pressure, dilation of arteries. It also helps to ease digestion, inhibit disorder like ulcers, gastritis, insomnia, stress and depression and thus overweight and excess body fat deterrence among others. In the studies of Bussmann et al., (2010)\cite{20}, the use of herbal medications have continually demonstrated an increase to about 60-80% of the world in developed and developing countries. It is used most as common form of alternative medicine.\cite{20}

RIDA herbal is a specially formulated concoction consisting of several herbs with synergistic and complimentary effects. RIDA herbal bitters are carefully made from a perfect combination of the finest herb and root extract Hunteria umbellate (9.18%), Colocynthis citrullus (24%), Uvaria chamae (9.41%), Curgulico pilosa (20.48%) and Senna alata (11.4%). Several studies have shown that number of diseases in the human body can be traced to a phenomenon referred to as Oxidative Stress\cite{21} which occurs when there’s an inequality between free radical activity than can be kept in balance by antioxidant activity in the body.\cite{21} The component of RIDA bitters have shown to manifest modifications in vast number of diseases over time traced to oxidative stress which causes damage to fatty tissue, DNA, and proteins in the body thus, leading to vast number of diseases over time such as diabetes, atherosclerosis, or hardening of the blood vessels, inflammatory conditions, heart disease and so on. Therefore, this study aimed at investigating the potential antidiabetic,
antihyperlipidemic and antioxidant safety and efficacy of RIDA herbal bitters in diabetes mellitus.

MATERIALS AND METHODS

ANIMALS
Thirty-two (32) male Wistar rats weighing 170 ± 30 g were used throughout the study. The animals were obtained from the animal house the Physiology Department, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. They were divided into four groups of eight (8) animals each in well-ventilated cages and were fed rat pelletised growers mash pellet and water ad-libitum. The animals were maintained under standardized conditions of 12h light/dark cycle, room temperature of 22-23°C, relative humidity 30-70% during the experiment. Two (2) weeks period of acclimatization was observed before the commencement of the experiment.

HERBAL BITTERS
RIDA herbal bitters (Marvel Natural Products Plc) was used throughout the study as a treatment drugs for the experimental treated subgroup. The bitters were purchased commercially at a store in Ogbomosho and were stored in the original package in a cool dry place below 30°C.

DRUGS AND CHEMICALS
Streptozotocin, STZ (Sigma Aldrich, Germany) obtained from Bridge Biotech Ltd; RC 1272466) was used for induction of diabetes. The reference drug Glimepiride 2mg tablets (May & Baker Nigeria Plc) was obtained from LAA-DEY Pharmacy, Ogbomoso, Oyo state, Nigeria. All chemicals used during the study were of analytical reagent grade.

ETHICAL CLEARANCE
The care and handling of rats were in accordance with the internationally accepted standard guidelines for use of animals. The study protocol was conducted as approved in compliance of institutional committee on animal care.

INDUCTION OF DIABETES
Rats were fasted overnight before induction of diabetes. A freshly prepared solution of STZ (50 mg/kg) in 0.1M cold citrate buffer (pH 4.5) was injected intraperitoneally for each experimental rat except for the control group. At 72hrs (3rd day) post-administration,
rats with stabilized diabetes, as indicated by a fasting blood glucose level of greater than 200 mg/dl, were selected for the study.

**TREATMENT PROTOCOL**

Treatment begins as hyperglycemia was confirmed by the elevated fasting glucose levels in blood determined at 72 h post i.p STZ injection. Treatment begins on the fourth (4th) day and continued for 7 days.

Rats with glucose levels >200 mg/dl were subdivided into three (3) subgroups. Those in the first subgroup remained without treatment and were considered diabetic. The second subgroup received the herbal bitters and was considered the RIDA -treated subgroup. Animals in the third subgroup were given glimepiride (May & Baker Nigeria Plc) 2mg/kg b.w and were considered the glimepiride-treated subgroup. The groupings for intervention are as follows:

**CONTROL:** Normal experimental rats on feed and water *ad libitum*

**STZ ONLY:** Diabetic rats administered Streptozotocin (50 mg/kg i.p) only

**STZ + RIDA:** Diabetic rats treated with RIDA herbal bitters (0.3ml p.o)

**STZ + GMP:** Diabetic rats treated with glimepiride (2mg/kg b.w p.o) as the reference drug

**DETERMINATION OF FASTING BLOOD SUGAR (FBS)**

Fasting blood glucose levels were determined using Accu-chek Active glucometer (Mannhelm, Germany). The blood glucose level of each animal was determined on the 1st, 3rd, and 10th days of the study. The animals were fasted 12hr over the night and given water *ad libitum*. Blood sample were collected by gentle prick on the tip of the animal tail. Drawn out blood were carefully drop onto the red field of the test strip inserted in the Accu-chek Active blood glucose meter (Mannhelm, Germany) and the result is read on 97.8 × 46.8 × 19.1 mm dimensional LCD display of the Accu-chek Active blood glucose meter.

**MONITORING OF BODY GROWTH RATE, DAILY WATER AND FEED CONSUMPTION**

The animals were individually weighed on the 1st and 3rd day 10th day of the experiment to record the changes in their body growth rates. A sensitive balance and restraining kits was used during the study. Animals were fed a known volume of water and growers rat chow pellet *ad libitum*. The daily feed remnants and water relic were collected and weighed using weighing scale and measuring cylinder respectively.
ANIMAL SACRIFICE AND TISSUE COLLECTION

On the 7th day post treatment, the animals were fasted overnight. Animals were sacrificed through cervical dislocation and blood samples was collected by cardiac puncture. Blood samples were transferred into lithium heparinised tubes and centrifuged at 3,500 rpm for 15min. The supernatant and plasma was separated and stored at a temperature of -4°C until it is required for assaying. Pancreas were harvested from each animal and fixed in a Bouin’s solution until it is required for histological assaying.

BIOCHEMICAL ASSAY

**Determination of Triglyceride, and Cholesterols**

Triglyceride level was determined by the method of Mochin and Leyva (1984).[25] The Serum triglyceride levels were measured by colorimetric enzymatic test using glycerol-3-phosphateoxidase[26] with Fortress reagent kits. The total cholesterol and HDL-cholesterol levels were determined by the method of De Hoff et al. (1978)[27] and LDL-cholesterol level was calculated by the method of Nauck et al. (2000). The serum cholesterol level was determined with a "CHOD-PAP" enzymatic photometric test[28] using Fortress reagent kits.

**Determination of Superoxide dismutase, Malondialdehyde (MDA), Glutathione (GSH), Glutathione peroxidase (GPx) and Catalase**

GPx activity was measured in accordance with the principle of Thomas et al., (1990)[29], Paglia and Valentine (1967)[30], and Pippenger et al., (1998)[31] to catalyze the oxidation of Glutathione (GSH) by cumene hydroperoxide. The level of plasma MDA was measured using Thiobarbituric Acid Reactive Substances (TBARS) as described by the principles of Armstrong and Browne (1994)[32], and Moore and Roberts, (1998).[33] The spectrophotometric procedures were used in GSH Assay and the procedure were based on the method of Ellman (Ellman, 1959)[34]. Catalase activity was determined by the method of Johannson and Borg (1988).[35]

**Determination of Urea, Uric acid, and Creatinine**

Serum urea was determined by colorimetric test[36] using Fortress reagent kits. Serum uric acid was measured by direct enzymatic assay[37] using Fortress reagent kits and Serum creatinine was determined by kinetic test without deproteinization[38] using Fortress reagent kits.
Determination of ALT, ALP, and AST
ALT, ALP, and AST was estimated in accordance with the Method recommended by the (IFCC, 1977; 1980).[39,40]

HISTOLOGICAL ANALYSIS
The Pancreas was excised out of each animal's body and were placed immediately in to their respective standard fixatives solution in a stoppered container for preservation until further process is required. The samples were then dehydrated, embedded (in paraffin), sectioned (with standard microtome) and stained (using Hematoxylin & eosin). The prepared slides were examined and photomicrographs were taken using a binocular microscope fixed with a microscope digital camera.

DATA ANALYSIS
All data were expressed as mean ± SEM (SEM: Standard error of mean). The data were analyzed using SPSS version 21.0 for Windows (Statistical Package for the Social Sciences Inc, Chicago, IL, USA). Means were compared by one-way analysis of variance (ANOVA), followed by Bonferroni post hoc test. A probability level less than 0.05 (p<0.05) were regarded as significant.

LEGEND
Fasting Blood Sugar Level of RIDA Treated Diabetic Wistar Rats
The Blood glucose level of STZ rats significantly increased (P< 0.05) when compared to control. There was significant reduction (P< 0.05) in the blood glucose level of RIDA treated rats when compared to STZ rats as presented in Figure 1.

Effects of RIDA bitters on Body Weight in Streptozotocin Induced Diabetic Wistar Rats
There was a significant reduction (P< 0.05) in body weight of STZ group compared to control. Administration of RIDA bitters for a period of 7 days resulted in significant increase in body weight of the RIDA treated rats when compared to STZ group as shown in Figure 2.

Estimation of Daily Water and Feed Intake in RIDA Treated Rats
Figure 3 shows water and feed intake in experimental rats. There was a significant (p<0.05) increase in water intake and significant (p<0.05) reduction in feed intake of the STZ rats compared to control. Administration of RIDA bitters resulted a significant reduction (p<0.05) in water intake and a significant (p<0.05) increase in feed consumption of the RIDA treated
group when compared with the diabetic group of rats which was comparable to the reference drug.

Effect of RIDA Bitters on Lipid Profile in Streptozotocin-Induced Diabetic Rats
Table 1 shows the effect of RIDA bitters on lipid profile in streptozotocin-induced diabetic rats. There were significant increases in total cholesterol (p<0.01), triglyceride (p<0.01) and low density lipoprotein concentrations (p<0.05) in the STZ group when compared to control. However, the high density lipoprotein concentration in the STZ group decreased significantly (p<0.05) compared to control. On administration of RIDA bitters, the high density lipoprotein concentration increased significantly while the total cholesterol (p<0.05), triglyceride (p<0.01) and low density lipoprotein (p<0.05) concentrations were significantly reduced in the RIDA group when compared to STZ, which was comparable to the GMP group.

Effects of RIDA bitters on Oxidative Stress Indices in Streptozotocin-Induced Diabetic Rats
The oxidative stress indices assessed in experimental rats were presented in Table 2. The superoxide dismutase activity, catalase level, glutathione peroxidase and reduced glutathione activity in the STZ group decreased significantly; malondialdehyde level were increased significantly at p<0.01 when compared to control. However, after treatment, there were significant increase in enzyme activity of glutathione peroxidase in the RIDA (p<0.05) and GMP (p<0.01) groups and superoxide dismutase, catalase and reduced glutathione levels increased significantly (p<0.01) in the RIDA and GMP treated groups when compared to STZ group. The MDA activity were found to significantly (p<0.01) reduced in the RIDA group compared to STZ, comparable to the GMP group.

Effects of RIDA Bitters on Markers of Liver Function in Diabetic Wistar Rats
There were significant (p<0.01) increases in aspartate aminotransferase, alkaline phosphatase and alanine transaminase in the STZ group compared to control as illustrated in Table 3. However, after treatment, apartate aminotransferase, alkaline phosphatase and alanine transaminase activities were significantly (p<0.01) reduced in the RIDA group compared to STZ, comparable to the GMP group.
Effects of RIDA bitters on some markers of kidney function in Streptozotocin-Induced Diabetic Rats

The markers of kidney function assessed in this study were presented in Table 4 below. The urea, uric acid and creatinine levels in the STZ group increased significantly (p<0.01) compared to control. However, there were significant (p<0.01) reduction in levels of urea, uric acid, and creatinine in the RIDA and GMP groups compared to STZ group after treatment.

Histological Study of Pancreas Section of Streptozotocin-Induced Diabetic Rats

There were many round and elongated islets in the photomicrograph of pancreas section of control rats stained by Haematoxylin and Eosin. The islets are evenly distributed throughout the cytoplasm with their nucleus slightly stain than the surrounding acinar cells seen as shown in plate 1 below.

Photomicrograph of a pancreas section stained by Haematoxylin and Eosin of diabetic rats is shown in Plate 2. The islets were damaged, shrunken in size and infiltration of lymphocytes was observed.

The plate 3 shows the photomicrograph of a pancreas section of diabetic RIDA treated rats. The islets were comparable to normal rats and there were reduction in shrinkage size of the islet although slight damage were observed.

In Plate 4 below, the pancreas architecture appears more or less like control. In this section, slight reduction in shrinkage size of the islet and minor damage were observed compared to diabetic rats.
FIGURES AND TABLES

Values are expressed as mean ± SEM (n=8). **- significant at p<0.01 compared with control, ##- significant at p<0.01 compared with STZ group.

LEGEND

CON- Control, STZ- Streptozotocin, RIDA- Rida bitters, GMP- glimepiride

Figure 1: Fasting blood sugar level in RIDA treated diabetic Wistar rats.

Values are expressed as mean ± SEM (n=8). *- significant at p<0.05 compared with control, #- significant at p<0.05 compared with STZ group.

LEGEND

CON- Control, STZ- Streptozotocin, RIDA- Rida bitters, GMP- glimepiride

Figure 2: Body weight in RIDA treated diabetic Wistar rats.
Values are expressed as mean ± SEM (n=8). *- significant at p<0.05 compared with control, #- significant at p<0.05 compared with STZ group.

LEGEND
CON- Control, STZ- Streptozotocin,
RIDA- Rida bitters, GMP- glimepiride,

Figure 3: Daily Food Consumption in RIDA Treated Diabetic Rats.

Values are expressed as mean ± SEM (n=8). *- significant at p<0.05 compared with control, #- significant at p<0.05 compared with STZ group.

LEGEND
CON- Control, STZ- Streptozotocin,
RIDA- Rida bitters, GMP- glimepiride,

Figure 4: Water intake in RIDA treated diabetic Wistar rats.
Table 1: Effects of RIDA Bitters on Lipid Profile in Streptozotocin-Induced Diabetic Rats.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CONTROL</th>
<th>STZ</th>
<th>RIDA</th>
<th>GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride (TG) (mg/dl)</td>
<td>20.1±2.0</td>
<td>106.0±8.3**</td>
<td>51.1±5.9##</td>
<td>38.1±3.5##</td>
</tr>
<tr>
<td>Total cholesterol (TC) (mg/dl)</td>
<td>66.7±8.1</td>
<td>119.3±23.7**</td>
<td>79.5±4.5*</td>
<td>66.4±2.4**</td>
</tr>
<tr>
<td>High density lipoproteins, HDL- cholesterol (mg/dl)</td>
<td>61.2±8.1</td>
<td>29.0±3.4*</td>
<td>32.0±1.7</td>
<td>44.4±5.7</td>
</tr>
<tr>
<td>Low density lipoproteins, LDL- cholesterol (mg/dl)</td>
<td>32.6±1.9</td>
<td>61.3±21.6*</td>
<td>37.2±2.4#</td>
<td>15.4±4.4##</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=8). *- significant at p<0.05 compared with control, **- significant at p<0.01 compared with control, #- significant at p<0.05 compared with STZ group; ##- significant at p<0.01 compared with STZ group.

LEGEND: STZ- Streptozotocin, RIDA- Rida bitters, GMP- glimepiride.

Table 2: Effects of RIDA Bitters on Antioxidant Enzymes and Oxidative Stress Parameters in Streptozotocin-Induced Diabetic Rats.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CON</th>
<th>STZ</th>
<th>RIDA</th>
<th>GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (µ/ml)</td>
<td>1.21±0.01</td>
<td>1.08±0.01**</td>
<td>1.34±0.01##</td>
<td>1.07±0.03</td>
</tr>
<tr>
<td>GPx (U/L)</td>
<td>9.00±1.21</td>
<td>1.87±0.27**</td>
<td>4.87±0.53*</td>
<td>9.00±0.80##</td>
</tr>
<tr>
<td>MDA (µM)</td>
<td>4.06±0.16</td>
<td>8.54±0.92**</td>
<td>3.21±0.39##</td>
<td>5.52±1.18##</td>
</tr>
<tr>
<td>GSH (mM)</td>
<td>3.96±0.54</td>
<td>2.80±0.13**</td>
<td>4.33±0.21##</td>
<td>4.28±0.25##</td>
</tr>
<tr>
<td>CAT (mol/ml/min)</td>
<td>53.1±2.83</td>
<td>28.4±0.32**</td>
<td>50.4±4.73##</td>
<td>53.4±3.23##</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=8). **- significant at p<0.01 compared with control, ##- significant at p<0.01 compared with STZ group.

LEGEND: CON- Control, STZ- Streptozotocin, RIDA- Rida bitters, GMP- glimepiride, SOD-Superoxide dismutase, MDA-Malondialdehyde, GSH-Glutathione, CAT-Catalase.

Table 3: Effects of RIDA Bitters on some markers of liver function in Streptozotocin-Induced Diabetic Rats.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CONTROL</th>
<th>STZ</th>
<th>RIDA</th>
<th>GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (U/L)</td>
<td>63.9±14.7</td>
<td>108.5±8.9</td>
<td>64.6±25.0</td>
<td>98.9±13.0</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>123.6±14.9</td>
<td>226.2±23.8**</td>
<td>142.1±7.5##</td>
<td>88.9±25.3##</td>
</tr>
<tr>
<td>ALP (mol/ml/min)</td>
<td>193.7±10.9</td>
<td>317.0±62.9**</td>
<td>153.2±16.0##</td>
<td>114.8±11.6##</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=8). **- significant at p<0.01 compared with control, ##- significant at p<0.01 compared with STZ group.

LEGEND: CON- Control, STZ- Streptozotocin, RIDA- Rida bitters, GMP- glimepiride, AST-Aspartate aminotransferase, ALT-Alanine Transaminase, ALP-Alkaline phosphatase.
Table 4: Effects of RIDA bitters on some markers of kidney function in streptozotocin-induced diabetic rats.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>CON</th>
<th>STZ</th>
<th>RIDA</th>
<th>GMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>UREA (mg/dl)</td>
<td>20.2±0.7</td>
<td>25.7±1.2**</td>
<td>21.7±1.1#</td>
<td>20.7±0.6##</td>
</tr>
<tr>
<td>URIC ACID (mg/dl)</td>
<td>3.6±0.25</td>
<td>5.0±0.45*</td>
<td>2.13±0.43##</td>
<td>3.09±0.38##</td>
</tr>
<tr>
<td>CREATININE (µmol/L)</td>
<td>20.5±3.62</td>
<td>143.5±25.4**</td>
<td>61.5±16.6##</td>
<td>107.6±16.6##</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=8). *- significant at p<0.05 compared with control, **- significant at p<0.01 compared with control, # - significant at p<0.05 compared with STZ group, ## - significant at p<0.01 compared with STZ group.

LEGEND: CON- Control, STZ- Streptozotocin, RIDA- Rida bitters, GMP- glimepiride.

Figure 5: Photomicrographs of H&E staining of pancreatic islets of normal rats (control), STZ, RIDA bitters treated and glimepiride treated rats (A, B, C and D respectively). Demarcated boundary (arrows) shows the observed islet cells in A, C and D when compared with B. In B, there is severe vacuolation and degranulation. C and D shows more noticeable islet pattern when compared with B. ×100

DISCUSSION

RIDA bitters have been described to contain *Hunteria umbellate* (9.18%), *Colocynthis citrullus* (24%), *Uvaria chamae* (9.41%), *Curgulico pilosa* (20.48%) and *Senna alata*
(11.4%) (Marvel Natural Products, Plc). The result of the phytochemical analysis of the bitters revealed the presence of antraquinones, flavonoids, saponin, terpenoids and alkaloids. However, the possible effects of this bitter on diabetes-induced oxidative stress are yet to be revealed. Thus, this study showed that RIDA bitters have antidiabetic, antihyperlipidemic and antioxidative effect.

In the present study, single intraperitoneal dose of STZ (50 mg/kg) in adult Wistar rats was effective initiating hyperglycaemia, thus manifesting the characterization of type 1 diabetes mellitus. The results obtained 4 days post administration of STZ shows significant (p<0.05) increase in plasma glucose levels (Fig. 1) in the STZ group of rats, therefore concluded a successfully created model as a result of the similarity supported by the findings of Sadeghi et al., (2017)\(^{41}\) and Sujithra et al., (2018).\(^{42}\)

The appropriate insulin function and essential energy sources have been attributed to indication of Blood glucose level.\(^{43}\) Thus, the insufficient insulin secretion or dysfunction of the signaling pathway results in a disturbance of glucose homeostasis. Subsequently, the body begins the use of other macromolecules such as lipids and proteins as energy source.\(^{44}\) This can further accompanied by a rapid weight loss in diabetic rats due to shrinking of muscle tissue.\(^{44,45}\) Data from the current study showed significant (p<0.05) reduction in fasting blood glucose in RIDA treated group when compared with diabetic rats (Figure 1). The diabetic rats treated with RIDA bitters shows clear regain of body weights (Figure 2) with an improved weight changes. The reduction in body weight in untreated diabetic rats might due to the hydrolysis of proteins into peptides and amino acids that oxidized when the cells could not absorb blood glucose for use as a metabolic energy source.\(^{46,47,48}\) Result from this findings are in agreement with the study of Adaramoye and Lawal (2013)\(^{49}\), who conveyed significant increase in Kolaviron treated rats when compared to the untreated diabetic counterparts. Also, the observed increase in the blood glucose level in the STZ groups may be a result of gluconeogenesis induction in the absence of insulin.\(^{50}\)

Lipids have been shown to play a vital role in the pathogenesis of diabetic mellitus.\(^{51,58}\) Result from this current study shows intense alterations in the plasma lipid, triglycerides and lipoprotein profile. Thus, there were significant increases in plasma triacylglycerol, total cholesterol, very low-density lipoprotein (VLDL) cholesterol and low-density lipoprotein (LDL) cholesterol, and significant decrease in high density lipoprotein (HDL) as shown in Table 1. Similar results were obtained in several studies in animal or experimental
The intense alterations in the plasma lipid, triglycerides and lipoprotein profile in the diabetic rats may be due to inactivation of lipoprotein lipase enzyme as a result of insufficient insulin actions and inability to degrade proteins and complex sugars to triglycerides. However, triacylglycerols, total cholesterol and LDL exhibited a significant reduction in RIDA treated rats and an upturn in HDL as shown in Table 1. This could be attributed to promoting an improved glycaemic control by a mechanism involving enhanced insulin action.\textsuperscript{55,56}

Diabetes has been shown to have an influence in the level of production and activity of the antioxidant enzymes.\textsuperscript{57,58} In the present study, there were significant reduction in SOD, CAT, and GPx activities in diabetic rats compared to the control rats. The observed reduction in antioxidant enzyme activities could be due to the oxidative inactivation of the enzyme by ROS or by the glycation of the enzymes.\textsuperscript{44,59} However, the administration of RIDA bitters influenced the activity of SOD, CAT, and GPx compared to the diabetic rats (Table 2). Furthermore, diabetes-induced oxidative stress is further supported by the elevated levels of Malondialdehyde (MDA). The increased levels are thus a sign of oxidative damage.\textsuperscript{60,61} It has been shown that LPO prompts disturbance of fine structures and functional loss of biomembranes which modifies low density lipoprotein (LDL) to proatherogenic and proinflammatory forms.\textsuperscript{60} The results clearly indicate a significant increased expression of MDA in the plasma of the diabetic rats when compared to the control. These results are in agreement with previous study of Adaramoye and Lawal, (2013).\textsuperscript{49} The findings from this present study indicate that diabetic rats treated with RIDA bitters attenuates the effect as observed through the decrease of MDA level to values close to standard of control rats (Table 3).

Aspartate aminotransferase, alanine aminotransferase, albumin and bilirubin are considered as part of liver toxicity markers.\textsuperscript{62} In this study, there were significant increases in ALP, AST and ALT activities in diabetic rats. Studies have been reported that the increased ALP, AST and ALT activities under insulin deficiency were responsible for the increased synthesis of glycogen from glucose and the metabolic breakdown of fatty acids in production of ketone bodies during diabetes as observed in this study.\textsuperscript{63} The increase in the activities of these enzymes in plasma of diabetic rats as shown (table 3) might be induced due to liver dysfunction. However, administration of RIDA bitters caused significant reduction in the
activities of ALP, ALT and AST in the plasma. This might consequently be due to lessening of liver damage caused by STZ– induced diabetic mellitus.\[^{64}\]

Current data revealed significant p<0.05 increases in plasma urea in streptozotocin-induced diabetic rats. A similar conclusion was recorded as previously reported in the study of Gawronska-Szklarz et al., (2003).\[^{65}\] The increase in plasma urea could be a result of improved protein catabolism and acceleration of amino acid deamination for gluconeogenesis. Similarly, plasma uric acid and creatinine were increased in diabetic rats. This could be imaginable defects in tubular reabsorption and probably decreased excretion may explain such increases in plasma uric acid and creatinine. Studies revealed that creatinuria follows starvation and poorly controlled diabetes condition associated with extensive muscle breakdown.\[^{66}\][73] However, RIDA treated group when compared with the diabetic rats’ shows a remedy that returned such changes in urea, uric acid, and creatinine towards normal as indicated in table 4. This may be enhancement of insulin secretion and improvement of metabolism both by pancreatic and extrapancreatic mechanisms.\[^{67,68}\]

The pancreatic tissue is shown to contribute an important role in metabolic regulation (Alese et al., 2013).\[^{69}\] In STZ groups, the histological analysis of the pancreas showed a cessation of micro-anatomical features including necrotic changes, shrinkage and condensation of β-cell nuclei, and intense formation of large membrane-bound vesicle (Plate 2) in the islet; thus showing normal structure of the pancreatic acinar epithelium, ductal and connective tissues. Also, fat and amyloid deposition occurs in the islets, and extreme reduction in the numbers of beta cells in diabetic rats was also noticed. Although slight damage were observed in the treated groups, but the islets of the RIDA treated rats was comparable to normal rats and there were reduction in shrinkage size of the islet as observed in the study of Alese et al., (2013).\[^{69}\]

**CONCLUSION**

Administration of RIDA bitters offered protection in terms of lipid profile, oxidative stress indices, liver and renal function, indicating that it has antidiabetic, antihyperlipidemic and antioxidative potential that can be of important in the management of diabetes mellitus. Further studies are required to be conceded out to seclude and ascertain the active ingredient(s) in RIDA as well to elucidate its potential effects claimed.
ACKNOWLEDGEMENT
The authors appreciate Marvel Natural Product Plc, for the opportunity given to carry out the research using their product (RIDA bitters).

Conflict of interest
The authors have no conflict of interest to declare.

REFERENCES


